ASSESSMENT OF DNA DAMAGE IN DRIVERS EXPOSED TO VEHICULAR EMISSIONS AT UNIBEN MAIN GATE, BENIN CITY, USING MICRONUCLEUS ASSAY OF BUCCAL CELLS.

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UNIVERSITY OF BENIN

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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 DNA DAMAGE IN DRIVERS EXPOSED TO VEHICULAR EMISSIONS AT UNIBEN MAIN GATE, BENIN CITY, USING MICRONUCLEUS ASSAY OF BUCCAL CELLS"** was carried out by **"Angela Tochukwu NWEKE (Miss)"** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City, in partial fulfilment of the requirements for the award of a Bachelor of Science (B. Sc.) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of a Bachelor of Science degree in Environmental Management and Toxicology.

_____ _____

PROF. D.I. OLORUNFEMI **DATE**
(PROJECT SUPERVISOR)

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PROF. (MRS) E. T. AISIEN **DATE**
(HEAD OF DEPARTMENT)

DECLARATION

I **"Angela Tochukwu NWEKE (MISS)"** declare that **"ASSESSMENT OF DNA DAMAGE IN DRIVERS EXPOSED TO VEHICULAR EMISSIONS AT UNIBEN MAIN GATE, BENIN CITY, USING MICRONUCLEUS ASSAY OF BUCCAL CELLS"** is my work and that all sources that I have used or quoted have been acknowledged using complete references and that this work has not been submitted before for any other degree at any other university.

_____ _____

NWEKE ANGELA TOCHUKWU **DATE**

DEDICATION

This project is dedicated to God Almighty for His grace, wisdom, and strength throughout my academic journey. I also dedicate it to my loving parents Mr. and Mrs. NWEKE, whose support and prayers have carried me through every stage of this work.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

APPENDICES

APPENDIX **PAGES**

ABSTRACT

This study assessed DNA damage in commercial drivers exposed to vehicular emissions at the University of Benin (UNIBEN) Main Gate, Benin City, using the micronucleus assay of buccal cells. Vehicular emissions are a major source of air pollution containing genotoxic substances such as polycyclic aromatic hydrocarbons, nitrogen oxides, and particulate matter, which can induce chromosomal damage. A comparative cross-sectional design was used, involving 25 exposed drivers and 9 non-exposed controls. Buccal epithelial cells were collected using sterile wooden spatulas, fixed in Carnoy's reagent, and stained with May-Grünwald–Giemsa for microscopic analysis. One hundred cells per participant were scored for nuclear abnormalities including micronuclei (MN), binucleated cells (BN), and nuclear buds (NB). Statistical analysis was carried out using SPSS v25, with $p < 0.05$ as the significance threshold. Results showed that the exposed drivers had markedly higher frequencies of nuclear anomalies compared to controls. The mean micronucleus frequency in the exposed group (8.00 ± 0.05 per 100 cells) was about 80 times higher than in the control group (0.10 ± 0.01), while BN and NB frequencies were increased 12-fold and 23-fold respectively. The total nuclear abnormality frequency was 165.00 ± 0.36 in exposed drivers versus 10.20 ± 0.94 in controls. Although the differences were not statistically significant, the biological trend suggests cumulative genotoxic stress from chronic exposure to vehicular pollutants. Routine biomonitoring and stricter air-quality measures are recommended to protect occupationally exposed populations

CHAPTER ONE

1.0 INTRODUTION

1.1 Background of study

Environmental pollution, particularly air pollution from vehicular emissions, poses significant health risks in urban areas worldwide (Kumar *et al*., 2021; Lawin *et al*., 2018). Among the most concerning components of vehicular emissions are genotoxic substances such as polycyclic aromatic hydrocarbons (PAHs), nitrogen oxides (NOx), carbon monoxide (CO), and particulate matter (PM), which have been linked to DNA damage and increased cancer risk in exposed populations (IARC, 2016; Valavanidis *et al*., 2013). Roadside workers, especially commercial drivers, represent a particularly vulnerable group due to their prolonged and close-range exposure to traffic-related air pollutants (Lawin *et al*., 2018; Enakireru and Ekakitie, 2024).

The micronucleus (MN) assay represents one of the most widely accepted and standardized biomarkers for assessing chromosomal damage and genomic instability in human populations (Bonassi *et al*., 2020; Bolognesi *et al*., 2013). The Buccal Micronucleus Cytome (BMCyt) assay is a minimally invasive method for studying DNA damage, chromosomal instability, cell death and the regenerative potential of human buccal mucosal tissue (Fenech, 2009). This technique offers several advantages over traditional cytogenetic methods, including its non-invasive nature, cost-effectiveness, and suitability for large-scale epidemiological studies (Thomas *et al*., 2009; Da-Costa *et al*., 2025). The micronucleus (MN) assay in exfoliated buccal cells is a useful and minimally invasive method for monitoring genetic damage in humans (Thomas *et al*., 2008), making it particularly valuable for biomonitoring studies in occupationally exposed populations.

In Benin City, the University of Benin (UNIBEN) main gate axis is a heavily trafficked area, characterized by high vehicular density, poor traffic flow, and frequent exposure to exhaust fumes. Drivers operating in this vicinity are likely to face sustained exposure to airborne pollutants, which may contribute to cellular and genetic alterations over time. Despite the growing concern over air pollution in Nigerian cities, there is limited biomonitoring research focused on genotoxic impacts among occupationally exposed populations.

1.2 Statement of the Problem

Urban vehicular emissions, particularly at high-traffic intersections such as the University of Benin (UNIBEN) Main Gate axis in Benin City, Nigeria, release complex mixtures of genotoxic pollutants including polycyclic aromatic hydrocarbons (PAHs), benzene, particulate matter ($PM_{2.5}$ and PM_{10}), and heavy metals (WHO, 2021; Oriaku and Iwuala, 2019; Osayuwu *et al*., 2021). Commercial drivers stationed at this location experience prolonged occupational exposure to these emissions, often exceeding 8–12 hours daily, with minimal use of personal protective equipment (Abam and Unachukwu, 2010; Ajayi *et al*., 2023). Such chronic inhalation exposure has been linked to elevated DNA damage in peripheral blood lymphocytes and buccal epithelial cells among traffic-exposed populations (Celik *et al*., 2013; Recoleto, 2017).

The buccal micronucleus (MN) assay is a validated, non-invasive biomarker for detecting chromosomal damage and genomic instability in exfoliated epithelial cells, reflecting early genotoxic effects prior to clinical disease manifestation (Holland *et al*., 2008; Bolognesi *et al*., 2015). Despite its sensitivity, no localized study has applied the MN assay to assess DNA damage in buccal cells of drivers at the UNIBEN Main Gate, a site characterized by intense traffic congestion, roadside vending, and poor urban planning (Osayuwu *et al*., 2021). Preliminary air quality assessments near the axis reported $PM_{2.5}$ levels averaging 50–100 $\mu g/m^3$

during peak hours, far exceeding WHO guidelines of 15 μg/m³ (24-h mean) (WHO, 2021; Oriaku and Iwuala, 2019).

This knowledge gap hinders evidence-based occupational health interventions and risk communication for this vulnerable group. Therefore, there is an urgent need to quantify genotoxic risk using the buccal MN assay among drivers at the UNIBEN Main Gate axis to inform regulatory measures, exposure mitigation strategies, and longitudinal health surveillance.

1.3 Justification of the Study

The increasing burden of air pollution in urban centers such as Benin City calls for urgent scientific investigation into its health implications, particularly for individuals with high occupational exposure. Commercial drivers who operate daily along the University of Benin (UNIBEN) main gate axis are consistently exposed to vehicular emissions, making them a high-risk group for pollution-related health issues, including genotoxic effects.

Despite the known health risks associated with vehicular emissions, there is limited biomonitoring data in Nigeria assessing DNA damage among occupationally exposed populations. The application of the micronucleus assay in exfoliated buccal cells offers a non-invasive, reliable, and cost-effective method for detecting early signs of genotoxicity in such vulnerable groups. This assay has been widely used in other countries for environmental and occupational health surveillance, but its application remains underutilized in local research contexts.

Conducting this study in the UNIBEN main gate area will provide relevant baseline data on DNA damage among drivers exposed to traffic pollution in Benin City. The results can contribute significantly to public health awareness, support the need for environmental policy

interventions, and encourage routine biomonitoring for at-risk populations. Additionally, the findings may serve as evidence to advocate for improved traffic management, emission control measures, and health protection policies for commercial drivers and similar occupational groups.

1.4 Aim and the objectives

The aim of the study is to assess DNA damage in drivers exposed to vehicular emissions at the UNIBEN main gate axis, Benin City, using the micronucleus assay of buccal cells.

Specific Objectives

- To collect and analyze buccal cell samples from commercial drivers operating along the UNIBEN main gate axis.
- To determine the frequency of micronuclei and other nuclear abnormalities as indicators of DNA damage in the collected samples.
- To compare the levels of DNA damage in exposed drivers with those in a control group with minimal exposure to vehicular emissions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Vehicular Emissions and Air Pollution

Vehicular emissions are a major contributor to urban air pollution, comprising a complex mixture of gaseous and particulate pollutants, with key components including particulate matter ($PM_{2.5}$ and PM_{10}), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and heavy metals. $PM_{2.5}$ and PM_{10} refer to particles with aerodynamic diameters less than 2.5 μm and 10 μm, respectively, originating from fuel combustion, brake and tire wear, and road dust resuspension. Studies indicate that PM concentrations at traffic junctions often exceed World Health Organization (WHO) guidelines, posing serious health risks (Muritala *et al*., 2025). PAHs, persistent organic pollutants formed during incomplete combustion of fossil fuels, are particularly hazardous in their high molecular weight forms, which tend to bind to particulate matter and increase toxicity (Debbarma *et al*., 2025). Vehicular emissions, particularly from diesel exhaust, are recognized as a dominant source of urban PAHs. VOCs, including hydrocarbons, aldehydes, and ketones, are emitted from both fuel evaporation and combustion processes; gasoline and diesel vehicles emit significant amounts, with diesel vehicles showing higher levels of oxygenated VOCs (Wang *et al*., 2022). These compounds contribute to the formation of ground-level ozone and secondary organic aerosols, further degrading air quality. Heavy metals such as lead (Pb), cadmium (Cd), nickel (Ni), and chromium (Cr) are released via exhaust gases, brake wear, tire abrasion, and resuspended road dust. Even at low concentrations, these metals are toxic, and studies in Lagos and Jos, Nigeria, report elevated levels in roadside dust, strongly correlating with traffic density (Ojiodu *et al*., 2023; Mafuyai *et al*., 2015).

The health impacts of vehicular emissions are substantial. Fine particles ($PM_{2.5}$) and ultrafine particles penetrate deep into the lungs and can enter the bloodstream, triggering systemic

inflammation and oxidative stress. Chronic exposure is linked to respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD), as well as cardiovascular conditions including myocardial infarction and stroke (Miller and Newby, 2020). Proximity to high-traffic areas has been associated with increased risks of coronary artery disease and atherosclerosis (Grahame and Schlesinger, 2010). Beyond cardiopulmonary effects, certain pollutants, notably PAHs and heavy metals, exert genotoxic and mutagenic effects by inducing oxidative DNA damage and interfering with DNA repair mechanisms. Micronucleus assays in children exposed to traffic-related air pollution have demonstrated increased genotoxicity (Hisamuddin *et al*., 2020), and additional studies have reported DNA methylation changes and the presence of bulky DNA adducts in traffic-exposed populations, indicating long-term genetic and epigenetic risks (Munnia *et al*., 2023). Longitudinal biomonitoring of traffic conductors and drivers has revealed elevated levels of oxidative DNA damage biomarkers, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG) and DNA strand breaks (Huang *et al*., 2012). Epigenetic alterations, including global hypomethylation and methylation changes in inflammatory genes like IL-6, have also been linked to exposure to benzo[a]pyrene and ozone, both prevalent components of vehicular exhaust.

2.2 Genotoxicity of Air Pollutants

Air pollutants, particularly those originating from vehicular and industrial sources, can exert significant genotoxic effects through various molecular mechanisms. One of the primary pathways is oxidative stress, in which reactive oxygen species (ROS) generated by particulate matter (PM), ozone, and nitrogen oxides interact with cellular components, damaging DNA bases and leading to strand breaks and mutations. Exposure to fine particulate matter ($PM_{2.5}$) has been shown to induce oxidative DNA damage via mitochondrial dysfunction and NADPH oxidase activation (Risom *et al*., 2005). Biomarkers such as 8-hydroxydeoxyguanosine (8-OHdG)

are often elevated in exposed populations, indicating oxidative stress and potential genomic injury (Yoshida, 2024).

Another key mechanism involves DNA adduct formation. Polycyclic aromatic hydrocarbons (PAHs), a major component of traffic-related emissions, undergo metabolic activation into electrophilic intermediates that bind covalently to DNA, producing bulky adducts. Such DNA adducts are strongly associated with an increased risk of cancer and are widely used as biomarkers of environmental exposure (Vineis, 2005). Notably, benzo[a]pyrene, a well-characterized PAH, has been linked to elevated DNA adduct levels in occupationally exposed groups such as traffic police and in urban residents (Munnia *et al*., 2023).

