



## Chromosomal Aberrations Induced in Onion Bulbs (*Allium cepa*) Exposed to Coal Mine Effluent in Ejenma, Ankpa, Kogi State, Nigeria

\*<sup>1</sup>Sanni, M.Q., <sup>2</sup>Alege, G.O., & <sup>3</sup>Michael, A.A.

Biological Science Departments, Faculty of Science, Federal University Lokoja, Kogi State Nigeria

\*Corresponding author email: [queenmariam450@gmail.com](mailto:queenmariam450@gmail.com)

### Abstract

The Enjema area in Ankpa LGA, Kogi State, Nigeria, is profoundly impacted by coal mining activities. This current study evaluated the chromosomal aberrations induced by coal mine effluent from this region using the *Allium cepa* meristem assay. The sampled Onion bulb (*Allium cepa*) were exposed to varying concentrations of the effluent (0%, 25%, 50%, 75%, and 100%) for 24 hours. Root tips were harvested between 7:30 and 8:30 a.m. WAT for microscopic analysis of mitotic phases and chromosomal aberrations under X4, X10 and X40 magnification. Data were analyzed using Analysis of Variance (ANOVA), and the results on concentration dependent variables were presented in bar chart. The effluent induced several chromosomal abnormalities, including sticky chromosomes, C-mitosis, spindle disturbances, binucleate cells, vacuolated cells, and fragmented chromosomes, indicating its genotoxic potential. A concentration-dependent increase in aberrations was observed, with the highest frequency (16.30%) recorded at 100% effluent concentration. These findings confirm that coal mine effluent from Enjema possesses significant genotoxic and phytotoxic properties. It is therefore crucial to enforce environmental regulations and control effluent discharge in the Enjema region to safeguard ecological and public health.

**Keywords:** Aberrations, Chromosomal, Effluent, Induced, Genotoxic

### Introduction

Chromosomal aberrations are structural or numerical changes in chromosomes that occur when the genetic material of an organism is damaged. In plants, these aberrations can serve as important bioindicators, revealing the mutagenic and cytotoxic effects of environmental pollutants. They are particularly useful in environmental monitoring because their occurrence is often directly linked to the presence of genotoxic agents such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), and other industrial contaminants commonly released in coal mining regions. Broadly, chromosomal aberrations can be divided into two major categories: numerical and structural abnormalities. Numerical aberrations involve changes in chromosome number. This includes aneuploidy, where there is a loss or gain of one or more chromosomes, and polyploidy, where entire sets of chromosomes are duplicated. Aneuploidy often results from spindle apparatus disruptions during mitosis or meiosis, leading to unequal segregation of chromosomes into daughter cells. Polyploidy, on the other hand, may arise from failures in cell division, resulting in cells containing multiple complete sets of chromosomes (Roşculete et al., 2018; Kim et al., 2022).

In bioassays using *Allium cepa*, a common plant model for cytogenetic studies, several specific aberrations are frequently observed following exposure to environmental toxicants. These include chromatid breaks, where a single chromatid is broken; fragments, where parts of chromosomes separate entirely; lagging chromosomes, which fail to migrate properly during cell division; and micronuclei, which are small extranuclear bodies containing chromosomal fragments or whole chromosomes excluded from the main nuclei during telophase (Roşculete et al., 2018). Liu (2025) and Musilová et al. (2023) reported that onion root cells exposed to untreated coal mining wastewater exhibited elevated rates of chromatid breaks, stickiness, and micronuclei formation. These aberrations not only signal direct DNA damage but also reflect secondary effects such as oxidative stress, which can interfere with spindle apparatus function and chromosome condensation.

Research using *Allium cepa* has been particularly illuminating in this regard. Yang et al. (2022) showed that exposing onion roots to different concentrations of industrial wastewater produced a dose-dependent pattern in the frequency of chromosomal aberrations. At low concentrations, minor alterations such as a slight decrease in mitotic index and occasional chromatid breaks were observed. However, as pollutant levels increased, more severe and diverse abnormalities appeared, including multiple fragments, sticky metaphases, and a dramatic reduction in overall cell division. The high sensitivity of *Allium cepa* root tip cells to pollutants makes them particularly effective in detecting even low levels of contamination. This relationship has significant implications for environmental monitoring, because the frequency of chromosomal aberrations reflects pollutant levels, bioassays can serve as biological dosimeters, effectively quantifying environmental contamination without requiring sophisticated chemical analyses. This is especially valuable in regions like Enjema, where laboratory facilities for advanced chemical testing may be limited, yet the need for ongoing environmental assessment is high.

### Aim and Objectives of the Study

The aim of this study is to assess the occurrence of chromosomal aberrations induced in Onion bulb (*Allium cepa*) exposed to Coal mine effluent. The objectives of the study are to:

1. determine the concentration of effluent with highest chromosomal aberrations
2. examine the type and frequencies of chromosomal abnormalities induced by coal mine effluent from Enjema on Onion (*Allium cepa*) root tip cells

### Materials and Methods

#### Description of Study Area

The study area for this investigation is the Enjema coal mining region, located in the north-central part of the country at coordinates 12°15'N, 6°30'E. Enjema is a major coal mining hub within the Enjema Local Government Area, Kogi State, in Nigeria (Enyigwe et al., 2021). The region is characterized by a mix of rural and semi-urban settlements, with the majority of the population directly or indirectly employed in coal mining and related activities. The Enjema coal mine effluent, which is the focus of this study, is the wastewater discharged from the various coal extraction and processing operations in the area. This effluent is known to contain a complex mixture of heavy metals, chemicals, and other pollutants that can have significant impacts on the surrounding environment and the communities that rely on the local water resources for agricultural, domestic, and industrial purposes. Given the economic importance of the coal industry to the Enjema Local Government Area and the Kogi State, as well as the potential environmental and health concerns, it is crucial to investigate the genotoxic potential and heavy metal composition of the Enjema coal mine effluent.

