UMYU Scientifica, Vol. 2 NO. 1, March 2023, Pp 106 – 114
https://doi.org/10.56919/usci.2123.013
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Evaluation of Cyto-Genotoxicity of Pharmaceutical Industrial Effluent in Kano Metropolis, Kano State, Nigeria, Using *Allium Cepa L.* Assay

Jibril Sani Mohammed[1]*, Yahaya Mustapha[2], Usman Abubakar[2], Eghobor Sunday[1], Bashir Mohammed Mayaki[2], Yahaya Sadiq Abdulrahman[3], Mohammed Isa Auyo[1] and Aisha Wada Abubakar[1]

[1]Department of Biological Sciences Federal University Dutse, Nigeria.
[2]Department of Plant Biology Bayero University Kano, Nigeria.
[3]Nigeria Institute for Oil Palm Research, Nigeria.

ABSTRACT

An *Allium cepa* root cells assay was used to assess cytotoxic and genotoxic impacts on Pharmaceutical industrial effluent in Kano Metropolis. An industrial effluent's physicochemical characteristics and heavy metal composition were assessed, and the readings were found to be higher than the required levels, demonstrating that it had not been treated before disposal. A set of 45 onion bulbs were grown for 96 hours in pharmaceutical effluent that included 2.5, 5.0, 7.5, and 10.0% (v/v), with distilled water serving as the control. All three root tips from each replication's treated bulbs were plucked at 96 hours and prepared for cytogenetic analysis using the aceto-carmine squashed procedure. At higher doses of industrial effluents, the root tips were highly cytotoxic, and their growth was strongly retarded. Exposure to the effluents inhibited root growth with an EC50 value of 6.3%. An analysis of variance (ANOVA) revealed a significant difference (P 0.05) in the average root growth of *Allium cepa* subjected to various pharmaceutical effluent concentrations. Mitosis Index (MI) rapidly reduced when effluent concentrations rose compared to control, whereas mitotic inhibition rose with rising effluent concentrations compared to controls. The pharmaceutical effluent triggered chromosomal abnormalities in *Allium cepa* root tip cells, particularly sticky chromosomes, Binucleated cells, and Bridge chromosomes being most commonly seen at lower doses of 2.5%. It was discovered that the compounds present in effluent might harm living things and, if left untreated, could poison the environment. Industrialists need to be legally required to switch their operations to environmentally friendly technology after it was determined that industrial effluents pose an environmental danger and can result in a number of human illnesses..

ARTICLE HISTORY

Received February 27, 2023
Accepted March 28, 2023
Published March 30, 2023

KEYWORDS

Allium cepa, Chromosomal aberration, Cytotoxicity, Pharmaceutical effluent, Genotoxicity, Mitotic index

INTRODUCTION

The *Allium* genus is a member of the family Alliaceae subf. Allioideae. More than 800 species make up this extensive genus (Li *et al.*, 2010a) and can be found all over the Northern Hemisphere. *Allium cepa*, a member of section *cepa*, was found to be closely related to *A. vavilovii* in internal transcribed spacer (ITS)-based phylogenetic analysis (Friesen *et al.*, 2006) and an established location through sequences of chloroplasts (Li *et al.*, 2010a).

Allium's ancestral karyotype appears to be x = 8. According to information gathered from various sources and stored in the Index to Plant Chromosome Numbers database (IPCN) at http://www.tropicos.org/Project/IPCN, *Allium cepa* has 2n = 16 chromosomes. On the other hand, it is fascinating to note that the variety *A. cepa* var. *Alef viviparum* According to Puizina and Papea (1996), it would be a triploid if it had 2n = 24.

The value of the *Allium cepa* test adds knowledge for preventing environmental toxicity. According to Firbas and Amon (2013), the onion (*Allium cepa* L.), a possible biomarker for genotoxic research, is frequently employed for genotoxicity in different aquatic environments. This assay assists in identifying potentially dangerous substances in an environment and evaluating mutagens (El-Shahaby *et al.*, 2003). Moreover, this is therefore acknowledged as a significant biomarker for assessing environmental contamination (Bagatini *et al.*, 2009; Leme and Marin-Morales, 2009). The consequences of these damages could be described via photomicrographs of cells with chromosomal abnorma-

Correspondence: Jibril Sani Mohammed. Department of Biological Sciences Federal University Dutse, Nigeria. ✉ **jibrilmohammedsani@gmail.com** Phone Number; +234 810 274 8831
How to cite: Jibril S. M., Yahaya M., Usman A., Eghobor S., Bashir M. M., Yahaya S. A., Mohammed I. A. and Aisha W. A. (2023). Evaluation of Cyto-Genotoxicity of Pharmaceutical Industrial Effluent In Kano Metropolis, Kano State, Nigeria, Using *Allium Cepa L.* Assay. UMYU Scientifica, 1(2), 106 – 114. https://doi.org/10.56919/usci.2123.013

-lities. One approach for determining and evaluating the extent of systemic alterations brought on by carcinogens, mutagens, or harmful compounds is the *Allium cepa* plant (Tedesco and Laughinghouse, 2012). Higher plants could be utilised as bioindicators using several biomarkers to evaluate the toxic effect of pesticide compounds (De Souza *et al.*, 2016). International initiatives like the International Program on Plant Bioassays (IPPB) of the United Nations Environment Program (UNEP) (Ma, 1995) and Geno-Tox of the US Environmental Protection Agency (USEPA) have standardised higher plants being used as indicator species. USEPA and the World Health Organization (WHO) agree that the data gathered from these toxicity tests helps incite environmental genotoxicity (Palmieri *et al.*, 2016). Genotoxicity assessments began to use molecular cytogenetic methods at the start of the twenty-first century. Genotoxicity investigations have used techniques like TdT-mediated dUTP nick end labelling (TUNEL test), single-cell gel electrophoresis (comet assay), and fluorescent in situ hybridisation (FISH), and the results have proven to be reliable (Silveira *et al.*, 2017; de Souza *et al.*, 2017b).

