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Determination of Cytotoxic Impact of Heavy Metals on Plant Cells Using *Allium cepa* (Onion).

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Abstract

The environment faces significant global health challenges due to the presence of heavy metals, with harmful substances being discharged into the lithosphere, hydrosphere, and atmosphere. This study focused on assessing the cytotoxic effects of heavy metals recovered from dumpsites on the meristematic tissue of root tips of Allium cepa L. Soil samples, collected from central dumpsites containing heavy metals, underwent standard procedures. The concentrations of heavy metals in the samples were determined using the Association Official Analytical Chemist (AOAC) method. Each sample was digested with acids, neutralized with Calcium Oxide (CaO), and used to grow Allium cepa at varying concentrations, determining the LD_{50} of each sample. The LD_{50} concentrations were then employed to investigate cytotoxic effects on Allium cepa using standard techniques. Processed soil samples yielded Pb, Cr, Cd, Ni, Co, Zn, Fe, and Cu at different concentrations, many of which exceeded the WHO standard values. The mitotic index ranged from 5.34 ± 0.01 to 10.36 ± 0.00, 5.34 ± 0.00 to 13.16 ± 0.01, 3.36 ± 0.01 to 6.81 ± 0.01, 2.48 ± 0.01 to 13.16 ± 0.01, with the control at 13.32 ± 0.01. Chromosomal aberrations, including chromosome break, fragmentation, bridge, vagrant, lesion, and sticky C-mitosis, were observed. These alterations indicated that toxic substances significantly impact DNA sequence.

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Keywords: Chromosomal aberration, Deoxyribonucleic acid, Meristematic, Mitotic index, Root tips

INTRODUCTION

Developing Countries, such as Nigeria are confronted with three main problems: pollution, population and destitution. Due to the rise of industry and expansion, pollution has become a global problem. Environmental pollution occurs when the environment cannot convert pollutants into harmless substances (Odutayo, *et al.*, 2016). The effects of these pollutants have a negative impact on the ecosystem, both plants and animals (Mariya, *et al.*, 2016). When released into the environment in excess, these pollutants cause great harm to human and animal health, as well as to plants and trees, including rainforest areas. Industrial wastewater, both liquid and solid, is the main source of direct and often continuous input of pollutants/toxins into the hydrosphere, which has long-term effects on the functionality of the ecosystem, not excluding the lithosphere. Non-selective discharge of untreated wastewater directly or indirectly into water bodies or land can make water resources and the environment unsafe for humans and other living organisms (Tayachew and Bizuayehu, 2022; Arthur and Bamford, 2015; Okereke *et al.*, 2016).

Sources of heavy metal pollution include natural processes and human activities (Imtiaz and Tariq, 2022). Heavy metals can be derived from parent materials like metals - enriched rocks, including serpentine and black shale. Human activities that can cause heavy metal pollution include mining, smelting, fossil fuel burning, waste disposal, corrosion and agricultural practices (Martin and Hosam, 2018; Chrysoula, *et al.*, 2022). A typical example is when a farmer uses industrial wastewater for irrigation, contaminating a large area of farmland with heavy metals while simultaneously contaminating millions of tons of crops worldwide each year. The main group includes metals that occur in the earth's crust as free metals, such as gold, silver, copper, iron, lead, mercury and tin (Martin and Hosam, 2018; Chrysoula, *et al.*, 2022).

Allium cepa, commonly known as onion, is a widely used bioindicator of heavy metal pollution due to its sensitivity to heavy metal toxicity. It has been used in several studies to assess heavy metal pollution in different parts of the world. It can also be used to study genetic variations in response to heavy metal exposure (Komolafe, *et al.*, 2018; Dimuthu and Minola, 2020). *Allium cepa L.* possesses a diploid genome (2n = 2x = 16) characterized by monocentric chromosomes with a base number of 220 chromosomes (x = 8). These chromosomes, being relatively large, are well-suited for detecting karyomorphological changes. The chromosomal arrangement includes metacentric (1, 4, 7 pairs), submetacentric (2, 3, 5, 8 pairs), and subtelocentric (6 pairs) configurations. Karyotypes, such as 225, offer insights into cytotoxic relationships, evolutionary origins of species, and genomic aberrations, as noted in AT (Valavanidis and Vlachogianni, 2010). The use of metacentric chromosomes in Allium cepa cells facilitates microscopic assessments.

