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Effects of Liquid Effluents from Textile Industries on the Root Tips of *Allium Cepa*

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Abstract: In Nigeria, water pollution is largely caused by municipal dumpsites, where leachates contaminate soil and release toxic substances. Industries, especially oil, gas, and petrochemical sectors, also contribute by discharging waste into the environment. This study examined the phenotypic and cytotoxic effects of textile industry effluents on the meristematic tissue of *Allium cepa* L. Samples were collected and analyzed for their impact on root tips over 24 to 120 hours in Lagos State. Results revealed a reduced mitotic index compared to the control group, though no chromosomal abnormalities were observed. Root growth was consistently greater in the control group (TW) compared to the experimental samples. The root lengths and total average lengths of marked roots were as follows: TW ($1.06 \pm 0.09a$ to $5.25 \pm 0.92e$ and $76.50 \pm 18.80a$ to $121.06 \pm 23.71e$), UEA ($1.20 \pm 0.32a$ to $1.58 \pm 1.82e$ and $70.75 \pm 31.05a$ to $99.50 \pm 46.25e$), UESF ($1.16 \pm 0.25a$ to $1.68 \pm 2.05e$ and $65.00 \pm 8.44a$ to $85.00 \pm 11.75e$), TEA ($1.30 \pm 0.32a$ to $3.23 \pm 2.31e$ and $57.25 \pm 11.81a$ to $86.00 \pm 23.82e$), and TESF ($1.30 \pm 0.36a$ to $3.53 \pm 2.37e$ and $57.25 \pm 12.82a$ to $86.00 \pm 23.83e$). The study showed minor cell damage, indicating the presence of toxic substances, useful for environmental monitoring and risk assessment.

Keywords: Environment, Toxic substances, Textile Industries, Liquid Effluents, Mitotic Index

Introduction

Groundwater, known as an aquifer, is water located beneath the land surface in fissures and voids within soil, sand, and rocks ¹ Aquifers serve as crucial water sources for plants and are vital to human civilization, constituting the primary reservoir of potable water in habitable regions. However, this water can become unsuitable for use by plants and humans due to the introduction of impurities, often originating from anthropogenic sources such as heavy metals, petroleum products, fertilizers, human and animal waste, and industrial chemicals (effluents), when they infiltrate into the ground ^{2,3}

It has been observed that when these pollutants are absorbed by plants or animals through groundwater, they can have various effects. Certain industries generate effluents containing high concentrations of contaminants that contribute to soil pollution. The predominant contaminants in these effluents are heavy metals such as cadmium, zinc, nickel, and mercury, which have densities exceeding 5g/cm^3 ^{4,5} In Nigeria, significant sources of environmental pollution in water bodies include municipal dumpsites, from which contents leach into the soil and release heavy metals ⁶. Industries such as oil, gas, and petrochemicals also contribute to environmental pollution by releasing their wastes into the environment ⁷. Over the years, various experiments have been developed to assess the potential toxicity of pollutants using biological indicator organisms. These experiments typically involve a range of organisms, from plant meristems to mammalian cells in tissue culture ⁸. One classical test for evaluating the effects of chemicals on plant chromosomes is the Allium test, pioneered by ⁹, which utilizes root tips from bulbs of *Allium cepa* L. ($2n=16$) as the assay system. Other plants within the same genus as *A. cepa*, including *A. carinatum* L. ($2n=16$), *A. cepa* var. *proliferum* Targioni-Tozzetti (tree or Egyptian Onion, $2n=16$), *A. fistulosum* L. (Welsh leek, $2n=16$), and *A. sativum* L. (garlic, $2n=16$), have also been used in similar studies. However, among these, *Allium cepa* remains the most widely recognized ^{10,11}

Damage to cultivated crops grown in polluted water bodies is significant; for example, high concentrations of cadmium have been found to hinder root growth and induce chromosome aberrations in *A. cepa*, Scientists conducted investigations on vegetable crops, illustrating that elevated cadmium levels inhibit root growth by disrupting mitosis ^{8,9}.

Industrial waste from mining has introduced large amounts of zinc into the soil, resulting in immediate die-off of plants in affected areas ¹³. The Allium test demonstrated that growth of *A. cepa* in normal tap water was nearly continuous, whereas growth in distilled water ceased completely after a few days ¹⁴. To assess environmental risks from discharged wastes, the Allium test was employed to investigate; unknown compounds in the water inhibited root growth, prompting chemical analysis to identify the present compounds ^{15,16}.