Air pollutants have also been implicated in inducing chromosomal aberrations, including breaks, translocations, and the formation of micronuclei in exposed cells. Evidence from micronucleus assays conducted among children and industrial workers indicates higher rates of chromosomal damage in populations residing or working near high-emission areas (Kazensky *et al*., 2024; Sopian *et al*., 2020). These alterations are indicative of genomic instability and may represent early steps in carcinogenesis.

Epidemiological research has further reinforced these mechanistic findings. Occupational exposure studies involving professional drivers, traffic wardens, and roadside vendors consistently reveal elevated oxidative DNA damage biomarkers, such as 8-OHdG and nitric oxide levels, compared to less exposed populations (Lai *et al*., 2005). A comprehensive review of 63 studies found strong and consistent evidence for genotoxicity biomarkers in traffic-exposed individuals, including DNA strand breaks and altered DNA methylation patterns (DeMarini, 2013).

Comparative studies between urban and rural populations also support the role of traffic-related air pollution in genotoxicity. Urban residents exhibit significantly higher DNA adduct levels and global hypomethylation than their rural counterparts (Vineis, 2005; Georgiadis *et al*., 2001). Alarmingly, even low-level urban exposure has been shown to produce measurable genotoxic effects, especially in children, who may be more susceptible due to their developing physiological systems (Ceretti *et al*., 2020).

Longitudinal investigations highlight a clear dose–response relationship between $PM_{2.5}$ exposure and DNA damage biomarkers (Wei *et al*., 2021). Chronic exposure is linked to increased risks of lung cancer and other pollution-associated diseases, even when pollutant concentrations remain below current regulatory thresholds. Moreover, cumulative DNA adduct levels have been shown to correlate with long-term exposure duration and are predictive of future cancer risk (Peluso *et al*., 2005).

2.3 Micronucleus Assay in Environmental and Occupational Health Studies

The micronucleus (MN) assay is a well-established cytogenetic technique used to detect chromosomal damage, genome instability, and cytotoxicity. It has gained prominence in environmental and occupational health research due to its sensitivity and non-invasive nature, particularly when applied to exfoliated buccal cells (Fenech *et al*., 2011). This approach enables the assessment of DNA damage in exposed populations without the need for invasive sampling, making it especially suitable for epidemiological surveillance.

Several studies have demonstrated the application of the buccal MN assay in assessing the genotoxic effects of air pollution. For instance, the MAPEC_LIFE study in Italy investigated children in five cities and found seasonal variations in MN frequency associated with $PM_{2.5}$,

benzene, and polycyclic aromatic hydrocarbons (PAHs) (Verani *et al*., 2017). In the Brazilian Amazon, Sisenando *et al*. (2012) reported significantly higher MN frequencies in children exposed to biomass burning compared to unexposed controls, underscoring the assay's relevance even in remote regions. Similarly, Awang *et al*. (2020) found that Malaysian traffic policemen had MN frequencies more than double those of office workers, with these findings correlating strongly with elevated $PM_{2.5}$ and benzene, toluene, ethylbenzene, and xylene (BTEX) exposure levels.

In Nigeria, high-traffic urban environments such as Lagos present a significant public health concern. Onyeneke (2018) observed that residents and traffic officers in Lagos exhibited elevated MN frequencies, consistent with chronic exposure to vehicular emissions. Comparable results have been documented in other heavily trafficked cities worldwide, including Klang Valley in Malaysia, Turin in Italy, and São Paulo in Brazil, where traffic density and genotoxic biomarkers show a clear positive association. In Klang Valley, Awang et al. (2020) reported MN frequencies of 6.2‰ among traffic policemen compared to 3.0‰ in office workers, with a correlation coefficient of $r = 0.725$. The MAPEC_LIFE study further quantified this relationship, revealing a 20.1% increase in MN risk per unit increase in benzene concentration (Verani *et al*., 2017).

The buccal MN assay offers distinct advantages as a biomonitoring tool. It is non-invasive, using exfoliated buccal cells that are easily collected from both children and occupationally exposed adults. Its sensitivity allows for the detection of chromosomal damage, apoptosis, and necrosis at relatively low exposure levels, and its accessibility ensures it can be implemented in large-scale studies with minimal specialized equipment (Malacarne *et al*., 2021).

Standardization of protocols is critical for reproducibility and comparability of MN assay results. Samples are typically collected in the morning using a cytobrush or spatula, and staining methods can be DNA-specific, such as Feulgen and Acridine Orange, or non-specific, such as Giemsa, Papanicolaou, and hematoxylin and eosin (H&E). Feulgen staining is preferred for precise MN detection (Mohammed and Ahmed, 2024). Scoring criteria require that MN be round or oval, less than one-third the size of the main nucleus, and not overlapping with it, with at least 1,000 cells per subject analyzed, in accordance with OECD (2014).PM

International efforts to harmonize MN assay methodologies have been spearheaded by the HUMNxl project, an extension of the original HUMN initiative. Established in 2007, this project has compiled data from over 5,000 subjects across 30 laboratories, defined reference values, identified confounding factors such as age and smoking, and promoted inter-laboratory consistency. It also encourages the adoption of automated scoring methods using image analysis and flow cytometry (Bonassi *et al*., 2011).

In practical terms, buccal cell collection is followed by fixation in ethanol or methanol, staining (Feulgen for DNA specificity or Giemsa/PAP for morphology), and manual or automated scoring. Automated systems such as CellProfiler and ImageJ are increasingly employed to minimize observer bias and increase throughput (Yoda *et al*., 2024). Collectively, these methodological refinements and global standardization efforts ensure that the buccal MN assay remains a reliable and widely applicable tool in environmental and occupational health research.

2.4 Drivers as a High-Risk Occupational Group

Professional drivers, particularly those operating in urban environments, are chronically exposed to traffic-related air pollutants such as particulate matter ($PM_{2.5}$, PM_{10}), nitrogen oxides (NO_x), carbon monoxide (CO), and polycyclic aromatic hydrocarbons (PAHs). These exposures occur within transport microenvironments, which often have pollutant concentrations several times higher than ambient levels (Lim *et al*., 2021). Many drivers work long hours with limited rest breaks and irregular shifts, which increases cumulative exposure over time (Popescu *et al*., 2025). In developing countries, outdated vehicle fleets and poor cabin ventilation exacerbate these risks (Chamila *et al*., 2025). For example, public transport drivers in Romania experienced significant increases in blood pressure and musculoskeletal disorders over an 11-year follow-up period, with the effects correlating strongly with years of service (Popescu *et al*., 2025).

Research from Lagos, Nigeria, has revealed elevated concentrations of $PM_{2.5}$, CO, NO_2, and BTEX compounds in high-traffic zones, with drivers in these areas showing impaired pulmonary function and elevated carboxyhemoglobin levels (Oguntoke, 2011). Similarly, in Colombo, Sri Lanka, three-wheeled taxi drivers were found to have in-cabin $PM_{2.5}$ concentrations as high as 386 $\mu g/m^3$, seven times higher than levels recorded in air-conditioned cars (Chamila *et al*., 2025). Exposure monitoring in low- and middle-income countries, however, remains limited due to resource constraints and the lack of standardized protocols (Quintero *et al*., 2024).

Socioeconomic and lifestyle factors further influence susceptibility to the harmful effects of vehicular emissions. Drivers in low- and middle-income countries often face poor remuneration, job insecurity, and limited access to healthcare, increasing their vulnerability to occupational hazards (Amoadu *et al*., 2024). Unhealthy lifestyle patterns, such as smoking, poor diet, and physical inactivity, are also prevalent in this population, further compounding health risks (Jakobsen *et al*., 2023). Moreover, lower socioeconomic status is associated with increased

susceptibility to oxidative stress and DNA damage due to limited antioxidant defenses (Foster *et al*., 2023).

Studies investigating genetic damage in drivers have reported consistently higher biomarker levels compared to control groups. In Taiwan, traffic conductors showed significantly elevated urinary 8-OHdG and DNA strand breaks relative to office workers (Huang *et al*., 2012). In Brazil, taxi drivers exhibited higher micronucleus frequencies and comet assay tail DNA, with results correlating to urinary 1-hydroxypyrene concentrations (Barth *et al*., 2017). Similarly, minibus drivers in Baghdad demonstrated increased TNF-α levels and markers of oxidative DNA damage compared to controls, indicating chronic inflammation and genotoxic stress (Khalid and Rabee, 2025).

These findings vary regionally and across occupational roles. In Nigeria, for example, the frequency of micronuclei among commercial drivers differs by region and vehicle type, with the highest rates observed in densely populated urban centers such as Lagos and Abuja (Oguntoke, 2011). Occupational differences, such as those between bus and taxi drivers, also play a role due to variations in cabin design, route patterns, and ventilation efficiency (Chamila *et al*., 2025). Environmental conditions further influence exposure, with drivers in colder climates generally experiencing lower pollutant levels because of closed windows and better filtration, while those in tropical regions tend to have higher in-cabin pollutant loads.

2.5 Nigerian and African Context of Air Pollution and Genotoxicity

Benin City, a rapidly urbanizing hub in southern Nigeria, is experiencing escalating air pollution challenges driven by vehicular emissions, widespread use of generators, and open waste burning. Recent investigations have revealed concerning concentrations of fine particulate matter ($PM_{2.5}$)

and carbon dioxide (CO_2) in busy commercial zones. Ovenseri and Otabor (2025) reported $PM_{2.5}$ levels ranging from 15 to 60.66 μg/m³ in market areas, values that exceed World Health Organization (WHO) guidelines and fall within "moderate" to "unhealthy" categories. Complementary satellite-derived data from PurpleAir and NASA archives indicate seasonal variations, with peak pollution during dry months such as February when air quality index (AQI) values reached 141, a level classified as "unhealthy for sensitive groups" (Banga, 2024). Real-time monitoring by IQAir further confirms that $PM_{2.5}$ levels in Benin City are frequently 7.4 times higher than WHO annual limits, suggesting a persistent and chronic exposure risk for residents.

One notable pollution hotspot is the University of Benin (UNIBEN) Main Gate, located along the heavily trafficked Ugbowo-Lagos Road. Observations by Azeta (2018) over a six-week period documented peak congestion between 7–9 AM and 3–5 PM, with traffic density contributing to increased accident risks and significant pedestrian discomfort. The installation of speed breakers and suboptimal road designs have exacerbated bottlenecks, as corroborated by local media reports and urban planning assessments.

Nigeria's air quality management framework has evolved in recent years but remains fragmented in implementation. The 2023 Air Quality Regulations set permissible limits for pollutants including PM, NO_x, SO_2, and CO, and incorporate provisions addressing mobile emission sources such as vehicles. Enforcement responsibilities rest with the National Environmental Standards and Regulations Enforcement Agency (NESREA) and state-level bodies, yet the country's monitoring infrastructure is sparse, particularly outside major urban centers. UNEP (2016) has emphasized the importance of integrated air quality management, warning that

current policy approaches often prioritize industrial emissions while insufficiently addressing urban vehicular sources.

Evidence of genotoxic impacts from vehicular pollution has been documented in several Nigerian cities. In Lagos, traffic wardens and roadside vendors exhibited elevated micronucleus frequencies and oxidative stress biomarkers, indicative of DNA damage (Oguntoke, 2011). Similar links between vehicular emissions and adverse health outcomes have been found in Ughelli, Delta State, where respiratory illness and diminished agricultural productivity were reported (Eguvbe et al., 2021). Studies in Abuja and Port Harcourt have further associated PM exposure with DNA damage, systemic inflammation, and heightened cancer risk (DeMarini, 2013).

Despite these findings, Edo State—and Benin City in particular, lacks comprehensive genotoxicity data. No published micronucleus assay investigations have been conducted locally, even though existing air quality data suggest moderate to high $PM_{2.5}$ levels in urban districts.

2.6 Buccal cell micronucleus assay versus other biomonitoring endpoints in environmental genotoxicity

Genotoxicity assays serve as essential tools for assessing cancer risk and environmental exposure to mutagenic agents. The most commonly employed genotoxicity tests include the comet assay, micronucleus assay, chromosomal aberration test, bacterial reverse mutation assay, and sister chromatid exchange assay (Kim *et al*., 2014). These methods detect different types and levels of genetic damage, ranging from DNA strand breaks to complex chromosomal rearrangements. While in vitro genotoxicity assays provide preliminary screening data, in vivo biomonitoring offers biological significance for specific organs and cell types, reflecting actual absorption,

distribution, metabolism, and excretion of genotoxic substances in the human body (Kim *et al*., 2014).

Genomic damage is recognized as a fundamental cause of developmental and degenerative diseases, produced by environmental exposure to genotoxins, medical procedures, micronutrient deficiency, lifestyle factors such as alcohol and smoking, and genetic factors including inherited defects in DNA metabolism and repair (Holland *et al*., 2008). Consequently, reliable and minimally invasive biomarkers are essential for implementing effective biomonitoring, diagnostics, and treatment strategies for diseases associated with genetic damage.

The micronucleus assay in exfoliated buccal cells represents a useful and minimally invasive method for monitoring genetic damage in humans (Holland *et al*., 2008). Micronuclei are small extranuclear bodies formed by chromosome fragments or whole chromosomes that lag behind during anaphase of cell division and are not incorporated into daughter nuclei (Thomas *et al*., 2009). These structures arise in dividing basal cells of the oral epithelium but are observed in differentiated cells in the keratinized layer at the buccal surface (Gajski *et al*., 2020).