The study investigates the impact of heavy metal exposure from dumpsites on the root tips of *Allium cepa*. By analyzing the effects of these contaminants, the research aims to identify gaps in existing knowledge regarding the specific mechanisms and consequences of heavy metal toxicity in plant roots. This investigation intends to contribute valuable insights into the understanding of environmental risks associated with dumpsite pollutants and provide a foundation for developing effective mitigation strategies. The mechanism for extracting metals from samples differed from conventional methods for obtaining heavy metals. It

involved digesting soil samples with concentrated acids and neutralizing them with calcium oxide to achieve a solution with a pH between 6.8 and 7.2.

METHODOLOGY

Study Area

This study selected four prominent central dumpsites situated in Southwestern states in Nigeria: Ojota, Lagos-Ibadan express road, Lagos State (6.5873° N, 3.3786° E); Gate dump site, Ibadan, Oyo State (7.7827° N, 4.5418° E); Iwo - Osogbo road dump site, Osogbo, Osun State (7.3775° N, 3.9470° E); and Ile Oluji - Akure road dump site, Ondo State (7.2017° N, 4.8676° E).

Collection and Preparation of Dumpsite Soil Samples

Soil samples were collected randomly from four different dumpsites using stainless steel soil auger at a depth of 5cm to 30 cm as described by group of researchers (Adeyemi-Ale *et al.*, 2018). These were aseptically taken to the laboratory for heavy metals analysis using standard procedure described by Association Official Analytical Chemist (AOAC, 2019).

Soil samples gathered randomly from different spots within a dumpsite were blended, air-dried in the laboratory for a two-week period, and then stored in black polythene bags. To prevent contamination, these bags were securely placed inside large brown envelopes. This standardized process was replicated for other dumpsites, and the samples were stored in the laboratory at room temperature (37°C) following the method outlined by Martí *et al.*, 2016.

Acquisition and Preparation of *Allium cepa L.* (Test Organism)

Fresh onion bulbs (*Allium cepa*) were procured from markets in Masifa Ile, Ejigbo Local Government of Osun State, and Sabo in Ogbomosho. The selection of these locations was based on the availability of diverse fresh onion bulb varieties cultivated without the use of herbicides, fungicides, or chemical fertilizers. The obtained onion samples underwent authentication at the Department of Botany, University of Lagos, Nigeria. To expose the primordial cells at the roots' apices, the loose outer scales, dried-up roots, and the brownish bottom plate of the bulbs were initially removed.

Calculation of LD_{50}

The standard LD_{50} procedure, as outlined by Richard, 2016 was employed to assess the short-term poisoning potential (acute toxicity) of heavy metals on the test organism (*Allium cepa*). In this context, LD_{50} represents the concentration at which half of the maximal growth of *Allium cepa* roots is inhibited (Komolafe *et al*., 2018; AOAC, 2019; Richard, 2016).

Onion bulbs with a diameter of 7cm neatly fitted into 6.8 cm-wide containers, each containing prepared soil digest of the respective samples at concentrations of 20%, 40%, 60%, 80%, and 100%, with each concentration replicated three times. Deionized-distilled water served as the experiment's control. Mean root lengths were measured at 24-hour intervals over 72 hours. LD_{50} values for each sample were calculated by plotting graphs of mean root lengths against concentrations. The derived LD_{50} for each sample served as the basis for setting up the actual experiments (Fiskejo, 1985; Ukaegbu and Odeigah, 2009; Obidi Njoku and Akinmolayan, 2017).

Cytotoxic Analysis

Allium cepa L. bulbs were cultivated using soil digest from each sample, following the previously described method. Between 24-72 hours, the roots matured for cytotoxic analysis,

employing the standard procedure outlined by Mehmet and Abubakar (Mehmet, 2016; Abubacker and Sathya, 2017; Obidi Njoku and Akinmolayan, 2017).