Allium cepa, human lymphocytes, lysogenic bacteria, and the Chinese hamster cell line V79 were utilized to assess the effects of two organic mercury compounds: MMC (Methyl mercury chloride) and MOEMC (Methoxyethyl Mercury Chloride). The findings indicated that *A. cepa* exhibited results consistent with those of human lymphocytes¹⁷ suggesting that results from *A. cepa* can be extrapolated to humans due to their close similarities. When testing wastewater and complex mixtures like phenoxyacetic acids and phenols using aquatic animals, aquatic plants, and *A. cepa*, the results highlighted the sensitivity of *A. cepa* to these compounds, which was more accurate compared to other aquatic plants⁸

The growth sensitivities of bacteria, fish, crustaceans, unicellular algae, and *A. cepa* were compared when exposed to wastewater from industries producing various chemicals. In these comparisons, *A. cepa* exhibited sensitivity levels similar to those of crustaceans and algae. However, bacteria and fish did not consistently respond to the parameters of mutagenicity and survival in some test waters¹⁸

The *Allium* test is utilized in laboratories for both macroscopic and microscopic studies. It has been used to investigate and compare the effects of agricultural chemicals on plant and animal systems, demonstrating a strong correlation between *A. cepa* and the mammalian system¹⁷

The process of cell growth in tissues is known as mitosis, which encompasses several stages, which are: prophase, prometaphase, metaphase, anaphase, telophase, and cytokinesis.

During prophase, following interphase which prepares the cell for division, distinct events are visible under the microscope. The most prominent event is the condensation (coiling) of chromatin threads into distinct chromosomes. Initially long and thin, these chromosomes gradually become shorter and thicker, forming chromatids. The nuclear membrane breaks down during this stage, which can last for several hours.

Prometaphase

After the nuclear envelope breaks down, the spindle microtubules and chromosomes are no longer separated by a double membrane boundary. The microtubules start interacting with the chromosomes, initiating congression where centromeres align at the metaphase plate in the middle of the spindle. Metaphase: Chromosomes align themselves along the equator of the spindle, supported by fibers extending from each kinetochore to the poles. Anaphase: Chromatids begin to move apart after the chromosomes split at the centromeres. Each daughter chromosome moves towards the spindle pole it is connected to, departing from the metaphase plate. Telophase: Chromosomes migrate close to the spindle pole regions, and the spindle mid zone starts to disappear. Vesicles accumulate in this central spindle region. The accumulation of vesicles marks the beginning of a new

wall assembly at the spindle's equator, which will eventually serve as a boundary between the newly formed daughter cells. Cytokinesis, meanwhile, involves the division of cytoplasm, ¹⁹.

Spontaneous aberrations in chromosome shape, size, and structure can occur and are often linked to environmental factors such as radiation, temperature variations, and effluents from chemical industries ²⁰. Changes in chromosome number, known as heteroploidy, can also occur, deviating from the normal diploid (2n) or haploid (n) chromosome count, discovered aneuploid cells in root tips of *A. cepa*, ^{21,22} investigated the effects of carbamazepine and observed chromosome stickiness, bridge formation, and disturbances in prophase, metaphase, and anaphase in algae and *A. cepa* root tips. Algae exhibited lagging chromosomes, chromosome fragments, and micronuclei, whereas *A. cepa* showed depolymerized chromosomes, also found that treatment of *A. cepa* bulbs with wastewater led to cytological abnormalities such as c-mitosis and anaphase with lagging chromosomes. Scientists conducted investigation revealing that heavy metals led to a decrease in the mitotic index in treated cells of *A. cepa*, with some causing C-mitosis and a few inducing chromosome breaks⁸.

The objective of this study is to assess the effects of liquid effluents from two textile industries on the growth of root tips of *A. cepa*, mitotic index and the process of mitosis in these root tips.

Methodology

Study Areas

This study selected two significant textile industries from Lagos State (Afprint and Sunflag). Both untreated and treated effluents were collected from the listed textile industries.

Collection and Preparation of the Effluents

Liquid samples, including treated (effluent treated with chemicals within the company premises to remove harmful compounds before release into public sewage) and untreated water, were collected from two different textile industries: Afprint and Sunflag, both located along Ericmoore Road, Surulere, Lagos, as well as tap water from Araromi Quarters, Mile-12, Ketu, Lagos. These samples were carefully treated and transported to the laboratory under aseptic conditions for analyses, following the standard procedure outlined by the Association of Official Analytical Chemists²³.

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

Equal-sized bulbs of *Allium cepa* (2n=16) were purchased from local market at Masifa Ile in Osun State and Mile-12, Ketu in Lagos State for the experiment. 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.

