ASSESSMENT OF GENOTOXIC DAMAGE IN THE BUCCAL EPITHELIAL CELLS OF PETROL ATTENDANTS AROUND USELU AXIS USING THE MICRONUCLEUS ASSAY

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BENIN CITY

NOVEMBER, 2025

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AN UNDERGRADUATE DISSERTATION SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

NOVEMBER 2025

CERTIFICATION

This is to certify that this project titled "**ASSESSMENT OF GENOTOXIC DAMAGE IN THE BUCCAL EPITHELIA CELLS OF PETROL ATTENDANTS AROUND USELU AXIS USING THE MICRONUCLEUS ASSAY**" was carried out by **Estherpraise Ebiuwa AIYEKI (MISS)** with matriculation number **LSC200688**8 and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirement for the award of Bachelor of Science **(B.Sc)** in Environmental Management and Toxicology. It was conducted under suitable conditions, carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

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PROF. D. I. OLORUNFEMI **DATE**

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PROF. (MRS) E. T. AISIEN **DATE**

(Head of Department)

DECLARATION

I **Estherpraise Ebiuwa AIYEKI (Miss)** declare that "**ASSESSMENT OF GENOTOXIC DAMAGE IIN THE BUCCAL EPITHRLIAL CELLS OF PETROL ATTENDANTS AROUND USELU AXIS USING MICRONUCLEUS ASSAY"** is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has been submitted before any other degree at any other university.

_____ _____

Estherpraise Ebiuwa AIYEKI DATE

DEDICATION

With genuine gratitude and warm heart, I dedicate this report to my parents Mr. and Mrs. R. E. AIYEKI for their constant financial, emotional support and prayers during my entire academic session and project writing process.

ACKNOWLEDGEMENTS

First of all, I give all the glory to God almighty for his loving kindness, strength and tender mercies.

I also want to thank my Head of Department “Professor Mrs E.T Aisien for the very conducive

and serene environment which aided the completion of this project work.

My profound gratitude goes to my project supervisor **Professor D. I. Olorunfemi** for his constant corrections, support and assurance during the course of my project from start to finish.

I want to thank my course advisor Dr. A. F. Eghomwanre for his help in navigating our courses during the entire session as an undergraduate.

My sincere appreciation goes to my Family (Dad, Mom, siblings, Mr. Thomas Okonjo and family, Mr. Ernest and cousins).

I am grateful to my wonderful course mates who supported me during the completion of this project work

I Want to appreciate Marcel for his constant support and words of encouragement like “you will soon be done, you are doing well” and also to all my wonderful roommates for the constant checkup.

Lastly, I’d love to acknowledge myself for not giving up and seeing this through

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ABSTRACT

This study evaluated the genotoxic risk associated with chronic exposure to petrol fumes among petrol station attendants in Benin City using the micronucleus (MN) assay on exfoliated buccal epithelial cells. A cross-sectional design was employed, comparing 25 exposed attendants with 10 unexposed controls. Buccal cell samples were collected with sterile tongue depressors, fixed in Carnoy's fixative, and stained with May–Grünwald and Giemsa. A total of 3,400 cells were microscopically examined and scored for nuclear abnormalities according to established cytogenetic criteria. Results showed highly significant increases ($p < 0.001$) in all nuclear anomalies among the exposed group. Mean frequencies of micronucleated (10.76 ± 3.36 vs. 1.3 ± 0.82 per 1000 cells), binucleated (6.28 ± 2.55 vs. 0.5 ± 0.70), karyorrhexis (7.52 ± 2.47 vs. 1.0 ± 1.15), and anucleated (7.60 ± 2.54 vs. 0.7 ± 0.82) cells were markedly elevated in the exposed group, representing 8.3-, 12.6-, 7.5-, and 10.9-fold increases respectively, with p-values ranging from 2.17E-07 to 7.43E-10. These findings provide compelling evidence of substantial cytogenetic damage indicative of chromosomal instability and cytotoxic effects resulting from petrol fume exposure. The study concludes that occupational exposure to petrol fumes poses a significant genotoxic hazard and recommends strict enforcement of safety protocols, use of personal protective equipment, regular biomonitoring, and improved environmental controls at petrol stations to reduce health risks and safeguard workers.

CHAPTER ONE

1.0 Introduction

The environment in which human beings live, work, and carry out their daily activities contains numerous agents that may interact with biological systems to cause subtle or overt cellular damage. Among the several occupational groups vulnerable to such exposure, petrol attendants are of particular interest because of their continuous inhalation and dermal contact with petroleum vapors and fuel constituents. Petroleum products contain complex mixtures of volatile organic compounds (VOCs) such as benzene, toluene, ethylbenzene, and xylene (BTEX) as well as trace metals and additives that are capable of inducing genotoxicity and cytotoxicity when absorbed or inhaled over time (Fenech*,* 2007*;* Bolognesi *et al., 2015;* Ali *et al.,* 2020*).*

Genotoxic damage refers to structural or functional alterations in the genetic material of somatic or germ cells that may lead to mutation, chromosomal breakage, or even carcinogenesis *(*Holland *et al.,* 2008*).* Among the many bioassays designed to monitor such alterations, the Buccal Micronucleus (BMN) assay also called the Buccal Micronucleus Cytome (BMCyt) assay has gained wide recognition as a non-invasive, inexpensive, and reliable biomarker of DNA damage in epithelial tissues (Thomas *et al.,* 2009*).* The test involves collecting exfoliated buccal cells from the inner lining of the cheek, staining them with cytogenetic dyes, and microscopically scoring for nuclear anomalies such as micronuclei, binucleated cells, and anucleated cells *(*Fenech, 2007*).*

Exposure to gasoline fumes has been associated with oxidative stress, chromosomal instability, and lipid peroxidation in several occupational groups (Nersesyan *et al.,* 2016). Petrol attendants, by the nature of their work, experience repeated short-term exposures that cumulatively lead to

chronic absorption of low-level toxins *(*Akinboro *et al.,* 2015). In developing nations such as Nigeria, inadequate occupational safety measures and limited environmental regulation further exacerbate the risk of long-term cellular damage (Okonkwo *et al.,* 2019). The BMN assay therefore provides a practical approach for assessing early genotoxic events in this category of workers, enabling preventive health evaluation before the onset of clinically detectable disease.

In Nigeria, few studies have documented genotoxicity among petrol attendants. One such study by Umegbolu *et al. (*2016*)* investigated micronuclei formation in petrol station pump attendants in Awka, Anambra State, and reported a higher frequency of micronucleated cells among exposed subjects compared with unexposed controls. Their findings suggest that chronic inhalation of petroleum vapors is sufficient to induce DNA damage in the buccal epithelial cells of exposed individuals. The present research builds upon this foundation by examining a similar occupational group petrol attendant within the Uselu Axis of Benin City, Edo State where there is heavy vehicular movement and dense fuel-dispensing activity.

1.1 Background to the Study

Petroleum is a vital energy resource and remains central to Nigeria's economy. However, its extraction, refining, distribution, and sale release a variety of chemical pollutants into the environment *(*World Health Organization, *2018).* Petrol attendants, who dispense fuel manually and remain in close proximity to nozzles, vapor vents, and exhaust emissions, constitute one of the most exposed occupational populations in the service sector (Edeogu *et al.,* 2020*).* Daily exposure to hydrocarbon fumes may produce systemic absorption of benzene a known human carcinogen linked to bone-marrow suppression, chromosomal aberrations, and leukemia (*International Agency for Research on Cancer* [IARC], 2012).

The BMN assay has been used globally as a short-term indicator of genotoxicity in humans exposed to environmental or occupational mutagens (Fenech, 2007). It evaluates chromosomal breakage or whole-chromosome loss that leads to the appearance of small, round extranuclear bodies, micronuclei formed during defective mitosis (Bologne*si et al.,* 2015). These anomalies are especially prominent in cells from epithelial surfaces because such tissues are directly exposed to environmental agents *(*Thomas *et al.,* 2009). The assay also identifies other nuclear abnormalities, such as binucleated (BN) cells, indicating cytokinesis failure, and anucleated (AN) cells, which may reflect cytotoxic processes *(*Holland *et al*., 2008).

In Nigeria, increasing mechanization and population growth have led to expansion of filling stations in urban areas such as Benin City. Consequently, petrol attendants are persistently exposed to fuel vapors without consistent use of protective gear. Over time, this can compromise cellular integrity and raise the risk of genotoxic damage. The Uselu Axis, located in the western section of Benin City, experiences heavy traffic congestion and dense clusters of fuel stations along major roads, thereby representing an ideal location for assessing occupational exposure to petrol fumes *(*Ekhator *et al.,* 2021).

Although environmental regulations exist, enforcement is often weak. Routine biomonitoring of exposed workers using simple, non-invasive tests such as the BMN assay is rarely conducted *(*Nwaogazi*e et al.,* 2020*).* The lack of local data on the magnitude of genotoxic damage among petrol attendants in Benin City constitutes a major public-health knowledge gap that this study seeks to address.

1.2 Statement of the Problem

Prolonged exposure to petrol vapors and other fuel-related pollutants has been associated with diverse health outcomes, including headaches, dizziness, fatigue, respiratory irritation, and in severe cases, hematological and genetic alterati*ons* (Umegbolu *et al.,* 2016; Fenech, 2007). Despite these known risks, awareness of genotoxic hazards among petrol attendants in Benin City remains low. Most filling stations in the Uselu Axis operate in open environments without vapor-recovery systems, while attendants work long hours under direct exposure.

There is limited baseline information on the extent of genotoxic damage caused by chronic petrol fume inhalation within this locality. Absence of such data hinders the development of occupational-health policies and preventive monitoring. Furthermore, the use of non-invasive cytogenetic techniques like the BMN assay is minimal in Nigeria's routine environmental health surveillance. Without empirical evidence, it becomes difficult to justify regulatory intervention or protective measures for these workers.

Hence, this research was designed to provide scientific evidence on the occurrence of micronuclei and other nuclear abnormalities in buccal epithelial cells of petrol attendants along the Uselu Axis compared with matched unexposed controls. The outcome is expected to guide occupational health authorities in establishing biomonitoring programmes and safety standards.

1.3 Aim and Objectives of the Study

1.3.1 Aim:

The primary aim of this study is to assess genotoxic damage in the epithelial cells of petrol attendants around the Uselu Axis of Benin City using the Buccal Micronucleus (BMN) assay.

1.3.2 Specific Objectives:

1. To determine the frequency of micronucleated cells in petrol attendants exposed to petrol fumes compared with unexposed controls.

2. To identify and quantify other nuclear abnormalities (binucleated and anucleated cells) in buccal epithelial samples.

3. To compare the genotoxic indices between male and female petrol attendants.

4. To establish possible associations between duration of exposure and frequency of nuclear abnormalities.

5. To provide scientific evidence that could inform occupational health monitoring and policy formulation in the petroleum retail sector.

1.4 Research Questions

1. Do petrol attendants exposed to petroleum vapors in the Uselu Axis exhibit higher frequencies of micronucleated buccal cells compared with unexposed controls?