Slides Preparation

Root tips measuring 0.3 cm were extracted for slide preparation. Any remaining part of the root was discarded. The root tip was placed at the center of a clean slide, and a drop of 1M HCl was added to soften the tissue. After 5 minutes, the HCl was removed using filter paper, and a dissecting needle was used to cut the root tip into small pieces, enhancing stain uptake. A drop of lactic acetic orcein was applied to the macerated tissue, left for 15-20 minutes, and then covered with a clean cover slip. The slides, prepared six times per sample per day for three days, were examined under high power magnification (40x objective) of a camera microscope. Good slides were temporarily preserved by sealing them with clear nail polish to reduce drying. Examination and counting of dividing cells at prophase, metaphase, anaphase, and telophase stages, as well as non-dividing cells at interphase, were conducted on each slide to calculate the Mitotic Index (Abubacker and Sathya, 2017; Michael *et al.,* 2018).

$$M.I = \frac{\text{No of dividing cells per field} \quad (\%)}{\text{Total no of cells per field}} \quad \ldots\ldots \quad 1$$

Mitotic stages (prophase, metaphase, anaphase, and telophase) were considered and compared with the control and previous works to identify any emergence of chromosomal aberrations.

RESULTS

Table 1 displays the concentrations of heavy metals extracted from the samples gathered at various dump sites across the states. The Pb concentration varied from 60.58 mg/kg to 202.50 mg/kg. Cr concentrations ranged from 23.50 mg/kg to 59.00 mg/kg, with Cd absent in Lagos but present in other states at concentrations between 1.5 mg/kg and 2.5 mg/kg. Additionally, Ni, Co, Zn, Fe, and Cu were detected in all four states covered, varying between 6.5 mg/kg and 19.2 mg/kg, 71.2 mg/kg and 212.0 mg/kg, and 1.1 mg/kg and 27.0 mg/kg, respectively, except for Co, which was not found in Lagos. The observed concentrations of all heavy metals, except cobalt and copper, exceeded the WHO permissible limits.

The LD_{50} represents the concentration at which half of the maximal growth of *Allium cepa* roots is inhibited. Figure 1 illustrated the determination of LD_{50} used for subsequent analyses. The average root length of *Allium cepa* was plotted against different concentrations of soil digest and control, leading to the extrapolation of LD_{50}. For dump sites A, B, C, and D, LD_{50} values were 39%, 36%, 32%, and 20%, respectively, while the control (de-ionized distilled water) showed 100% (Figure 1).

Table 1: Mean Concentrations (mg/kg) of Heavy metals in each Dump Site

	Dumpsite				
Heavy metal	A	B	C	D	permissible limits (WHO)
Lead (Pb)	60.58	140.50	202.50	65.00	0.1
Chromium (Cr)	44.50	28.00	59.00	23.50	0.1
Cadmium (Cd)	BDL	2.50	2.00	1.50	0.003
Nickel (Ni)	6.50	18.60	19.20	12.00	0.05
Cobalt (Co)	BDL	14.50	26.00	9.50	100.0
Zinc (Zn)	10.50	270.00	78.50	150.00	50.0
Iron (Fe)	98.60	197.00	71.20	212.00	0.30
Copper (Cu)	1.10	27.00	22.00	24.00	36.0
Manganese (Mn)	13.20	290.10	69.40	68.60	1 – 45

Key: BDL means below detection limit

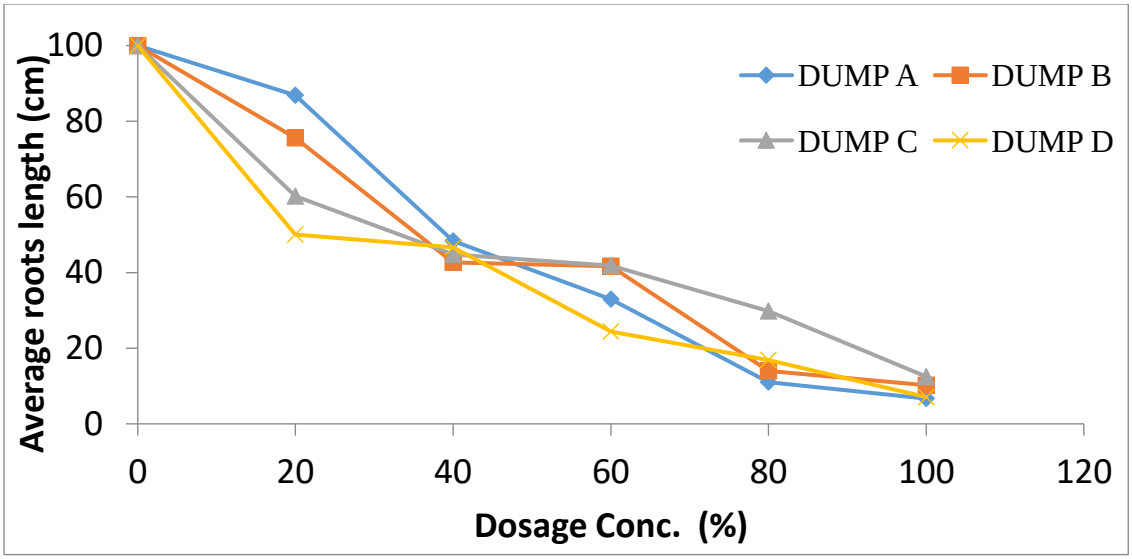

Figure 1: Growth Curves of Roots of *Allium cepa* Following Exposure to Different Concentration of Soil Digest and Control to Determine LD_{50}

