Genotoxicity Effects of Heavy Metal Pollution on Mangifera indica L. (Mango) Exposed to Roadside Traffic in Makurdi, Nigeria

Jose V. Addy

Ella A. Boniface

Buukume T. Benjamin

Licia M. Addy

Department of Environmental Sustainability, Federal University of Agriculture, Makurdi, North Central, Nigeria. Department of Environmental Sustainability, Federal University of Agriculture, Makurdi, North Central, Nigeria. Department of Environmental Sustainability, Federal University of Agriculture, Makurdi, North Central, Nigeria. Department of Microbiology, Benue State University,

Makurdi, Nigeria.

Objective: The study aimed to assess the genotoxicity effects of heavy metals pollution on $Mangifera\ indica\ L.$ (Mango) exposed to roadside traffic from NNPC Petroleum Depot, Makurdi-Nigeria.

Methods: Thirty (30) samples of *Mangifera indica* (Mango) parts comprising of fruits, bark and roots were washed with distilled water, dried at 40 °C and digested with trioxonitrate (v) acid HNO₃ at 120 °C. Six heavy metals nickel, lead, zinc, copper, cadmium, and chromium were analyzed using Shimadzu AAS 6300 Atomic Absorption Spectrophotometer (AAS).

Results: The mean concentration of Nickel, Zinc, Lead, Copper, Cadmium and chromium in fruits, barks, and roots of Mangifera~indica~L (Mango) were above the permissible limit of 0.03, 3.00, 0.1, 0.08, 0.03 and 0.05 mg/kg (WHO, 2022) for Nickel, Zinc, Lead (Pb2+), Copper, Cadmium and Chromium respectively except the mean concentration of Zinc in fruit, bark, and root that was within the permissible limit of 3mg/kg (WHO, 2022). The DNA amounts in fruits and roots at the NNPC Petrol Depot were 555 and 422 respectively, while from the control site were 208 and 180, respectively. The DNA content $(ng/\mu l)$ in fruits and roots at the NNPC Depot were 940 and 840, respectively, while the DNA amounts in fruits and roots from the control site were 202 and

210, respectively. The DNA content in fruits and roots after the NNPC Depot, Apir-Makurdi were 748 and 540, respectively, while the DNA amounts in fruits and roots from the control site were 211 and 201 respectively.

Discussion: One-way ANOVA showed that the concentration of heavy metals in parts of *Mangifera indica* L (Mango) is statistically significant at (P<0.05). Genotoxic effects of heavy metals pollution from roadside traffic on mango plants cause oxidative stress, which plays a major role in DNA-damage induction with more DNA content, fragmentation as well as more smearing of DNA. The DNA fragmentation index from all the polluted sites was above forty percent (40 %), indicating the level of severity. From these findings, people that consume these Mango fruits are prone to metal pollution capable of causing cancer.

Conclusion: Policies on reducing air pollution should be enforced while creating awareness that food crops cultivated near roadside should not be consumed.

Introduction

Fossil fuel combustion and exhaust emissions have been identified as primary sources of atmospheric metallic nuisance [1, 2]. Burnt petrol from motor vehicles releases significant quantities of sulphur dioxide, nitrogen oxides, carbon monoxide, lead, and suspended particulate matter [3, 4]. Particulate matters (PMs) are airborne particles that may contain toxic substances that can endanger the health of both human and animal populations, such as heavy metals [5]. The emissions of metal particles related to road traffic can take place via different ways, such as fuel and oil combustion, corrosion of metallic car parts, abrasion of tires, and particle re-suspension from the road surface [6]. The metal particles that are airborne can precipitate directly on parts of plants and the surrounding ground surfaces. They can also be deposited on these surfaces during rainwater after accumulating in the atmosphere or absorbed through the roots [7]. High vehicular emissions in the cities make trees in cities more prone to heavy metal airborne pollution due to the pervasive pressure of vehicular emissions. The increasing concern for the welfare of both humans and ecosystems requires the detection of the genotoxic risk of heavy metals at an early stage because heavy metals are capable of undergoing bioaccumulation along the food chain owing to their carcinogenic potentials [8, 9].