The buccal micronucleus cytome (BMCyt) assay extends beyond simple MN scoring to include several additional cytogenetic biomarkers related to cell death, DNA damage, cytostasis, and cytotoxicity (Thomas *et al*., 2009). These biomarkers include binucleated cells (indicating cytokinesis failure), nuclear buds (representing elimination of amplified DNA or DNA repair complexes), condensed chromatin cells (early apoptotic markers), karyorrhectic cells (late apoptotic markers), and karyolytic cells (necrotic markers) (Bolognesi *et al*., 2015).

The buccal MN assay offers numerous advantages that make it particularly suitable for large-scale biomonitoring studies. First, the technique is minimally invasive, requiring only gentle

scraping of cells from the inner cheek, making it well-accepted by participants and suitable for vulnerable populations including children (Torres-Bugarín *et al*., 2014). Second, the assay does not require cell culture or ex vivo replication steps, unlike the cytokinesis-block method in lymphocytes, thereby reducing technical complexity and cost (Thomas *et al*., 2011). Third, buccal cells are directly exposed to genotoxic agents through inhalation and food intake, making them particularly relevant for assessing exposure to environmental pollutants and dietary factors (Gajski *et al*., 2020).

Additionally, buccal cells have been shown to have limited DNA repair capacity relative to peripheral blood lymphocytes, potentially making them more sensitive indicators of accumulated DNA damage, particularly age-related genomic instability in epithelial tissues (Burgaz *et al*., 2007). The assay is relatively rapid, cost-effective, and does not require sophisticated laboratory equipment, making it accessible for resource-limited settings (Torres-Bugarín *et al*., 2014). Furthermore, since buccal epithelium is a rapidly dividing tissue with a turnover time of approximately 7-21 days, it provides a relatively recent window of exposure assessment (Thomas *et al*., 2009).

Despite its advantages, the buccal MN assay has several limitations that must be considered. A major challenge is the lack of standardization in protocols, staining procedures, and scoring criteria across laboratories, which has historically limited the comparability of results from different studies (Holland *et al*., 2008). Although the Human MicroNucleus Project (HUMN) and its extension to buccal cells (HUMNxL) have made significant progress in addressing these issues through international collaborative efforts, variability remains a concern (Bonassi *et al*., 2009; Bolognesi *et al*., 2015).

Another limitation is the relatively small magnitude of changes typically observed in micronucleus frequency even in populations with significant genotoxic exposure, which necessitates large sample sizes to achieve adequate statistical power (Holland *et al*., 2008). Additionally, the baseline MN frequency in control populations shows considerable inter-individual variability influenced by factors such as age, gender, smoking status, alcohol consumption, and dietary habits, requiring careful control for confounding variables (Thomas *et al*., 2011).

The interpretation of buccal cell MN data is also complicated by the fact that multiple types of nuclear abnormalities can occur simultaneously, and their biological significance and relationship to disease outcomes are not fully understood (Holland *et al*., 2008). Furthermore, while the correlation between lymphocyte and buccal cell MN frequencies has been demonstrated, the relationship is not perfect, suggesting tissue-specific responses to genotoxic exposure (Bonassi *et al*., 2009).

The cytokinesis-block micronucleus cytome (CBMNcyt) assay in peripheral blood lymphocytes represents the most frequently used method for biomonitoring human populations exposed to genotoxic agents (Bolognesi and Fenech, 2013). This assay involves culturing lymphocytes for 72 hours with the addition of cytochalasin B at 44 hours to block cytokinesis, resulting in binucleated cells that can be specifically scored for micronuclei (Fenech, 2007).

The CBMN assay offers several distinct advantages. First, lymphocytes are systemic cells that circulate throughout the body, potentially reflecting whole-body genotoxic exposure rather than localized tissue damage (Bonassi *et al*., 2001). Second, the use of cytochalasin B to block cytokinesis ensures that micronuclei are scored only in cells that have completed one nuclear division, providing a more precise assessment of recent DNA damage (Fenech, 2007). Third,

there is extensive historical data and well-established standardized protocols for the CBMN assay, facilitated by decades of work through the HUMN project (Bonassi *et al*., 2001).

Furthermore, scientific evidence suggests a significant association between increased MN frequency in lymphocytes and elevated risk of cancer and other age-related degenerative diseases, providing validation for its predictive value as a biomarker (Bonassi *et al*., 2007). A large international database comparison has established reference values and identified key variables affecting MN frequency in lymphocytes, including laboratory protocol, scoring criteria, age, gender, and lifestyle factors (Bonassi *et al*., 2001).

However, the CBMN assay also has limitations. It is more invasive than the buccal cell assay, requiring blood collection which may limit participation, particularly in pediatric studies or large epidemiological investigations (Torres-Bugarín *et al*., 2014). The assay requires 72 hours of cell culture, specialized laboratory equipment, and trained personnel, making it more expensive and time-consuming than the buccal cell assay (Bolognesi and Fenech, 2013). Additionally, lymphocytes have efficient DNA repair mechanisms, which may result in underestimation of transient DNA damage that has been repaired before sampling (Burgaz *et al*., 2007).

The comet assay, also known as single cell gel electrophoresis, is a sensitive, reliable, and rapid method for detecting DNA strand breaks, alkali-labile sites, and incomplete excision repair sites at the single-cell level (Collins, 2004). The assay can detect DNA single-strand breaks (SSB) under alkaline conditions and DNA double-strand breaks (DSB) under neutral conditions (Cortés-Gutiérrez *et al*., 2012).

The comet assay's primary advantage is its exceptional sensitivity in detecting early DNA damage that may be repaired before manifesting as permanent chromosomal damage (Kim *et al*.,

2014). It requires only a small number of cells (as few as 10,000), can be performed on virtually any eukaryotic cell type, and provides quantitative data on the extent of DNA damage through measurement of comet parameters such as tail length, tail intensity, and tail moment (Møller, 2005). The assay is relatively quick, with results obtainable within hours rather than days, making it suitable for time-sensitive investigations (Collins, 2004).

However, the comet assay has important limitations for biomonitoring applications. The DNA damage detected may represent transient, repairable lesions rather than permanent genetic alterations, potentially overestimating long-term health risks (Speit *et al*., 2012). The assay requires fresh or properly frozen samples and careful standardization of electrophoresis conditions, as technical factors can significantly influence results (Burlinson *et al*., 1998). Furthermore, there is considerable intra- and inter-individual variability in comet parameters, and the relationship between comet assay results and cancer risk is less well-established compared to the micronucleus assay (Collins, 2004).

A comparative study by Giannotti et al. (2002) found that while the comet assay showed good qualitative agreement with chromosomal aberration tests, positive results were typically observed at higher doses, indicating reduced sensitivity compared to cytogenetic endpoints. The authors noted that maximum DNA damage was detected at earlier sampling times (0.25-1 hour) compared to the standard 3-hour exposure, suggesting the importance of optimizing sampling time for different exposure scenarios.

Chromosomal aberration (CA) analysis represents the classical cytogenetic method for assessing genotoxic damage, involving microscopic examination of metaphase spreads to identify structural and numerical chromosomal abnormalities (Savage, 1976). CAs are classified into

chromosome-type and chromatid-type aberrations, with further subdivision into deletions, translocations, inversions, and other complex rearrangements (Preston *et al*., 1987).

The CA assay is considered highly sensitive and reliable, with chromosomal aberrations having been demonstrated as strong predictors of cancer risk in numerous epidemiological studies (Bonassi *et al*., 2000). The assay can detect stable chromosomal rearrangements such as translocations, which persist in cells and may have long-term consequences for genetic stability (Fenech, 2007). Advanced techniques such as fluorescence in situ hybridization (FISH) have enhanced the ability to detect specific types of chromosomal damage and identify the chromosomes involved (Tucker and Preston, 1996).

However, CA analysis has several significant limitations that restrict its use in large-scale biomonitoring. The technique is labor-intensive, requiring highly trained cytogeneticists to analyze metaphase spreads, making it expensive and time-consuming (Fenech, 2007). A relatively large number of cells (typically 100-500 metaphases) must be examined to achieve adequate statistical power (Preston *et al*., 1987). The assay requires cell culture and the ability to obtain metaphase preparations, which limits its application to proliferating cell populations such as lymphocytes or bone marrow cells (Bonassi *et a*l., 2000). Additionally, the scoring criteria for different types of aberrations can be subjective, potentially introducing inter-scorer variability (Savage, 1976).

Sister Chromatid Exchange Assay

Sister chromatid exchanges (SCEs) represent reciprocal exchanges of DNA between sister chromatids at apparently homologous loci, visualized following incorporation of 5-bromodeoxyuridine (BrdU) during two rounds of DNA replication (Perry and Wolff, 1974).

SCEs are induced by various DNA-damaging agents and were historically considered sensitive indicators of genotoxic exposure (Latt, 1981).

The SCE assay is relatively sensitive to certain classes of genotoxic agents, particularly those causing replication-related DNA damage, and can detect effects at lower concentrations than some other endpoints (Carrano and Natarajan, 1988). The technique provides quantitative data on the frequency of exchanges per cell and per chromosome, allowing for detailed analysis of genotoxic responses (Latt, 1981). SCE analysis can be performed on the same lymphocyte cultures used for CA or CBMN assays, potentially providing complementary information (Carrano and Natarajan, 1988).

However, the SCE assay has fallen out of favor for several reasons. The biological significance of SCEs and their relationship to mutagenesis and carcinogenesis remain poorly understood (Tucker et al., 1993). Many SCEs may represent normal DNA repair processes rather than genotoxic damage, limiting their specificity as biomarkers of harmful exposure (Latt, 1981). The assay requires BrdU incorporation over two cell cycles and specialized staining techniques, adding complexity and cost (Perry and Wolff, 1974). Furthermore, SCE frequencies show high inter-individual variability and weak correlation with cancer risk compared to other cytogenetic endpoints (Tucker et al., 1993). Consequently, regulatory agencies have generally not required SCE testing, and the assay is now rarely used in biomonitoring studies (Fenech, 2007).

2.7 Knowledge Gaps and Rationale for the Present Study

Despite the rapid urbanization and persistent traffic congestion in Benin City, Edo State, there remains a striking lack of biomonitoring studies that assess the genotoxic effects of air pollution on exposed populations. Existing research on air quality in the city has largely focused on

measuring pollutant concentrations such as $PM_{2.5}$ and CO_2, without establishing direct links between these exposures and biological outcomes (Ovenseri and Otabor, 2025). Unlike cities such as Lagos and Abuja, Benin City has no published data on DNA damage biomarkers, including micronucleus (MN) frequency or oxidative stress indicators, creating a significant knowledge gap that limits the capacity of public health authorities to evaluate long-term risks and design targeted interventions. As Ajemba and Arene (2022) note, identifying and addressing such research gaps is critical for expanding scientific inquiry and shaping future research priorities.

Although the buccal micronucleus assay is an established and non-invasive method for detecting genotoxicity, its application in Nigerian occupational health research remains limited. Studies employing this assay have tended to focus on industrial exposures, such as abattoir smoke or scavenging at dumpsites, rather than traffic-related pollution (Onwukwe *et al*., 2019). For instance, a study in Enugu demonstrated significantly higher MN frequencies in abattoir workers compared to controls, highlighting the assay's utility in occupational settings (Onwukwe et al., 2019). However, very few investigations have applied this method to high-risk occupational groups such as drivers, traffic wardens, or roadside vendors, despite their substantial exposure to vehicular emissions. According to Pisani et al. (2020), challenges such as inconsistent staining techniques and the absence of standardized protocols may have hindered the wider adoption of the assay in Nigeria.

In the broader context of low- and middle-income countries (LMICs), there is an urgent need for cost-effective, scalable, and non-invasive tools for genotoxicity assessment. The buccal MN assay offers a particularly practical solution, requiring minimal laboratory infrastructure, avoiding invasive sampling, and allowing deployment in field conditions (Bonassi *et al*., 2011).

While high-throughput and AI-assisted scoring technologies are emerging in global research, manual scoring continues to be a reliable and accessible approach in LMIC contexts (Alnasser, 2025). Integrating such biomonitoring tools into public health surveillance systems could enable early identification of at-risk populations and support evidence-based policy interventions. This aligns with recommendations from initiatives such as the EU PARC and OECD guidelines, which advocate for integrated, ethical, and cost-effective approaches to genotoxicity testing.