### Sample Collection and Preparation

The effluent sample was collected into sterile bottles at the point of discharge from Enjema coal mine, Kogi State Nigeria; while the Onion bulbs (*Allium cepa*) of fairly equal sizes were purchased from Kpata market Lokoja, Kogi State. Twenty-five healthy *Allium cepa* bulbs of fairly equal sizes were rinsed with water and the dry scaly leaves were removed. In order to hasten the development of roots, dried roots were cautiously removed. The reduced stems in lower parts of the bulbs were thereafter partially immersed in distilled water in the beakers after dissecting the onion bulbs transversely, and the upper parts of the dissected onion bulbs were discarded.

To maintain uniform genotypes, the dissected onions were allowed to sprout shoots and only onions bulbs that sprouted up to five small onions sets were selected for further study. The onion set were transferred into the beakers containing different concentrations of the coal effluent (i.e., 25 %, 50 %, 75 % and 100 %). The roots were removed from the treatments after 24 hours and washed thoroughly. Only the roots measuring about 1cm in length were harvested into test tubes between the hours of 8:00 to 9:00am WAT. Each treatment had five replicates and followed Complete Randomized Design (CRD) arrangement (Alege et al., 2020). The roots were fixed in 1:3 glacial acetic and ethanol, hydrolysed in 1N HCl at 60°C, after which they were stained with Aceto-carmines and each slide carefully studied under the Celestron Digital model of the light microscope. Three different objectives (X4, X10, X40) were used to examine the slides. Photomicrographs of different stages of cell division and abnormal cells were taken using X40 magnifications.

**Data Analysis**

Data obtained were subjected to Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) using Post hoc test with PAST. The Mitotic Index (MI) and Percentage of Aberrant Cells (PAC) for the effluent treated cells and control were calculated using the formulae outlined by Malode et al. (2012) and presented in a bar chart.

**Results**

**Mitotic Index (MI):**

$$MI = \frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}} \times 100$$

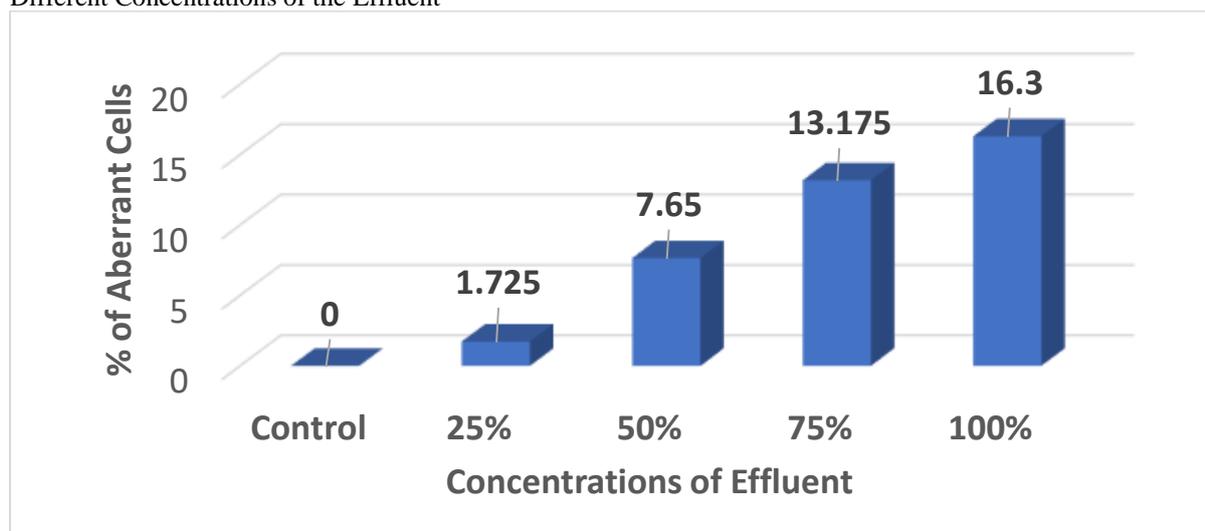
**Percentage of Aberrant Cells (PAC):**

$$PAC = \frac{\text{Total number of abnormal cells}}{\text{Total number of cells examined}} \times 100$$

Treatments	Sticky Chromosomes	Spindle Disturbance	C-Mitosis	Vacuolated Cells	Binucleate Cells	Fragmented Chromosomes
Control	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>
25%	2.20 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	1.00 <sup>c</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>
50%	3.20 <sup>b</sup>	0.80 <sup>b</sup>	1.80 <sup>ab</sup>	5.00 <sup>b</sup>	4.00 <sup>a</sup>	0.00 <sup>b</sup>
75%	8.40 <sup>a</sup>	3.00 <sup>a</sup>	3.60 <sup>a</sup>	5.60 <sup>b</sup>	5.00 <sup>a</sup>	1.60 <sup>a</sup>
100%	9.00 <sup>a</sup>	3.60 <sup>a</sup>	4.20 <sup>a</sup>	10.60 <sup>a</sup>	4.20 <sup>a</sup>	2.60 <sup>a</sup>
LSD Value	0.92	0.39	0.48	0.89	0.62	0.30