Experimental techniques to measure cytotoxicity are constantly being created and enhanced along with the development of modern cell biology. The biological evaluation method *in vitro* uses the cytotoxicity test as one of its most important indicators to track how substances affect cellular proliferation, division, and morphology (Li *et al.*, 2015b). Chemical cytotoxicity has been evaluated using various bioassays and cell lines. Countries must implement the necessary provisions for the associated cytotoxicity tests in accordance with their unique circumstances when enacting cytotoxicity *in vitro* regulations (Rudra *et al.*, 2020). As cytotoxicity tests have continued to advance, new techniques have emerged that have gradually transitioned from qualitative evaluation to quantitative analysis, including the identification of cell damage through morphology, as well as the measurement of cell damage, cell expansion, and metabolic characteristics (Piao *et al.*, 2011; Damas *et al.*, 2011). Testing for environmental water contamination and drinking water quality is frequently done using the sensitive and standardised "*Allium* test." More than 40 years have passed since the government accepted this strategy. It is particularly helpful for monitoring wastewater, testing contaminated streams, rivers, rain, snow, soil, herbicides like atrazine and benzo(a) piren, Pharmaceutical effluents, hospital outflows, and potentially radioactive wastes. The *Allium* test correlates well with other plant and animal assays (Peter and Tomaz, 2013).

There are many harmful chemicals in the environment, most of which are released by industrial facilities into the water, air, and soil. Many chemical industries have been established worldwide due to widespread chemical use. Both natural and artificial processes allow substances to infiltrate our environment (Nimita and Sonia, 2013). They disrupt numerous biochemical processes, which can have catastrophic consequences, and are challenging to remove from the environment once they have entered our biological system. Many compounds that have the potential to produce mutations have been researched and have been found to alter the genetic code and cause damage. Several thousand deadly substances, including medications, household and industrial by-products, insecticides, and petrochemical products, are present in the environment, and new compounds are being developed every year (Palmieri *et al.*, 2016). Without question, the chemical industry's rapid development has benefited the economy and society, but it has also made social and environmental issues more prominent. Environmental biologists are concerned with protecting people from chemical exposure (Nimita and Sonia, 2013).

Industries are essential to a country's growth. However, because of their enormous environmental relevance, the effects of industrial waste fluids on the biotic and abiotic environments have received considerable focus globally (Olorunfemi *et al.*, 2011). Hence, the majority of industries are situated close to water sources. Industrial wastewater has a far higher potential for pollution than home wastewater since it is both concentrated and abundant. In Nigeria, over 80% of companies release gaseous pollutants, liquid effluents, and solid wastes into the surrounding area untreated (Federal Ministry of Water Resources, 1994). These harmful substances are absorbed by the roots and moved to the aerial section of plants, where they cause cytogenotoxicity and phytotoxicity (chlorophyll degradation) and may contaminate crops grown in irrigation zones. The health of people and other animals consuming these items could potentially be put in direct or indirect jeopardy. In addition to their direct negative impacts on health, pollutants also pose a hidden threat since they may be poisonous or mutagenic and cause a number of human diseases like cancer, atherosclerosis, cardiovascular problems, and early aging. (Grover and Kaur, 1999). Water source eutrophication may also produce environmental conditions that favour the growth of cyanobacteria that produce toxins. Animals exposed repeatedly to these poisons may develop gastroenteritis, liver damage, nervous system impairment, skin rashes, and liver cancer (Okereke, 2016). Numerous microbial infections found in wastewater can lead to chronic illnesses with expensive long-term consequences, like heart disease and stomach ulcers. Depending on the severity and frequency of the infection, the density and diversity of these contaminants can change. Different forms of microbiological contaminants in wastewater are usually challenging to find, isolate, and identify. These tasks are also costly and time-consuming. In order to prevent this, indicator organisms are always employed to assess the relative risk of the potential presence of a certain disease in wastewater effluents. (Okereke, 2016). In this investigation, the cytogenotoxicity impacts of pharmaceutical industry wastewater discharge in the Kano metropolitan were assessed using the *Allium cepa* root

cell assay. The objectives of this research were to determine the physico-chemical properties of the pharmaceutical industrial effluent, evaluate the effects of different concentrations of the effluent on root growth of *Alium cepa*, determine the effects of different concentrations of the effluent on cytotoxic parameters of *Alium cepa* root cells (mitotic index and mitotic inhibition) and to identify the different types of chromosomal aberrations induced by different concentrations of the effluents in *Alium cepa* root cell

METHODOLOGY

Area of Study

From the very beginning of time, Kano City has served as the state's capital. It is situated 840 kilometres from the Sahara desert's edge at latitude 12.000N and longitude 8.300E in West Africa's semi-arid Sudan savannah region. Kano is located at a mean elevation of 472.45 meters above sea level. Although it occasionally drops as low as 10^0 C during the harmattan (Muhammad, 2019). Kano's temperature typically fluctuates from a maximum of 33^0 C to a minimum of 15.8^0 C.