The global concern for the welfare of both humans and the environment necessitates the beam light to be focused on thorough searching for genotoxic agents and their effects in the environment, which requires early assessment and detection of environmental genotoxic risk [10]. Bioindicators are living organisms used to possibly detect forms of pollution or contamination that are difficult to measure or analyze in the field [11]. The significant roles plants play in the food chain make them to be classified as good bioindicators. Bio-monitors are cost-effective and are alternatives to analyze the quality of air in order to obtain related information to any population's exposure to air pollutants. In this way, it allows the evaluation of different sampling sites simultaneously [12]. The plant fruit is the most sensitive part to be affected by air pollutants, as major physiological processes are concentrated in the fruit. Plants found near the roadside have a relative increase of lead (Pb) deposition as a result of exhaust fumes from vehicles using petrol containing lead [13].

At low doses, some heavy metals are very essential micro-nutrients but at higher doses, might cause inhibition of growth and metabolic disorders [14]. The use of DNA marker(s) in the detection of genotoxicity is very advantageous over other markers [15]. Recent developments in molecular biology provide novel ways of DNA damage detection on plants [16]. Studies have shown that heavy metals such like Pb, Cu, Mn and Cd are capable of altering the DNA components in plant cells [17]. Among heavy metals, lead and cadmium toxicity has become important due to their constant increase in the environment. This study intends to research on genotoxicity effects of *Mangifera indica* L. (Mango) exposed to heavy metal pollution by roadside traffic (heavy duty vehicles).

Materials and Methods

Study area

The study was conducted within areas with high traffic within the Makurdi Local Government Area of Benue State, which is the capital of Benue State in Nigeria (Figure 1).

Figure 1. Map of Benue State in Nigeria. Source, [18].

Makurdi is located at 7.73 latitude and 8.52 longitudes, it is situated at an elevation of 104 m above sea level. The annual total rainfall and diurnal temperature in Makurdi range from 1200 to 2000 mm (from April to October) and an average of 28°C to 35°C, respectively [19]. Three sampling sites were identified within Makurdi metropolis, namely, Kanshio, Wadata and North Bank (Figure 2).

Figure 2. Map of Benue State Showing Makurdi Metropolis the Sample Sites.

Sample collection for Heavy metal analysis

A total of 30 samples were obtained by random sampling. Five mango plants were randomly selected from each designated area (Around the NNPC Depot and Control). Six samples (two fruits, two bark and two roots) were collected from five different mango plants, making a total of thirty (30) Mango samples taken for laboratory analysis. The samples were placed in sterile containers before been transported to the Biological Science laboratory of Federal University of Agriculture Makurdi for analysis. The samples of mango fruits (Mangifera indica) were washed thoroughly with distilled water and dried overnight in an oven at 40°C to obtain constant weight. The samples were ground and homogenized for heavy metal analysis. Digestion was carried out in triplicate for each sample by weighing 0.5 g of dry samples. 0.5 g of dry samples was digested by the addition of trioxonitrate (v) acid HNO₃ at 120 °C and then diluted with deionized water to 25 ml. The resulting filtrate was submitted to heavy metal analysis using the Shimadzu AAS 6300 atomic absorption spectrophotometer. For reference, a blank digestion solution was made. For the purpose of calibration, a standardized solution for each heavy metal under study was prepared. Heavy metal nickel (Ni), zinc (Zn), lead (Pb), copper (Cu), cadmium (Cd), and chromium (Cr) concentrations on the fruit samples were determined by the use of Direct Reading atomic absorption spectrophotometer (DR/2000) from HACH Company, Loveland, Colorado, USA. Ni, Pb, Zn, Cu, Cd, and Cr hollow cathode lamps at specific wavelengths were used [20]. The dilution factor for solid samples = concentration (mg/kg) = AAS reading x dilution factor/ ass of weight digested.