\*Means with the same alphabets in the same column are not significantly different at 5% level of significant

Table 1: Showing Chromosomal Aberrations Induced in Onion bulb (*Allium cepa*) Root Tip Cells Grown with Different Concentrations of the Effluent



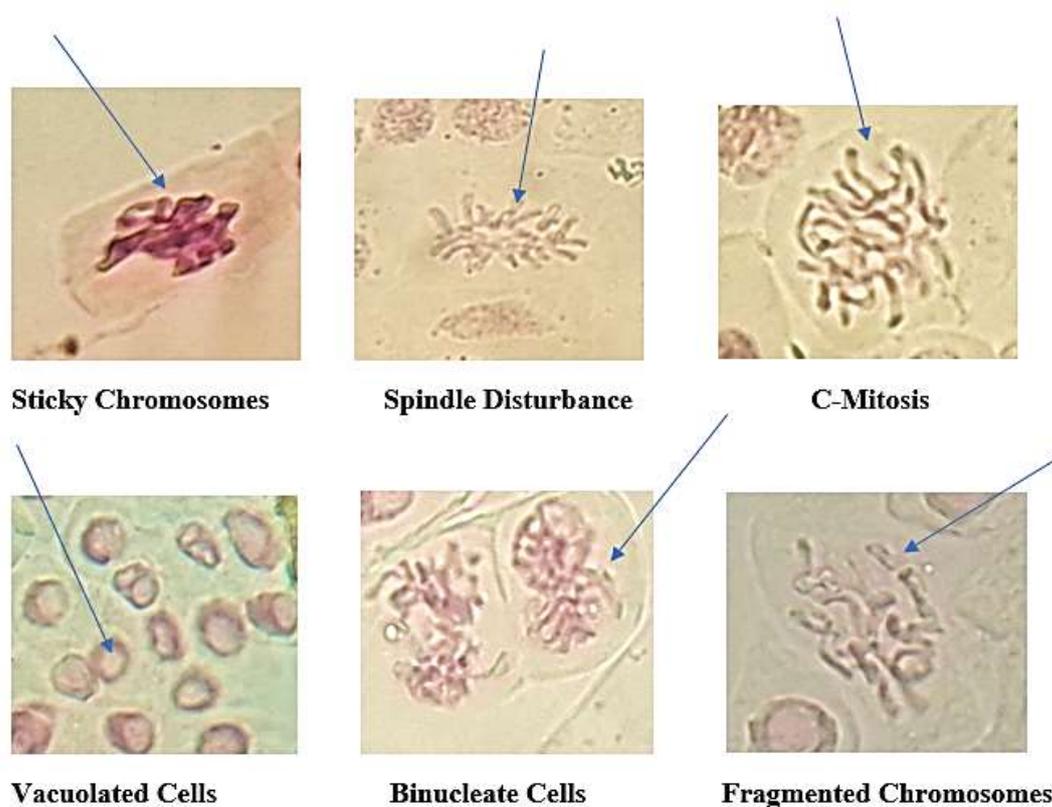
Bar chart Showing Percentage of Chromosomal Aberrations Induced by Different Concentrations of Effluent from Ejenma Coal Mine.

Chromosomal aberrations induced in Onion bulb (*Allium cepa*) root tip cells exposed to varying concentrations of effluent

Chromosomal aberrations induced in *Allium cepa* root tip cells exposed to varying concentrations of effluent as shown in the table above. Sticky chromosomes were absent in the control but increased with effluent concentration, reaching 9.00 at 100%. Spindle disturbances were only observed from 50% upwards, with the highest value of 3.60 recorded at 100%. C-mitosis appeared at 50% (1.80), peaking at 4.20 in the 100% treatment. Vacuolated cells increased from 1.00 at 25% to 10.60 at 100%. Binucleate cells were observed at concentrations  $\geq 50\%$ , with the highest count (5.00) at 75%. Fragmented chromosomes were absent in the control through 50% but were recorded at 75% (1.60) and 100% (2.60).

Photomicrographs of chromosomal aberrations observed in Onion bulb (*Allium cepa*) root tip cells treated with coal mine effluent

The photomicrographs of chromosomal aberrations observed in *Allium cepa* root tip cells treated with coal mine effluent is shown in Plate II. The images show various forms of abnormalities including sticky chromosomes, which appear clumped and fused, and spindle disturbance characterized by disoriented spindle fiber arrangement. Also shown is C-mitosis, with scattered and disorganized chromosomes, indicating spindle inhibition. Vacuolated cells exhibit prominent cytoplasmic vacuolation, while binucleate cells are identified by the presence of two nuclei within a single cell. Fragmented chromosomes appear as broken and dispersed chromatin materials within the cell. These visual evidences correspond to the quantitative data presented in and confirm the table above cytogenotoxic effects of effluent exposure at the cellular level in *A. cepa* root meristem.



Micrograph Chromosomal Aberrations Induced by Coal Mine Effluent in Onion bulb (*Allium cepa*) Root Tip Cells.