Determination of LD₅₀

This was carried out using standard LD₅₀ procedure described by⁸ measure the short term poisoning potential (acute toxicity) of the effluents on the test organism (*Allium cepa*). i. e the LD₅₀ is the concentration at which half maximal growth of *Allium cepa* roots are inhibited⁸.

Bulbs with a diameter of 7cm were well-suited for the containers with a mouth diameter of 6.8cm and a length of 10cm. Container A held de-ionized water as a control, while container B held untreated effluent from Afprint, container C held untreated effluent from Sunflag, container D held treated effluent from Afprint and container E held treated effluent from Sunflag. The bulbs were suspended in the containers so that only their bases came into contact with the test liquids, each sample at concentrations 20%, 40%, 60%, 80% and 100%, each was replicated three times. De ionized-Distilled water serves as control of the experiment. Mean root lengths were taken at 24 hours intervals for 120 hours. LD₅₀ was calculated for all the samples by plotting graphs of mean root lengths against concentrations. The derived LD₅₀ for each sample was used to set up the real experiments^{8,24}.

Determination of Phenotypic Variation

Phenotypic variation in the *Allium cepa* roots was carried out using results obtained from LD₅₀ to grow *A. cepa* and roots growth was determined and recorded by counting and measuring the root lengths at intervals of 24, 48, 72, 96, and 120 hours. The values obtained from the experiments were compared with the control to determine variation in the mean root lengths of the test organisms^{8,25}.

Cytotoxic Analysis

Allium cepa bulbs were grown with effluents of each sample as described above. Between 48 and 120 hours, the roots matured for cytotoxic analysis, using the standard procedure of^{8,25}.

Slide Preparation

The slides were prepared by removing the root tips of 0.3 cm, any part of the root left was discarded. This root was placed on the centre of a clean slide and a drop of 1N HCl was added to soften the tissue. After 5 minutes, a piece of filter paper was used to remove the HCl. A dissecting needle was the used to cut the root tips into tiny pieces. This serves to enhance stain uptake by the cells.

A drop of lactic acetic orcein solution (2g orcein in 22.5ml lactic acid, 22.5ml acetic acid, and 55ml distilled water) was applied to the macerated tissue and left for 15 to 20 minutes. A clear cover slip was carefully placed over the stained material, pressed down diagonally with the forefinger and thumb or with the tip of a dissecting needle to eliminate air bubbles. The slide was gently tapped with the base of the dissecting needle to spread and flatten the cells. Excess stain was removed by gently pressing the slide between folds of filter paper using the base of the dissecting needle. This process was repeated to prepare four slides from each sample per day over five days.

All prepared slides were examined under high-power magnification (40x objective) using a light microscope. Slides of satisfactory quality were temporarily preserved by sealing them with clear fingernail polish to prevent drying. Examination and counting of both dividing cells (cells in prophase, metaphase, anaphase, and telophase) and non-dividing cells (cells in interphase) were performed on each slide. Five different fields were observed on each slide to determine the mitotic index^{8,10,18}.

Mitotic index was calculated as:

$$\text{M.I.} = \frac{\text{No. of dividing cells per field} \times 100}{\text{Total no. of cells per field}}$$

Photomicrographs of selected high-quality slides were captured under an oil immersion lens (100x objective) using a Wild Photoautomat microscope with MPS 55 system.

Results

Determination of LD₅₀

LD₅₀ is the concentration at which half maximal growth of *Allium cepa* root were inhibited. Figure 1 depicts the determination of LD₅₀ which was used for further analyses. The average root length of *Allium cepa* was plotted against the different concentrations of the liquid effluents and control. The LD₅₀ obtained from the graph was 52%, 55%, 76% and 78% while that of control with de-ionized water was 100%.

Fig. 1: Growth Curves of Roots of *Allium cepa* Following Exposure to Different Concentration of Liquid Effluents and Control to Determine LD₅₀