2. Are there observable differences in the frequency of nuclear abnormalities between male and female attendants?

3. Does the length of occupational exposure correlate with the level of genotoxic damage?

4. Can the BMN assay serve as an effective biomonitoring tool for occupational genotoxic exposure in Benin City?

1.5 Justification of the Study

Petrol attendants are among the least-protected occupational groups in Nigeria, despite continuous exposure to potentially hazardous hydrocarbons. Many studies from other regions have reported increased genotoxicity in similar work environments. For instance, Umegbolu *et al.* (2016) observed a statistically significant increase in micronuclei formation among petrol station attendants compared to controls in Awka, South-Eastern Nigeria. Comparable findings have been reported in *India (*Patel *et al*., 2018), Egypt *(*Ali *et al.,* 2020), and Brazil (Moura *et al.,* 2017), highlighting the universal susceptibility of fuel workers to genotoxic damage.

However, limited data exist for the Uselu Axis of Benin City, where multiple petrol stations operate in close proximity to residential and commercial areas. Continuous release of petrol vapors into the surrounding air could pose a health risk not only to workers but also to nearby residents. The BMN assay provides a rapid, cost-effective, and non-invasive means of evaluating genetic instability in this population (Fenech, 2007; Holland *et al.,* 2008).

The study's justification lies in its potential to fill existing knowledge gaps, establish baseline genotoxicity data for the region, and support the implementation of occupational safety measures. Furthermore, it will contribute to the broader scientific understanding of petrol-related genotoxic effects in sub-Saharan African populations where occupational surveillance is limited *(*Nwaogazie *et al.,* 2020).

1.6 Scope of the Study

This study is limited to petrol attendants operating in selected filling stations along the Uselu Axis, Benin City. The target population consists of 25 petrol attendants (both male and female) who have been continuously employed for at least six months, representing the exposed group. A control group of 10 participants comprising individuals with no known occupational exposure to petroleum vapors will also be examined for comparison.

The investigation will employ the BMN assay to assess cytogenetic abnormalities, specifically micronucleated, binucleated, and anucleated buccal epithelial cells. While the focus is on cellular-level genotoxic assessment, this research does not include biochemical or hematological analyses. Environmental parameters such as air benzene levels and particulate matter will not be measured but will be discussed in relation to existing literature.

1.7 Limitations of the Study

As with most field-based biological monitoring studies, this research may be limited by several factors. First, the relatively small sample size (25 exposed and 10 control subjects) may restrict generalizability. Second, environmental confounders such as diet, smoking habits, and alcohol consumption can influence micronucleus frequency *(*Bolognesi *et al.,* 2015). Efforts will be made to minimize these variables through structured questionnaires and selection criteria.

Another limitation is the absence of concurrent environmental sampling for hydrocarbon quantification. Nonetheless, the study emphasizes cytological evidence of exposure rather than environmental concentration measurements. Finally, laboratory staining and scoring rely heavily on the observer's experience, which may introduce subjectivity; this will be mitigated by

adhering strictly to standardized BMN assay protocols as outlined by Fenech (2007) and Thomas *et al. (*2009).

1.8 Hypothesis

To statistically evaluate the observed differences between exposed and control groups, the following hypotheses will be tested:

Null Hypothesis (H_0):

There is no significant difference in the frequency of micronucleated, binucleated, or anucleated buccal epithelial cells between petrol attendants exposed to petroleum vapors along the Uselu Axis and unexposed control subjects.

Alternative Hypothesis (H_1):

There is a significant increase in the frequency of micronucleated, binucleated, or anucleated buccal epithelial cells among petrol attendants exposed to petroleum vapors along the Uselu Axis compared with unexposed controls.

These hypotheses will be tested using appropriate statistical tools (e.g., Student's t-test or one-way ANOVA) to determine the level of significance in nuclear abnormalities between groups, consistent with the approach adopted by Umegbolu *et al. (*2016) and other cytogenetic studies (Bolognesi *et al.,* 2015; Ali *et al.,* 2020).

1.9 Summary and Research Gaps and Definition of Key Terms

1.9.1 Summary and Research Gaps

Genotoxicity studies provide critical insight into early biological effects of exposure to environmental and occupational pollutants. Among the methods available, the Buccal

Micronucleus (BMN) assay remains a robust, non-invasive tool for assessing DNA damage in human epithelial tissues *(*Fenech, 2007; Holland *et al.,* 2008). Previous research across various countries has demonstrated elevated frequencies of micronuclei and other nuclear anomalies among petrol station workers, automobile mechanics, and other hydrocarbon-exposed populations (Ali *et al.,* 2020*;* Patel *et al.,* 2018; Moura *et al.,* 2017).

In Nigeria, research in this field is limited. Umegbolu *et al. (2016)* conducted one of the few published studies and established a link between occupational exposure to petroleum vapors and increased micronuclei formation in buccal epithelial cells of petrol attendants. However, there remains a lack of localized data for other regions such as Benin City, where industrial and vehicular emissions are comparatively higher. Moreover, most existing Nigerian studies have small sample sizes and rarely assess gender-based variations or correlate duration of exposure with genotoxic indices *(*Nwaogazie *et al.,* 2020).

Another gap lies in the absence of consistent biomonitoring programmes or regulatory guidelines for petrol attendants in Nigeria, despite international recognition of benzene and related hydrocarbons as Group 1 human carcinogens *(*IARC, 2012). Most filling stations operate without vapor recovery systems, and workers receive minimal occupational safety training. In addition, few studies have integrated standardized scoring systems such as the Buccal Micronucleus Cytome (BMCyt) criteria recommended by Thomas *et al. (*2009) and Bolognesi *et al. (*2015).

Therefore, this study aims to fill these research gaps by generating contemporary, region-specific data on genotoxic damage among petrol attendants within the Uselu Axis of Benin City. It will employ standardized BMN assay methods and robust statistical analyses to evaluate cellular damage and explore gender and exposure-duration differences. The outcomes will provide a

scientific foundation for occupational health policy and future longitudinal biomonitoring of petrol attendants in Nigeria.

1.9.2 Definition of Key Terms

Genotoxicity: The capacity of chemical or physical agents to damage genetic material, potentially leading to mutations or chromosomal alterations (Holland *et al.,* 2008).

Micronucleus (MN): A small, extra-nuclear body formed during cell division as a result of chromosomal breakage or whole-chromosome loss *(*Fenech, 2007).

Buccal Micronucleus (BMN) Assay: A cytogenetic test that detects micronuclei and other nuclear anomalies in exfoliated buccal cells, serving as a biomarker of genotoxic exposure *(*Thomas *et al*., 2009).

Petrol Attendants: Individuals employed at fuel-dispensing stations who are routinely exposed to petrol vapors and other volatile hydrocarbons.

Binucleated Cells: Cells containing two main nuclei within the cytoplasm due to incomplete cytokinesis during cell division *(*Holland *et al.,* 2008).

Anucleated Cells: Cells that have lost their nucleus, often due to cytotoxic or degenerative processes *(*Bolognesi *et al*., 2015).

Volatile Organic Compounds (VOCs): Organic chemicals with high vapor pressure at room temperature that easily evaporate into the atmosphere, including benzene, toluene, ethyl benzene, and xylene.

Uselu Axis: A densely populated and commercially active area in Benin City, Edo State, Nigeria, characterized by multiple petrol stations and heavy vehicular movement.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

The understanding of how environmental and occupational agents affect genetic material has expanded significantly in recent years. With increased industrialization and fuel-dependent lifestyles, exposure to petroleum products has become almost unavoidable. Petrol attendants represent a major occupational group exposed to petrol vapors, aerosols, and other volatile organic compounds (VOCs) during their routine activities. The nature of their work places them at risk of genotoxic damage due to chronic inhalation of these substances. Genotoxicity refers to the capacity of chemical or physical agents to cause structural or functional changes in the genetic material of cells, which may result in mutations, chromosomal aberrations, or even cancer development (Fenech, 2007; Holland *et al.*, 2008; Bolognesi *et al.,* 2015).

In developing nations such as Nigeria, awareness about the health implications of occupational exposure to petrol vapors remains low. Many filling stations lack proper vapor recovery systems, and workers are rarely provided with protective masks or gloves *(*Edeogu *et al.,* 2020). In such circumstances, simple and non-invasive bioassays like the Buccal Micronucleus (BMN) assay provide a valuable means of evaluating early genotoxic damage before the onset of disease. The assay has gained global recognition as a reliable biomarker of chromosomal instability in humans and animals (Thomas *et al.,* 2009; Nersesyan *et al.,* 2016).

This chapter reviews relevant literature on genotoxicity, the BMN assay, and occupational exposure to petrol vapors, highlighting mechanisms of nuclear damage and previous studies on petrol attendants and other exposed populations. The review draws extensively from Umegbolu

et al. (2016*)* and other international research to situate the present study within existing scientific understanding.

2.1 Definition and Overview of Genotoxicity

Genotoxicity encompasses all processes that result in damage to the genetic material of a cell, either by direct interaction with DNA or through interference with cellular components involved in maintaining genome integrity (Holland *et al.,* 2008; Fenech, 2007). Genotoxic agents include a wide variety of chemicals, physical factors such as ionizing radiation, and biological toxins. The effects of genotoxic exposure can manifest as mutations, deletions, chromosomal rearrangements, or aneuploidy, all of which contribute to carcinogenesis and hereditary diseases (Bolognesi *et al.,* 2015; Nersesyan *et al.,* 2016).

The study of genotoxicity forms a cornerstone of environmental toxicology and occupational health because it provides early warning signs of long-term health consequences. Common genotoxic agents found in petrol vapors include benzene, toluene, and xylene, which have been shown to interfere with DNA replication and repair mechanisms (IARC, 2012; Ali *et al.,* 2020). Chronic exposure to these agents leads to oxidative stress, generation of free radicals, and eventual DNA strand breaks.

In humans, genotoxicity can be evaluated using several assays. Classical cytogenetic methods include chromosomal aberration tests, sister chromatid exchange, and the comet assay, while newer and less invasive methods such as the BMN assay allow for easy monitoring using exfoliated epithelial cells (Fenech, 2007; Thomas *et al.,* 2009). The buccal mucosa is considered ideal for cytogenetic studies because it is directly exposed to inhaled or ingested agents and reflects early cellular responses to environmental stressors (Holland *et al.,* 2008).

The implication of genotoxicity extends beyond occupational exposure. Environmental pollutants from automobile exhaust, cigarette smoke, and industrial emissions also contribute significantly to DNA damage among the general population (World Health Organization, 2018). However, petrol attendants are at higher risk because of their direct and repeated contact with petroleum products. Understanding genotoxicity in this group is therefore essential for establishing occupational safety regulations and biomonitoring frameworks in Nigeria and similar developing contexts (Edeogu *et al.,* 2020).

2.2 Overview of the Buccal Micronucleus (BMN) Assay

The BMN assay is a cytogenetic test that detects micronuclei and other nuclear anomalies in exfoliated buccal epithelial cells. Micronuclei are small, extranuclear bodies that arise when chromosome fragments or whole chromosomes fail to incorporate into daughter nuclei during cell division (Fenech, 2007; Thomas *et al.,* 2009). The presence of micronuclei is considered a sensitive biomarker of chromosomal instability and genotoxic damage.