#### Mitotic Index of the Different Concentrations of Effluent from Enjema Coal Mine

As shown in Figure 3, the mitotic index decreased progressively with increasing concentrations of effluent from Enjema Coal Mine. The control group exhibited the highest mitotic index at 53.2%, followed closely by the 25% effluent concentration with a mitotic index of 52.8%. A noticeable decline was observed at 50% concentration, recording a mitotic index of 40.9%. Further reductions were noted at 75% and 100% concentrations, with mitotic indices of 31.4% and 26.4%, respectively. This pattern indicates a concentration-dependent decline in mitotic activity. The observed trend demonstrates a consistent reduction in mitotic index across increasing effluent concentrations, as illustrated in the figure. These values reflect a dose-responsive effect of the coal mine effluent on cell division activity.

#### Discussion

Mitotic Phases of Onion bulb (*Allium cepa*) Root Tip Cells Grown with Different Concentrations of the Effluent

The impact of different concentrations of coal-mine effluent on the mitotic phases of *Allium cepa* root tip cells exhibited pronounced variations, illustrating the detrimental effects of heavy metal exposure on plant cellular processes. The increase in interphase frequency correlates with the stress response mechanisms that plants employ when faced with toxic substances (Jalmi et al., 2018). In contrast, the observed decline in the frequencies of prophase, metaphase, anaphase, and telophase as effluent concentration increased highlights the cytotoxic effects of the heavy metals present in the effluent. This phenomenon is well documented in the literature. For instance, Riyazuddin et al. (2021) discuss how heavy metal toxicity leads to chromosomal aberrations and mitotic irregularities in plant cells, reflecting the data presented. Likewise, another research corroborates these observed mitotic disturbances thus, emphasizing the crucial role of environmental stressors in influencing cellular division and plant health (Singh et al., 2022). The progressive decrease in mitotic phases correlates with previous observations across various studies indicating heavy metal exposure as a significant contributor to mitotic inhibition and cellular apoptosis in plant systems (Okereafor et al., 2020).

The significant differences ( $p < 0.05$ ) observed in the study reinforce the notion that heavy metals induce oxidative stress, which ultimately hampers plant growth and development. The findings of Xu et al. (2018) support this, indicating that heavy metal contamination significantly impacts plant physiological and developmental traits. These insights suggest that remediation efforts must address both the constituents of effluent and their biological impacts on surrounding vegetation. Continuous research and monitoring of heavy metal contamination are critical for developing viable mitigation strategies, similar to methodologies suggested by Maiga-Yaleu et al. (2020) focusing on the assessment of agricultural influences on water quality. As such, this study's revelations not only contribute to the understanding of heavy metals' impact on plant biology but also emphasize the urgent need for comprehensive environmental policies and effective governance structures aimed at reducing both the prevalence of heavy metals in effluents and their biological repercussions.

#### Stages of Mitotic Division in *Allium cepa* Root Tip Cells Treated with Coal Mine Effluent

The treatment of *Allium cepa* root tip cells with coal mine effluent provides essential insights into the normal stages of mitotic division. Observations of interphase show distinct nuclei with uncondensed chromatin, indicative of normal cellular functioning. As cells transition into prophase, chromatin condenses into visible chromosomes. This orderly progression is crucial, as it forms the foundation for successful cell division, a mechanism necessary for growth and development (James et al., 2015). In this study, the identification of distinct mitotic phases suggests that, despite exposure to coal mine effluent, the basic processes of cell division are retained, aligning with findings from Alege et al. (2020) who reported noticeable mitotic progression in conjunction with effluent exposure in their experiments. Further analysis of metaphase reveals an orderly alignment of chromosomes along the metaphase plate, a pivotal process ensuring accurate chromosome segregation. This formation is critical to preventing aneuploidy, which could potentially arise from inefficient chromosome alignment, a concern highlighted in studies assessing the genotoxic effects of various effluents on *Allium cepa* (Mohammed et al., 2023). The successful segregation of sister chromatids during anaphase, in which the chromatids migrate toward opposite poles, underscores the efficiency of the mitotic spindle apparatus. However, it is crucial to consider potential alterations in the functionality of this apparatus under stress conditions induced by the effluent, a subject of ongoing research (Freitas et al., 2017).

The completion of the mitotic cycle is marked by telophase, where nuclear membranes reform around the separated chromatids, indicating that despite the presence of effluent, the fundamental characteristics of the mitotic cycle are preserved. Such findings are essential for understanding the implications of industrial effluent on plant development, particularly in relation to toxicity and stress responses. Studies indicate that while secondary toxic effects may not manifest instantly, persistent exposure can lead to long-term cellular adaptations or malfunctions (Roa et al., 2012; Lee et al., 2015). Moreover, histological evaluations through microscopy have corroborated these findings, demonstrating that cellular structures remain morphologically distinct and identifiable. These conclusions align with broader literature, which highlights that alteration in mitotic processes can serve as biomarkers for assessing environmental toxicity levels (Admas & Kerisew, 2022; Mansilha et al., 2021). The integrity of mitotic features signals the resilience of *Allium cepa*, reflecting a certain level of adaptability to the harsh conditions introduced by coal mine effluent. This insight resonates with research that has indicated the potential of plant bioassays, such as *Allium cepa*, in environmentally monitoring toxic exposures stemming from industrial activities (Lee et al., 2015; Wijeyaratne & Wickramasinghe, 2020). In environmental management, the ability of a plant like *Allium cepa* to sustain normal mitotic progression despite exposure to certain toxin levels underscores the importance of employing such bioassays in assessing the environmental impact of industrial waste. Future investigation should focus on determining the underlying mechanisms by which *Allium cepa* adapts to effluent exposure, possibly facilitating improved bioremediation strategies for contaminated water sources (Hemachandra & Pathiratne 2017; Abdullahi & Ibrahim, 2020).