Deoxyribonucleic acid (DNA) extraction and analysis The fruits and roots were washed thoroughly. The mesocarps of mango fruits were removed using an alcohol-sterilized knife. 400 µl of DNA isolation buffer containing [0.15 M Sorbitol, Tris-base (0.125 M), Ethylene diamine tetra acetic acid (EDTA) 0.05 M, 8.3M of sodium bissulfide, 0.83 M NaCl, 2 % w/v cetyltrimethly-ammonium bromide (CTAB), 0.83 % v/v sodium Sarkosyl, 1% w/v polyvinylpyrollidone (PVP) (MW 40,000), 1% v/v betamercaptoethanol] was added into each 1.5 ml microfuge tube. 100 base pair DNA marker was used as standard. About 0.20 g of fruit and root samples were weighed and pulverized in liquid nitrogen and transferred into microfuge tubes containing buffer. After a quick spin, another 300 µl of DNA isolation buffer was added. All tubes were gently flicked and inverted thoroughly to mix the sample and were incubated at 65°C for 40 min. 700 µl of chloroform:isoamyl alcohol (24:1) was added to each microfuge tube and gently inverted until emulsion was formed. Centrifugation of samples was at 10,000 rpm for about 15 min, and the aqueous phase was gently transferred into a new 1.5 ml microfuge tube. Repetition of the chloroform:isoamyl alcohol procedure was performed. Half the volume of 5 M NaCl was added, and then two volumes of cold isopropanol and contents were gently inverted before incubating on ice for 10 min. Centrifugation of samples was at 10,000 rpm for a period of 5 min. Supernatant liquid was gently discarded. DNA pellets were washed twice with 500 µl of cold 70% ethanol. The resulting DNA pellets were air-dried at room temperature and dissolved in 30 µl of TE buffer. The RNA from crude DNA was eliminated by treating the sample with RNase (10 mg/ml) for 30 min at 37 °C. Storage of samples was at. The coloration of the extracted DNA pellets from each growth stage of the fruit was checked visually. Extracted DNA samples were quantified and purified by using a spectrophotometer measuring the absorbance of diluted DNA solution at 260 and 280 nm [20].

Polymerase chain reaction (PCR) amplification

The PCR reaction was carried out in a 20 μ l volume of master mixture. In the master mixture, 30 ng of template DNA and 0.25 μ M primers (forward: 5'CGGAGAATTAGGGTTCGATTC-3',' reverse:

5'CCAGAACATCTAAGGGCATCA-3') were added in each tube. Tubes were vortexed and centrifuged after adding DNA and primer together. The amplification was carried out on the PCR machine. The first denaturation was done at 94 °C for 3 min, segment denaturation at 94 °C for 1 min, annealing

at 35.5 °C for 30 s, extension at 72 °C for 1 min, and final extension at 72 °C for 3 min was performed for amplification [21].

Agarose gel electrophoresis

The quality of the DNA was determined using agarose gel electrophoresis. The amplified products were separated on a 1.5% agarose gel in 1X Tris acetate-EDTA (TAE) buffer for electrophoresis at 100 V for 48 min and then visualized by staining the gel with 0.5 µg/mL of ethidium bromide [22].

DNA fragmentation index (DFI) was determined using the formula:

DFI (%) = $100 \times \text{(number of fragmented DNA)/ (number of DNA counted)}$ (2)

Statistical analysis

A statistical treatment of the data was carried out using the Microsoft Excel and SPSS version 2010. Descriptive statistics was performed and Analysis of Variance (ANOVA) to separate the means with the view to assess heavy metals varied significantly between parts of Mango at NNPC Depot, Makurdi and the Control site. Results were expressed as mean, \pm standard deviation. The level of probability p< 0.05 was considered significant while p> 0.05 was considered insignificant.

Results and Discussion

Heavy metals concentration in parts of Mangifera indica

Findings displayed in Table 1 revealed the mean value of heavy metals analyzed: nickel, lead, zinc, cadmium, chromium, and copper in mg/kg from the NNPC Depot Apir–Makurdi. The fruits, bark, and roots of *Mangifera indica* were selected from the mango plants situated at and after the NNPC Depot, Apir-Makurdi and control. Heavy-duty vehicles (trucks) are seen parked within and outside of the premises of the depot to load petroleum products.

Heavy	FAD	BAD	RAD	CF	СВ	CR	24	p-value
Metal								
Ni	$1.4^{a} \pm 0.5$	$1.2^{\rm b} \pm 0.4$	$0.8^{\circ} \pm 0.2$	$0.4^{a} \pm 0.2$	$0.2^{\rm b} \pm 0.1$	$0.1^{\rm c} \pm 0.3$	0.03	< 0.05
Zn	$5.6^{a} \pm 0.7$	$4.4^{\rm b} \pm 1.8$	$3.6^{\circ} \pm 1.6$	$0.4^{a} \pm 0.3$	$0.5^{\rm b} \pm 0.3$	$0.6^{\rm c} \pm 0.3$	3	< 0.05
Pb	$1.1^{a} \pm 1.3$	$0.9^{\rm b} \pm 0.5$	$0.7^{\rm c} \pm 0.3$	$0.4^{a} \pm 0.1$	$0.2^{\rm b} \pm 0.1$	$0.1^{\rm c} \pm 0.1$	0.01	< 0.05
Cu	$1.6^{a} \pm 0.1$	$1.4^{\rm b} \pm 0.4$	$1.1^{\circ} \pm 0.6$	$0.5^{a} \pm 0.2$	$0.4^{\rm b} \pm 0.1$	$0.2^{c} \pm 0.1$	0.08	< 0.05
Cd	$1.3^{a} \pm 0.6$	$1.0^{\rm b} \pm 0.6$	$0.8^{\mathrm{c}} \pm 0.4$	$0.5^{a} \pm 0.2$	$0.3^{\rm b} \pm 0.5$	$0.1^{\rm c} \pm 0.1$	0.03	< 0.05
Cr	$1.2^{a} \pm 0.6$	$1.0^{\rm b} \pm 0.3$	$0.7^{\rm c} \pm 0.2$	$0.4^{a} \pm 0.3$	$0.2^{\rm b} \pm 0.1$	$0.5^{\rm c} \pm 0.4$	0.05	< 0.05