The mean mitotic index was computed using the provided formula in the methodology. Notably, the mitotic index of the control experiment surpassed that of the test experiments. Additionally, the test experiments exhibited diverse mitotic indexes across all collected samples (Table 2). Data with different superscripts within the row are deemed significantly different ($p > 0.05$).

Various mitotic stages (prophase, metaphase, anaphase, and telophase) were observed in bulbs treated with soil digests of the samples. Plates 1 to 7 present representative aberrations identified in samples exposed to heavy metals. Chromosomal aberrations included chromosome bridge, laggard chromosome, bi-nucleated cell, ring chromosome, sticky chromosome, and nuclear lesions on chromosomes. Tables 3 to 6 summarize the occurrences of observed aberrations. In sample A, observations encompassed chromosome bridge, laggard, binucleated, ring, sticky, and nuclear lesions (Table 2).

Sample B exhibited observations of stickiness, bridge, bi-nucleation, and lesion (Table 3). In sample C, occurrences of stickiness, C-mitosis, bridge, bi-nucleation, and lesion were noted (Table 4), while sample D showed observations of stickiness, bridge, bi-nucleation, and lesion (Table 5).

Table 2: Mean Mitotic Index of the roots of *A. Cepa*

	Sample A			Sample B			Sample C			Sample D		
Concentration (%)	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
Control	347	45	13.32 ± 0.01^{e}	347	45	13.32 ± 0.01^{e}	347	45	13.32 ± 0.01^{e}	347	45	13.32 ± 0.01^{e}
20	227	19	10.11 ± 0.01^{a}	218	12	5.34 ± 0.00^{b}	136	9	5.91 ± 0.05^{bc}	110	5	6.00 ± 0.01^{d}

40	261	25	10.23 ± 0.01[a]	207	14	7.17 ± 0.01[b]	203	13	6.42 ± 0.01[c]	89	10	13.16 ± 0.01[d]
60	295	26	10.36 ± 0.00[a]	186	14	8.31 ± 0.01[b]	117	8	6.81 ± 0.01[c]	211	20	10.71 ± 0.01[ad]
80	215	43	8.96 ± 0.00[a]	166	18	13.16 ± 0.01[b]	161	9	6.03 ± 0.01[c]	264	16	7.05 ± 0.01[d]
100	223	12	5.34 ± 0.01[a]	169	14	7.89 ± 0.01[b]	192	6	3.36 ± 0.01[c]	172	5	2.48 ± 0.01[d]

I=mean no. of cells, II=mean no. of dividing cells, III=mean no ± SD of mitotic index

The results are presented as mean ± standard deviation. Significantly different data within the same row are indicated by different superscripts ($p > 0.05$).

Table 3: Chromosomal aberrations observed in samples collected from Dumpsite A.

Concentration (%)	Stickiness	C- mitosis	Bridge	Bi-nucleated	Laggard	Lesion	Ring
Control	1	0	0	0	0	1	0
20	3	0	4	2	1	1	0
40	6	1	2	6	0	8	1
60	1	0	1	4	0	5	0
80	2	0	3	2	0	3	0
100	2	0	2	3	0	10	0

Table 4: Chromosomal Aberration Observed in Samples from Dumpsite B

Concentration (%)	Stickiness	C- mitosis	Bridge	Bi nucleated	Laggard	lesion	Ring
Control	1	0	0	0	0	1	0
20	4	0	1	0	0	0	0
40	3	0	2	1	0	0	0
60	0	0	1	0	0	0	0
80	3	0	1	0	0	4	0
100	3	0	0	0	0	0	0

Table 5: Chromosomal Aberration Observed in Samples from Dumpsite C

Concentration (%)	Stickiness	C- mitosis	Bridge	Bi nucleated	Laggard	Leision	Ring
Control	1	0	0	0	0	1	0
20	0	0	1	0	0	0	0
40	4	0	0	0	0	0	0
60	0	0	0	0	0	4	0
80	0	4	0	0	0	0	0
100	0	0	0	5	0	7	0