### Chromosomal Aberrations Induced in Onion bulb (*Allium cepa*) Root Tip Cells Exposed to Varying Concentrations of Effluent

The induction of chromosomal aberrations in *Allium cepa* root tip cells is a critical indicator of genotoxicity associated with exposure to coal mine effluent. The rate of chromosomal defects, such as sticky chromosomes and spindle disturbances, was correlated with effluent concentration, marking an alarming trend. The absence of sticky chromosomes in the control group contrasted sharply with the significant induction observed at higher effluent concentrations, with values reaching 9.00 at 100% concentration. The presence of sticky chromosomes is often associated with chromatin alterations due to DNA damage, a phenomenon well-documented in the context of environmental stressors (Ogunyemi et al., 2018). Spindle disturbances, observed starting from the 50% effluent concentration, indicate potential disruptions to the mitotic spindle apparatus, which can significantly affect chromosome segregation. This finding is consistent with studies that have reported spindle-related defects as a consequence of chromosome damage induced by toxic substances, thereby highlighting the complexities of mitotic fidelity under pollution stress (Rodrigues et al., 2010). The peak observation of spindle disturbances at 100% effluent concentration, with a recorded value of 3.60, emphasizes the need to evaluate toxic impacts on cell division mechanisms further.

C-mitosis, indicative of abnormal mitotic processes, emerged in the treatment groups starting at 50%, reaching 4.20 at 100%. C-mitosis is known to arise due to metabolic disturbances and aberrant spindle function, prompting questions regarding the underlying biological mechanisms responsible for these alterations. High concentrations of heavy metals, frequently found in coal mine effluents, have been implicated in causing chromosomal aberrations through oxidative stress and subsequent cellular damage (Kannangara & Pathiratne, 2015; Mohammed et al., 2023; Rossato et al., 2010). Significantly, the increase in vacuolated cells from 1.00 at 25% to 10.60 at 100% demonstrates that exposure to effluent impairs normal cellular function and contributes to adverse cytological changes, supporting findings from other studies assessing the cytotoxic effects of various industrial effluents (Babatunde & Anabuiké, 2015; Abdullahi & Ibrahim, 2020). Furthermore, the appearance of binucleate cells at concentrations  $\geq 50\%$  underscores the potential for altered cell cycle regulation and failed cytokinesis—factors documented in environmental toxicology (Hemachandra & Pathiratne, 2017).

The absence of fragmented chromosomes up to 50% effluent treatment, with subsequent appearances at 75% and 100%, indicates that concentrations above a certain threshold initiate irreversible chromosomal damage. This nuanced relationship between concentration and type of chromosomal aberration exemplifies the critical need for continuous monitoring of heavy metal content and its biological implications in surrounding ecosystems (Santos et al., 2020; Wijeyaratne & Wickramasinghe, 2020; Admas & Kerisew, 2022). Statistical linkage between number of aberrations and effluent concentration emphasizes the *Allium cepa* assay's efficacy in evaluating environmental toxicity, providing a reliable bioindicator for assessing the genetic integrity of individuals exposed to contaminated environments (Roa et al., 2012; Mansilha et al., 2021). As the global community faces increasing environmental pollution challenges, such bioassays are essential in formulating conservation and remediation strategies, potentially shaping policies for sustainable industrial practices and environmental health preservation.

Photomicrographs of Chromosomal Aberrations Observed in Onion bulb (*Allium cepa*) Root Tip Cells Treated with Coal Mine Effluent. The examination of photomicrographs for *Allium cepa* root tip cells subjected to coal mine effluent elucidates the cytogenotoxic effects induced by the effluent on cellular integrity. The observed chromosomal aberrations, including sticky chromosomes, C-mitosis, vacuolated cells, and binucleate cells, signify marked disruptions in normal mitotic processes. Sticky chromosomes, which appear clumped and fused, are indicative of severe chromosomal damage. They frequently arise from interference in DNA replication or repair processes, potentially caused by oxidative stress attributable to the high concentration of heavy metals present in the coal mine effluent (Souza et al., 2022; Alias et al., 2023). Moreover, the documented spindle disturbances, characterized by disoriented arrangements of spindle fibers, could suggest a direct impact of the effluent on microtubule assembly and function, leading to ineffective segregation of chromosomes during mitosis. This aligns with findings from studies involving environmental pollutants and their detrimental impact on spindle apparatus functionality, which also underscored similar chromosomal abnormalities ((Roşculete et al., 2018; Ogunyemi et al., 2018).

C-mitosis features, evidenced by scattered and disorganized chromosomes, reinforce the notion that effluent exposure inhibits normal spindle formation and function. Such aberrations signal a disturbance in the metabolic pathways governing cell division, echoing the conclusions drawn in studies evaluating the effects of various environmental pollutants on plant species (Roşculete et al., 2018; Alias et al., 2023). Vacuolated cells, characterized by prominent cytoplasmic vacuolation, further signify a response to stress that may involve the activation of cellular degradation pathways as a means to cope with toxic exposure. This observation aligns with other studies that outlined similar vacuolation in response to heavy metal stress (Souza et al., 2022; Alias et al., 2023). The presence of binucleate cells in this context points to potential failed cytokinesis, a critical step following mitosis, when nuclear division should be followed by cytoplasmic division. This indicates severe

dysregulation of the cell cycle, which can lead to developmental defects at the organismal level, as highlighted previously in research linking these cellular anomalies to environmental toxicity (Roşculete et al., 2018; Karaismailoğlu, 2015; Alias et al., 2023). Fragmented chromosomes within treated cells present additional evidence of lethal genotoxic effects, where DNA integrity is compromised significantly; this is in accordance with studies that underscore the importance of assessing chromosomal integrity to gauge toxic exposure in plants (Alias et al., 2023; Karaismailoğlu, 2015).