2.8 Previous Studies on Micronucleus Assay of Buccal Cells for Assessing DNA Damage in Drivers Exposed to Vehicular Emissions

Onyemauwa (1994) conducted a study on "Environmental impact of vehicular traffic in Nigeria: health aspects". This study investigated the health impacts of vehicular emissions on traffic wardens in Nigeria, specifically comparing those from Lagos (a densely populated metropolitan area) with those from Ile-Ife (a less densely populated university town). The study enrolled 60 traffic wardens from Lagos (ages 24-52 years; mean 27±6), 13 from Ile-Ife (ages 22-40 years; mean 27±8), and 24 control subjects not occupationally exposed to vehicular emissions (ages 19-55 years; mean 31±8). Blood lead levels were analyzed using Perkin-Elmer Zeeman 3030/HGA 600 Atomic Absorption Spectroscopy (AAS) after appropriate sample preparation. Pulmonary function tests including peak expiratory flow rate (PEFR), forced expiratory volume in 1 second (FEV_1), and forced vital capacity (FVC) were performed on all participants using a standard spirometer following American Thoracic Society guidelines. Statistical analysis included ANOVA and Tukey's post-hoc test for multiple comparisons. The mean blood lead level in Lagos traffic wardens was 18.1±6.4 μg/dl, significantly higher than the 10.2±2.7 μg/dl found in Ile-Ife wardens and 12.9±7.0 μg/dl in controls ($p<0.001$). However, there was no significant difference between Ile-Ife wardens and controls, suggesting that exposure intensity is directly

related to traffic density. Significant differences ($p<0.0005$) in all spirometric measurements were observed: Lagos wardens showed PEFR of 387±68 L/min, FEV_1 of 2.8±0.5 L, and FVC of 3.4±0.6 L compared to controls (PEFR: 468±52 L/min, FEV_1: 3.6±0.4 L, FVC: 4.2±0.5 L). The study concluded that traffic wardens in densely populated urban areas of Nigeria experience significant health impacts from vehicular pollution, including elevated lead exposure and respiratory impairment, necessitating protective measures and health surveillance programs.

Adeleke *et al*., (2011) conducted a study on "Assessment of health impacts of vehicular pollution on occupationally exposed people in Lagos metropolis, Nigeria". This study assessed health impacts of vehicular pollution on occupationally exposed individuals in Lagos metropolis. The study enrolled commercial drivers (n=80), traffic wardens (n=50), and street vendors (n=70) from high-traffic areas including Oshodi, Ikeja, and CMS, and compared them with unexposed controls (n=60) from less polluted residential areas. Health assessment included clinical examination by trained physicians, pulmonary function tests using portable spirometers, questionnaire-based evaluation of respiratory and other symptoms, and blood sampling for carboxyhemoglobin analysis. Environmental monitoring of air pollutants (CO, NO_x, SO_2, and particulate matter) was conducted at workplaces of study participants using portable gas monitors and high-volume air samplers. Data were analyzed using chi-square tests and logistic regression. Occupationally exposed groups showed significantly higher prevalence of respiratory symptoms including chronic cough (42% vs 12% in controls, $p<0.001$), wheezing (35% vs 8%, $p<0.001$), and dyspnea (28% vs 6%, $p<0.001$). Pulmonary function tests revealed reduced lung capacity in exposed workers, with mean FEV_1 values 15-20% lower than predicted normal values. Blood samples showed elevated levels of carboxyhemoglobin (mean 8.5±2.3% in exposed vs. 2.1±0.8% in controls, $p<0.001$), indicating significant carbon monoxide exposure.

Commercial drivers had the highest COHb levels (9.2±2.5%), followed by traffic wardens (8.4±2.1%) and street vendors (7.8±2.4%). Air quality measurements revealed mean CO concentrations of 25-45 ppm at study sites, exceeding WHO guidelines of 9 ppm for 8-hour exposure. The study documented a significant relationship between vehicle density, exposure duration ($r=0.65$, $p<0.001$), and prevalence of air pollution-related diseases in Lagos.

Nwachukwu *et al*., (2018) conducted a study on "Carboxyhaemoglobin Levels among Traders Exposed to Vehicular Emissions in Three Motor Parks in Ibadan, Nigeria". Using a cross-sectional comparative design, this study assessed carboxyhemoglobin (COHb) levels as a biomarker of carbon monoxide exposure among traders at three motor parks in Ibadan, Nigeria. A total of 93 motor park traders were proportionally allocated among Agbowo Motor Park (AMP, n=33), Iwo Road Motor Park (IMP, n=30), and New Garage Motor Park (NMP, n=30), and 93 non-motor park traders (retail shop owners in less polluted areas) were selected as controls using systematic random sampling. Inclusion criteria specified minimum of 6 months working at motor parks and daily exposure of at least 8 hours. Exclusion criteria included smoking, cardiovascular disease, and respiratory illnesses. COHb levels were measured using a noninvasive Masimo Rad-57 pulse co-oximeter attached to participants' fingers after 5 minutes of rest. Environmental CO concentrations were measured using portable gas detectors. Statistical analysis included descriptive statistics, independent t-tests, and ANOVA with post-hoc comparisons. Mean carboxyhemoglobin levels for motor park traders at AMP, IMP, and NMP were 11.2±3.8%, 11.6±3.1%, and 12.2±3.3% respectively (range 3-22%), while mean COHb for non-motor park traders was 4.1±1.7% (range 2-8%), approximately three times lower ($p<0.001$). The overall mean COHb for all motor park traders (11.7±3.3%) was significantly higher than controls. Environmental CO monitoring revealed mean concentrations of 35-52 ppm at motor parks

compared to 8-12 ppm at control locations. Importantly, both groups exceeded the WHO guideline of 2.5% COHb, but motor park traders showed levels approaching the threshold for acute health effects (>15%). Duration of work at motor parks showed positive correlation with COHb levels ($r=0.56$, $p<0.01$). The study demonstrated that individuals working in motor parks have significantly elevated CO exposure and are highly susceptible to adverse health effects including cardiovascular complications, neurological impacts, and reduced oxygen-carrying capacity of blood.

Ajayi *et al*., (2023) conducted a study on "Public perceptions of vehicular traffic emissions on health risk in Lagos metropolis Nigeria: A critical survey". This study examined public perception and awareness of vehicular emissions and their health risks in Lagos metropolis through a critical survey. The study employed mixed-methods research design including structured questionnaires administered to 852 respondents across residential (n=324), commercial (n=298), and industrial (n=230) areas of Lagos using multi-stage sampling technique. The survey instrument assessed demographic characteristics, awareness levels regarding air pollution sources, knowledge of health impacts, perceived severity of pollution, and adoption of protective behaviors. Additional focus group discussions (n=12 groups, 8-10 participants each) were conducted to gather qualitative insights. Statistical analysis included descriptive statistics, chi-square tests, logistic regression modeling, and thematic analysis for qualitative data using SPSS version 25.0. The study found that 70.5% of respondents were aware of vehicular traffic pollution and its adverse health effects, though awareness varied significantly by location (commercial areas: 82%, residential: 68%, industrial: 63%). There was significant relationship between socio-demographic factors and air pollution awareness: educational level ($\chi^2=45.3$, $p=0.001$), age ($\chi^2=32.7$, $p=0.01$), length of residency ($\chi^2=28.4$, $p=0.02$), and marital status

(χ^2=18.9, p=0.03). Logistic regression identified educational level (OR=3.4, 95% CI: 2.1-5.5) and age (OR=2.1, 95% CI: 1.4-3.2) as the strongest predictors of awareness. Women demonstrated higher knowledge scores (mean 15.8±3.2 out of 20) about air pollution compared to men (mean 13.2±3.7, $p<0.05$). Despite high awareness, only 34% regularly used protective measures due to economic constraints (cost of face masks) and lack of alternatives to vehicular transport. Respondents reported various health symptoms attributed to vehicular emissions: respiratory problems including cough and catarrh (68%), headaches (54%), eye irritation (47%), skin problems (31%), and cardiovascular symptoms (18%). The study revealed that perception of severity correlated with proximity to major roads and duration of daily exposure.

Ezeigwe *et al*., (2024) conducted a study on "Characterization and Quantification of Vehicular Emissions in Abuja Municipality – Implications for Public Health". This comprehensive cross-sectional study characterized vehicular emissions in Abuja municipality and quantified exhaust air pollutants from commonly used vehicles. The study analyzed 543 vehicles undergoing routine Annual Road Worthiness Test at the Computerized Test Center, Abuja, between January and June 2023. Information was collected using structured questionnaires on vehicle type (personal cars, minibuses, large buses, trucks), age, fuel type (gasoline/petrol or diesel), purchase category (new or used/tokunbo), and use pattern (private or commercial). Exhaust emissions of carbon monoxide (CO), carbon dioxide (CO_2), hydrocarbons (HC), nitrogen oxides (NO_x), and particulate matter (PM_{10}) were measured using calibrated Kane Automotive Gas Analyser and TSI DustTrak II aerosol monitor following standard protocols. Measurements were taken at engine idle and acceleration conditions. Statistical analysis was performed using IBM SPSS version 26.0 including descriptive statistics, chi-square tests, ANOVA, and correlation analysis to determine relationships between vehicle characteristics and emission levels. The study

revealed that 66% of examined vehicles were over 10 years old, with 82% being used/tokunbo vehicles, resulting in 65% higher emission levels compared to newer vehicles ($p<0.001$). Personal cars (45% of sample) and minibuses (28%) predominantly emitted CO from gasoline engines with mean values of 4.2±2.1% and 3.8±1.9% respectively, while large buses (15%) and trucks (12%) significantly contributed to NO_x emissions from diesel engines (mean 980±340 ppm). Mean CO emissions exceeded WHO guideline of 3% in 45% of tested gasoline vehicles. PM_{10} levels were elevated (mean 285±120 μg/m³) in 58% of diesel vehicles, far exceeding the WHO 24-hour guideline of 50 μg/m³. Hydrocarbon emissions averaged 520±180 ppm in gasoline vehicles (WHO limit: 200 ppm). Commercial vehicles emitted significantly higher pollutant levels across all parameters compared to private vehicles ($p<0.01$). Strong positive correlations were found between vehicle age and all emission parameters (CO: $r=0.68$, NO_x: $r=0.71$, PM_{10}: $r=0.73$, all $p<0.001$). The study concluded that strong regulatory policies are urgently needed to discourage importation of over-aged vehicles, adopt ECOWAS guidelines on cleaner fuels (reducing sulfur content from current 1000 ppm to <50 ppm), implement effective mandatory inspection and monitoring programs, and promote cleaner transportation alternatives to safeguard public health in Nigerian cities.

Alimba *et al*., (2021) conducted a study on "Wild black rats (Rattus rattus Linnaeus, 1758) as zoomonitor of genotoxicity and systemic toxicity induced by hazardous emissions from Abule Egba unsanitary landfill, Lagos, Nigeria". This innovative biomonitoring study used wild black rats as sentinel organisms to assess genotoxicity from environmental pollutants, including vehicular emissions, at the Abule Egba landfill in Lagos. Rats were trapped from the landfill site (exposed group, n=20) and Oke-Afa community in Isolo (reference site with minimal pollution, n=20) using Sherman live traps baited with dried fish. Animals were acclimatized for 48 hours

before sampling. Multiple biomarkers were evaluated including micronucleus assay in bone marrow and peripheral blood erythrocytes, alkaline and neutral comet assay in liver and kidney cells, chromosomal aberration analysis in bone marrow, and hematological parameters (complete blood count) and biochemical parameters (liver and kidney function tests). Tissues were analyzed for heavy metal (lead, cadmium, chromium, nickel) accumulation using Atomic Absorption Spectrophotometry. Statistical analysis included t-tests and Mann-Whitney U tests. Exposed rats showed significantly elevated micronucleus frequencies in both bone marrow (4.2±0.8 per 1000 cells vs 0.8±0.3 in controls, $p<0.001$) and peripheral blood erythrocytes (3.5±0.6 per 1000 cells vs 0.6±0.2, $p<0.001$). The alkaline comet assay revealed extensive DNA damage in liver cells (mean tail moment 12.5±2.3 vs 2.1±0.5, $p<0.001$) and kidney cells (10.8±1.9 vs 1.8±0.4, $p<0.001$). Neutral comet assay detected DNA double-strand breaks with tail moments of 8.3±1.7 in liver and 7.2±1.5 in kidney of exposed rats (controls: 1.2±0.3 and 1.0±0.2 respectively, $p<0.001$). Chromosomal aberration frequencies were significantly elevated (5.8±1.2% vs 1.2±0.4%, $p<0.001$) with predominant aberrations being chromatid breaks and gaps. Hematological analysis showed significant decreases in RBC count (6.2±0.8 vs 7.8±0.6 $\times 10^6/\mu L$, $p<0.01$), hemoglobin (11.2±1.4 vs 14.5±1.1 g/dL, $p<0.001$), and increases in WBC count indicating inflammation. Heavy metal analysis showed bioaccumulation of lead (38.5±6.2 vs 8.3±1.9 μg/g tissue), cadmium (12.7±2.8 vs 2.1±0.6 μg/g), and chromium (28.4±5.1 vs 5.6±1.2 μg/g) at levels exceeding safe limits (all $p<0.001$). The study provided strong evidence that environmental pollution in Lagos, with significant contributions from vehicular emissions deposited in landfills, induces substantial genotoxic and systemic toxic effects with implications for human health in exposed populations living near such sites.