Effluent Collection

The pharmaceutical effluent was obtained from the Kano State Drug Supply and Consumable Products. The effluent was discharged into a big gutter near the factory, from where it flowed into major water bodies in the Kano metropolis, from the site of outflow into the environment, the effluent was obtained in a 15-litre container.

Collection and Preparation of plant material

The yan-albasa market along Katsina Road, Kano City, Nigeria, provided healthy, uniform-sized bulbs of common onion (*Allium cepa* L: 2n=16). Sorting and removing the unhealthy bulbs were done. A sharp knife was used to carefully shave off the desiccated roots at the bottom of the plant medium (Allium cepa) to reveal the fresh meristematic tissues. Two weeks were spent drying the plant media (Allium cepa) in the sun. After that, the bulbs were submerged in newly made distilled water to prevent the primordial cells from drying out. The bulbs were removed from the distilled water and placed on absorbent paper so they could dry (Rank, 2003).

Preparation of Effluents

Following the pilot testing, the pharmaceutical effluent was generated in four concentrations, namely 2.5%, 5%, 7.5%, 10%, and 0% (v/v). After that, distilled water was used to allow a series of bulbs to grow roots. The onion bulbs were placed on 100ml of beaker after two days, with varying concentrations of each effluent, for four days (96 hours). Every day, new test solutions were used (Fiskesjö and Levan, 1993).

Experimental design

Three replicates and a Completely Random Design (CRD) were used in the experiment. There are a total of forty-five (45) onion bulbs that were grown in test solutions contained in 100ml of a glass beakers.

Determination of physicochemical parameters

The physicochemical characteristics of the effluents sample, including temperature, PH, colour, dissolving oxygen, biological oxygen demand, total dissolved solids, and heavy metals, were determined using standard analytical methods (WHO, 1996; FEPA, 1991; USEPA, 1999) standards for effluents discharge regulation. The physicochemical analyses were completed at Bayero University Kano's core laboratory. However, at the Center for Dry Land Agriculture (CDA), heavy metals analysis was done using Atomic Absorption Spectroscopy (AAS). The samples were stored in a fridge until usage.

Cytological investigation

Cytological investigations were conducted at the Umaru Musa Yara'adua University's central laboratory in Katsina State. After 96 hours, two-centimetre root tips from the control and treatment groups were obtained, preserved in the fluid of Carnoy (Glacial acetic acid and Ethanol in 1 ratio 3 v/v), and maintained at 40 degrees Celsius in the refrigerator for 24 hours before being hydrolysed in 1N HCl at 600 degrees Celsius for 15 minutes. Around 2 to 5 mm of the tips of roots were cut off, macerated and put in glass slides with two drops of freshly made aceto-carmine stain. After that, it was left to stand for 4 minutes on the glass slides. Using distilled water to clean them, the stained slides were then covered with coverslips. Then the coverslips were gently pressed with the aid of a thumb. Transparent Canada balsams were applied at the edge of the coverslips to prevent air penetration, and each stained slide was carefully labelled with a masking tape. From replications, four slides for each treatment were created. Slides were microscopically examined using Motic bright field microscope under 100x objective lens with the addition of oil immersion. Photomicrographs of selected cells showing chromosomal aberrations were captured.

Determination of Root Growth/inhibition

The root growth inhibition tests were determined after four days (96 hours) of the experiment. For every group, root growth lengths (controls and treatment groups) were measured using a calibrated ruler after the experiment's 1^{st}, 2^{nd}, 3^{rd} and 4^{th} day and the average values were computed. It was assumed that the control group's mean root length was 100%, and the test concentrations and the root length of various treatment sets were plotted against one another to calculate Effective Concentration (EC50) at a point where growth was 50%, the percentages of root length inhibition and growth from all of the test effluents were calculated using a formula.

$$\text{Root length growth (\%)} = \frac{\text{root length of treatment group}}{\text{root length of control}} \times 100$$

$$\text{Root length inhibition (\%)} = \frac{\text{root length of control} - \text{root length of treatment}}{\text{root length of control}} \times 100$$

Determination of Cytotoxicity

Based on the mitotic parameters (Mp), such as the mitotic index and mitotic inhibition, in the root cells of *Allium cepa*, the cytotoxic level of evaluated chemical or compound was calculated. They were assessed using the methodology laid out by Rank (2003) and Bakare et al. (2000). Percentages were used to express the outcomes.

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells obsreved}} \times 100.$$

$$\text{Mitotic inhibition} = \frac{\text{Mitotic index in control group} - \text{mitotic index in test group}}{\text{Mitotic index in control group}} \times 100.$$

$$\text{Frequency of aberrations} = \frac{\text{total numbr of abberrant cells}}{\text{Total number cells count}} \times 100.$$

Determination of Genotoxicity (Chromosome aberrations)

In order to examine the various chromosomal abnormalities, Photomicrographs of selected cells showing chromosomal aberrations were captured throughout various phases of the cell cycle such as Bridge, Stickiness, Laggard, Vagrant, Binucleated cells, C-Mitosis.