Table 1. Heavy Metals Concentration (mg/kg) Mangifera Indica (Mango) and World Health Organization Standard.

Values presented are in Mean ± SD. Within the column, paired mean with different alphabets are statistically significant (p < 0.05), SD = Standard deviation. KEY: FAD= fruits of Mangifera indica around NNPC Depot; BAD= Bark of Mangifera indica around NNPC Depot; RAD= Root of Mangifera indica around NNPC Depot; CL=Control Fruit; CB=Control Bark; CR=Control Root.

Deoxyribonucleic acid (DNA) content in Mangifera indica (Mango)

Table 2 shows the DNA content (ng/µl) of *Mangifera indica* (Mango) samples from polluted and controlled sites.

Part of mango plant	NNPC Petrol Depot	Control Site	
A	555	208	
В	422	180	
С	920	202	
D	840	210	
E	748	211	
F	540	201	

Table 2. DNA Content (ng/µl) of Mangifera Indica Samples.

KEY: FAD= fruits of *Mangifera indica* around NNPC Depot; BAD= Bark of *Mangifera indica* around NNPC Depot; RAD= Root of *Mangifera indica* around NNPC Depot; CL=Control Fruit; CB=Control Bark; CR=Control Root

The DNA amounts in fruits and roots before the NNPC Petrol site, Apir-Makurdi, were 555 and 422, respectively, while the DNA amounts in fruits and roots from the control site were 208 and 180, respectively. The DNA content in fruits and roots at the NNPC Petrol site, Apir-Makurdi, was 940 and 840, respectively, while the DNA amounts in fruits and roots from the control site were 202 and 210, respectively. The DNA content in fruits and roots after the NNPC petrol site, Apir-Makurdi, was 748 and 540, respectively, while the DNA amounts in fruits and roots from the control site were 211 and 201, respectively. Table 3 indicates the plant DNA fragmentation index.

Part of Mango plant	t of Mango plant DNA fragmentation index (%)		Remarks	
	Polluted site	Control site	Polluted site	Control site
A	73	27	severe	moderate
В	70	30	severe	moderate
С	82	18	severe	moderate

Table 3. Plant DNA Fragmentation Index and Remarks.

DNA fragmentation grading, 0-10% = Mild, 11-40% = Moderate, > 40% = Severe. KEY: A= fruits of *Mangifera indica* at NNPC Depot, Apir & Control site, B= Stem of *Mangifera indica* at NNPC Depot, Apir & Control site, C= Roots of *Mangifera indica* after NNPC Depot, Apir & Control site

The DNA fragmentation index from all the polluted sites was above forty percent (40 %), indicating the level of severity.

Heavy metals concentration in parts of Mangifera indica L (Mango)

One-way ANOVA showed that the concentration of heavy metals in parts of *Mangifera indica* L (Mango) is statistically significant at (P<0.05). The result of this study also showed that cadmium concentration is found to be highest in leaves of sugar baby plant and least in pulp. The trend of heavy metals concentration in samples analyzed were fruit >bark > Root The result showed that the concentrations of cadmium were lower than the World Health Organization safe limit of 0. 2mg/kg. The overall total concentrations of heavy metals determined were in the following order; Zn > Cu > Ni > Cd > Cr > Pb.

Heavy-duty vehicles or trucks usually park within and outside the NNPC Depot Apir-Makurdi for petroleum- related activities, and the exhausts of these trucks produce thick black emissions. Traffic activities are one of the major sources leading to heavy metal contamination in roadside soils due to their long-term accumulation [8]. Vehicle exhausts, as well as several industrial activities, emit these heavy metals such that soils, plants, and even residents along roads with heavy traffic loads are subjected to increasing levels of contamination with heavy metals [23].