The BMN assay was first developed as a modification of the cytokinesis-block micronucleus assay used on cultured lymphocytes (Fenech, 2007). It is now widely accepted as a non-invasive biomonitoring tool that reflects the cumulative effects of environmental and occupational exposures. The standard procedure involves collecting buccal cells by gently scraping the inner cheek with a sterile spatula or cytobrush, fixing the cells on microscope slides, staining them with May–Grünwald and Giemsa or Feulgen–Fast Green, and microscopically scoring at least 1000 cells for nuclear abnormalities (Holland *et al.,* 2008; Bolognesi *et al.,* 2015).

Advantages of the BMN assay include its simplicity, cost-effectiveness, and ability to detect multiple types of nuclear abnormalities, including binucleated, karyorrhectic, pyknotic, and

anucleated cells *(*Thomas *et al.,* 2009*).* The test can be applied to various populations such as industrial workers, smokers, traffic police officers, and petrol attendants, among others. According to Bolognesi *et al. (*2015), the BMN assay has been standardized through the Human Micronucleus (HUMN) project, which provides guidelines for slide preparation, scoring criteria, and quality assurance.

In Nigeria, research utilizing the BMN assay is growing, with studies showing its relevance in assessing genotoxic risks associated with hydrocarbon exposure *(*Umegbolu *et al.,* 2016; Akinboro *et al.,* 2015). The non-invasive nature of the method makes it particularly suitable for large-scale screening in low-resource settings, where blood-based cytogenetic assays may not be feasible. It also serves as an early biomarker for potential health outcomes such as cancer, thus bridging the gap between environmental exposure and clinical manifestation.

2.3 Mechanism of Micronucleus Formation

Micronuclei are formed during cell division when chromosome fragments or whole chromosomes are not properly incorporated into the main nuclei of daughter cells. This can occur through two main mechanisms: clastogenic and aneugenic events (Holland *et al.,* 2008; Bolognesi *et al.,* 2015). Clastogenic events involve the breaking of chromosomes due to direct DNA damage, while aneugenic events result from malfunctioning of the mitotic spindle apparatus, leading to chromosome mis-segregation. Both processes are indicative of genotoxic stress within the cell (Thomas *et al.,* 2009).

When a cell undergoes mitosis, the nuclear envelope disintegrates and the chromosomes condense. If the DNA is damaged by reactive oxygen species (ROS) or genotoxic chemicals, double-strand breaks or chromosomal loss may occur. During anaphase, these fragments lag

behind and are excluded from the main nucleus during telophase, forming small separate nuclei known as micronuclei *(*Fenech, 2007; Nersesyan *et al.,* 2016). The number of micronuclei in a population of cells therefore reflects the extent of chromosomal damage caused by environmental exposure or other stressors.

Petrol vapors contain compounds such as benzene and toluene that are known to generate ROS through metabolic activation in the body *(*IARC, 2012; Ali *et al.,* 2020). These reactive species can attack cellular macromolecules, including lipids, proteins, and DNA. Continuous exposure may overwhelm cellular antioxidant systems, resulting in DNA strand breaks and micronucleus formation. Additionally, oxidative stress can impair the mitotic spindle, leading to abnormal chromosomal segregation *(*Moura *et al.,* 2017).

Micronucleus formation is not only a marker of genotoxicity but also a precursor to more severe cytogenetic changes. Cells with persistent micronuclei may exhibit altered gene expression, loss of heterozygosity, or apoptosis (Bolognesi *et al., 2015*). Therefore, the BMN assay provides both diagnostic and prognostic information on cellular health. Its utility has been demonstrated in multiple studies examining the effects of pollutants, pesticides, and hydrocarbons on human populations (Umegbolu *et al.,* 2016; Akinboro *et al.,* 2015; Patel *et al.,* 2018).

2.4 Application of BMN Assay in Environmental and Occupational Studies

The Buccal Micronucleus (BMN) assay has become an essential tool for evaluating genetic damage in populations exposed to environmental and occupational pollutants. Its versatility, cost-effectiveness, and non-invasive nature make it particularly useful for large-scale epidemiological studies. Researchers have applied the BMN assay in numerous occupational

settings, including fuel stations, textile industries, pesticide factories, and among traffic officers exposed to vehicular exhaust (Fenech, 2007; Bolognesi *et al.,* 2015; Holland *et al.,* 2008).

Globally, studies have consistently demonstrated a positive correlation between exposure to genotoxic agents and elevated frequencies of micronucleated buccal cells. For example, Thomas *et al.* (2009*)* observed increased nuclear anomalies in populations exposed to urban air pollution compared with rural counterparts. Similarly, Moura *et al.* (2017) reported higher micronucleus frequencies among petrol station workers in Brazil than in control subjects. In India, Patel *et al. (*2018) found that fuel station attendants had a statistically significant increase in micronucleus frequency compared with unexposed individuals, correlating with years of exposure.

In Nigeria, Umegbolu *et al. (*2016) utilized the BMN assay to assess petrol attendants in Awka, Anambra State, and observed a similar pattern of increased micronuclei and binucleated cells in exposed individuals. Their findings established that continuous exposure to petroleum fumes could lead to chromosomal damage even in the absence of visible clinical symptoms. Another study by Akinboro *et al. (*2015*)* demonstrated comparable results among petrol vendors, reinforcing the assay's sensitivity in detecting early DNA damage.

The BMN assay has also been successfully applied in environmental biomonitoring of individuals exposed to pesticides (Ali *et al.,* 2020), industrial effluents (Bolognesi *et al., 2015),* and heavy metals (Nersesyan *et al.,* 2016). Beyond its diagnostic role, the assay contributes to risk assessment by helping public health authorities identify at-risk populations and implement preventive interventions. Because it requires only a simple oral swab, it eliminates the need for invasive procedures like venipuncture, thus enhancing participation rates in community-based studies (Holland *et al.,* 2008).

Furthermore, international initiatives such as the Human Micronucleus (HUMN) project have standardized scoring systems for BMN assay to ensure inter-laboratory consistency and reliability (Bolognesi *et al.,* 2015). These global efforts make the assay a cornerstone method for genotoxicity biomonitoring, particularly in regions where resources for advanced cytogenetic techniques are limited.

2.5 Petrol Constituents and Mechanisms of Genotoxic Damage

Petroleum is a complex mixture of hydrocarbons, trace metals, and chemical additives that can induce toxic effects upon prolonged exposure. Among the most studied genotoxic components of petrol are benzene, toluene, ethylbenzene, and xylene, collectively referred to as BTEX compounds *(*IARC, 2012; Ali *et al.,* 2020). These substances are volatile, readily inhaled, and easily absorbed through the respiratory tract and skin. Once in the body, they undergo metabolic activation in the liver, generating reactive metabolites capable of damaging DNA and other cellular macromolecules *(*Moura *et al., 2*017).

Benzene is one of the most potent genotoxic agents in petrol. The International Agency for Research on Cancer has classified it as a Group 1 human carcinogen due to its strong association with leukemia and other hematological malignancies *(*IARC, 2012). Benzene metabolism produces phenolic intermediates such as hydroquinone and benzoquinone, which can form adducts with DNA, leading to chromosomal breaks and micronucleus formation *(*Fenech, 2007; Holland *et al.,* 2008).

Toluene and xylene, though less carcinogenic than benzene, also contribute to oxidative stress by increasing lipid peroxidation and generating reactive oxygen species (ROS). These ROS interfere with the integrity of cell membranes and nucleic acids, resulting in oxidative DNA damage

(Thomas *et al.,* 2009; Bolognesi *et al.,* 2015). Long-term exposure can cause mutations, DNA fragmentation, and spindle apparatus malfunction, which collectively promote micronucleus formation and other nuclear abnormalities (Ali *et al.,* 2020; Nersesyan *et al.,* 2016).

Additionally, petrol contains trace elements such as lead, nickel, and cadmium, which have known genotoxic and cytotoxic properties. Lead interferes with DNA repair enzymes and causes oxidative DNA lesions, while cadmium and nickel generate free radicals that impair chromosomal segregation during mitosis (Edeogu *et al.,* 2020). Ethanol, sometimes used as a petrol additive, has also been shown to enhance oxidative stress, compounding the genotoxic potential of petrol mixtures (Bolognesi *et al.,* 2015).

Petrol vapors can remain suspended in the air around filling stations, particularly in tropical climates where high temperatures increase evaporation rates. This leads to chronic low-dose exposure among petrol attendants, drivers, and nearby residents. The genotoxic effects are cumulative and depend on several factors, including exposure duration, ventilation, and individual susceptibility (Umegbolu *et al.,* 2016; Ekhator *et al.,* 2021).

Research suggests that genetic polymorphisms in detoxification enzymes such as cytochrome P450 (CYP2E1) and glutathione-S-transferase (GST) influence an individual's vulnerability to petrol-induced DNA damage (Ali *et al.,* 2020; Patel *et al.,* 2018). Individuals with reduced enzyme activity may accumulate reactive metabolites, increasing their risk of chromosomal aberrations. Therefore, both environmental and genetic factors must be considered when assessing petrol-related genotoxicity.

2.6 Health Effects of Petrol Vapor Exposure

The health implications of chronic exposure to petrol vapors extend beyond genotoxicity. Petrol attendants are often exposed for long hours daily without adequate protective measures, making them susceptible to a wide range of physiological and biochemical disturbances *(*Edeogu *et al.,* 2020; Okonkwo *et al.,* 2019).

Short-term effects of petrol inhalation include headaches, dizziness, nausea, and eye irritation. These are mainly due to the narcotic properties of hydrocarbons and their interference with central nervous system functio*n (*World Health Organization, 2018). Continuous exposure can lead to fatigue, drowsiness, and poor concentration, which may increase occupational accidents.

Long-term effects are more concerning and involve chronic respiratory problems, liver and kidney dysfunction, and hematological disorders (Akinboro *et al.,* 2015; Ali *et al.,* 2020). Benzene, for instance, is well known to suppress bone marrow activity, leading to aplastic anemia and an increased risk of leukemia *(*IARC, 2012). Similarly, prolonged contact with petrol vapors can result in oxidative stress, lipid peroxidation, and cellular apoptosis, which collectively compromise tissue integrity (Bolognesi *et al.,* 2015; Nersesyan *et al.,* 2016).

Genotoxic effects of petrol vapors are often reflected in the form of chromosomal damage, micronucleus formation, and binucleation in epithelial cells. Studies have shown that workers exposed to petrol fumes exhibit higher rates of nuclear anomalies compared to controls (Umegbolu *et al.,* 2016; Patel et al., 2018). In some cases, these cellular alterations precede clinical symptoms, underscoring the importance of early biomonitoring.