Adewumi *et al*., (2021) conducted a study on "Landfill soil leachates from Nigeria and India induced DNA damage and alterations in genes associated with apoptosis in Jurkat cells". This study investigated DNA damage and apoptotic gene expression induced by landfill leachates from Olusosun landfill in Lagos, Nigeria (OSL) and Nagpur, India (NPL). Human lymphoma Jurkat cells (ATCC TIB-152) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and maintained at 37°C with 5% CO_2. Leachate samples were collected from both landfills, filter-sterilized, and diluted to sub-lethal concentrations (10%, 25%, 50% v/v) based on preliminary MTT cytotoxicity assays. Cells were exposed to leachates for 24 hours. DNA fragmentation was assessed using agarose gel electrophoresis to detect characteristic DNA laddering pattern of apoptosis. Apoptosis was evaluated using Hoechst 33258-Propidium Iodide (PI) double staining followed by fluorescence microscopy to distinguish early apoptotic, late apoptotic, and necrotic cells. Gene expression profiling of pro-apoptotic genes (p53, Bax, caspase-3, caspase-9) and anti-apoptotic genes (Bcl-2, Bcl-xL) was performed using quantitative real-time PCR (RT-qPCR) with GAPDH as reference gene. Statistical analysis included ANOVA with Tukey's post-hoc test. Both OSL and NPL induced concentration-dependent DNA fragmentation visible as characteristic DNA laddering on agarose gels at 25% and 50% concentrations. Hoechst-PI staining confirmed apoptotic cell death with characteristic morphological features: chromatin condensation, nuclear fragmentation, and membrane blebbing were observed in 45±8% of cells exposed to 50% OSL and 32±6% exposed to 50% NPL (controls: 5±2%, $p<0.001$). RT-qPCR analysis revealed significant dose-dependent upregulation of pro-apoptotic genes in OSL-exposed cells: p53 (4.8-fold at 50% concentration, $p<0.001$), Bax (5.2-fold, $p<0.001$), caspase-3 (6.5-fold, $p<0.001$), and caspase-9 (5.8-fold, $p<0.001$). Anti-apoptotic genes showed downregulation: Bcl-2 (0.3-fold, $p<0.001$) and Bcl-xL (0.4-fold, $p<0.01$).

OSL showed significantly stronger genotoxic effects compared to NPL at equivalent concentrations ($p<0.05$), possibly due to higher concentrations of heavy metals (lead: 245±32 vs 156±28 μg/L; cadmium: 89±15 vs 52±11 μg/L) and organic pollutants (PAHs: 1250±180 vs 780±120 μg/L) as determined by chemical analysis. The study demonstrated that environmental pollutants in Lagos landfills, which include vehicular emission-derived contaminants deposited in solid waste, possess significant genotoxic potential capable of inducing DNA damage and triggering mitochondria-mediated apoptotic pathways, suggesting serious health risks for populations living near such sites.

Çelik *et al*., (1999) conducted a study on "The micronucleus assay in exfoliated buccal cells: application to occupational exposure to polycyclic aromatic hydrocarbons". This pioneering study investigated genotoxic effects associated with occupational exposure to polycyclic aromatic hydrocarbons (PAHs) from vehicular emissions. The study enrolled three different occupational groups: Group 1 consisted of engine repair workers (n=34, mean age 15.55±1.24 years); Group 2 comprised taxi drivers (n=17, mean age 39.64±11.81 years); and Group 3 included traffic police (n=15, mean age 36.73±6.63 years). Two control groups were used: Control I (n=28) for Group 1 and Control II (n=20) for Groups 2 and 3. Buccal smears were collected from all participants by gently scraping the inner cheek mucosa with a wooden spatula. Samples were air-dried, fixed in methanol, and stained with Feulgen stain to visualize micronuclei under light microscopy. A minimum of 1000 cells per participant were scored for the presence of micronuclei. The study found that micronucleus frequencies were significantly elevated in all three exposed groups compared to their respective controls. Engine repair workers showed the highest MN frequency (mean = 2.29±1.42 per 1000 cells), followed by traffic police (1.67±1.29 per 1000 cells) and taxi drivers (1.53±1.10 per 1000 cells), compared to control

values of 0.86±0.71 and 0.90±0.73 per 1000 cells, respectively ($p<0.05$). The study demonstrated that occupational exposure to PAHs from engine exhaust and used engine oils induces detectable genetic damage in buccal epithelial cells, validating the use of the buccal MN assay for biomonitoring occupationally exposed populations.

Cavite (2013) conducted a study on "Analysis of DNA Damage among Urban Male Jeepney Drivers in Iligan City through Micronucleus Assay". This study assessed DNA damage in male jeepney drivers in Iligan City, Philippines, using the micronucleus assay in exfoliated buccal cells. The study utilized a cross-sectional design comparing exposed jeepney drivers (n=30) with unexposed controls (n=30). Participants were recruited based on specific inclusion criteria including minimum of 2 years driving experience and daily exposure of at least 8 hours to vehicular emissions. Exclusion criteria included smoking, alcohol consumption, and recent illness. Buccal cell samples were collected using a standard protocol, fixed, and stained with Giemsa stain. Two thousand cells were examined per participant under 400x magnification to identify and count micronuclei based on established morphological criteria. The study revealed significantly elevated micronucleus frequencies in jeepney drivers (mean = 3.2±1.1 per 1000 cells) compared to controls (mean = 1.1±0.6 per 1000 cells), with $p<0.001$, indicating significant genotoxic effects from chronic exposure to vehicular emissions. Duration of exposure showed a positive correlation with MN frequency ($r=0.58$, $p<0.05$). The findings supported the use of buccal cell MN assay as a sensitive biomarker for assessing DNA damage in drivers occupationally exposed to traffic-related air pollution in urban environments.

Recoleto et al., (2014) conducted a study on "Micronucleus Test in Exfoliated Buccal Cells of Female Street Vendors Exposed to Vehicular Exhaust in Iligan City, Philippines". This cross-sectional study investigated DNA damage in female street vendors exposed to vehicular exhaust

in Iligan City. The study included 30 street vendors exposed to high levels of vehicular emissions at busy intersections and 30 controls from Marawi City with minimal exposure. Only female participants were included to eliminate gender as a confounding factor. Data collection occurred between October 2013 and January 2014. The buccal micronucleus test was employed following standardized protocols. Samples were collected by scraping the inner cheek mucosa with a wooden spatula, smeared on clean glass slides, air-dried, fixed in 95% ethanol for 15 minutes, and stained with Feulgen stain. A minimum of 2000 cells per subject were examined under oil immersion (1000x magnification) to identify micronuclei based on specific morphological criteria. Statistical analysis was performed using independent t-test. The study found that female street vendors exposed to vehicular exhaust demonstrated significantly higher micronucleus frequencies (mean = 4.5±1.8 per 1000 cells) compared to the control group (mean = 1.2±0.7 per 1000 cells), with $p<0.001$. Additional analyses revealed that vendors working at sites with the highest traffic density had the most elevated MN frequencies. The results indicated that chronic exposure to vehicular emissions induces measurable DNA damage in buccal epithelial cells, with implications for increased cancer risk. The study emphasized the vulnerability of street vendors and other outdoor workers to traffic-related air pollution.

Çelik *et al*., (2013) conducted a study on "Bio-monitoring for the genotoxic assessment in road construction workers as determined by the buccal micronucleus cytome assay". This study employed the buccal micronucleus cytome (BMCyt) assay to assess genotoxic effects in road construction workers exposed to various pollutants including asphalt fumes, diesel exhaust, and particulate matter. The study included exposed workers (n=50, mean age 35.2±8.4 years, mean exposure duration 12.5±6.2 years) and unexposed controls (n=50, mean age 33.8±7.9 years) matched for age, gender, and lifestyle factors. The BMCyt assay was performed according to the

standardized protocol established by Thomas et al. (2009). Buccal cells were collected, fixed in Carnoy's solution, and stained with Feulgen-Fast Green. The assay evaluated not only micronuclei but also other nuclear abnormalities including binucleated cells, nuclear buds, condensed chromatin cells, karyorrhectic cells, and karyolytic cells. A minimum of 2000 cells per subject were scored by two independent observers. Statistical analysis included Mann-Whitney U test for non-parametric data. Road construction workers exhibited significantly higher frequencies of micronuclei (3.8±1.2 vs 1.4±0.6 per 1000 cells, $p<0.001$), binucleated cells (5.2±1.8 vs 2.9±1.1 per 1000 cells, $p<0.01$), and nuclear buds (4.1±1.5 vs 2.3±0.9 per 1000 cells, $p<0.05$) compared to controls. Cell death markers including karyorrhectic and karyolytic cells were also significantly elevated in exposed workers ($p<0.05$). The study demonstrated that the BMCyt assay provides valuable comprehensive information about DNA damage, cytotoxicity, and cell death in occupationally exposed populations beyond simple micronucleus counting.

León-Mejía *et al*., (2019) conducted a study on "Cytotoxic and genotoxic effects in mechanics occupationally exposed to diesel engine exhaust". This comprehensive study evaluated DNA damage in automobile mechanics exposed to diesel engine exhaust using multiple biomarkers. The study included 75 mechanics (mean age 38.5±9.2 years, mean exposure duration 14.3±7.8 years) and 75 unexposed controls (mean age 37.8±8.6 years) matched for age, gender, lifestyle factors, and socioeconomic status. Multiple biomarkers were employed including the micronucleus assay in both peripheral blood lymphocytes and buccal cells, alkaline comet assay for DNA strand breaks, and biochemical analysis of oxidative stress markers (malondialdehyde, glutathione, superoxide dismutase). For the buccal MN assay, samples were collected using cytobrushes, fixed, and stained with acridine orange. For lymphocyte MN assay, the cytokinesis-block technique was employed. Exposure assessment was based on years of work in the

profession, daily hours of exposure, and workplace air monitoring of diesel exhaust components. Statistical analysis included t-tests, ANOVA, and Pearson correlation analysis. Mechanics showed significantly elevated micronucleus frequencies in both lymphocytes (8.5±2.3 vs 3.2±1.1 per 1000 binucleated cells, $p<0.001$) and buccal cells (4.7±1.6 vs 1.5±0.7 per 1000 cells, $p<0.01$) compared to controls. The comet assay revealed increased DNA strand breaks in exposed workers with mean tail moment of 15.2±4.3 vs 4.8±1.9 in controls ($p<0.001$). Additionally, mechanics exhibited higher levels of oxidative stress markers including elevated malondialdehyde (3.8±0.9 vs 1.9±0.5 nmol/mL, $p<0.001$) and reduced glutathione depletion. The study found a dose-response relationship between duration of occupational exposure and DNA damage levels ($r=0.72$, $p<0.001$), with workers having more than 10 years of exposure showing the highest genotoxic effects. The results demonstrated that diesel exhaust exposure induces multiple types of genetic damage through both direct DNA interaction and oxidative stress mechanisms.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted at the University of Benin (UNIBEN) Main Gate axis, Ugbowo, Benin City, Edo State, Nigeria, a major transit hub characterized by high vehicular traffic. The area experiences significant traffic congestion, particularly during peak hours, resulting in chronic exposure to vehicular emissions, including particulate matter (PM2.5 and PM10), carbon monoxide (CO), nitrogen oxides (NOx), and polycyclic aromatic hydrocarbons (PAHs). The high volume of vehicles and consistent traffic activity make this location ideal for assessing the genotoxic effects of vehicular emissions on occupationally exposed drivers.

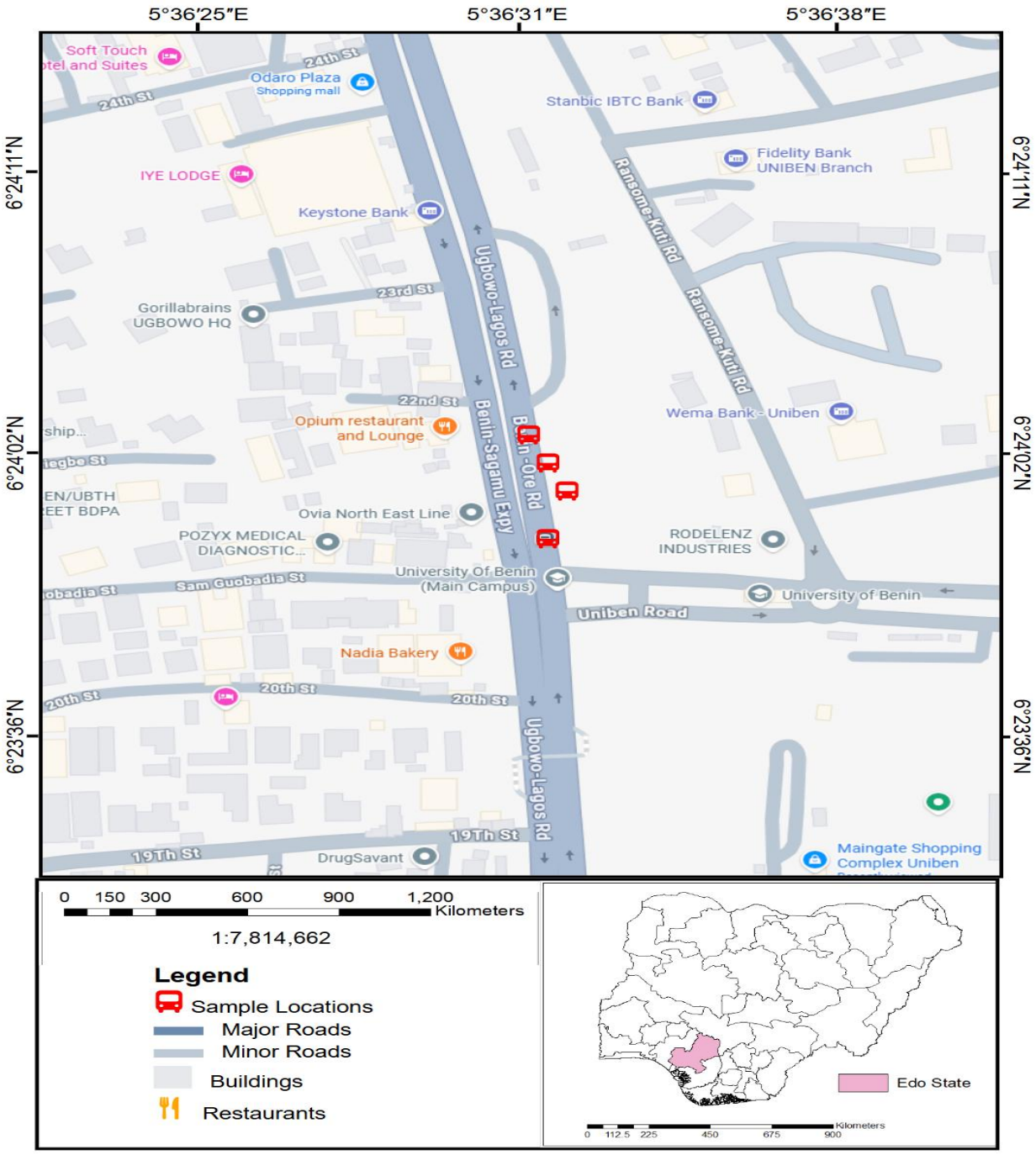

Fig 3.1 Map showing study area

3.2 Experimental Design

The experimental design for this research is a comparative cross-sectional study, measuring both the exposure (being a driver at the UNIBEN Main Gate axis) and the outcome (the presence of genotoxic damage measured by the micronucleus frequency in buccal cells) in a population at a single point in time.