Genotoxicity of heavy metals in Mangifera indica L (Mango)

The collective mutagenic effect of the heavy metals from roadside traffic in *Mangifera indica* L. may cause alterations in the expression of regulatory genes or structural genes that lead to the appearance or disappearance of new bands. The heavy metals assessed from this study have been reported to affect DNA repair enzymes (DNA glycosylases), either by altering the protein structure of the enzymes or by reducing the formation of the enzymes at the transcription stage, which could also lead to chromosomal aberrations in mitotic cells [24].

Nickel nanoparticles are responsible for systematic toxicity, inflammation, reactive oxygen stress (ROS), and cell apoptosis (programmed death of cells in all multicellular organisms), which include shrinking of the cell, fragmentation of the nucleus, condensation of chromatin, chromosomal DNA fragmentation and ribonucleic acid (RNA) decay. Apoptotic death, or the intrinsic (mitochondrial) pathway, is activated by signals within the cells as a result of stress, which triggers the release of cytochrome C from the mitochondria. The cell kills itself as a result of cellular stress [25]. The extrinsic pathway is activated by extracellular ligands that bind to cell surface of death receptors (Tumor Necrosis Factor), which leads to the formation of death-inducing signal complex. The signals received from other cells cause the cell to kill itself. Both intrinsic and extrinsic pathways cause cell death through the activation of caspases (cysteine proteases) or enzymes that degrade proteins. Excessive rates of cell death are the cause of many neurodegenerative diseases, whereas the inability to undergo apoptosis gives rise to immune diseases and uncontrolled increases in cell proliferation, such as cancer [26].

The intake of dissolved Zn^{2+} ions into the cell causes DNA damage [27, 28] showed that metallic nanoparticles that are chemically stable show negligible cellular toxicity, whereas nanoparticles that are unstable that are able to be oxidized or reduced lead cytotoxic and genotoxic processes for cellular organisms. Zn^{2+} is shown to induce DNA damage should usually by p53-associated apoptosis. A p53 knockdown fibroblast cell line exposed to zinc oxide nanoparticles was resistant to zinc oxide nanoparticle-mediated apoptosis and a progressive cellular proliferation, an indication of a likely first step towards carcinogenesis [28].

Lead (Pb) and its derivatives have the ability to distorting the spindle components of dividing cells, thereby leading to genotoxicity. Metal toxicity causes oxidative stress, which plays a major role in DNA-damage induction. Oxidative stress is the major mechanism of Pb toxicity which is primarily involved in the imbalance between the creation of reactive oxygen species (ROS) and the functionality of antioxidants. Pb possesses the ability of preventing the functions of antioxidant enzymes by disturbing antioxidant enzymes functional sulfhydryl (SH) group, for example, superoxide dismutase (SOD), aminolevulinic acid dehydrase. The condensation of delta-aminolaevulinic acid (-ALA) to porphobilinogen (PBG) is catalyzed by the inhibition of -ALAD for heme synthesis, which leads to the accumulation of -ALA. This process eventually activates ROS production and subsequently the production of 4,5-dioxovaleric acid, an efficient alkylating agent of the guanine moieties present between nucleoside and isolated DNA. Interestingly, the DNA repair machinery processes are vital in the protection of cells from DNA damage when exposed to cytotoxic agents, carcinogens, and heavy metals [29].

Cadmium may cause damage to the mitochondria, interfere with Ca^{2+} signaling, and also increases mitochondrial ROS formation. The respiratory chain of mitochondria plays a vital role in