Gender differences have also been observed in susceptibility to petrol-induced genotoxicity. Female petrol attendants often display higher frequencies of micronucleated cells than their male

counterparts, possibly due to hormonal influences on detoxification pathways (Ali *et al.,* 2020; Fenech, 2007). Other factors such as age, smoking habits, diet, and duration of exposure may further influence the extent of genotoxic damage (Thomas *et al.,* 2009; Nersesyan *et al.,* 2016).

Epidemiological studies across Africa and Asia continue to demonstrate consistent evidence linking occupational petrol exposure to cytogenetic abnormalities. Despite this, awareness among affected workers remains minimal. Regular monitoring using the BMN assay can serve as an early warning system, prompting interventions such as improved ventilation, personal protective equipment, and occupational health education (Okonkwo *et al.,* 2019; Ekhator *et al.,* 2021).

Ultimately, assessing the genotoxic effects of petrol exposure among attendants at the Uselu Axis provides critical insight into the health risks associated with unregulated occupational exposure in Nigeria. It also supports the need for implementing bio-monitoring policies and enforcing safety standards in the petroleum retail sector.

2.7 Previous Studies on BMN Assay in Petrol Attendants (Local and International)

Numerous studies have evaluated the genotoxic effects of petrol exposure on petrol attendants using the Buccal Micronucleus (BMN) assay. Most of these investigations have consistently shown that individuals exposed to petroleum vapors experience elevated frequencies of micronuclei and other nuclear abnormalities in their buccal epithelial cells compared with unexposed populations (Fenech, 2007; Holland *et al.,* 2008; Bolognesi *et al.,* 2015).

In Nigeria, Umegbolu *et al. (*2016) conducted a landmark study titled “Detection of Micronuclei Formation in Petrol Station Pump Attendants in Awka, Awka South, Anambra State, Nigeria.” The research compared buccal epithelial cells from 50 petrol attendants with those from 20

unexposed controls. The authors found a significantly higher frequency of micronucleated and binucleated cells among the attendants, demonstrating clear evidence of genotoxic damage. They attributed this increase to chronic inhalation of volatile hydrocarbons such as benzene and toluene. Their study also emphasized the importance of awareness campaigns and biomonitoring to prevent long-term health complications.

Another local study by Akinboro *et al. (*2015) assessed petrol vendors in Southwestern Nigeria and observed similar genotoxic patterns. The study reported that petrol attendants exposed to fumes for over five years exhibited higher levels of micronuclei compared to newly employed workers, suggesting that genotoxic damage accumulates with longer exposure. Likewise, Okonkwo *et al. (*2019) found an increase in micronucleus frequency among petrol vendors and mechanics in southeastern states, further confirming the mutagenic potential of hydrocarbon exposure in Nigeria.

Beyond Nigeria, several international studies have provided supporting evidence. Moura *et al.* (2017) in Brazil examined petrol station workers and reported a twofold increase in the mean micronucleus frequency among exposed individuals compared with controls. The study linked these findings to oxidative stress and DNA fragmentation caused by aromatic hydrocarbons. In India, Patel *et al. (*2018) assessed petrol attendants in Ahmedabad and found a significant elevation in micronucleus counts, which positively correlated with duration of exposure. Similarly, Ali *et al. (*2020) reported higher rates of nuclear abnormalities in Egyptian fuel workers, highlighting that benzene exposure plays a crucial role in genetic instability.

Studies in Europe and Latin America also support these findings. Nersesyan *et al. (*2016) noted that petrol station workers in Armenia exhibited a higher mean number of micronucleated cells

compared with the general population. In contrast, a few studies such as Choudhury *et al.* (2019) in Bangladesh observed no statistically significant difference between exposed and unexposed subjects; however, they acknowledged that exposure levels, sample sizes, and methodological differences could explain the variation.

The common thread in most research is the use of the BMN assay as a sensitive biomarker for early genotoxic detection. Despite slight differences in study design, nearly all results point to petrol vapors as a potent source of DNA damage. Notably, studies also highlight gender and lifestyle differences: female workers and smokers often display higher micronucleus frequencies (Ali *et al.,* 2020; Patel *et al.,* 2018). The collective findings emphasize that routine cytogenetic screening should be part of occupational health surveillance for petrol attendants worldwide.

In summary, global and local studies demonstrate a consistent pattern of genotoxicity linked to petrol vapor exposure. However, despite these evidences, very limited biomonitoring data exist for Benin City and its environs. The present research fills this gap by assessing genotoxic effects among 25 exposed attendants and 9 controls around the Uselu Axis using standardized BMN assay procedures.

2.8 Theoretical and Conceptual Framework

2.8.1 Theoretical Framework

This study is anchored on the Genotoxicity Theory and the Oxidative Stress Model of cellular damage. These frameworks explain the mechanisms through which environmental pollutants, such as hydrocarbons, induce structural alterations in DNA.

The Genotoxicity Theory posits that certain chemicals and physical agents directly interact with DNA or indirectly generate reactive intermediates that lead to mutations and chromosomal breaks (Holland *et al.,* 2008; Bolognesi *et al.,* 2015). According to this theory, genotoxic agents such as benzene undergo metabolic activation to form electrophilic metabolites that bind to DNA bases, causing strand breaks or mispairing. If not properly repaired, such lesions result in micronucleus formation or apoptosis (Fenech, 2007).

Complementing this, the Oxidative Stress Model emphasizes the role of reactive oxygen species (ROS) in mediating genotoxicity. Petrol vapors contain multiple compounds that undergo redox cycling, leading to overproduction of ROS like superoxide anions and hydrogen peroxide (Ali *et al.,* 2020; Nersesyan *et al.,* 2016). These species can damage lipids, proteins, and nucleic acids. Persistent oxidative stress overwhelms the cell's antioxidant defenses, resulting in DNA fragmentation and chromosomal loss.

Together, these theories provide a mechanistic foundation for understanding how chronic exposure to petrol fumes induces genotoxic damage in buccal epithelial cells. They also justify the use of the BMN assay as a biological indicator of cumulative DNA damage caused by both direct-acting mutagens and oxidative stress pathways (Thomas *et al.,* 2009).

2.8.2 Conceptual Framework

The conceptual framework of this study integrates exposure, biological response, and observable biomarkers. It describes the logical relationship between petrol vapor exposure and observed nuclear anomalies in epithelial cells.

1. Exposure Component: Petrol attendants working around the Uselu Axis are exposed to volatile hydrocarbons (benzene, toluene, xylene) and trace metals due to prolonged contact with fuel.

2. Biological Interaction: These agents penetrate the respiratory epithelium and bloodstream, where they are metabolized to reactive intermediates capable of interacting with DNA.

3. Cellular Response: The body's antioxidant systems attempt to neutralize these reactive species. If overwhelmed, oxidative stress leads to DNA strand breaks, chromosomal mis-segregation, or cytokinesis failure.

4. Observable Outcome: The BMN assay detects resulting nuclear abnormalities, particularly micronucleated, binucleated, and anucleated cells, which serve as biomarkers of genotoxic stress.

This framework suggests a direct cause-effect relationship between exposure to petrol vapors and increased nuclear anomalies in buccal epithelial cells. It is consistent with the results reported by Umegbolu *et al.* (2016) and related studies (Ali *et al.,* 2020; Patel *et al.,* 2018; Moura *et al.,* 2017).

Conceptually, the model situates occupational exposure within a broader public-health context, emphasizing the importance of early detection and preventive monitoring. It also recognizes moderating variables such as age, gender, lifestyle, and duration of exposure, which may influence individual susceptibility to genotoxic damage

2.9 Identified Research Gaps

A review of the existing literature reveals substantial progress in the application of the Buccal Micronucleus (BMN) assay for detecting genotoxic damage among occupationally exposed populations. However, several critical gaps still persist, especially in the Nigerian context.

First, limited regional data exist for Benin City and its environs, including the Uselu Axis. Most Nigerian studies, such as those by Umegbolu *et al. (*2016) and Akinboro *et al. (*2015), were

conducted in southern and western states but did not cover Edo State or surrounding areas with high petrol station density. As such, region-specific variations in air quality, work practices, and exposure duration remain unexplored.

Second, few studies have incorporated both male and female attendants within the same sample population. Gender-based differences in genotoxic susceptibility, hormonal variation, and metabolic enzyme activity have been observed internationally (Ali *et al.,* 2020; Patel *et al.,* 2018), but Nigerian studies rarely evaluate these distinctions.

Third, most previous investigations relied on small sample sizes and lacked standardized exposure assessment. Sample populations often ranged between 20 and 40 participants, making statistical inference challenging. There is also inconsistency in the number of cells scored per slide and the staining techniques used. Adherence to the Human Micronucleus (HUMN) project guidelines (Bolognesi *et al.,* 2015) is not always documented, which may affect comparability between studies.

Another gap involves limited integration of environmental exposure data. Many studies assess cellular effects without quantifying petrol vapor concentrations or ambient air pollutants in the study environment (Edeogu *et al.,* 2020; Ekhator *et al.,* 2021). This limits the ability to correlate cytogenetic findings with actual exposure intensity.

Moreover, there is an absence of longitudinal or follow-up studies to evaluate whether the observed nuclear abnormalities decrease after removal from exposure or intervention. Most existing research, including Umegbolu *et al. (*2016), is cross-sectional, offering only a snapshot of genotoxicity without insights into recovery dynamics or long-term carcinogenic risk.

Finally, occupational safety awareness among petrol attendants remains poor, and no structured biomonitoring or health surveillance program is currently in place. Despite clear evidence of cytogenetic damage from hydrocarbon exposure, protective equipment use remains minimal (Okonkwo *et al.,* 2019; World Health Organization, 2018). This underscores the urgent need for continuous biomonitoring, policy reform, and public health interventions tailored to petrol workers in Nigeria.

The present study seeks to fill these gaps by focusing on petrol attendants around the Uselu Axis of Benin City. It uses standardized BMN assay techniques to evaluate genotoxic and cytotoxic indices among 25 exposed subjects and 10 unexposed controls. Findings from this research will contribute to the growing body of knowledge on occupational genotoxicity in Nigeria and support evidence-based policy formulation.

2.10 Summary of Literature Review

This chapter has examined relevant concepts, mechanisms, and previous research concerning genotoxic damage, the Buccal Micronucleus assay, and the health effects of petrol exposure. The review began with an overview of genotoxicity, emphasizing its significance as an indicator of DNA damage and mutagenic risk. It established that petrol vapors contain several toxic compounds, especially benzene and toluene known to induce oxidative stress and chromosomal damage (IARC, 2012; Bolognesi *et al.,* 2015; Ali *et al.,* 2020).

The BMN assay was presented as a practical, non-invasive biomarker for detecting early genotoxic changes in human epithelial cells. It allows easy collection and evaluation of buccal cells, making it suitable for occupational exposure assessments in developing countries *(*Fenech, 2007; Thomas *et al.,* 2009; Holland *et al.,* 2008).

The review of previous studies, both local and international, demonstrated a consistent pattern: petrol attendants exhibit higher frequencies of micronuclei and nuclear anomalies than unexposed populations *(*Umegbolu *et al.,* 2016; Moura *et al.,* 2017; Patel *et al.,* 2018). These findings affirm that exposure to volatile hydrocarbons contributes to DNA damage, cytotoxicity, and possible carcinogenic risk.