Statistical Data Analysis

Data were reported as the three replicates per sample mean ± standard error (SE) at 5% probability level. Variations between exposure treatments and controls were declared statistically significant. Using the Excel application. The measured parameters in *Allium cepa* roots were subjected to analysis of variance (ANOVA) and post-hoc least significance difference (LSD) tests to see whether there had been any significant changes. Using Paleontological Statistics and Software (PAST), a plot of root length as a percent of control versus the effluent concentrations was used to compute the EC50 and regression equation. The root length and effluent concentrations were tested for a significant association (Positive or Negative) using a Pearson correlation analysis.

RESULTS AND DISCUSSIONS

Physicochemical properties

The physical-chemical characteristics of pharmaceutical effluent are presented in table 01 below. Most of the physical-chemical characteristics were discovered to be more than the World Health Organization-recommended environmental criteria (WHO, 1996), Federal Environmental Protection Agency (FEPA, 1991), and United State Environmental Protection Agency (USEPA, 1999). In contrast, some were within the allowed limit for effluents discharge into the environment. The PH of Pharmaceutical effluent was strongly acidic when compared to acceptable requirements, the dissolved oxygen and biological oxygen demand values were quite low, indicating that many organic wastes with a high oxygen requirement were being produced in these sectors (Emongor *et al.*, 2005). The effluent contained heavy metals such as lead, zinc, copper, iron, and chromium, and the values were determined to be outside of the acceptable limit, indicating that the effluents had not been treated before being discharged. Metal contamination is a serious environmental issue, particularly in the aquatic environment. If certain metals enter the food chain, they could be harmful to humans since they can potentially be toxic or carcinogenic even at extremely low concentrations. In pharmaceutical effluent, heavy metals like Zn, Cr, Cu, and Pb can bind to specific proteins in fish and plants, causing disruptions in membrane integrity, metabolism of cells, and minerals transportation in living cells. The poisonous substances occasionally even result in aquatic organisms' death (Bobmanuel *et al.*, 2006; Yadav *et al.*, 2007; Ekweozor *et al.*, 2010).

Table 01: Physicochemical properties of Pharmaceutical effluent obtained from Kano drugs supply and consumable products on 10th February, 2021.

Parameters	Pharmaceutical	FEPA[a]	USEPA[b]	WHO[c]
Colour	Brownish red	NS	NS	NS
Odour	Unpleasant	NS	NS	NS
PH	3.60±0.058	6.0-9.0	6.0-9.0	6.5-9.5
Temp	28.73±0.088	<40	NS	NS
DO	0.90±0.058	NS	NS	NS
TDS	689±0.577	2000	500	<1200
BOD	0.80±0.058	50	NS	NS
COND	10690±1.155	NS	NS	1200
Zn	0.63±0.0577	<1	0.12	NS
Fe	18.47±0.577	2.0	0.009	3.0
Cu	1.37±0.0058	<1	0.30	1.0
Pb	0.23±0.0058	<1	0.003	0.01
Cr	2.30±0.058	<1	0.30	0.01

Save for temperature; all variables were expressed in Mg/L. There is no unit in PH. [a]Federal Environmental Protection Agency (1991), [b]United State Environmental Protection Agency (1999), [c]World Health Organization (1996). NS: Not stated

Root growth parameters (Phytotoxicity)

When compared to the control, the tips of roots of *Allium. cepa* subjected to various pharmaceutical effluent concentrations exhibited a few unique deformations, including swollen, twisted, and crotchet roots. It has been demonstrated that the development of root deformity in *Allium cepa* is a reliable toxicity indicator (Silva *et al.*, 2003; Bagatini *et al.*, 2009). The results of a statistical study using ANOVA showed that the average root growth lengths of the *Allium cepa* subjected to various concentrations of the Pharmaceutical effluent differed significantly (p 0.05). In general, examination of the Pearson correlation's effect on root growth (r = - 0.99332, Y = -8.2551 +103.88X) was seen to be negatively correlated to concentration, demonstrating that root development retardation was considerably ($p<0.05$) concentration-dependent, implying that a significant growth rate was seen when the effluent content decreased.

The roots of *Allium cepa* experienced cytotoxic effects from pharmaceutical effluent, according to the findings of EC50 estimation and the root growth study. The low EC50 value of 6.3% discovered in *Allium cepa* root cells exposed to varied concentrations of the effluent led to the conclusion that Pharmaceutical effluent is exceedingly toxic and harmful. Hence, if cell division is suppressed, there can be a phytotoxic impact.

Table 02: Root growth length and EC50 of *Allium cepa* root cells subjected to various concentrations of Pharmaceutical effluent obtained from Kano drugs supply and consumable products at 96hrs.