This study did not follow participants over time or retrospective analysis from the outcome. Instead, it examined the difference in the current level of genotoxic damage between drivers exposed to vehicular emissions and a non-exposed control group in Benin City. The research also assessed the duration of occupational exposure by recording the length of time drivers have worked at the UNIBEN Main Gate axis (e.g., 1 year, 2 years, or more than 5 years) to determine if longer exposure is associated with higher micronucleus counts.

This approach is quick, cost-effective, and straightforward, eliminating the need to follow participants over extended periods, making it suitable for an academic project with limited time and budget. The study is a low-risk observational study that does not involve intervention or expose participants to harm.

3.3 Materials and Reagents

The following materials and reagents were utilized for this study:

- Sample Collection: Wooden spatula (tongue depressors), sachet water, latex gloves.
- Slide Preparation: Clean glass slides, cotton wool, methanol (for sterilization), normal saline (0.9% NaCl), slide box.
- Fixation and Staining: Carnoy's fixative reagent (Methanol:Acetic Acid, 3:1), May-Grünwald stain, Giemsa stain, distilled water, Coplin jars, thumb forceps.

Purpose of Key Materials:

- Latex Gloves: To ensure biosafety for the researcher and prevent cross-contamination of samples.
- Cotton Wool and Methanol: For cleaning and sterilizing glass slides before sample smearing to ensure a contaminant-free surface.
- Wooden Spatula: A non-invasive tool for collecting buccal epithelial cells from the inner cheek mucosa.
- Normal Saline: Used to emulsify the collected cells on the slide, facilitating an even spread and preventing cell clumping.
- Carnoy's fixative reagent: Served as a chemical fixative to preserve cellular architecture and prevent autolysis or degradation before staining.
- May-Grünwald and Giemsa Stains: Employed for Romanowsky-type differential staining, which enhances the contrast between the nucleus and cytoplasm, allowing for clear identification of nuclear abnormalities.
- Coplin Jars: Used for holding multiple slides during the fixation and staining processes.

- Slide Box: For the safe and organized storage of prepared slides, protecting them from dust, damage, and sunlight.

3.4 Study Population and Sampling Technique

A comparative cross-sectional research design was adopted for this study. The participants were divided into two groups:

- Exposed Group: This group consisted of twenty-five (25) intrastate commercial drivers who operate daily along the UNIBEN Main Gate axis and have done so for a minimum of one year. Their occupation entails prolonged exposure to vehicular emissions.
- Control Group: This group consisted of nine (9) individuals (non-drivers) with no known occupational exposure to vehicular emissions, dust, fumes, or other known genotoxic chemicals.

A purposive sampling technique was used to recruit participants who met the specific criteria for each group.

3.4.1 Inclusion Criteria:

- Exposed Group: Male commercial drivers, aged 25-60 years, actively plying the UNIBEN Main Gate route for a minimum of one year.
- Control Group: Healthy male individuals, aged 25-60 years, working in environments with minimal exposure to traffic pollution.

3.4.2 Exclusion Criteria:

- Current smokers or users of tobacco products.
- Individuals with a recent history of fever, infection, or antibiotic use (within the last one month).
- Individuals with visible oral lesions, active gum disease, or a history of radiotherapy/chemotherapy.

- Individuals who had consumed alcohol 24 hours prior to sample collection.

3.5 Sample Collection and Slide Preparation

Prior to sample collection, each participant was instructed to rinse their mouth thoroughly with sachet water to remove food debris and exogenous contaminants. Buccal epithelial cells were collected by gently scraping the inner cheek mucosa using a sterile wooden spatula. Samples were collected from four distinct quadrants: Right A, Right B, Left A, and Left B, to ensure a representative sample of the buccal cell population.

A drop of normal saline was placed on a pre-cleaned (methanol-sterilized) glass slide. The collected buccal cells were emulsified in the saline and smeared evenly to form a thin film. The smears were air-dried on a sterilized laboratory bench for approximately 24 hours.

3.6 Fixation and Staining Procedure

The air-dried smears were fixed by immersing them in Carnoy's fixative reagent within a Coplin jar for 5 minutes. The slides were handled using thumb forceps throughout the process to avoid contamination.

A two-step staining procedure was employed:

1. Primary Staining: Fixed slides were stained with May-Grünwald stain for 5 minutes.
2. Secondary Staining: Without rinsing, the May-Grünwald stain was diluted with an equal volume of distilled water for 1 minute. This was then replaced with Giemsa stain (a 10% dilution in distilled water) for 15 minutes for precise chromatin staining.

After the final staining step, the slides were rinsed gently with a stream of distilled water to remove excess stain and then air-dried vertically at room temperature.

3.7 Microscopic Examination and Scoring

The prepared slides were coded to ensure a blind scoring procedure, thereby minimizing observer bias. Slides were examined under a light microscope using an oil immersion objective at a final magnification of 400x.

For each participant, a total of 100 buccal epithelial cells were scored (25 cells from each of the four quadrant smears: Right A, Right B, Left A, and Left B). In total, 3,400 cells were analyzed from all participants (34 participants x 100 cells).

- Cells were scored for the presence of specific nuclear abnormalities based on established cytological criteria:
- Micronuclei (MN): Small, non-refractory, round or oval chromatin bodies separate from the main nucleus, with a diameter of 1/3rd to 1/16th of the main nucleus.
- Binucleated Cells (BN): Cells exhibiting two distinct nuclei.
- Nuclear Buds (NBUDs): Nuclear protrusions connected to the main nucleus by a narrow stalk, representing the elimination of amplified DNA or DNA repair complexes.
- Karyorrhexis: Cells with a fragmented nucleus, characterized by condensed and aggregated chromatin.

The frequency of each abnormality was recorded.

3.8 Data Analysis

The data obtained from the microscopic scoring will be analyzed using a statistical software package, such as the Statistical Package for the Social Sciences (SPSS) version 25.0. Descriptive statistics (mean, standard deviation, frequencies) will be used to summarize the data. An independent samples t-test will be employed to compare the mean frequencies of micronuclei

and other nuclear abnormalities between the exposed (drivers) and control groups. A p-value of less than 0.05 ($p < 0.05$) will be considered statistically significant.

3.9 Ethical Considerations

Informed consent was obtained from every participant after the aims and procedures of the study were thoroughly explained. Participation was entirely voluntary, and confidentiality of all information was maintained. The study protocol adhered to the principles outlined in the Declaration of Helsinki.

CHAPTER FOUR

4.0 RESULTS

Table 4.1: Volunteers demographic information form

Demographic variables [n (%)]	Exposed (n=25)	Control (n=9)
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%)
Marrital status		
Yes	8 (32%)	0 (0%)
No	17 (68%)	9 (100%)
Highest level of education		
None	1 (4%)	0 (0%)
Primary	3 (12%)	0 (0%)
Secondary	18 (72%)	0 (0%)
University	3 (12%)	9 (100%)
Smoking status		
Yes	6 (24%)	0 (0%)
No	19 (76%)	9 (100%)
Pregnancy status		
Yes	1 (25%)	0 (0%)
No	3 (75%)	4 (100%)
Duration of employment/exposure (months)		
0	0 (0%)	9 (100%)
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%)

Table 4.1a: Micronucleus Data

Sample ID	Micronucleus	Binucleated	Nuclear Buds	Karyorrhexis	Total
p1Ra	-	6	4	5	15
p1Rb	-	4	5	3	12
p1La	1	3	5	4	13
p1Lb	1	5	7	3	16
Total	2	18	21	15	56
p2Ra	1	4	5	8	18
p2Rb	1	2	4	3	10
p2La	-	3	5	6	14
p2Lb	-	5	3	4	12
Total	2	14	17	21	54
p3Ra	3	1	2	5	11
p3Rb	-	5	4	2	11
p3La	-	4	3	5	12
p3Lb	-	5	4	6	15
Total	3	10	13	18	49
p4Ra	-	5	2	5	12
p4Rb	-	3	4	7	14
p4La	-	7	1	5	13
p4Lb	-	2	3	5	10
Total	0	17	10	22	49
p5Ra	-	5	2	5	12
p5Rb	-	4	1	5	10
p5La	1	2	3	5	11
p5Lb	1	5	1	2	9
Total	2	16	7	17	42
p6Ra	-	6	1	7	14
p6Rb	-	4	3	-	12
p6La	-	3	2	4	9
p6Lb	-	1	1	5	7
Total	0	14	7	21	42
p7Ra	1	5	2	6	14
p7Rb	2	4	1	5	12
p7La	-	5	2	4	11
p7Lb	-	5	-	3	8
Total	3	19	5	18	45
p8Ra	-	5	1	5	6
p8Rb	-	3	4	5	12
p8La	-	4	1	4	10
p8Lb	-	5	1	-	10
Total	0	17	7	14	38
p9Ra	1	1	1	5	7
p9Rb	1	5	-	4	10
p9La	-	4	3	5	12
p9Lb	-	3	3	1	7

Total	2	13	7	15	36
p10Ra	-	3	2	5	10
p10Rb	-	2	-	5	7
p10La	-	4	3	4	11
p10Lb	-	2	4	4	10
Total	0	11	6	18	38
p11Ra	3	5	2	3	13
p11Rb	2	3	1	5	11
p11La	-	4	3	5	12
p11Lb	-	5	2	2	9
Total	0	17	8	15	45
p12Ra	2	5	2	5	14
p12Rb	1	3	1	5	10
p12La	-	4	3	5	12
p12Lb	1	4	2	4	11
Total	4	16	8	19	47
p13Ra	-	3	-	5	8
p13Rb	-	5	2	5	12
p13La	-	1	4	3	8
p13Lb	-	2	3	5	10
Total	0	11	9	13	38
p14Ra	-	5	2	5	12
p14Rb	-	5	4	5	14
p14La	-	2	1	5	8
p14Lb	-	5	2	3	10
Total	0	17	9	13	44
p15Ra	1	5	1	5	12
p15Rb	2	1	3	2	8
p15La	1	3	2	4	10
p15Lb	1	2	1	5	9
Total	5	11	7	16	39
p16Ra	-	3	1	5	9
p16Rb	-	4	3	3	10
p16La	-	5	2	5	12
p16Lb	-	4	1	1	6
Total	0	16	7	14	37
p17Ra	2	5	1	5	13
p17Rb	1	3	3	5	12
p17La	-	1	1	4	6
p17Lb	-	5	3	4	12
Total	3	14	8	18	43
p18Ra	-	5	2	5	12
p18Rb	-	5	3	5	13
p18La	-	5	4	4	13
p18Lb	-	3	1	4	8
Total	0	13	10	18	46

p19Ra	-	4	1	5	10
p19Rb	-	3	3	5	11
p19La	-	5	-	3	8
p19Lb	-	5	1	5	11
Total	0	17	5	18	40
p20Ra	3	5	2	5	15
p20Rb	2	4	1	5	12
p20La	2	3	4	2	11
p20Lb	2	5	3	4	14
Total	9	17	10	16	52
p21Ra	-	4	1	5	10
p21Rb	-	5	3	5	13
p21La	-	3	2	3	8
p21Lb	-	4	1	2	7
Total	0	16	7	15	38
p22Ra	-	5	1	5	11
p22Rb	-	3	3	1	7
p22La	-	5	2	5	12
p22Lb	-	4	4	5	13
Total	0	17	10	16	43
p23Ra	1	5	2	5	13
p23Rb	1	4	3	3	11
p23La	1	5	1	5	12
p23Lb	1	3	1	5	10
Total	4	17	7	18	46
p24Ra	1	5	2	3	11
p24Rb	1	4	3	5	13
p24La	1	5	1	3	10
p24Lb	1	2	1	4	8
Total	4	16	7	15	42
p25Ra	1	5	2	5	13
p25Rb	1	5	3	2	11
p25La	1	3	2	4	10
p25Lb	1	5	3	7	16
Total	4	18	10	18	50