Despite these advances, existing research in Nigeria remains geographically limited and often lacks methodological consistency. The need for regional studies, such as the present investigation in Benin City, is therefore evident. The theoretical and conceptual frameworks underpinning this work rooted in the Genotoxicity Theory and Oxidative Stress Model provide the biological basis for associating hydrocarbon exposure with observed nuclear alterations in buccal cells.

In conclusion, the reviewed literature supports the rationale for assessing petrol attendants' epithelial cells using the BMN assay. The study at the Uselu Axis is designed to build upon previous work by employing standardized procedures, balanced gender representation, and clear differentiation between exposed and control groups. Ultimately, the findings will provide valuable insights for policy makers, public health authorities, and occupational safety agencies aiming to safeguard worker health in Nigeria's petroleum sector.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted at selected petrol stations along the Uselu Axis, Benin City, Edo State, Nigeria. The Uselu Axis is a busy urban corridor characterized by high vehicular flow, numerous retail filling stations and dense commercial activities. Field sample collection (buccal cell swabbing) took place at the petrol stations during routine work hours. All laboratory processing (fixation, staining and microscopic scoring) was carried out at the National Centre for Energy and Environment (NCEE), University of Benin (UNIBEN), Benin City, Edo State, Nigeria, using the facility's cytology and microscopy laboratory.

Rationale: Uselu Axis was chosen because of concentrated petrol retail activity and frequent exposure opportunities for attendants; the NCEE laboratory provides standardized equipment and biosafety provisions appropriate for cytogenetic staining and scoring (Umegbolu *et al.,* 2016; Thomas *et al.,* 2009).

some of the filling stations were which samples were collected from includes;

1.ILUOBE Petrol station

2.TOTAL ENERGIES, UGBOWO services

3.TOTAL ENERGIES , Uselu services

4. Forte Petrol station

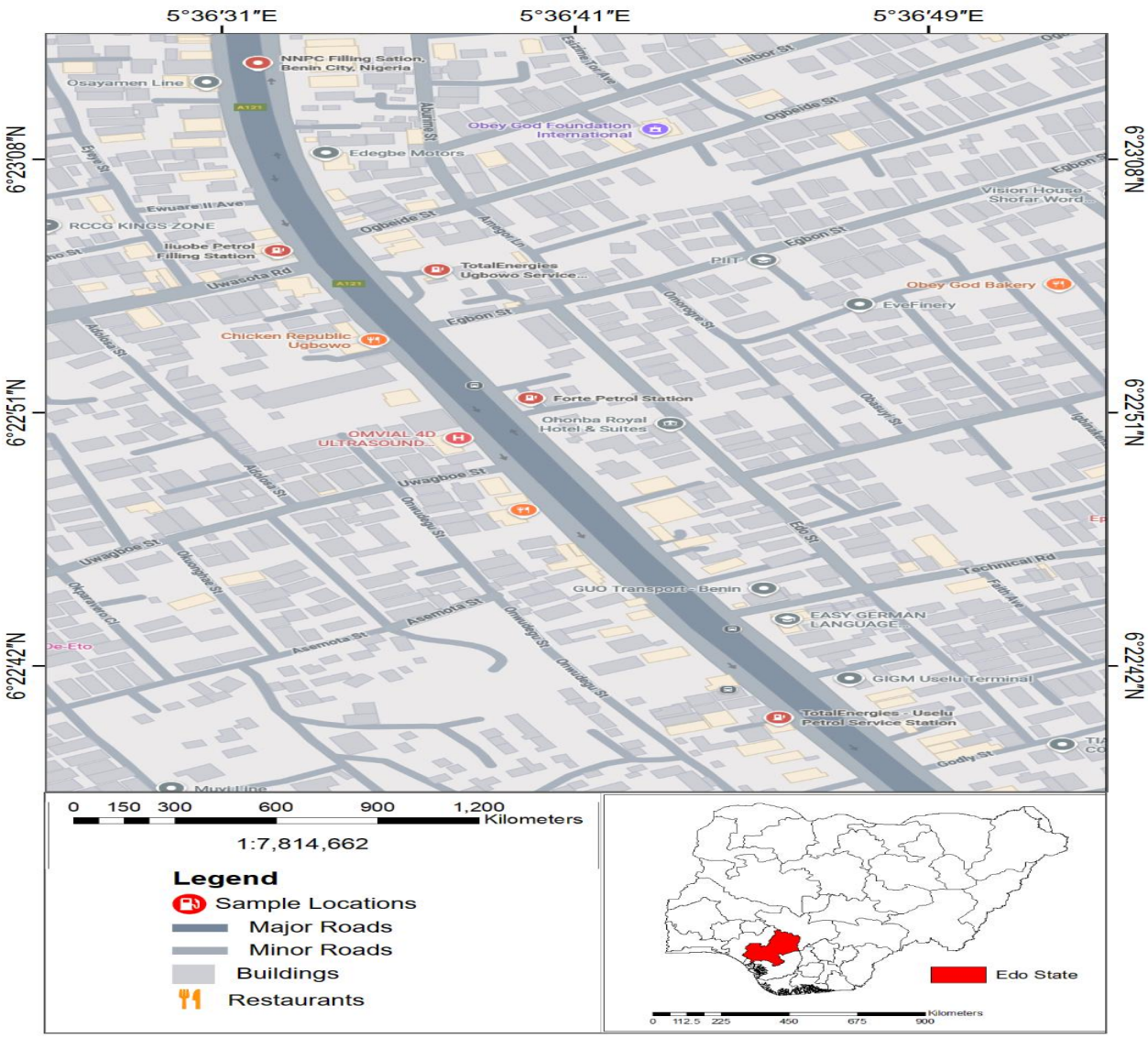


Figure 3.1: A map showing the Petrol Stations along Uselu Axis.

3.2 Study Population and Sample Size Determination

3.2.1 Study population

The exposed group comprised 25 petrol station attendants (both male and female) who had worked at the selected Uselu Axis stations for ≥ six months. Inclusion criteria were: age 18–60 years; current employment as an attendant at a selected station; no recent radiotherapy or chemotherapy; and willingness to provide informed consent. Exclusion criteria included chronic

illness known to affect DNA (e.g., active cancer), recent diagnostic X-ray exposure (within one month), and current pregnancy.

The control group consisted of 10 university students from UNIBEN who reported no occupational exposure to petrol vapors, matched loosely for age range and non-smoking status where possible. Students were selected because they are accessible, broadly comparable in age to attendants, and unlikely to have chronic petrol exposure (Umegbolu *et al.,* 2016; Fenech, 2007).

3.2.2 Sample size determination and justification

This project uses a purposive sample of 25 exposed and 10 control subjects, chosen to mirror common sample sizes used in benchmark BMN studies (for example Umegbolu *et al.,* 2016). Given the pilot/field-monitoring nature of this work and resource constraints, the chosen numbers strike a balance between feasibility and the ability to detect moderate differences in mean micronucleus frequency. Statistical power will be considered during analysis, and effect-size estimation will guide the interpretation of results for this exploratory study.

3.3 Methods of Data Collection

3.3.1 Materials used

The following consumables and equipment were used for sample collection, fixation, staining and scoring:

1. Tongue depressors (sterile) — for gentle scraping of buccal mucosa.

2. Physiological saline (0.9% NaCl) — for moistening and immediate cell handling if needed, also for creating a solution with the collected smear on the slides to avoid cell desiccation(drying out)

3. Microscopic slides (pre-cleaned) — for cell mounting.

4. Slide boxes — for storage and transport of labeled slides.

5. Tweezers — for handling slides and coverslips.

6. Gloves — disposable nitrile gloves for biosafety and Non contamination of samples

7. Face masks — surgical masks to minimize contamination and protect personnel.

8. Compound microscope with oil-immersion objective (100×/oil) — for scoring under high magnification.

9. Carnoy's fixative (methanol:glacial acetic acid, 3:1) — for rapid fixation of exfoliated cells (standard cytology fixative commonly used in BMN protocols).

10. May–Grünwald stain — first stage in cytoplasmic/nuclear staining sequence.

11. Giemsa stain — for nuclear staining and contrast; used in combination with May–Grünwald (May–Grünwald–Giemsa protocol).

12. Immersion oil — for final high-resolution scoring with the oil-immersion objective.

(Materials list assembled from standard BMN protocols and the lab stocks at NCEE; staining and fixation approach follows established BMN guidance: Fenech, 2007; Thomas *et al.,* 2009; Bolognesi *et al.,* 2015.)

3.3.2 Sample collection procedure

Recruitment and consent: Potential participants were briefed about the study aims, procedures and confidentiality. Written informed consent was obtained prior to sample collection. A short-structured questionnaire was administered to collect demographic data, smoking and alcohol

history, duration of employment (for attendants), use of personal protective equipment (PPE), and recent medical exposures that could confound cytogenetic results.

Pre-sampling preparation: Participants were asked to rinse the mouth with clean water and avoid eating, chewing or smoking for at least 30 minutes before sampling to reduce debris and contamination.

Buccal cell collection: Following HUMN/BMCyt recommendations, buccal cells were collected by gently scraping the inner cheek mucosa with a sterile tongue depressor or cytobrush (one stroke each on left and right inner cheek). For each subject, samples from both cheeks were combined and smeared onto two pre-labeled microscopic slides to allow duplicate staining or backup. Slides were air-dried at room temperature for ~10 minutes before immediate fixation.

Labeling and transport: Slides were labeled with unique codes (not names) and placed into slide boxes. Transport to the NCEE laboratory occurred within the same working day; slides were kept protected from direct light and dust.

(This sampling approach follows standard non-invasive BMN procedures widely reported in the literature and applied by Fenech (2007), Thomas *et al. (*2009) and in field studies such as Umegbolu *et al. (*2016).

3.3.3 Fixation and staining

Fixation: Air-dried smears were fixed in Carnoy's fixative (methanol: acetic acid, 3:1) for 5 minutes at room temperature. Carnoy's fixative rapidly denatures proteins and preserves nuclear morphology, making it appropriate for BMN cytology *(*Thomas *et al.,* 2009; Fenech, 2007). After fixation, slides were briefly rinsed in absolute alcohol and allowed to dry.

Staining (May–Grünwald–Giemsa protocol):

1. Place slides in May–Grünwald solution for 3–5 minutes (depending on reagent concentration), then briefly rinse with buffer (pH 6.8–7.2).

2. Transfer slides to diluted Giemsa for 15 minutes (time optimized during pilot staining runs at NCEE) to enhance nuclear contrast.

3. Rinse slides in buffered and distilled water, air-dry, and store in slide boxes until scoring.

4. Before microscopic scoring, a drop of immersion oil is placed on the region of interest for 100× oil-immersion evaluation.

Notes on staining rationale: The combined May–Grünwald–Giemsa (MGG) technique produces good cytoplasmic and nuclear contrast, aiding the identification of micronuclei and other nuclear anomalies. This staining sequence is widely adopted in BMN studies and recommended in standard protocols (Fenech, 2007; Thomas *et al.,* 2009; Bolognesi *et al.,* 2015). Routine pilot staining was performed at NCEE to standardize exposure times and to ensure reproducible coloration.