Concs	Mean RL (cm)	Mean NR	%RL Growth	Root LI(%)	EC50
0%(contl)	6.53 ± 0.32^{a}	18.7 ± 0.33^{a}	100	0	
2.5%	5.50 ± 0.15^{b}	13.7 ± 0.33^{b}	84	16	
5.0%	4.47 ± 0.26^{c}	10.0 ± 0.58^{c}	68	32	6.3%
7.5%	2.87 ± 0.43^{d}	6.0 ± 0.57^{d}	43	56	
10%	1.23 ± 0.30^{e}	3.67 ± 0.33^{e}	18	81	
LSD	0.96	1.40			

Key: The means that are denoted by a distinct superscript letter in the column are statistically different ($p< 0.05$). RL: root length, NR: number of roots, LI: length inhibition, EC50: Effective Concentration at 50%. LSD: Least Significant Difference

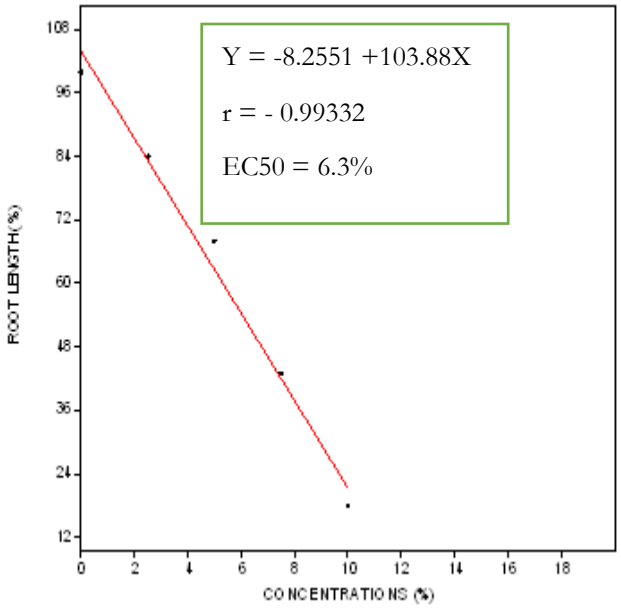

Figure 1: *Allium cepa* roots subjected to various pharmaceutical effluent concentrations showed growth inhibition based on a Pearson correlation coefficient.

Mitotic Index and Mitotic Inhibition (cytotoxicity effects)

It was observed that the mitotic index of *Allium cepa* root cells decreased with an increase in the concentrations of the industrial effluent (The mitotic index ranges from 32, 11, 8, 5, 0% in 0%, 2.5%, 5%, 7.5% and 10% of the Pharmaceutical effluent as shown in table 03). The results of Babatunde, *et al.* (2016), who found that the mitotic index showing the proportion of diving cells reduced with increasing concentration of UTH effluents, are consistent with this result. The mitotic index of control groups was found to be greater than that of the treatment groups. There was zero mitotic inhibition discovered in the control groups (treated with distilled water). 100% mitotic inhibition was observed at 10% concentration of Pharmaceutical effluent, this may be as a result of necrosis. Unlike the mitotic index, mitotic inhibition increased with the effluent concentrations. The mitotic index is an excellent parameter to gauge cell growth and estimate the proportion of cells going through mitosis. The mitotic index (MI) is a valuable tool in biomonitoring to evaluate the impact of various contaminants on cell division (Leme

and Marin-Morales, 2009). M.I. calculates the percentage of cells going through the cell cycle's mitotic phase; its blockage could be considered cellular death. (Rojas *et al.*, 1993). The limit value is believed to be a 50% reduction in the mitotic index relative to control; a decline below 50% results in sublethal effects on the test organism, while a decrease below 22% results in lethal effects (Mesi *et al.*, 2012; Singh, 2000). The pharmaceutical effluent sample in this investigation lowered the mitotic index, demonstrating both sublethal and lethal effects in onions root tip cell lines. Pharmaceutical effluent was very fatal, especially around concentrations of 7.5 and 10.0%, which were more cytotoxic.

Table 03: Mitotic Index (MI %) and Mitotic Inhibition (Mih %) of *Allium cepa* root cells subjected to different concentrations of Pharmaceutical effluent at 96hrs

Concs	TNC	NDC	MI (%)	Mih (%)
0%(control)	300	95[P_{40} M_{30} A_{15} T_{10}] ±2.91^{a}	32	0
2.5%	300	34[P_{15} M_{9} A_{6} T_{10}] ±1.86^{b}	11	66
5%	300	24[P10 M6 A6 T2] ±2.33^{c}	8	75
7.5%	300	14[P_{6} M_{3} A_{4} T_{1}] ±2.08^{d}	5	84
10%	300	0[P_{0} M_{0} A_{0} T_{0}] ±0^{e}	0	100
LSD		6.55		

A substantial difference exists between the means with the various superscript letters throughout the column ($p< 0.05$). NDC: number of dividing cells, TNC: total number of cells count.