maintaining energy homeostasis via oxidative phosphorylation, energy generation in the form of adenosine triphosphate (ATP), which is energy vital for life. Additionally, mitochondria are important in the production of amino acids, phospholipids, lipids, ion, motility, homeostasis and apoptosis (programmed death). Five to seven genomes of the mitochondria have been stabilized by DNA-binding proteins that vary from the nuclear histones. The thiol groups (-SH) of cysteines present in proteins are the critical targets of cadmium. The blocking of the mitochondrial electrontransfer chain by affecting electron flow through complex III (cytochrome bc1 complex or cytochrome c oxidoreductase) is the main toxic effect related to cadmium. Cadmium inhibits adenosine diphosphate and uncouples stimulated respiration, thus inducing an increase in ion permeability in the inner mitochondrial membrane by creating the mitochondrial permeability transition pore opening [30]. Cadmium may change many mitochondrial protein processes within and outside the membranes by preventing respiratory chain enzymes, collapsing the ability of the mitochondrial membrane, and swelling thee mitochondria, which then causes the inhibition of respiration. Cadmium could increase permeability and decrease mitochondrial membrane potential directly, a situation that causes the release of cytochrome C and the subsequent activation of the caspase pathway. Furthermore, cadmium inhibits lactate dehydrogenase (LDH), ATPase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities, thereby enhancing the levels of ROS and lipid peroxidation. Cadmium may alter redox status of the cell by reacting with both endogenous and exogenous antioxidants, such as glutathione GSH. Cadmium ions act as xenobiotic by preventing the activity of complex II (succinate dehydrogenase) and complex III (cytochrome bc1 complex) of the electron transfer chain. The ROS accumulation activates a sequence of events; including apoptosis (programmed and genetically encoded cell death-self cellular destruction mechanism). The extrinsic apoptotic pathway triggers apoptosis in response to external stimuli, while the intrinsic apoptotic pathway activates apoptosis in reaction to internal stimuli, for example, DNA damage. Stress stimuli (ROS, UV radiation, ionizing radiation, Ca2+, and Cd²⁺ lead to permeability of the mitochondrial outer membrane with the release of cytochrome C into the cytosol, thereby initiating caspase-8 and causing apoptosis. The intrinsic pathway, which is activated by signals from cellular stress such as DNA damage, activates caspase-9. Extrinsic and intrinsic pathways activate their respective initiators, such as caspase-8 and caspase-9 that initiates the executioner caspase-3 and caspase-7. The caspases possess cysteine that creates proteolytic activity that can cleave proteins after an aspartate residue. The execution pathway results in noticeable cell features such as cell shrinkage, formation of cytoplasmic blebs, chromatin condensation, and apoptotic bodies. In addition, apoptosis causes phagocytic recognition, protein cross-linking, protein cleavage, and DNA breakdown [30].

Chromium (VI) compounds are highly toxic and carcinogenic (cancer-causing compounds). Chromium VI) compounds can cause gene mutations, exchanges in sister chromatids, and chromosomal aberrations and are thus said to be genotoxic. Constituents of the cell must be present for Cr (VI) to react with DNA to cause damage to macromolecules. Cr reacts with bases of the DNA and phosphodiester framework where binding takes place through the coordinate covalent binding and ionic interactions. Protein crosslinks of DNA are also one of the ways by which cytotoxic effects are induced by chromium. Cr (VI) compounds are clastogens chemical agents that promote the rate of gene mutation through interfering with the role of nucleic acids). Reports also indicate that abnormalities of the chromosomes (micronuclei) and instability of the genome (microsatellite instability) are likely involved in inducing cancer by Cr (VI). Inter strand crosslinks of DNA are caused by Cr (III) interrupting reaction sites on the DNA complementary strand. These couplings initiate DNA repair processes when not repaired before replication in the DNA; it might lead to substitutions of nucleotidess, rearrangements of the chromosome, and subsequent deletions, complexation of Cr with GSH and other metabolites of ROS causes DNA adduct formation leading to genotoxicity [31].

Copper is a heavy metal capable of undergoing redox reactions, which induce oxidative stress, which is possible by increased formation of reactive oxygen species (ROS) and a decrease in the antioxidant capacity of the cell. The non-specific binding of Cu^{2+} cations to vital sites in the DNA repair causes inhibition. This shows the formation of micronuclei as a result of DNA damage and

prevention of repair initiated by Cu compound [27]. The polyunsaturated fatty acids undergo peroxidative degeneration in the membrane of lipids when the production of ROS exceeds the antioxidant defense mechanisms and produces histopathological abnormalities due to the generation of reactive oxygen stress (ROS) and DNA damage [26, 32]. The increase in quantity of DNA is believed to be induced by the presence of heavy metals in NNPC Depot Apir-Makurdi (Table 3). The increase in DNA contents maybe a mechanism to overcome heavy metal toxicity in response to the mango plant's heavy metal stress.