Table 6: Chromosomal Aberration Observed in Samples from Dumpsite D

Concentration (%)	Stickiness	C- mitosis	Bridge	Bi nucleated	Laggard	Lesion	Ring
Control	1	0	0	0	0	1	0
20	3	0	0	0	0	3	0
40	2	0	0	0	0	0	0
60	2	0	3	2	0	1	0
80	4	0	1	3	0	2	0
100	0	0	0	5	0	7	0

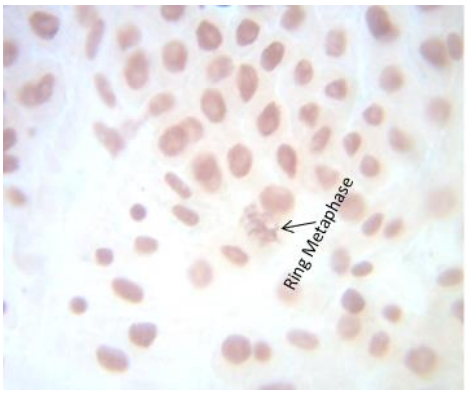

Plate 1: Representative Microscopic View of Ring Chromosome at Metaphase Stage

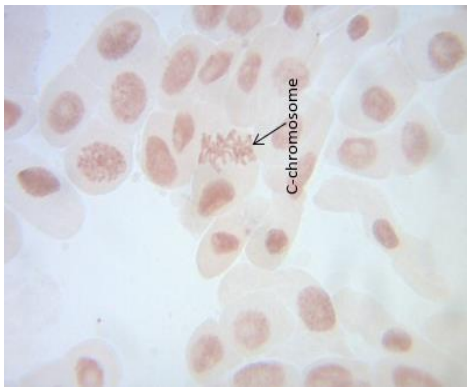

Plate 2: Representative Microscopic View of C- Chromosome at Metaphase Stage

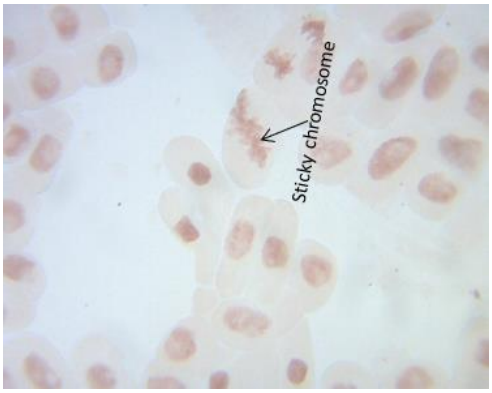

Plate 3 Representative Microscopic View of Sticky Chromosome at Metaphase Stage

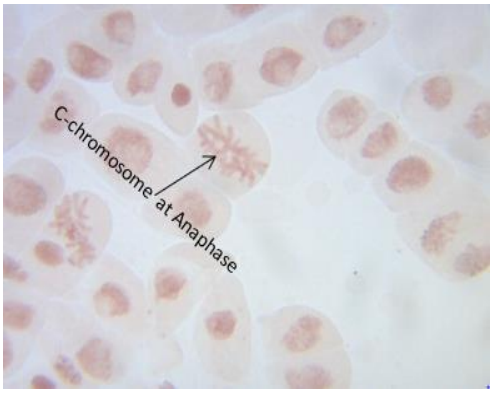

Plate 4 Representative Microscopic View of C-Chromosome at Anaphase Stage

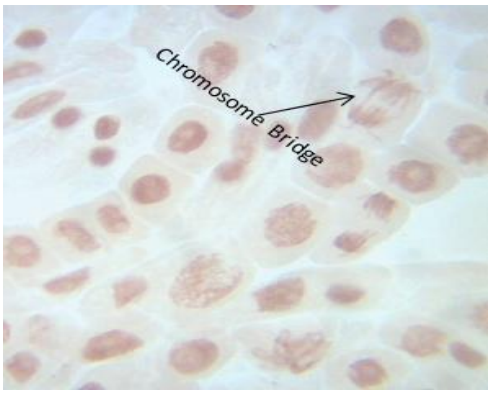

Plate 6 Representative Microscopic View of Chromosome Bridge at Anaphase

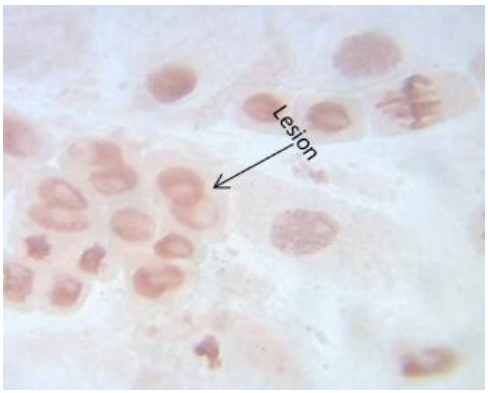

Plate 5 Representative Microscopic View of

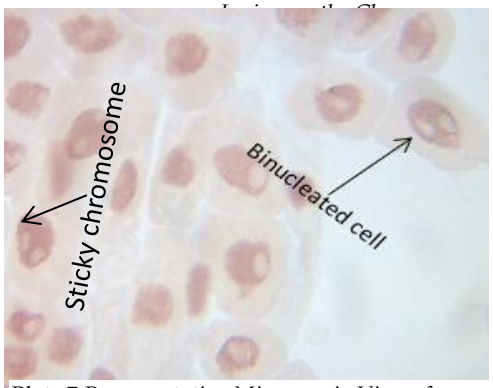

Plate 7 Representative Microscopic View of Binucleated Cell

DISCUSSION

The concentrations of metals obtained from almost all the samples except sample A were discovered to be higher than the permissible limits of WHO standards. The results obtained are in line with that of results obtained by Michael *et al.,* 2018 who reviewed heavy metals contamination food crops in Nigeria (Tobias, *et al.,* 2013). Another result that is in line with the present one is by Tobias *et al.,* 2013 that considered Environmental metals pollutants load of density populated and heavily industrialized commercial city of Aba, Nigeria (Irina, *et al.,*2020).

Metals that disturb division of cells do so by blocking of active division during interphase, which may be as a result of a prolonged G_2 Period or to the inhibition of DNA synthesis (Maria, *et al.,* 2020). There was no uniformity in the mitotic index of all the samples, this may be as a result of non-heavy metal ions such K^+ present in the digests (Michael, *et al.,* 2018). Two commonalities among all samples were evident: at 100% concentration, the Mitotic Index (MI) experienced a significant reduction, and the MI obtained from the control surpassed all other concentrations. A lower