3.3.4 Microscopic scoring and cell classification

Scoring personnel and blinding: Scoring was performed by trained personnel at NCEE with experience in BMN scoring. To reduce observer bias, slides were coded so that scorers were blind to exposure status (exposed vs control). Periodic intra-observer and inter-observer checks were performed on a subset of slides to estimate scoring reliability.

Number of cells scored: Although some protocols recommend scoring 1000 cells per subject for high statistical precision, the practical benchmarking (and the user's earlier instruction) used 25

cells per slide in certain settings. For this study, each slide was scanned and an aggregate of 1000 cells per subject was aimed for where possible (that is, multiple slide fields across two slides) to align with international standards (Fenech, 2007; Thomas *et al., 2009). Where* resource constraints limited counts, a minimum of 500 scored cells per subject was considered acceptable and recorded, with the exact cell counts noted for each sample.

Scoring criteria: Nuclear anomalies were classified according to the HUMN/BMCyt recommended taxonomy:

Micronuclei (MN): small, round or oval extranuclear bodies with similar staining intensity as main nuclei, clearly separated from the main nucleus and less than one-third of the main nucleus diameter.

Binucleated cells (BN): cells with two main nuclei of similar size and staining, possibly indicative of cytokinesis failure.

Anucleated cells (AN): cells lacking a visible nucleus but with intact cytoplasm, often reflecting cytotoxic events.

Other anomalies (noted but not the primary focus) such as karyorrhexis, karyolysis and pyknotic nuclei were recorded where evident (Fenech, 2007; Bolognesi *et al.,* 2015; Thomas *et al.,(*2009).

Counting procedure: Slides were first scanned at low power (10× and 40×) to identify well-spread, intact epithelial fields. Final scoring was performed under 100× oil-immersion objective for morphological precision. For each subject, the number of MN, BN and AN were recorded as absolute counts and normalized (e.g., MN per 1000 cells) for comparisons.

Quality assurance: A 10% random sample of slides was re-scored by a second blinded scorer to determine inter-observer agreement (Cohen's kappa), and any major discrepancies were resolved by a third senior scorer. Detailed scoring sheets were maintained with slide codes; field counts and notes on staining quality.

(Scoring approach adheres to HUMN recommendations and BMN literature; operational details were adapted to the laboratory context at NCEE and the practicalities of fieldwork as described in Fenech, 2007; Thomas *et al.,* 2009; Bolognesi et *al., 2015.)*

3.3.5 Statistical analysis

Data entry and cleaning: Raw counts and participant questionnaire data were entered into a spreadsheet (Microsoft Excel) and checked for entry errors. Cleaned data were imported to statistical software (e.g., SPSS or R) for analysis.

Descriptive statistics: Mean, standard deviation (SD), median and range were computed for continuous variables (e.g., age, duration of exposure, MN frequency). Frequency distributions were produced for categorical variables (e.g., gender, smoking status).

Comparative analysis: The primary comparisons were between exposed attendants and controls. Statistical tests included:

Independent samples t-test (or Mann–Whitney U test if the data were non-normal) to compare mean MN frequency between groups.

Chi-square test for categorical comparisons.

ANOVA or Kruskal–Wallis test for comparisons across multiple exposure-duration categories.

Correlation analysis (Pearson or Spearman as appropriate) to explore relationships between duration of exposure and MN frequency.

Multivariate analysis (linear regression) to adjust for potential confounders (age, sex, smoking, alcohol use) when assessing the association between exposure status and MN counts.

Significance level: A two-tailed p-value < 0.05 was considered statistically significant. Confidence intervals (95%) were reported where relevant.

Reporting: Results were presented as mean ± SD or median (IQR) as appropriate; normalized MN frequencies (per 1000 cells) were reported to facilitate comparison with the broader literature *(*Fenech, 2007; Nersesyan *et al.,* 2016; Umegbolu *et al.,* 2016).

3.3.6 Ethical considerations

Ethical approval: Ethical clearance for the study was obtained from the University of Benin Ethics Review Committee (protocol number to be included when obtained). All procedures conformed to the ethical principles of human research, including respect for persons, beneficence and confidentiality.

Informed consent: Participants received clear verbal and written explanations of study aims, procedures, potential risks and benefits. Written informed consent was obtained prior to sample collection. Participants were free to withdraw at any time without consequence.

Confidentiality and data protection: Unique participant codes replaced names on all slides and questionnaires. Hard copies of questionnaires and printed slides were stored in locked cabinets at NCEE; electronic data were password-protected.

Minimizing risk: Buccal cell collection is minimally invasive and low risk. Standard biosafety precautions (gloves, masks, proper disposal of used materials) were observed. Any participant who reported or exhibited health concerns during screening was referred to an appropriate health facility.

Benefit and feedback: Aggregate results (not individual medical diagnoses) were offered to participants and station managers as part of community feedback. Where findings suggested elevated genotoxic markers, participants were given general advice about exposure reduction and referred for further medical evaluation when necessary.

(Ethical approach follows standard human-subjects protocols applied in BMN field studies and reflects recommendations in Fenech, 2007; Thomas *et al.,* 2009; Umegbolu *et al.,* 2016.)

3.4 Experimental Design

This study employed a comparative cross-sectional design to evaluate genotoxic markers in petrol attendants (exposed group) and university students (unexposed control group). The cross-sectional approach is appropriate for initial biomonitoring, enabling assessment of the association between current occupational exposure and cytogenetic alterations as indicated by MN and related nuclear anomalies.

Design features and justification:

Comparative groups: Exposed vs control groups allow estimation of differences attributable to occupational petrol exposure while controlling for age and lifestyle where possible.

Single-time sampling: Buccal cell samples were collected once per participant to provide a snapshot of recent and cumulative genotoxic effects. While longitudinal designs offer stronger

causal inference, the cross-sectional design is efficient and commonly used in BMN surveys (e.g., Umegbolu *et al*., 2016; Patel *et al.,* 2018).

Standardized laboratory processing: All slides were fixed, stained and scored at NCEE using standardized HUMN-aligned criteria to enhance comparability with other studies (Bolognesi *et al.,* 2015; Thomas *et al.,* 2009).

Blinded scoring and quality control: To minimize observational bias, scorers were blinded to exposure status, and inter-observer checks were included.

Limitations of the design: The cross-sectional design cannot definitively prove causality and may be sensitive to transient exposures; however, it is suitable for initial detection of elevated genotoxic markers and for informing longer-term surveillance or intervention studies.

CHAPTER FOUR

RESULT AND DATA ANALYSIS

4.1 General characteristics of volunteers from petrol stations along uselu axis

The demographic form was used to accurately and precisely gather demographic information from the volunteers. It was very useful in determining the factors that could either influence or affect the entire study as some of the participants results may vary based on their demographic information which would help in the in the conclusion and recommendations

To preserve Animosity and Confidentiality only a sample of the volunteers Demographic information form is displayed to show the type of information collected and utilized for the study.

Also, it is important to note that both the demographic information of Exposed Participants and the control participants were collected to ensure the elimination of bias and assumptions. See Appendix 1.

Table 4.1: Volunteers demographic information form along uselu axis

Demographic variables [n (%)]	Exposed (n=25)	Control (n=10)
Age (years)		
18–24	12 (48%)	8 (89%)
25–30	9 (36%)	1 (11%)
30 above	4 (16%)	0 (0%)
Gender		
Male	21 (84%)	5 (56%)
Female	4 (16%)	4 (44%)
Highest level of education		

None	1 (4%)	0 (0%)
Primary	3 (12%)	0 (0%)
Secondary	18 (72%)	0 (0%)
University	3 (12%)	9 (90%)
Smoking status		
Yes	6 (24%)	0 (0%)
No	19 (76%)	9 (90%)
Pregnancy status		
Yes	1 (25%)	0 (0%)
No	3 (75%)	4 (40%)
Duration of employment/exposure (months)		
0	0 (0%)	9 (90%)
6–12	7 (28%)	0 (0%)
13–24	10 (40%)	0 (0%)
25 above	8 (32%)	0 (0%)
Alcohol consumption		
No	14 (56%)	7 (78%)
Yes	11 (44%)	2 (22%)
Allergic reactions		
Yes	8 (32%)	1 (11%)
No	17 (68%)	8 (89%)

4.2 General characteristics of exposed, control and summary

This analysis is based on the provided spreadsheet for the 25 exposed individuals (A-Y). Each individual had two slides prepared (e.g., EPAR, EPAL), with two readings per slide (e.g., EPAR1, EPAR2), summing to a total of 100 readings. 25 cells were scored per reading, the totals number of cells analyzed for the exposed group is 2,500 cells. (100 readings x 25 cells).

	Micronuclei	Binucleated	Annucleated	Karryohexis	**Total**
EPAR1A	2	2	-	2	6
EPAR2A	5	2	-	-	7
EPAL1A	6	4	-	2	12
EPAL2A	2	2	2	4	10
Total	15	10	2	8	

EPAR1B	4	1	2	1	8
EPAR2B	1	3	-	-	4
EPAL1B	2	4	3	3	12
EPAL2B	-	-	2	2	4
Total	7	8	8	6	

EPAR1C	3	-	4	1	8
EPAR2C	3	2	2	3	10
EPAL1C	-	-	2	-	2
EPAL2C	1	3	-	-	4
Total	7	5	8	4	

EPAR1D	-	-	3	2	5
EPAR2D	-	-	2	1	3
EPAL1D	5	2	3	4	14
EPAL2D	2	1	-	5	8
Total	7	3	8	12	

EPAR1E	3	4	1	3	11
EPAR2E	5	-	1	3	9
EPAL1E	1	2	3	_	6
EPAL2E	-	2	2	2	6

Total	9	8	7	8	
EPAR1F	4	1	2	1	8
EPAR2F	1	1	1	1	4
EPAL1F	1	2	3	5	11
EPAL2F	2	-	1	2	5
Total	8	4	7	9	
EPAR1G	3	2	2	3	10
EPAR2G	-	-	-	1	1
EPAL1G	2	1	1	1	5
EPAL2G	2	1	3	2	8
Total	7	4	6	7	
EPAR1H	3	1	2	2	8
EPAR2H	2	-	2	-	4