In conclusion, the result of the study established that the studied tree species, Mangifera indica, situated at the NNPC Depot Apir, were negatively affected by higher vehicular air pollutant concentrations as compared to the standard set by regulatory bodies like the World Health Organization (WHO, 2022). The result of the study revealed that the concentration of heavy metals decreased as the distance away from the edge of the road increased on both sides. The pollution of soils along the highways indicated the presence of carcinogenic heavy metals. Heavy metals have significantly negative effects on plant growth and through the process of accumulation and food-chain transfer, may reach humans the ultimate consumer of fruit trees. Most of the heavy metals had low BAF, indicating their bioaccumulation properties in the plant. Genotoxic effects of heavy metal pollution from roadside traffic on mango plants around the NNPC Depot Apir-Makurdi had more DNA content and fragmentation as well as more smearing of DNA than those from the control site.

Acknowledgements

The researchers are grateful to the Departments of Biological Science, University of Agriculture, Makurdi, Nigeria, for giving us access to their facilities. This research was carried out in collaboration among all authors.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article from any funding agencies in public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors solemnly declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

References

References

- 1. Boamponsem LK, Hopke PK, Davy PK. Long-term trends and source apportionment of fine particulate matter (PM2.5) and gaseous pollutants in Auckland, New Zealand. *Atmospheric Environment*. 2024; 322DOI
- 2. Gul H, Das BK. The Impacts of Air Pollution on Human Health and Well-Being: A Comprehensive Review. *Journal of Environmental Impact and Management Policy*. 2023; 3(06)DOI
- 3. Christou A, Giechaskiel B, Olofsson U, Grigoratos T. Review of Health Effects of Automotive Brake and Tyre Wear Particles. *Toxics*. 2025; 13(4)DOI

- 4. Gómez Yepes ME, Villamizar Vargas RH, Rendón García OL, Cremades LV. Evaluation of Particulate Emissions During Braking Tests in Technical-Mechanical Overhaul Workshops in Armenia-Quindío (Colombia). *Environments*. 2025; 12(2)DOI
- 5. Flood-Garibay JA, Angulo-Molina A, Méndez-Rojas MA. Particulate matter and ultrafine particles in urban air pollution and their effect on the nervous system. *Environmental Science: Processes & Impacts.* 2023; 25(4)DOI
- 6. Du W, Li X, Chen Y, Shen G. Household air pollution and personal exposure to air pollutants in rural China A review. *Environmental Pollution*. 2018; 237DOI
- 7. Shahid M., Pinelli E., Pourrut B., Silvestre J., Dumat C.. Lead-induced genotoxicity to Vicia faba L. roots in relation with metal cell uptake and initial speciation. *Ecotoxicology and Environmental Safety*. 2011; 74(1)DOI
- 8. Uka UN, Belford EJD, Elebe FA. Effects of road traffic on photosynthetic pigments and heavy metal accumulation in tree species of Kumasi Metropolis, Ghana. *SN Applied Sciences*. 2021; 3(1)DOI
- 9. Okunromade O, Yin J, Ray C, Adhikari A. Air Quality and Cancer Prevalence Trends across the Sub-Saharan African Regions during 2005-2020. *International Journal of Environmental Research and Public Health*. 2022; 19(18)DOI
- 10. Drzymała J., Kalka J.. Assessment of genotoxicity, mutagenicity, and cytotoxicity of diclofenac and sulfamethoxazole at environmental concentrations on Vicia faba. *International Journal of Environmental Science and Technology*. 2024; 21(4)DOI
- 11. Zaghloul A, Saber M, Gadow S, Awad F. Biological indicators for pollution detection in terrestrial and aquatic ecosystems. *Bulletin of the National Research Centre*. 2020; 44(1)DOI
- 12. Naveed NH. Fruits of roadside plants as bioindicator of traffic related lead pollution during different seasons in Sargodha, Pakistan. *African Journal of Environment Science anf Technology*. 2010; 4(11):770-774. DOI
- 13. Mulay JR. Roadside Plants as Bio-indicators of Urban Air Pollution", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN: 2395-602X, Print ISSN: 2395-6011. 2020; 7(4):321-326. DOI
- 14. Wacławek M, Świsłowski P, Rajfur M. The Biological Monitoring as a Source of Information on Environmental Pollution with Heavy Metals. *Chemistry-Didactics-Ecology-Metrology*. 2023; 27(1-2)DOI
- 15. Dawodu O, Erinle Q. Extraction of Genomic DNA from Different Plant Tissues through Phenol-chloroform Method. *Annual Research & Review in Biology.* 2024; 39(11)DOI
- 16. Kumar M., Ponnuswami V., Nagarajan P., Jeyakumar P., Senthil N.. Molecular characterization of ten mango cultivars using simple sequences repeat (SSR) markers. *African Journal of Biotechnology*. 2013; 12(47)DOI
- 17. Tomuter, Dzenda, Oche CY. Analysis of Onset and Cessation of Rainfall and its Effects on Groundnut Planting and Yield in Kyado, Ukum LGA, Benue State, Nigeria. *International Journal of Research Publication and Reviews.*. 2025; 5(12):1107-1115. DOI
- 18. HACH. Water Analysis Guide, Edition 1. HACH Company, Loveland, Colorado, USA, 62pp. 2013.
- 19. Ogli A, Ahmed M, Itodo A, Wuana R, Sha'Ato, R. GIS mapping, biomonitoring and distribution of heavy metal concentration using Neem (Azadirachta indica) bark in Makurdi Town, Benue State, Nigeria. *Journal of Geoscience and Environmental Protection*. 2023; 11(7):213-226. DOI
- 20. Obiakor MO, Okonkwo JC, Ezeonyejiaku CD, Ezenwelu CO. Genotoxicology: Single and Joint Action of Copper and Zinc to Synodontis clarias and Tilapia nilotica. 2010; 14(2):59-64. DOI
- 21. Kiss T, Karácsony Z, Gomba-Tóth A, Szabadi KL, Spitzmüller Z, Hegyi-Kaló J, Cels T, Otto M, Golen R, Hegyi AI, Geml J, Váczy KZ. A modified CTAB method for the extraction of high-quality RNA from mono-and dicotyledonous plants rich in secondary metabolites. *Plant Methods*. 2024; 20(1)DOI
- 22. Kamala Jayanthi PD, Rajinikanth R, Sangeetha P, Ravishankar KV, Arthikirubha A, Devi Thangam S, Abraham V. Rapid isolation of high molecular weight DNA from single dry preserved adult beetle of Cryptolaemus montrouzieri for polymerase chain reaction (PCR)