Visual assessments through photomicrographs serve as compelling evidence that chronic exposure to coal mine effluent negatively influences chromosomal architecture and cellular health. By illustrating these morphological changes, the photographs reinforce quantitative data previously gathered, highlighting the cumulative impact of effluent concentration on chromosomal integrity (Bhat et al., 2016; Alias et al., 2023). As a widely recognized bioindicator, *Allium cepa* provides a robust model to elucidate the cytotoxic and genotoxic repercussions of environmental pollutants, including those from coal mining operations, thus emphasizing the need for ongoing research and improved management practices to mitigate such detrimental environmental impacts (Zhang and Wang, 2017; Çavuşoğlu et al., 2017).

#### Percentage of Chromosome Aberrations Induced in *Allium cepa* Root Tip Cells Treated with Different Concentrations of Effluent from the Enjema Coal Mine

The study of chromosome aberrations in *Allium cepa* root tip cells treated with varying concentrations of coal mine effluent elucidates clear dose-dependent increase in cytogenotoxic effects. The absence of aberrant cells (0.00%) in the control group establishes a baseline for comparative analysis with treated groups (Souza et al., 2022; Roşculete et al., 2018). At 25% effluent concentration, a modest elevation in the percentage of aberrant cells to 1.73% signifies the initiation of detrimental effects at relatively low pollutant levels, setting the stage for more pronounced effects as the concentration increases. Notably, the percentage of aberrations escalates to 7.65% at 50% concentration, further intensifying to 13.18% and 16.30% in 75% and 100% concentrations, respectively. This clear progressive trend reflects a consistent correlation between effluent concentration and the induction of chromosomal abnormalities, reinforcing the concept that increased exposure to environmental pollutants yields greater genotoxic potential, as extensively documented in previous studies (Roşculete et al., 2018; Karaismailoğlu, 2015; Alias et al., 2023).

Research consistently supports the hypothesis that higher levels of contaminants will incite more substantial biological responses, particularly in sensitive plant systems like *Allium cepa*. For example, studies involving pesticide and industrial effluents have reported similar findings wherein increased concentrations led to higher levels of chromosomal aberrations, as manifested through a variety of factors, including microtubule disruption and oxidative damage (Roşculete et al., 2018; Karaismailoğlu, 2015; Alias et al., 2020; Alias et al., 2023). This correlation is significant as it implies a pathway linking exposure to effluent concentrations with observable genetic damage, which reflects broader ecological implications for biodiversity and public health.

Furthermore, the escalating percentage of chromosomal aberrations observed in the current analysis complements insights from other studies assessing impacts of various environmental pollutants on *Allium cepa* and other plant species. Findings indicate that effluent, with its attendant heavy metal load, disrupts cellular processes, leading to significant alterations in mitotic index and fostering conditions conducive to chromosomal fragmentation and aberrations (Souza et al., 2022; Karaismailoğlu, 2015; Roşculete et al., 2018; Alias et al., 2020). This supports the broader narrative that pollution control is essential in maintaining both environmental health and agricultural productivity. The findings underscore the vital role of monitoring chromosomal aberrations as a bioindicator of environmental toxicity. Given that chromosomal aberrations often correlate with potential carcinogenic effects in higher trophic levels, the results from *Allium cepa* studies carry implications extending beyond the immediate environmental context (Souza et al., 2022; Roşculete et al., 2018; Karaismailoğlu, 2015; Alias et al., 2020).

#### Conclusion

Cytogenetic evaluation using the Onion bulb (*Allium cepa*) assay demonstrated that the effluent induced diverse chromosomal aberrations such as sticky chromosomes, spindle disturbances, C-mitosis, binucleate cells, vacuolated cells, and fragmented chromosomes. The frequency of these abnormalities increased in a concentration-dependent manner, with the highest percentage of aberrant cells (16.30%) recorded at 100% effluent concentration. This confirms the effluent's strong genotoxic potential.

#### Recommendation

Coal mining companies in Enjema community should adopt effective effluent treatment technologies before discharging waste into the environment. Also, regular environmental impact assessments should be mandated for mining operations, integrating biological assays including *Allium cepa* test as early-warning tools for genotoxicity. Community awareness programs should be implemented to educate local populations about the potential health and ecological risks posed by untreated mining effluents.