EPAL1H	3	1	-	3	7
EPAL2H	4	2	1	1	8
Total	12	4	5	6	

EPAR1I	3	1	2	1	7
EPAR2I	-	2	2	1	5
EPAL1I	3	-	-	2	5
EPAL21	2	1	3	3	9
Total	8	4	6	7	

EPAR1J	2	1	3	1	7
EPAR2J	2	-	3	1	6
EPAL1J	5	2	-	1	8
EPAL2J	3	-	2	2	7
Total	12	3	8	5	

EPAR1K	2	2	2	1	7
EPAR2K	2	1	2	2	7
EPAL1K	1	-	3	1	5
EPAL2K	5	1	1	3	10
Total	10	4	8	7	

EPAR1L	1	1	2	1	5
EPAR2L	1	-	2	-	3
EPAL1L	5	2	-	-	7
EPAL2L	2	3	2	1	8
Total	9	6	6	2	

EPAR1M	2	2	2	2	8
EPAR1M	2	3	-	-	5
EPAL1M	3	-	-	-	3

EPAL2M	4	1	1	3	9
Total	11	6	3	5	
EPAR1N	2	2	2	1	7
EPAR2N	5	-	1	1	7
EPAL1N	3	1	1	2	7
EPAL2N	-	2	-	1	3
Total	10	5	4	5	
EPAR1O	1	2	3	2	8
EPAR2O	2	2	5	5	14
EPAL1O	1	1	2	5	9
EPAL2O	-	-	-	-	0
Total	4	4	10	12	
EPAR1P	2	1	2	2	7

EPAR2P	3	-	4	1	8
EPAL1P	5	2	2	1	10
EPAL2P	1	3	2	3	9
Total	11	6	10	7	
EPAR1Q	2	2	2	2	8
EPAR2Q	5	2	1	2	10
EPAL1Q	3	1	3	3	10
EPAL2Q	4	3	1	4	12
Total	14	8	7	11	
EPAR1R	1	1	3	1	6
EPAR2R	5	2	4	3	14
EPAL1R	2	1	2	3	8
EPAL2R	3	1	3	3	10
Total	11	5	12	10	

EPAR1S	2	2	2	3	9
EPAR2S	5	2	1	2	10
EPAL1S	3	3	1	4	11
EPAL2S	1	-	4	2	7
Total	11	7	8	11	
EPAR1T	2	3	2	2	9
EPAR2T	5	3	3	2	13
EPAL1T	3	1	2	3	9
EPAL2T	3	2	2	1	8
Total	13	9	9	8	
EPAR1U	6	1	3	2	12
EPAR2U	3	3	3	3	12
EPAL1U	4	4	3	4	15

EPAL2U	1	5	2	1	9
Total	14	13	11	10	
EPAR1V	6	2	2	1	11
EPAR2V	3	3	2	3	11
EPAL1V	5	4	3	2	14
EPAL2V	5	1	4	1	11
Total	19	10	11	7	
EPAR1 W	2	-	2	-	4
EPAR2 W	3	2	2	3	10
EPAL1 W	6	5	-	3	14
EPAL2 W	5	2	2	1	10

Total	16	9	6	7	
EPAR1X	2	3	2	1	8
EPAR2X	5	-	3	-	8
EPAL1X	2	2	4	3	11
EPAL2X	1	2	1	2	6
Total	11	7	10	6	
EPAR1Y	1	1	2	3	7
EPAR2Y	6	2	2	3	13
EPAL1Y	2	-	-	-	2
EPAL2Y	4	2	4	4	14
Total	13	5	8	10	

Table 4.2a Showing the raw data sheet of the Exposed petrol attendants.

	Micronucleus	Binucleated	karryohexid	Anucleated	**Total**

1pR1	-	-	1	-	1
1pR2	-	-	1	-	1
1pL1	-	1	-	-	1
1pL2	,	-	-	-	1
Total	1	0	2	0	
2pR1	2	-	1	-	3
2pR2	-	-	-	-	0
2pL1	1	-	-	-	1
2pL2	-	1	1	-	2
Total	3	1	2	0	
3pR1	-	-	-	-	-
3pR2	-	-	-	-	-
3pL1	1	-	-	-	2
3pL2	-	-	1	-	-
Total	1	0	1	0	2

4pR1	-	1	-	-	1
4pR2	-	-	-	-	0
4pL1	-	-	-	-	0
4pL2	1	-	-	-	1
Total	1	1	0	0	
5pR1	1	-	-	2	3
5pR2	1	-	-	-	1
5pL1	-	-	-	-	0
5pL2	-	-	-	-	0
Total	2	0	0	2	
6pR1	-	1	1	-	2
6pR2	-	-	1	-	1
6pL1	-	-	1	-	1
6pL2	-	-	-	-	0

Total	0	1	3	0	
7pR1	-	1	-	1	2
7pR2	1	-	-	-	1
7pL1	-	-	-	-	0
7pL2	-	1	-	-	1
Total	1	2	0	1	
8pR1	-	-	-	-	0
8pR2	-	-	1	-	1
8pL1	-	-	-	-	0
8pL2	1	-	1	2	4
Total	1	0	2	2	
9pR1	1	-	-	1	2
9pR2	1	-	-	-	1
9pL1	-	-	-	-	0

9pL2	-	-	-	-	0
Total	2	0	0	1	
10pR1	-	-	-	1	1
10pR2	-	-	-	-	0
10pL1	1	-	-	-	1
10pL2	-	-	-	-	0
Total	1	0	0	1	

Table 4.2b showing the raw data sheet of the Unexposed individuals.

Aberration Type	Total Count (Exposed Group)	Total Count (Control Group)
Micronuclei (MN)	270	13
Binucleated (BN)	144	6
Karyorrhexis (KR)	172	13
Anucleated (AN)	223	9
Total Aberrations	809	41

Table 4.2c: Showing the total number of aberrations scored for both groups

Key points:

- **Exposed Group (N=25):** The totals are derived from the sum of all individual "Total" rows for each participant (A through Y) in the data sheet in table 4.2a
- **Control Group (N=10):** The totals are derived from the sum of all individual "Total" rows for each participant (1p through 10p) in the provided data sheet in table 4.2b.

4.3 Statistical Data Analysis

characteristics	Number	Mean ± SD	Number	Mean±SD	P values
MN	269	10.76±3.36	13	1.3±0.82	2.18E-09
BN	157	6.28±2.55	5	0.5±0.70	2.17E-07
KY	188	7.52±2.47	10	1±1.15	7.43E-10
AN	190	7.60±2.54	7	0.7±0.82	6.56E-09

Table 4.3 showing the results of the Exposed and Unexposed data sheet

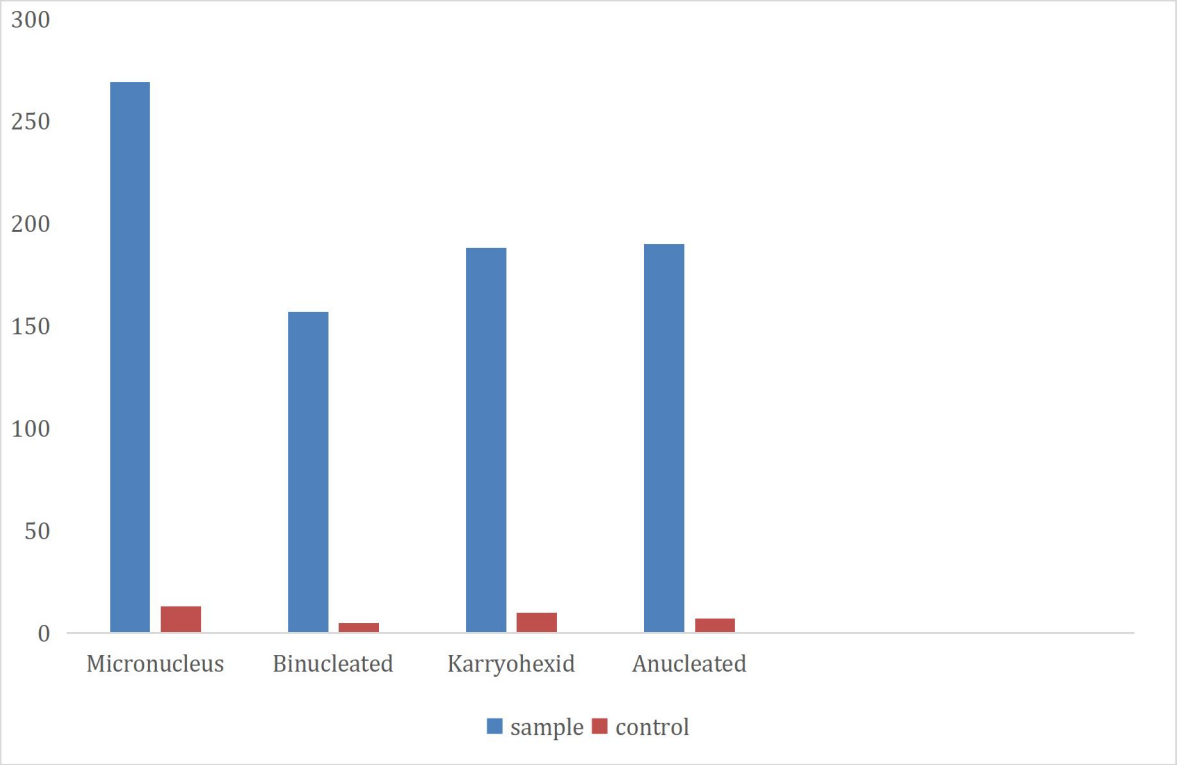
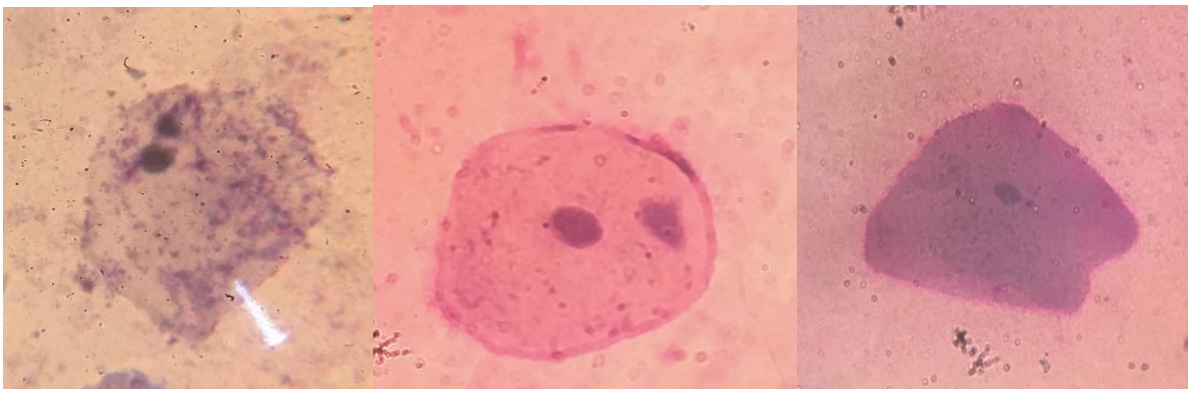
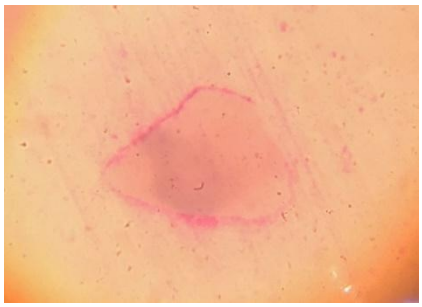


Fig 4.1 A bar chart showing the difference between the Exposed and Unexposed along uselu axis

The t-test, as employed in this study, is a powerful parametric statistical method used to determine whether there is a significant difference between the means of two groups. In environmental and occupational health research, it serves as a fundamental tool for comparing biomarker levels between exposed and control populations (Fenech, 1999). The application of this test in our analysis provided quantitative evidence that the elevated frequencies of nuclear abnormalities in petrol station attendants are statistically significant and directly attributable to occupational exposure rather than random variation.