Chromosomal aberrations (Genotoxicity)

Table 04 summarises the microscopic investigation of *Allium cepa* root cells subjected to various Pharmaceutical effluent concentrations. Chromosome analysis revealed that, as compared to the control, the effluents caused chromosomal abnormalities (Stickiness, Bridge, Laggard, Vagrant, Scattered and Binucleated cells) that were statistically significant ($P>0.05$). The chromosome of the Allium cepa plant subjected to the control had no abnormalities and also 10% of pharmaceutical effluents (due to the high presence of toxicant leading to the death of cells). C-mitosis was not observed. The sticky chromosome was observed to be the most frequent aberration at 2.5% of pharmaceutical effluent (6.0±0.58), followed by the binucleated cells (5.0±0.58); at the same concentrations of effluent and then followed by the bridge chromosome. The least number of aberrations were laggard, vagrant and scattered chromosomes. The findings from this study are consistent with other studies on industrial effluents by Samuel *et al.* (2010), Olorunfemi and Ehwre (2011). The high number of sticky chromosomes at the anaphase and metaphase stages demonstrated that industrial effluents contain hazardous compounds. A sticky chromosome is a sign of poisoned chromosomes, which may cause cell death (Antonise-Wiez, 1990). A disrupted nucleic acid metabolism may cause stickiness (Sudhakar *et al.*, 2001). The genotoxic effect was mostly noticed at the anaphase, prophase and metaphase cell division phases. The frequency of aberrations was concentration dependent (that is, the frequency of aberrations decreases as the concentrations increase down the effluent sample). This might be due to apoptosis, this contradicts Qian's (2004) claim that the aberrant rate increases as concentrations do.

Table 04: Chromosomal aberrations (Genotoxicity) of *Allium cepa* root cells subjected to various concentrations of Pharmaceutical effluent at 96hrs.

Concs	BC	ST	BRG	LAG	VAG	SC	CMT	FOA%
Contl(0)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.5%	5.0±0.58	6±0.58	3.3±0.33	1.3±0.88	1.3±0.88	1.0±0.58	0.0	6.0
5.0%	3.7±0.67	4.7±0.67	2.7±0.67	1.3±0.33	1.3±0.33	1.7±0.67	0.0	5.1
7.5%	1.7±0.33	2.3±0.88	1.0±0.58	1.0±0.58	1.0±0.57	1.0±0.58	0.0	2.7
10%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

BC: binucleated cell, ST: Stickiness, BRG: Bridge, LAG: Laggard VAG: Vagrant: SC: Scattered, CMT: C-mitosis, FOA (%): Percentage frequency of aberrations.

Plate A: Root length of *Allium cepa* exposed to Pharmaceutical effluent at 2.5% conc.

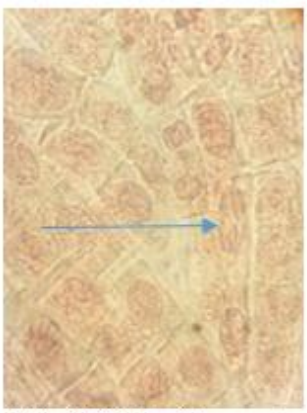

Plate B: Bridge Fragment

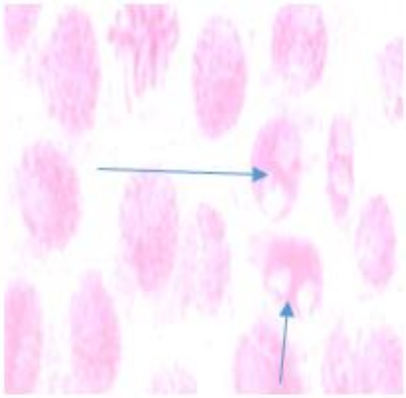

Plate C: Binucleated Cells

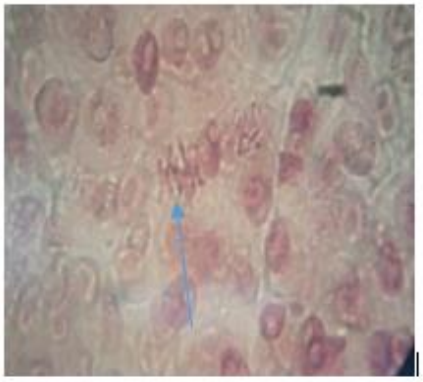

Plate D: Stickiness at Metaphase

Plates: Photomicrographs of cells showing Chromosomal Aberration

CONCLUSSION AND RECOMMENDATION

The present research indicates that the Pharmaceutical effluent contains abnormally high levels of total dissolved solids (TDS), conductivity, biological oxygen demand (BOD), dissolved oxygen (DO), and pH, as well as elevated concentrations of iron (Fe) and significant amounts of other heavy metals such as chromium (Cr), copper (Cu), zinc (Zn), and lead (Pb). These levels are above the recommended permissible limit set by environmental regulatory agencies, making the effluents toxic. The study also revealed that increasing concentrations of Pharmaceutical effluent induced cytogenotoxicity, leading to a decrease in the number of dividing cells, root length, mitotic index, and frequency of aberration. To promote environmental sustainability, it is necessary to enforce legal measures that compel industries to adopt eco-friendly operations.

ACKNOWLEDGEMENT

The researchers appreciated the work of Prof. Yahaya Mustapha of the Department of Plant Biology at Bayero University Kano for his significant contribution in the areas of experimental design and photomicrographs, as well as the staff at the Centre for Dry Land Agriculture (CDA) and the Department of Biology at UMYU for their technical assistance in microscopy and Atomic absorption spectroscopy.

REFERENCES

Antonise-Wiez D. (1990). Analysis of the cell cycle in root meristem of *Allium cepa* under the influence of Ledakrin. *Folia Histochemical Cytobiologia* 26:79-96.