## References

- Abdullahi, U. and Ibrahim, S. (2020). Heavy Metals Analysis and Toxicity Evaluation of Some Textile and Dyeing Effluents in Kano, Nigeria using *Allium cepa* Bioassay. Bayero Journal of Pure and Applied Sciences, 12(1), 344-351. <https://doi.org/10.4314/bajopas.v12i1.52s>
- Alege, G., Anyoku, C., Olubiyo, C., Olubiyo, G., Adejoh, B., and Onemayin, D. (2020). Chromosomal Aberrations Induced by Cassava Industrial Effluent using *Allium cepa* assay. GSC Biological and Pharmaceutical Sciences, 13(3), 97-104. <https://doi.org/10.30574/gscbps.2020.13.3.0386>
- Alias, C., Feretti, D., Benassi, L., Abbà, A., Gelatti, U., Sorlini, S. and Piovani, G. (2020). The Release of Contaminants from Steel Slags and Natural Aggregates: Evaluation of Toxicity and Genotoxicity. Environmental and Molecular Mutagenesis, 62(1), 66-77. <https://doi.org/10.1002/em.22407>
- Alias, C., Piovani, G., Benassi, L., Abbà, A., Sorlini, S., Gelatti, U., and Feretti, D. (2023). Evaluation of Toxicity and Genotoxicity of Concrete Cast with Steel Slags Using Higher Terrestrial Plants. Environmental Toxicology and Chemistry, 42(10), 2193-2200. <https://doi.org/10.1002/etc.5709>
- Alias, C., Zerbini, I., and Feretti, D. (2023). A Scoping Review of Recent Advances in the Application of Comet Assay to *Allium cepa* Roots. Environmental and Molecular Mutagenesis, 64(5), 264-281. <https://doi.org/10.1002/em.22553>
- Babatunde, B. and Anabuiké, F. (2015). In vivo Cytogenotoxicity of Electronic Waste Leachate from Iloabuchi Electronic Market, Diobu, Rivers State, Nigeria on *Allium cepa*. Challenges, 6(1), 173-187. <https://doi.org/10.3390/challe6010173>
- Bhat, S., Singh, J., and Vig, A. (2016). Genotoxicity Reduction in Bagasse Waste of Sugar Industry by Earthworm Technology. Springerplus, 5(1). <https://doi.org/10.1186/s40064-016-2882-1>
- Çavuşoğlu, D., Tabur, S., and Çavuşoğlu, K. (2017). Physiological and Cytogenetical Effects of Royal Jelly (honey bee) in *Allium cepa* L. Seeds Exposed to Salinity. Cytologia, 82(2), 115-121. <https://doi.org/10.1508/cytologia.82.115>
- Çavuşoğlu, K., Cadıl, S., and Çavuşoğlu, D. (2017). Role of Potassium Nitrate (kno<sub>3</sub>) in Alleviation of Detrimental Effects of Salt Stress on Some Physiological and Cytogenetical Parameters in *Allium cepa* L. Cytologia, 82(3), 279-286. <https://doi.org/10.1508/cytologia.82.279>
- Enyigwe, M T., Onwuka, O S., and Egbueri, J C. (2021). Geochemical distribution, statistical and health risk assessment of toxic elements in groundwater from a typical mining district in Nigeria. Taylor and Francis, 23(5-6), 469-481. <https://doi.org/10.1080/15275922.2021.1907822>
- Freitas, L., Rambo, C., Franscescon, F., Barros, A., Lucca, G., Siebel, A. and Magro, J. (2017). Coal extraction causes sediment toxicity in aquatic environments in Santa catarina, Brazil. Ambiente E Agua - An Interdisciplinary Journal of Applied Science, 12(4), 591. <https://doi.org/10.4136/ambi-agua.2036>
- Hemachandra, C. and Pathiratne, A. (2017). Cytogenotoxicity screening of Source Water, Wastewater and Treated Water of Drinking Water treatment Plants Using Two in vivo Test systems: *Allium cepa* Root Based and Nile Tilapia Erythrocyte-based Tests. Water Research, 108, 320-329. <https://doi.org/10.1016/j.watres.2016.11.009>
- Jalmi, S. K., Bhagat, P. K., Verma, D., Noryang, S., Tayyeba, S., Singh, K., Sharma, D., and Sinha, A. K. (2018). Traversing the Links Between Heavy Metal Stress and Plant Signaling. Frontiers in Plant science, 9, 12. <https://doi.org/10.3389/fpls.2018.00012>
- James, O., Oluwaleye, S., Olufunmilayo, A., and Adebisi, O. (2015). Cytotoxic effects and Genotoxic Screening of Pharmaceutical Effluents Using Onion bulbs (*Allium cepa* L.). Journal of Advances in Biology and Biotechnology, 2(1), 51-58. <https://doi.org/10.9734/jabb/2015/12962>
- Kannangara, D. and Pathiratne, A. (2015). Toxicity Assessment of Industrial Wastewaters Reaching Dandugan oya, Sri lanka Using a Plant-based Bioassay. Journal of the National Science Foundation of Sri Lanka, 43(2), 153-167. <https://doi.org/10.4038/jnsfsr.v43i2.7943>
- Karaismailoğlu, M. (2015). Investigation of the Potential Toxic Effects of Prometryne Herbicide on *Allium cepa* Root-tip Cells with Mitotic Activity, Chromosome Aberration, Micronucleus Frequency, Nuclear DNA Amount and Comet Assay. Caryologia, 68(4), 323-329. <https://doi.org/10.1080/00087114.2015.1109927>
- Kim, Y., Lee, J., Cho, Y., Choi, Y., Lee, Y., and Chung, H. (2022). Chromosome Damage in Relation to Recent Radiation Exposure and Radiation Quality in Nuclear Power Plant Workers. Toxics, 10(2), 94-109. <https://doi.org/10.3390/toxics10020094>
- Liu, W. (2025). Trends and Emerging Hotspots in Toxicology of Chironomids: a Comprehensive Bibliometric Analysis. Insects, 16(6), 639-647. <https://doi.org/10.3390/insects16060639>
- Maiga-Yaleu, S., Hahiou, A., and Guel, B. (2020). Assessment of Surface Water Contamination by Heavy Metals Due to Agricultural Practices in the Northern Part of Burkina-Faso. International Research Journal of Pure and Applied Chemistry, 85-98. <https://doi.org/10.9734/irjpac/2020/v2i1i1030211>