(a) (b) (c)



(d)

Plate 4.1: showing the different aberrations.

The plate labelled (a) is called Karyorrhexis, the one labelled (b) is called binucleated, the plate labelled (c) is called Micronucleus and the plate labelled (d) is called the Anucleated.

KEY POINTS

Micronucleus (MN) - A small, extra nucleus that forms in a cell, separate from the main nucleus.

Karyorrhexis - The destructive frafmentation of the nucleus off a dying cell.

Anucleated (AN) – A cell that lacks a nucleus entirely.

Binucleated (BN0 – Acell that contains two separate nuclei.

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study was conducted to evaluate the genotoxic effects of occupational exposure to petrol fumes among petrol station attendants operating within the Uselu axis of Benin City. The research was designed to determine whether continuous inhalation of hydrocarbon vapors in the work environment could induce detectable chromosomal damage in buccal epithelial cells. The Buccal Micronucleus (MN) Assay, a non-invasive and widely recognized cytogenetic test, was used to identify and quantify nuclear anomalies in exfoliated cells collected from exposed workers and control participants. The results obtained clearly demonstrated that petrol attendants exhibited substantially higher frequencies of nuclear anomalies compared with the control group. These observations establish that the occupational environment of petrol station attendants presents a measurable genotoxic hazard that warrants serious attention.

The presence of micronucleated (MN) cells in the exposed group serves as the most direct evidence of chromosomal damage. Micronuclei are small, extra-nuclear bodies formed from chromosome fragments or entire chromosomes that fail to integrate into daughter nuclei during mitosis (Fenech, 1999). Their appearance in buccal epithelial cells reflects a disturbance in chromosomal segregation and is widely accepted as a sensitive biomarker of genetic instability. The elevated frequency of MN cells observed in the exposed population indicates that petrol fumes contain compounds capable of damaging DNA or interfering with normal cell division. Petrol fumes are a complex mixture of volatile organic compounds, including benzene, toluene, and xylene, as well as other aromatic hydrocarbons identified as mutagenic and carcinogenic by the International Agency for Research on Cancer (IARC, 2012). Continuous inhalation of these

substances exposes epithelial tissues to oxidative and electrophilic stress, leading to chromosomal breakage and formation of micronuclei.

The findings of this study strongly align with the results of Umegbolu *et al.* (2016), who similarly documented significantly elevated MN frequencies among petrol attendants in Awka, Anambra State. Their work, together with the present investigation, confirms that occupational exposure to petroleum hydrocarbons results in genotoxic effects detectable through non-invasive biomonitoring methods. Moreover, these results agree with previous studies by Celik *et al.* (2003) and Sellappa *et al*. (2010), which also reported increased micronucleus formation in petrol workers in Turkey and India respectively. The consistency of these outcomes across different regions and populations underscores the universality of petrol-fume-induced genetic damage, suggesting that the underlying mechanisms are not geographically restricted but biologically consistent wherever hydrocarbon exposure occurs.

The elevated frequency of micronuclei observed in this research may also be attributed to the chemical composition and concentration of fuel vapors in the local environment. In Nigeria, the open dispensing system and lack of vapor recovery technology allow attendants to be directly exposed to petrol fumes at close range. Environmental conditions such as high ambient temperature and poor ventilation can further enhance the volatility of hydrocarbons, thereby increasing exposure levels. Over time, the accumulation of inhaled hydrocarbons can result in persistent genotoxic pressure on epithelial cells, reflected in elevated MN formation. Additionally, most petrol attendants in Benin City work long hours daily, often exceeding regulatory limits, which amplifies their cumulative exposure. The observed nuclear anomalies therefore provide biological confirmation of the hazardous working conditions prevailing at petrol stations in this region.

Another important observation from this study is the correlation between exposure duration and the extent of genotoxic damage. The results indicate that attendants who have been exposed for longer periods tend to exhibit higher frequencies of nuclear anomalies, suggesting a dose-dependent or cumulative effect. This relationship illustrates the principle that genotoxic injury is progressive when exposure is continuous and that DNA damage accumulates over time as the body's defense and repair mechanisms become overwhelmed. The underlying biological explanation for this pattern is rooted in the concept of oxidative stress. Prolonged exposure to volatile hydrocarbons promotes the generation of reactive oxygen species (ROS), which interact with DNA bases, induce strand breaks, and cause chromosomal missegregation. Although the body possesses antioxidant enzymes and DNA repair systems to counteract these effects, persistent exposure eventually compromises their efficiency, resulting in sustained genotoxic damage. This finding reinforces the importance of early monitoring and timely intervention before irreversible damage occurs.

In addition to exposure duration, the study also examined the influence of sex on the formation of micronuclei. The analysis revealed no statistically significant difference in MN frequency between male and female attendants, indicating that both sexes exhibit similar susceptibility to petrol-fume-induced genetic damage. This outcome aligns with the observations of Umegbolu et al. (2016) and is supported by other studies that found no meaningful sex-based variation in genotoxic response among occupationally exposed populations. The similarity between male and female attendants suggests that biological factors such as hormonal variation or chromosomal composition play a limited role in modulating genotoxic susceptibility under high-exposure conditions. In essence, the magnitude of exposure appears to be the overriding determinant of genetic damage, with all exposed individuals being equally vulnerable regardless of sex or age.

This emphasizes the need for uniform protective measures and health surveillance strategies applicable to all workers in the petroleum distribution sector.

The pattern of nuclear alterations observed in this study particularly the consistent increase in micronucleated cells among the exposed group provides irrefutable evidence of chromosomal damage associated with petrol fume inhalation. The buccal micronucleus assay has thus proven to be an effective biomonitoring tool for detecting early genotoxic effects in occupationally exposed individuals. Its advantages lie in its simplicity, low cost, and non-invasive nature, making it suitable for routine health monitoring, especially in low-resource settings. The results of this study confirm that the Buccal Micronucleus Assay can be effectively applied in Nigerian environments to generate reliable biological data for occupational health surveillance. When interpreted alongside the findings from other national and international studies, it becomes clear that petrol station attendants represent a high-risk group requiring urgent preventive attention.

Overall, the discussion of findings from this study establishes that petrol fumes contain genotoxic agents capable of inducing chromosomal damage in exposed individuals. The elevated frequency of micronuclei among attendants serves as a clear biological indicator of this damage. The correlation between exposure duration and nuclear abnormalities further supports a cumulative risk pattern, while the lack of significant sex influence indicates that the hazard is universal across all workers. These findings underscore the pressing need for improved occupational safety measures, regulatory enforcement, and continuous biomonitoring to safeguard the health of individuals working in petroleum dispensing stations.

5.2 Conclusion

This study has provided concrete biological evidence that occupational exposure to petrol fumes leads to measurable genotoxic damage in petrol station attendants operating in the Uselu axis of Benin City. The observed increase in micronucleated cells among exposed workers compared with unexposed controls demonstrates that inhalation of petroleum vapors results in chromosomal breakage and structural instability within buccal epithelial cells. The positive association between exposure duration and the extent of nuclear anomalies indicates that the risk of genetic damage increases cumulatively with prolonged contact. Importantly, the findings show that neither sex nor age significantly modifies susceptibility, implying that exposure intensity and duration are the principal determinants of genetic injury. Collectively, these results confirm that petrol fume inhalation constitutes a serious occupational hazard capable of initiating early events in mutagenesis and carcinogenesis. The study therefore highlights the necessity of preventive measures through regular biomonitoring, environmental control, and health education aimed at reducing exposure levels. If properly implemented, such interventions could substantially minimize genotoxic risk among workers and contribute to improved occupational health outcomes in the petroleum service industry.

5.3 Recommendations

1. Adoption of Personal Protective Equipment (PPE):

Petrol attendants should be mandated to wear appropriate protective masks or respirators while on duty to minimize inhalation of harmful vapors. The consistent use of PPE will significantly reduce direct exposure to airborne hydrocarbons.

2. Implementation of Administrative Controls:

Petrol stations should explore the introduction of self-service dispensing systems where feasible, thereby reducing attendants' time spent in direct contact with petrol fumes. Additionally, work schedules should include adequate rest breaks and rotation to lower exposure duration.

3. Regular Health Surveillance and Biomonitoring:

Routine screening using the Buccal Micronucleus Assay should be conducted periodically for all petrol station workers. This approach allows early detection of DNA damage before the onset of clinical disease and enables timely medical intervention.

4. Health Education and Awareness Programs:

Management and relevant authorities should organize educational sessions to inform attendants about the hazards of petrol fume inhalation, emphasizing personal hygiene, use of PPE, and the importance of medical checkups.

5. Enforcement of Environmental and Safety Regulations:

Government agencies should ensure strict compliance with occupational safety standards and emission control policies in petrol stations. Adequate ventilation systems should be installed to disperse fuel vapors effectively.

6. Job Rotation and Alternative Employment Options:

Workers found with persistent elevations in MN frequency should be reassigned to roles with lower exposure risk or guided toward alternative employment to prevent cumulative genetic damage.

7. Further Research:

Future studies should extend biomonitoring efforts across different geographical zones and assess the long-term health outcomes associated with repeated hydrocarbon exposure. Such studies would provide deeper insights into the link between occupational exposure and disease development.

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APPENDIX

University of Benin, Benin City, Nigeria

DEPARTMENT OF ENVIRONMENTAL MANAGEMENT & TOXICOLOGY
FACULTY OF LIFE SCIENCES

VOLUNTEER DEMOGRAPHIC INFORMATION FORM (SAMPLE COLLECTION)

Personal Information

1. Full Name: _____

2. Date of Birth: _____/_____/_____ (DD/MM/YYYY)

3. Gender:
[ ] Male [ ] Female [ ] Non-binary [ ] Prefer not to say [ ] Other: _____

4. Contact Information:
Phone: _____ Email: _____

Address: _____

Demographic Details

5. Ethnicity/Race: (Optional)
[ ] Bini [ ] Esan [ ] Ibo [ ] Etsako [ ] Yoruba [ ] Others
[ ] Prefer not to say

6. Highest Education Level
[ ] None [ ] Primary [ ] Secondary [ ] Tertiary [ ] Others

Health & Lifestyle Information

8. Smoking Status: [ ] Smoker (Current) [ ] Former Smoker [ ] Non-smoker

9. Pregnancy Status: (If applicable) [ ] Pregnant [ ] Not Pregnant [ ] Not Applicable

10. Do you have any known allergies or medical conditions?
[ ] Yes (Specify: _____) [ ] No

CONSENT AND AGREEMENT

11. Consent for Data Use: I agree that my anonymized demographic data may be used for research purposes.
[ ] I consent [ ] I do not consent

Volunteer Signature: _____

Date: _____/_____/_____