Babatunde B.B, Vincent-Akpu I.F, and Aiwerioghene A. O. (2016). Cytogenotoxicity Screening of Untreated Hospital Wastewaters Using the *Allium cepa* Test. *Journal of Applied Sciences and Environmental Management* 20(3): 724-732. **[Crossref]**

Bagatini M, Vasconcelos T, Laughinghouse IVHD, Martins A, and Tedesco S. (2009). Biomonitoring hospital effluentsby the *Allium cepa* L. test. *Bulletin of Environmental Contamination and Toxicology* 82:590-592. **[Crossref]**

Bakare A. A, Mosuro A. A, and Osinbajo O. (2000). Effect of simulated leachate on chromosomes and mitosis in roots of *Alium cepa* (L). *Journal of Environmental Biology*. 21(3): 263-271.

Bakare B.A, Wheater M.A, Bringas J.S, and Hoen M.M. (2011). Cytotoxicity comparison of mineral. *Cytologia*. 52:877–888

Bobmanuel, N., Gabriel, U., & Ekweozor, I. (2006). Direct toxic assessment of treated fertilizer effluents to Oreochromis niloticus, Clarias gariepinus and catfish hybrid(Heterobranchus

bidorsalis x Clarias gariepinus). African Journal of Biotechnology, Vol. 5, No. 8, pp 635-642, ISSN 1684-5315

Damas B.A, Wheater M.A, Bringas J.S, and Hoen M.M. (2011). Cytotoxicity comparison of mineral. *Cytologia*. 52:877–888

De Souza C.P., Guedes T.A., and Fontanetti C.S. (2016). Evaluation of herbicides action on plant bioindicators by genetic biomarkers: a review. *Environmental Monitoring Assessment*. 188:694. **[Crossref]**

De Souza R.P, de Souza C.P, Bueno O.C, and Fontanetti C.S. (2017b). Genotoxicity evaluation of two metallic-insecticides using Allium cepa and Tradescantia pallida: a new alternative against leaf-cutting ants. *Chemosphere*. 168:1093–1099. **[Crossref]**

El-Shahaby A.O., AbdelMigid H.M., Soliman M. I., and Mashaly I. A., (2003). Genotoxicity screening of industrial wastewater using the *Allium cepa* chromosome aberration assay. *Pakistan Journal of Biological Sciences*, 6(1): 23-28. **[Crossref]**https://doi.org/10.3923/pjbs.2003.23.28

Ekweozor, I., Bobmanuel, N., & Gabriel, U. (2010). Sublethal Effects of Ammoniacal Fertilizer Effluents on three Commercial Fish Species from Niger Delta Area, Nigeria. Journal of Applied Sciences and Environmental Management, Vol. 5, No. 1, ISSN 1119-8362

Emongor, V; Kealotswe, E; Koorapetse, I; Sankwansa, S.; and Keikanetswe, S. (2005). Pollution indicators in Gaberone effluent. *Journal of Applied Science* 5: 147- 150. **[Crossref]**

Federal Ministry of Water Resources (1994). The study of the National Water Resources master plan under Japan International Cooperation Agency (JICA).Abuja. Federal Ministry of Water Resources.

FEPA (Federal Environmental Protection Agency). (1991). Guidelines to Standards for Environmental Pollution Control in Nigeria, FG Press Lagos Nigeria.238pp

Firbas P., and Amon T. (2013). Allium chromosome aberration test for evaluation effect of cleaning municipal water with Constructed Wetland (CW) in Sveti Tomaž, Slovenia. *Journal of Bioremediation and Biodegradation*. 4(4): 189. **[Crossref]**

Fiskesjo G and Levan, A. (1993). Evaluation of the first ten MEIC chemicals in the *Allium* test. *ATLA* **21**: 139–149. **[Crossref]**

Friesen N., Fritsch R. M, and Blattner F. R. (2006). Phylogeny and new intrageneric classification of *Allium* (Alliaceae) based on nuclear ribosomal DNA ITS Sequences. *Aliso*. 22(1):372–395. **[Crossref]**

Grover I.S and Kaur S. (1999). Genotoxicity of wastewater samples from sewage and industrial effluent detected by the Allium root anaphase aberration and micronucleus assays. Mutation Research. 426: 183 – 188

Leme D. M, Marin-Morales M. A. (2009). *Allium cepa* test in environmental monitoring: A review on its Application. *Mutation Research*, 682: 71–81. **[Crossref]**

Li D, Huang Q, Lu M, Zhang L, Yang Z, Zong M, and Tao L. (2015b). The organophosphate insecticide chlorpyrifos confers its genotoxic effects by inducing DNA damage and cell apoptosis. *Chemosphere*. 135:387–393. **[Crossref]**

Li –Q.Q, Zhou S.D, He X.J, Yu Y, Zhang Y.C, and Wei X.Q. (2010). Phylogeny and biogeography of *Allium* (Amaryllidaceae: allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast rps16 sequences, focusing on the inclusion of species endemic to China. *Annals of Botany*. 106:709–733. **[Crossref]**

Ma T.H, Xu Z, Xu C, Mc Connell H, Rabago E.V, Arreola G.A, and Zhang H. (1995). The improved Allium/Vicia root tip micronucleus assay for clastogenicity of environmental pollutants. *Mutation Research*, 334:185–195. **[Crossref]**

Mesi A, Kopliku D, and Golemi S. (2012). The use of higher plants as bio-indicators of environmental pollution - a new approach for toxicity screening in Albania. *Malaysian Journal of Soil Sciences*; 3(8): 237-248.