- Mansilha, C., Melo, A., Flores, D., Ribeiro, J., Rocha, J., Martins, V. and Marques, J. (2021). Irrigation with Coal Mining Effluents: Sustainability and Water Quality Considerations (são pedro da cova, north portugal). *Water*, 13(16), 2157-2171. <https://doi.org/10.3390/w13162157>
- Musilová, P., Kadlčíková, D., Hradská, H., Vozdová, M., Selingerová, I., Černohorská, H. and Rubeš, J. (2023). Chromosome Damage in Regions with Different levels of Air Pollution. *Environmental and Molecular Mutagenesis*, 64(6), 326-334. <https://doi.org/10.1002/em.22562>
- Mohammed, J., Mustapha, Y., Him, M., and Danladi, Z. (2023). Assessment of Cytogenotoxicity of Plastic Industrial Effluent Using *Allium cepa* root tip cells. *International Journal of Cell Biology*, 2023, 1-7. <https://doi.org/10.1155/2023/5161017>
- Ogunyemi, A., Samuel, T., Amund, O., and Ilori, M. (2018). Toxicity Evaluation of Waste Effluent from Cassava-processing Factory in Lagos state, Nigeria Using the *Allium cepa* assay. *Ife Journal of Science*, 20(2), 305. <https://doi.org/10.4314/ijfs.v20i2.11>
- Okereafor, U., Makhatha, M., Mekuto, L., Uche-Okereafor, N., Sebola, T., and Mavumengwana, V. (2020). Toxic Metal Implications on Agricultural Soils, Plants, Animals, Aquatic Life and Human Health. *International Journal of Environmental Research and Public Health*, 17(7), 2204-2211. <https://doi.org/10.3390/ijerph17072204>
- Riyazuddin, R., Nisha, N., Ejaz, B., Khan, M., Kumar, M., Ramteke, P. and Gupta, R. (2021). A comprehensive review on the heavy metal toxicity and sequestration in plants. *Biomolecules*, 12(1), 43-47. <https://doi.org/10.3390/biom12010043>
- Roa, O., Yeber, M., and Venegas, W. (2012). Genotoxicity and toxicity Evaluations of Cellulose Bleaching Effluents Using the *Allium cepa* L. Test. *Brazilian Journal of Biology*, 72(3), 471-477. <https://doi.org/10.1590/s1519-69842012000300009>
- Rodrigues, F., Angeli, J., Mantovani, M., Guedes, C., and Jordão, B. (2010). Genotoxic Evaluation of an Industrial Effluent from an oil Refinery Using Plant and Animal Bioassays. *Genetics and Molecular Biology*, 33(1), 169-175. <https://doi.org/10.1590/s1415-47572010005000006>
- Roşculete, C., Bonciu, E., Roşculete, E., and Olaru, L. (2018). Determination of the Environmental Pollution Potential of some Herbicides by the Assessment of Cytotoxic and Genotoxic effects on *Allium cepa*. *International Journal of Environmental Research and Public Health*, 16(1), 75-91. <https://doi.org/10.3390/ijerph16010075>
- Rossato, L., Tedesco, S., Laughinghouse, H., Farias, J., and Nicoloso, F. (2010). Alterations in the Mitotic Index of *Allium cepa* Induced by Infusions of *Pluchea sagittalis* Submitted to three Different Cultivation Systems. *Anais Da Academia Brasileira De Ciências*, 82(4), 857-860. <https://doi.org/10.1590/s0001-37652010000400007>
- Santos, K., Almeida, V., Weiler, J., and Schneider, I. (2020). Removal of pollutants from an and from a Coal Mine by Neutralization/Precipitation Followed by “in vivo” Biosorption Step with the Microalgae *Scenedesmus* sp., *Minerals*, 10(8), 711. <https://doi.org/10.3390/min10080711>
- Singh, P., Singh, A., and Singh, V. (2022). Extreme Metal Research on the Water, Fish, and Vegetation of Lakhabanjara Lake, Sagar. *International Journal of Health Sciences*, <https://doi.org/10.53730/ijhs.v6ns1.8049>
- Souza, R., Souza, C., and Guimarães, J. (2022). Environmentally Realistic Concentrations of Eprinomectin induce Phytotoxic and Genotoxic Effects in *Allium cepa*. *Environmental Science and Pollution Research*, 29(53), 80983-80993. <https://doi.org/10.1007/s11356-022-21403-7>
- Wijeyaratne, W. and Wickramasinghe, P. (2020). Chromosomal Abnormalities in *Allium cepa* Induced by Treated Textile Effluents: Spatial and Temporal Variations. *Journal of Toxicology*, 20, 1-10. <https://doi.org/10.1155/2020/8814196>
- Xu, Q., Duan, D., Cai, Q., and Shi, J. (2018). Influence of Humic Acid on Pb Uptake and Accumulation in Tea Plants. *Journal of Agricultural and Food Chemistry*, 66(46), 12327-12334. <https://doi.org/10.1021/acs.jafc.8b03556>
- Yang, W., Jannatun, N., Zeng, Y., Liu, T., Zhang, G., Chen, C., and Li, Y. (2022). Impacts of microplastics on immunity. *Frontiers in Toxicology*, 4, 412-423 <https://doi.org/10.3389/ftox.2022.956885>