MI compared to the control suggested the presence of chemicals that may impede cell division. Similar results were obtained by group of scientists when *Allium cepa* assay based on study of selected vegetables and the chromosomal aberrations due to heavy metals accumulation on Potassium Nutrition in Plants and its interactions with other nutrients in Hydroponic Culture (Betül *et al.,* 2017; Ashutosh Yadav *et al.,* 2019; Dimuthu and Minola, 2020).

Analyzing chromosomal aberrations (CA) in the root tip cells of *Allium cepa* serves as an effective test for determining the cytotoxic potential of chemical agents and industrial solid effluents. CA, characterized by structural alterations in chromosomes, can result from exposure to physical or chemical agents (Obidi and Akinmolayan, 2017; Abubacker and Sathya, 2017; Ashutosh, *et al.,* 2019). Various types of chromosomal aberrations were observed across the four stages of the cell cycle (prophase, metaphase, anaphase, and telophase). The presence of aberrations at different mitotic divisions and concentrations suggests the potential involvement of toxic substances, possibly heavy metals(Dimuthu and Minola, 2020). This aligns with findings from a comparative study between *Allium cepa* assay and selected vegetables, showing chromosomal aberrations due to heavy metal accumulation (Maria Sabeen *et al.*, 2020). Similar results were obtained by Ashutosh, *et al.,* 2019 the cytotoxicity evaluation of organic and inorganic pollutants in tannery wastewater (Obidi and Akinmolayan, 2017; Ashutosh, *et al.,* 2019).

CONCLUSION

The study highlighted that the exposure to environmental toxic substances, particularly heavy metals from dump sites, can induce various biological effects, including molecular alterations that compromise the health of living organisms and pose risks to ecosystem integrity. The findings contribute to an enhanced understanding of the phenotypic and cytotoxic effects of toxic substances.

Given the observed damages resulting from the presence of toxic substances, it is advisable to avoid buying and consuming food crops from roadside sources without knowledge of their origin.

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