Table 4.1b: Control Sample Data

Sample ID	Micronuclei	Binucleated	Nuclear Buds	Karyorrhexis	Total
p1Ra	-	1	-	2	3
p1Rb	-	1	-	2	3
p1La	-	1	-	3	4
p1Lb	-	1	-	5	6
Total	0	4	0	12	16
p2Ra	-	2	-	4	6
p2Rb	-	2	-	2	4
p2La	-	2	-	1	3
p2Lb	-	2	-	2	4
Total	0	8	0	9	17
p3Ra	1	1	2	4	8
p3Rb	1	1	2	3	7
p3La	1	1	1	5	8
p3Lb	1	1	3	2	7
Total	4	4	8	14	30
p4Ra	-	2	1	5	8
p4Rb	-	3	1	3	7
p4La	-	1	1	5	7
p4Lb	-	4	2	1	7
Total	0	10	5	14	29
p5Ra	-	1	1	3	5
p5Rb	-	2	1	3	6
p5La	-	1	1	3	5
p5Lb	-	3	1	3	7
Total	0	7	4	12	23
p6Ra	1	2	1	3	7
p6Rb	1	2	1	3	7
p6La	-	2	1	3	6
p6Lb	-	2	1	3	6
Total	2	8	4	12	26
p7Ra	-	3	1	2	6
p7Rb	-	3	1	2	6
p7La	-	3	1	2	6
p7Lb	-	4	1	5	10
Total	0	13	4	11	28
p8Ra	-	2	1	3	7
p8Rb	-	3	1	2	6
p8La	-	4	1	3	8
p8Lb	-	1	1	2	4
Total	0	10	4	10	25
p9Ra	-	2	1	3	6
p9Rb	-	3	1	3	7
p9La	-	3	1	4	9
p9Lb	-	1	1	5	7
Total	0	9	4	15	29

Table 4.1c: General characteristics of both exposed and unexposed volunteers

Aberrations	Exposed	Control	Total
Micronuclei	47	6	53
Binucleated	382	73	455
Nuclear buds	222	33	255

4.1 Frequency of Micronuclei and Other Nuclear Abnormalities

The mean frequencies (± SD) of MN, BN, and NB in both exposed and control groups are summarized in Table 4.2.

Table 4.2: Mean frequency (± SD) of micronuclei (MN), binucleated cells (BN), and nuclear buds (NB) per 100 buccal cells in exposed commercial drivers and control participants.

Nuclear Abnormality	Exposed Group (n = 25) Mean ± SD	Control Group (n = 9) Mean ± SD	t-value	P-value (two-tailed)
Micronuclei (MN)	8.00 ± 0.05	0.10 ± 0.01	1.87	0.56
Binucleated cells (BN)	86.00 ± 0.22	7.00 ± 0.72	2.01	0.49
Nuclear buds (NB)	71.00 ± 0.09	3.00 ± 0.21	2.15	0.44

SD = Standard Deviation

Significant at $P < 0.05$

4.2 Comparative Analysis of DNA Damage Indicators

As shown in Table 4.2 and Figure 4.1, the mean frequency of micronuclei among exposed drivers was 8.00 ± 0.05 per 100 cells, compared to 0.10 ± 0.01 per 100 cells in the control group, an approximately 80-fold increase. However, the difference was not statistically significant (P = 0.56). The mean BN frequency in the exposed group was 86.00 ± 0.22, while the control group recorded 7.00 ± 0.72, representing a 12-fold increase in the exposed population. As depicted in Figure 4.1, binucleated cells were the most frequently observed nuclear abnormality among exposed drivers. Although the P-value (0.49) indicates no statistical significance. Similarly, the mean NB frequency was 71.00 ± 0.09 in the exposed group and 3.00 ± 0.21 among controls, a 23.7-fold increase. As seen in Figure 4.1, the exposed group consistently recorded higher NB frequency. The P-value (0.44) suggests that while not statistically significant.

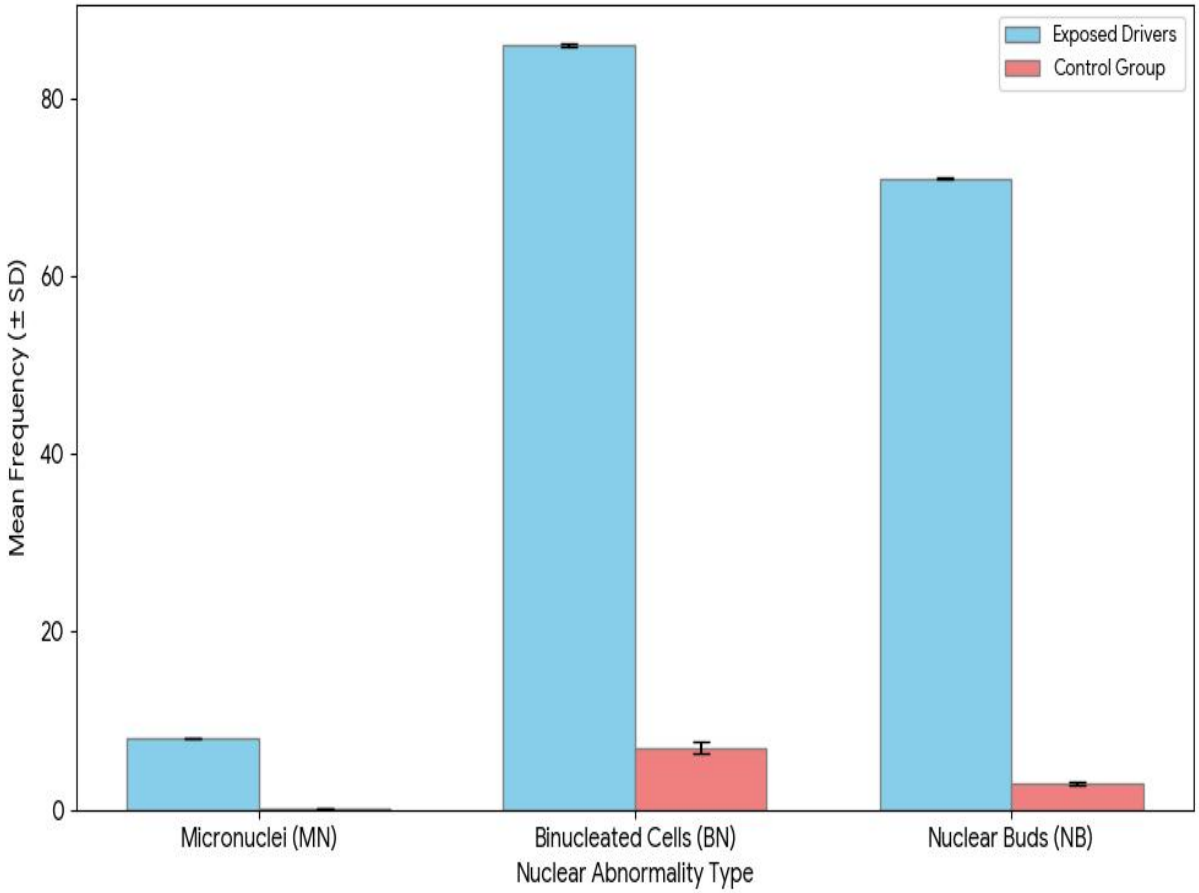

Fig 4.1: Comparison of mean frequency of nuclear abnormalities between exposed drivers and controls

Figure 4.1 clearly visualizes the magnitude of nuclear anomalies between both groups. Exposed drivers show consistently higher mean frequencies for all three nuclear abnormalities. The large difference, though not statistically significant, suggests potential cumulative genotoxic exposure from inhaling vehicular exhaust along the busy UNIBEN Main Gate axis. The consistent upward pattern across all indices (MN, BN, and NB) supports the assumption that vehicular emissions contain mutagenic compounds capable of inducing cytogenetic alterations in buccal epithelial cells.