Muhammad, I. H. (2019). Risk Assessment Associated with Pesticides Application on Selected Agricultural Farmland in Kano State, Nigeria (Doctoral dissertation, Kwara State University (Nigeria)).

Namita K, and Sonia S. (2013). The *Alium cepa* root chromosomal aberration assay: A review, *Indian Journal of Pharmaceutical and Biological Research,* Vol. 1 (3). **[Crossref]**

Okereke, J. N., Ogidi, O. I., & Obasi, K. O. (2016). Environmental and health impact of industrial wastewater effluents in Nigeria-A Review. Int J Adv Res Biol Sci, 3(6), 55-67.

Olorunfemi D I., Ogieseri U M., and Akinboro A. (2011). Genotoxicity Screening of Industrial Effluents using Onion bulbs (*Allium cepa* L.). *Journal of*

Applied Science and Environmental Management. Volume, 15 (1) 211 – 216. **[Crossref]**

Olorunfemi D. I . and Ehwre E . O. (2011). Chromosomal aberrations induced in root tips of *Allium cepa* by squeezed garri extracts. *Report and Opinion*. 2(12):166-171.

Palmieri M. J., Andrade-Vieira, L. F., Trento, M. V. C., Eleutério, M. W. F., Luber, J. and Davide, L. C. (2016). Cytogenotoxic effects of spent pot liner (SPL) and its main components on human leukocytes and meristematic cells of *Allium cepa*. *Water Air and Soil Pollution*. 227:1-10. **[Crossref]**

Peter F, and Tomaz A. (2013). *Allium* Chromosome Aberration Test for Evaluation Effect of Cleaning Municipal Water with Constructed Wetland (CW) in Sveti Tomaž, Slovenia. *Journal of Bioremediation and Biodegradation*. 4: 4.

Piao M.J., Kang K.A., Lee I.K., Kim H.S., Kim S., Choi J.Y., Choi J, and Hyun J.W (2011). Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced. *Cytologia, p.20-66*. **[Crossref]**

Puizina J, and Papea D. (1996). Cytogenetical evidences for hybrid structure and origin of diploid and triploid shallots (*Allium cep*a var. viviparum, Liliaceae) from Dalmatia (Croatia). *Plant Systematic and Evolution*. 199:203–215. **[Crossref]** https://doi.org/10.1007/BF00984905

Qian X.W (2004). Mutagenic effects of chromium trioxide on root tips of *Vicia faba*. *Journal of Zhejiang University of Sciences*. 5(12): 1570 – 1576. **[Crossref]**

Rank J. (2003). The method of *Alium* anaphase–telophase chromosome aberration assay. *Ekologija volume 1: page 38-315*.

Rojas E, Herrera LA, and Sordo M. (1993). Mitotic index and cell proliferation kinetics for identification of antineoplastic activity. *Anti-Cancer Drugs* 4(6): 637- 640. **[Crossref]**

Rudra, S., Tahamina, A., Emon, N. U., Adnan, M., Shakil, M., Chowdhury, M. H. U., ... & Uddin, S. B. (2020). Evaluation of various solvent extracts of Tetrastigma leucostaphylum (Dennst.) Alston leaves, a Bangladeshi traditional medicine used for the treatment of Diarrhea. Molecules, 25(21), 4994. **[Crossref]**

Rudra O.B., Osuala F, and Odeigah P.G.C. (2010). Cytogenotoxicity evaluation of two industrial effluents using *Allium cepa* assay. *African Journal of Environmental Science andTechnology*., 3:481-484.

Samuel O.B., Osuala F, and Odeigah P.G.C. (2010). Cytogenotoxicity evaluation of two industrial effluents using *Allium cepa* assay. *African Journal of Environmental Science andTechnology*., 3:481-484.

Silva J, Erdtmann B, and Henriques JAP. (2003). *Geneticatoxicologica*. Alcance, *Porto Alegre* Pp.70–75.

Silveira G.L, Lima MGF, Dos Reis G.B, Palmieri M.J, and Andrade-Vieira L.F. (2017). Toxic effects of environmental pollutants: comparative investigation using *Allium cepa* L. and *Lactuca sativa* L. *Chemosphere*. 178:359–367. **[Crossref]**

Singh R.J. (2000). Plant Cytogenetics. 2nd ed. New York: CRC Press;. p.12. **[Crossref]**

Sudhakar R., Gowda N., and Venu G. (2001). Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. *Cytologia*; 66: 235-239. **[Crossref]**

Tedesco S.B., and Laughinghouse H.D IV (2012). Boindicator of Genotixicity. The *Allium cepa* Test. *Journal ofEnvironmental Contamination*, 138-156.

United State Environmental Protection Agency (USEPA). (1999). National recommended water quality criteria-correction: EPA 822/Z-99-001, USEPA, and Washington DC

World Health Organization (WHO). (1996). Guideline for drinking water quality recommendations 2, World Health Organization.

Yadav, A., Neraliya, S., & Singh, R. (2005). Effect of fertilizer industrial effluent on the behavior and morphology of fresh water catfish, Heteropeneustes fossilis (Bloch). Proceeding of National Academy of Science India, Vol. 75, No. 111, pp 191–195.