- amplification. African Journal of Biotechnology. 2012; 11(90)DOI
- 23. Almeida A, Santos M, Oliveira J. Genotoxicity of Paraforcein rice seedlings: Evidence from comet assays. *Journal of Agricultural Science*. 2023; 15(12):145-147. DOI
- 24. Ostad-Ali-Askari K, Gholami H, Madadi M, Karimi A, Abdollahi S. Assessment of Heavy Metal Cu, Pb, Cd Contamination in Dust in Zahedan City using Pollution Indices and GIS and Geo Statistics Technologies, Zahedan, Iran. *American Journal of Engineering and Applied Sciences*. 2020; 13(4)DOI
- 25. Genchi G, Carocci A, Lauria G, Sinicropi MS, Catalano A. Nickel: Human Health and Environmental Toxicology. *International Journal of Environmental Research and Public Health*. 2020; 17(3)DOI
- 26. Dalhus B, Helle IH, Backe PH, Alseth I, Rognes T, Bjørås M, Laerdahl JK. Structural insight into repair of alkylated DNA by a new superfamily of DNA glycosylases comprising HEAT-like repeats. *Nucleic Acids Research*. 2007; 35(7)DOI
- 27. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*. 2014; 7(2)DOI
- 28. Heim J, Felder E, Tahir MN, Kaltbeitzel A, Heinrich UR, Brochhausen C, Mailänder V, Tremel W, Brieger J. Genotoxic effects of zinc oxide nanoparticles. *Nanoscale*. 2015; 7(19)DOI
- 29. Hemmaphan S, Bordeerat NK. Genotoxic Effects of Lead and Their Impact on the Expression of DNA Repair Genes. *International Journal of Environmental Research and Public Health*. 2022; 19(7)DOI
- 30. Yan L, Allen DC. Cadmium-Induced Kidney Injury: Oxidative Damage as a Unifying Mechanism. *Biomolecules*. 2021; 11(11)DOI
- 31. Castro MP, Moraes FR, Fujimoto RY, Cruz C, Belo MADA, Moraes JRE. Acute toxicity by water containing hexavalent or trivalent chromium in native Brazilian fish, Piaractus mesopotamicus: anatomopathological alterations and mortality. *Bulletin of Environmental Contamination and Toxicology*. 2014; 92(2)DOI
- 32. Mandil R, Prakash A, Rahal A, Koli S, Kumar R, Garg SK. Evaluation of oxidative stress-mediated cytotoxicity and genotoxicity of copper and flubendiamide: amelioration by antioxidants in vivo and in vitro. *Toxicology Research*. 2023; 12(2)DOI