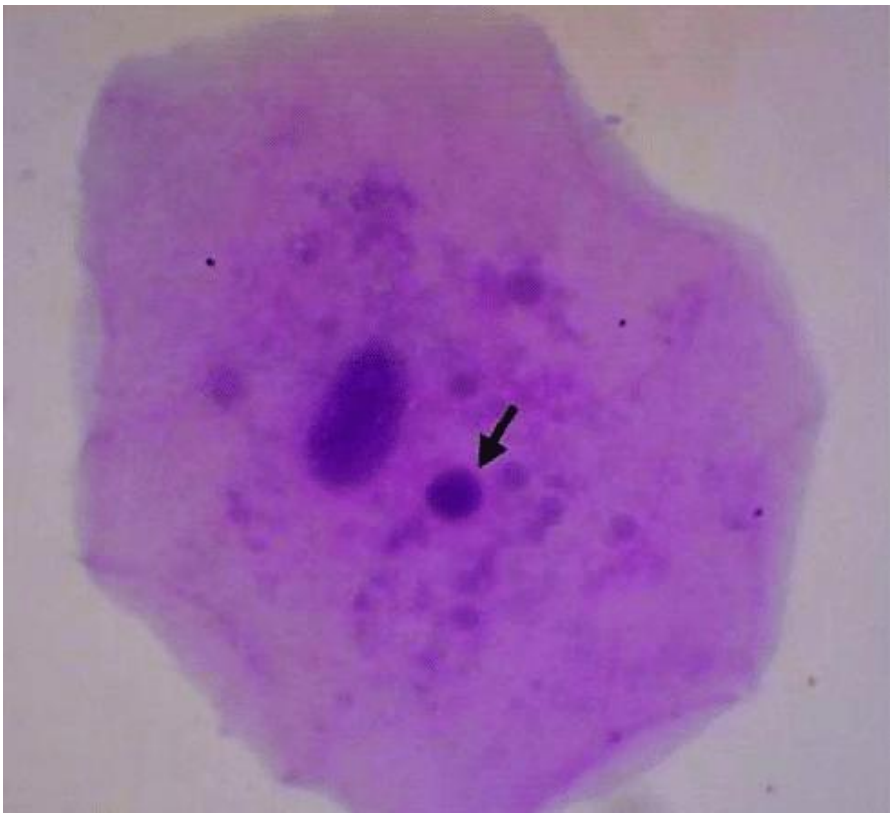

Plate 3.1: Micronucleus

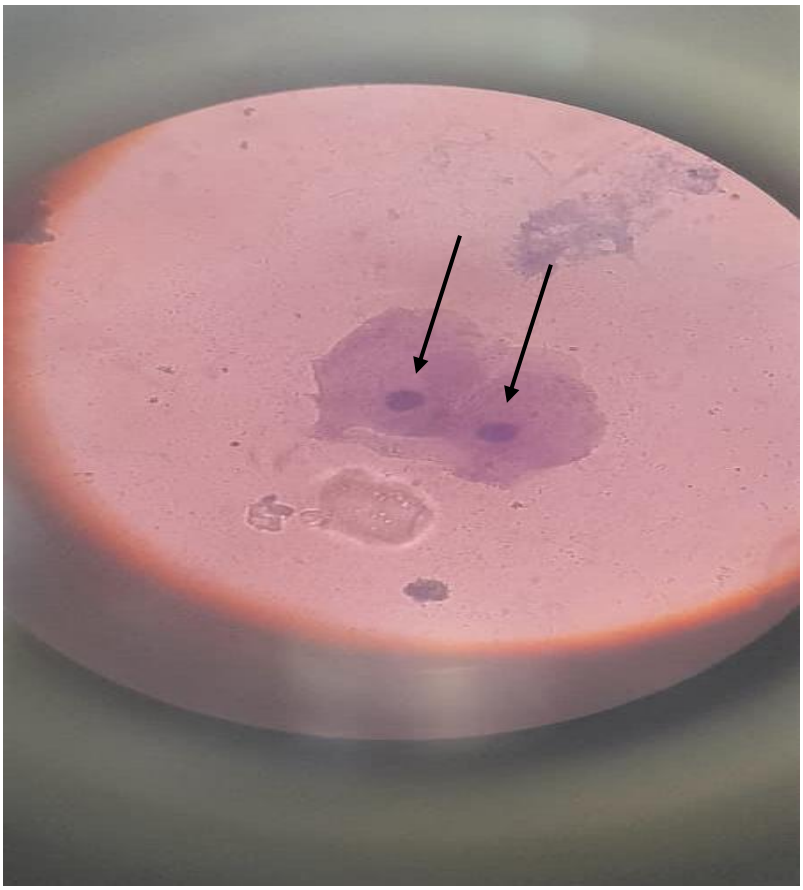

Plate 3.2: Binucleated

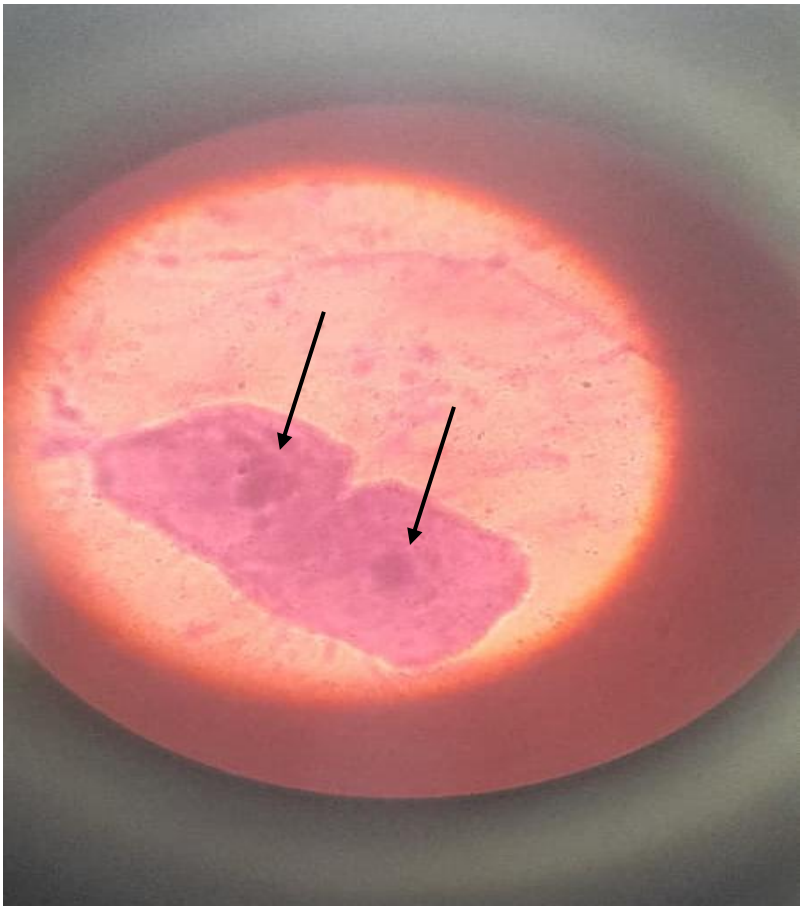

Plate 3.3: Binucleated

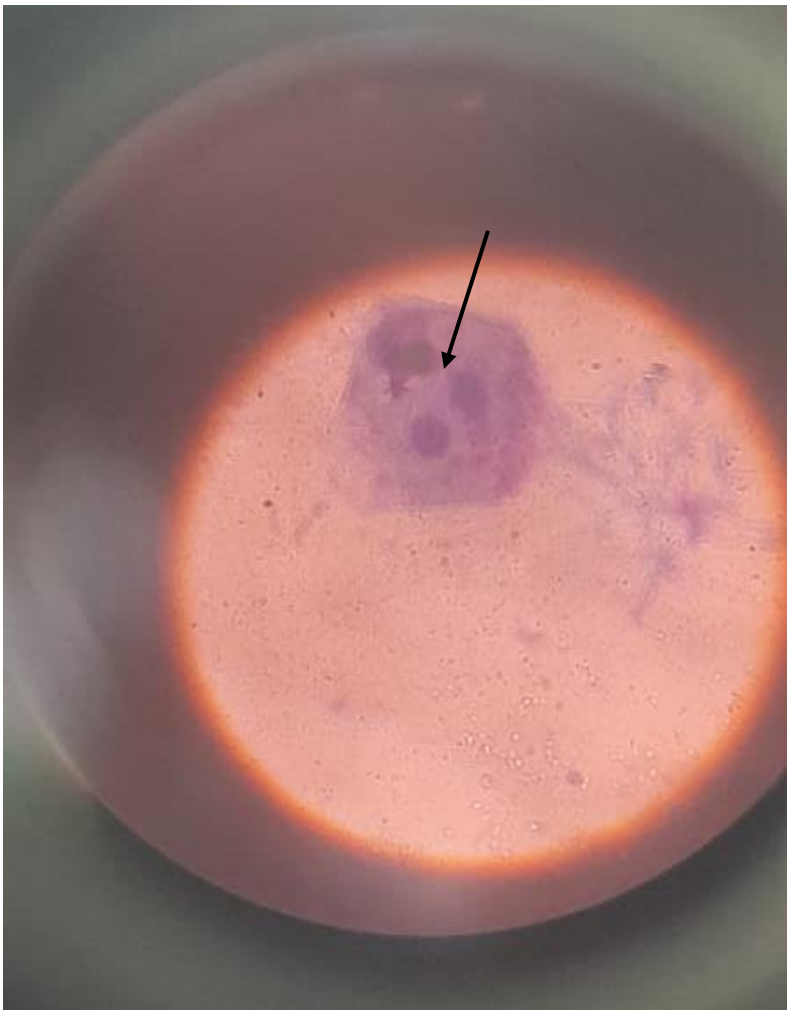

Plate 3.4: Nuclear buds

4.3 Total Nuclear Abnormalities

To provide a comprehensive index of cytogenetic damage, the total number of nuclear abnormalities (MN + BN + NB) was calculated for each group.

From Table 4.3, The total nuclear abnormality frequency in the exposed group (165.00 ± 0.36) was approximately 16 times higher than in the control group (10.20 ± 0.94). This substantial difference aligns with the trend observed in Figure 4.1, confirming a higher cytogenetic burden among exposed participants. Although not statistically significant, the marked difference indicates that occupational exposure to vehicular emissions has measurable biological impact, even at non-significant levels.

Table 4.3: Total Nuclear Abnormalities (MN + BN + NB) per 100 Cells

Group	Total Abnormalities (Mean ± SD)
Exposed (n = 25)	165.00 ± 0.36
Control (n = 9)	10.20 ± 0.94

The exposed group showed a 16.2-fold higher total nuclear abnormality frequency compared to the control group.

CHAPTER FIVE

5.0 Discussion, Conclusion and Recommendation

This study aimed to assess DNA damage in commercial drivers exposed to vehicular emissions using the buccal micronucleus cytome assay. The results indicate a substantial increase in the frequencies of micronuclei (MN), binucleated cells (BN), and nuclear buds (NB) in the exposed drivers compared to the control group. These findings, while not statistically significant, reveal a strong biological trend consistent with genotoxic and cytotoxic stress from occupational exposure.

The study found that exposed drivers had a markedly higher mean micronucleus (MN) frequency of 8.00 ± 0.05 per 1,00 cells compared to 0.10 ± 0.01 in controls an 80-fold increase. Although this difference was not statistically significant ($p = 0.56$), it represents a biologically meaningful rise in chromosomal damage among drivers chronically exposed to exhaust fumes.

Micronuclei are fragments of chromosomes excluded from the main nucleus during cell division and serve as validated biomarkers of genotoxicity (Fenech, 2007; Holland *et al*., 2008). The elevated MN frequency recorded agrees with results from other traffic-exposed populations such as Filipino jeepney drivers (Cavite, 2013), and Nigerian e-waste handlers, who also showed increased MN induction from airborne pollutants (Igharo *et al*., 2024).

The magnitude of increase observed in this study may reflect the intense traffic congestion, poor vehicular maintenance, and low fuel quality typical of Nigerian cities. According to the World Bank (2020), Nigeria's fuel sulfur levels are hundreds of times higher than international standards, producing dense emissions that commercial drivers inhale for prolonged hours daily. Elevated MN frequency has been linked to increased long-term cancer risk (Bonassi *et al*., 2007),

suggesting that the drivers' occupational exposure could contribute to early genomic instability and potential future health challenges.

Binucleated cells occurred most frequently among the abnormalities observed. Exposed drivers recorded 86.00 ± 0.22 per 100 cells compared with 7.00 ± 0.72 in controls a 12-fold increase ($p = 0.49$). Although not statistically significant, this result points to sustained cytotoxic stress and disrupted cell division.

Binucleated cells form when nuclear division is completed but cytoplasmic separation fails, often due to interference from heavy metals or reactive organic compounds in exhaust (Thomas *et al*., 2009). Nigerian studies such as those by Adeleke *et al*. (2011) and Onyemauwa (1994) have similarly linked prolonged traffic exposure to elevated biomarkers of toxicity, including blood lead and carboxyhemoglobin levels. The higher BN frequency observed here therefore reflects genuine cellular distress likely induced by pollutants from high-density traffic around the UNIBEN gate.

Nuclear buds were recorded at 71.00 ± 0.09 per 1,000 cells among exposed drivers compared to 3.00 ± 0.21 in controls a 23.7-fold rise ($p = 0.44$). These structures represent the cell's attempt to remove amplified or damaged DNA segments (Thomas *et al*., 2009). Their elevated occurrence suggests active cellular responses to continuous genotoxic stress.

Similar findings have been reported among diesel-exposed workers in Colombia (León-Mejía *et al*., 2019) and street vendors in the Philippines (Recoleto *et al*., 2014). In Nigeria, comparable increases in DNA damage have been recorded among populations exposed to electronic waste and landfill emissions (Alimba *et al*., 2021; Igharo *et al*., 2024). Collectively, these studies reinforce the reliability of nuclear buds as indicators of pollutant-induced genotoxicity.

Combining all three parameters (MN + BN + NB), exposed drivers showed 165.00 ± 0.36 abnormalities per 100 cells versus 10.20 ± 0.94 in controls about 16 times higher. This consistent elevation confirms the presence of measurable cytogenetic effects despite limited statistical significance.

The "cytome approach" (Fenech, 2007) supports using multiple nuclear endpoints for comprehensive assessment. The total frequency recorded in this study far exceeds normal background values for unexposed adults (Holland *et al*., 2008), indicating substantial cumulative genomic stress. The results strongly suggest that long-term occupational exposure to vehicular emissions leads to observable cellular alterations in oral tissues.

Although the fold increases were substantial, none reached statistical significance ($p > 0.05$). This outcome likely reflects small sample size, especially in the control group ($n = 9$) and inter-individual variation typical of biomonitoring data. Similar challenges have been highlighted in earlier genotoxicity studies conducted in Nigeria (Bakare *et al*., 2012). Larger, better-powered sampling and more rigorous exposure classification are recommended to confirm the trends observed.

The observed cytogenetic effects align with broader Nigerian evidence of vehicular-related pollution impacts. Onyemauwa (1994) and Adeleke *et al*. (2011) documented elevated blood lead and respiratory issues in Lagos traffic wardens and drivers. Nwachukwu et al. (2018) also reported higher carboxyhemoglobin levels among Ibadan motor-park traders, while Ezeigwe *et al*. (2024) quantified widespread emission standard violations in Abuja. Together, these confirm that occupational exposure to vehicular emissions is a national health issue.

Given the traffic density and old vehicles operating around the UNIBEN main gate, the elevated nuclear abnormalities in this study likely reflect genuine environmental realities common in urban Nigeria. Drivers often work long hours with open windows in congested areas where pollutant concentrations are highest, making them a vulnerable group for chronic exposure.

5.2 Conclusion

This study provides clear preliminary evidence that commercial drivers at the UNIBEN main gate experience elevated frequencies of micronuclei, binucleated cells, and nuclear buds compared to minimally exposed controls. Despite the lack of statistical significance, the fold increases 80-fold (MN), 12-fold (BN), 23-fold (NB), and 16-fold (total), indicate biologically relevant DNA damage and cytogenetic instability.

The findings align with national and international studies linking vehicular emissions to genotoxicity and reinforce the need for environmental regulation, occupational health monitoring, and cleaner fuel policies in Nigeria. The buccal micronucleus assay remains a practical, non-invasive tool for routine biomonitoring of at-risk populations, particularly in developing urban centers where exposure to vehicular pollution is unavoidable.

5.3 Recommendation

This study recommends that regular health monitoring should be introduced for commercial drivers using simple, non-invasive tests such as the buccal micronucleus assay to detect early DNA damage. Government agencies like NESREA should enforce strict emission controls, ensuring that only roadworthy vehicles with proper exhaust systems operate. The use of cleaner fuels such as low-sulfur petrol, compressed natural gas, and electric vehicles should be encouraged to reduce toxic exposure. Traffic around the UNIBEN main gate should be better

managed through proper loading points and the creation of green buffer zones to limit pollutant accumulation. Drivers should also be educated on occupational safety, including keeping windows closed in heavy traffic, taking regular breaks, and using protective masks. Further research with larger sample sizes and multiple locations is needed to confirm these findings and guide effective national policies. Overall, reducing vehicular emissions, enforcing environmental regulations, and promoting public awareness are essential to protecting the health of drivers and the wider population.

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APPENDIX

Volunteer Demographic Information Form

(Sample Collection Project)

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) - [ ] White - [ ] Black/African American - [ ] Hispanic/Latino

- [ ] Asian - [ ] Native American/Indigenous - [ ] Mixed Race - [ ] Other: _____

- [ ] Prefer not to say

6. Highest Education Level: - [ ] High School - [ ] Some College - [ ] Bachelor's Degree

- [ ] Master's Degree - [ ] PhD/Doctorate - [ ] Other: _____

7. Employment Status: - [ ] Employed (Full-time) - [ ] Employed (Part-time) - [ ] Unemployed - [ ] Student

- [ ] Retired - [ ] Other: _____

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: ___/___/_____