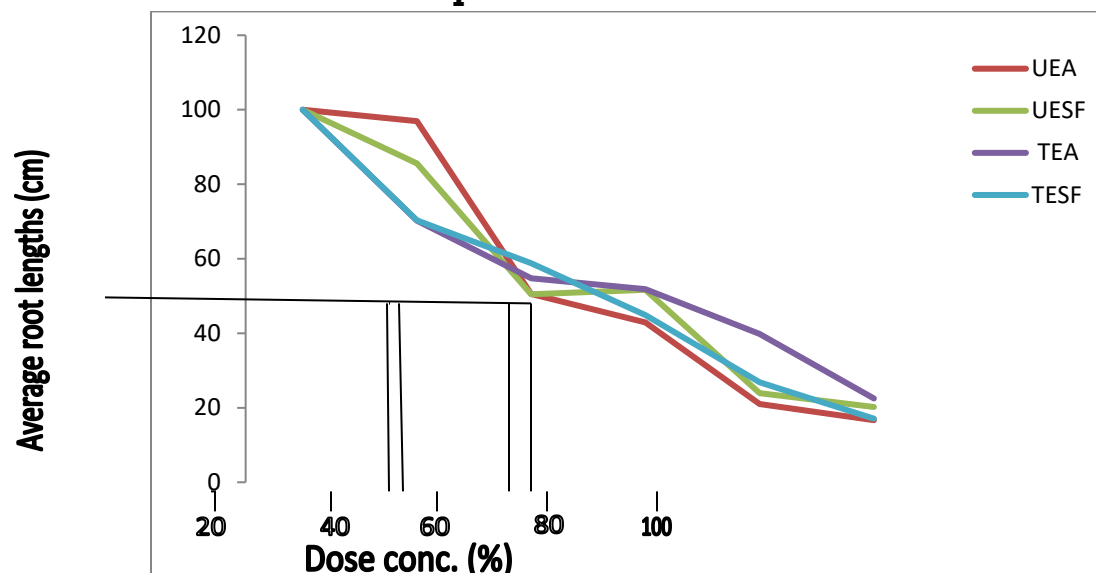


Table 1: Average Length (cm) and Total Number of Roots of *A. cepa* Measured at Different Intervals in Tap Water as control (TW)

Water Source	Number of Hours	Average Number of Roots	Total Average Length of Marked Roots in all Containers Per Period (cm)	Remarks
TW	24	76.50 ± 18.80 ^a	1.06 ± 0.09 ^a	
	48	96.00 ± 21.86 ^b	2.23 ± 0.66 ^b	
	72	104.25 ± 24.39 ^c	3.40 ± 0.81 ^c	
	96	113.75 ± 26.25 ^d	4.45 ± 0.58 ^d	
	120	121.00 ± 27.31 ^e	5.25 ± 0.92 ^e	

Values are expressed as mean ± standard deviation. Data having different superscripts across the row are significantly different ($p < 0.05$).

Key: TW-Tap Water

Table 2: Average Length (cm) and Total Number of Roots of *A. cepa* Measured at Different Intervals of Untreated Effluent from Afrprint (UEA)

Water Source	Number of Hours	Average Number of Roots	Total Average Length of Marked Roots in all Containers Per Period (cm)	Remarks
UEA	24	70.75 \pm 31.05 ^a	1.20 \pm 0.32 ^a	
	48	88.00 \pm 48.26 ^b	2.13 \pm 0.17 ^b	
	72	93.00 \pm 46.88 ^c	2.00 \pm 1.41 ^c	U8
	96	97.25 \pm 47.38 ^d	2.08 \pm 1.45 ^d	
	120	99.50 \pm 46.25 ^e	1.58 \pm 1.82 ^e	U10

Values are expressed as mean \pm standard deviation. Data having different superscripts across the column are significantly different ($p < 0.05$).

Key: UEA- Untreated Effluents Afrprint

U – Death root.

Table 3: Average Length (cm) and Total Number of Roots of *A. cepa* Measured at Different Intervals of Untreated Effluent from Sunflag (UESF)

Water Source	Number of Hours	Average Number of Roots	Total Average Length of Marked Roots in all Containers Per Period (cm)	Remarks
UESF	24	65.00 \pm 8.44 ^a	1.16 \pm 0.25 ^a	
	48	72.75 \pm 12.09 ^b	2.00 \pm 0.28 ^b	
	72	76.50 \pm 10.15 ^c	1.45 \pm 1.74 ^c	U72
	96	80.25 \pm 10.87 ^d	1.60 \pm 1.96 ^d	U18
	120	85.00 \pm 11.75 ^e	1.68 \pm 2.05 ^e	

Values are expressed as mean \pm standard deviation. Data having different superscripts across the column are significantly different ($p < 0.05$).

Key: UESF- Untreated Effluents Sunflag

U – Death roots

Table 4: Average Length (cm) and Total Number of Roots of *A. cepa* Measured at Different Intervals of Treated Effluent from Afrprint (TEA)

Water Source	Number of Hours	Average Number of Roots	Total Average Length of Marked Roots in all Containers Per Period (cm)	Remarks
TEA	24	57.25 \pm 11.81 ^a	1.30 \pm 0.32 ^a	
	48	66.75 \pm 17.58 ^b	2.35 \pm 0.66 ^b	
	72	76.50 \pm 23.56 ^c	2.35 \pm 1.73 ^c	U12
	96	82.75 \pm 24.76 ^d	2.70 \pm 1.91 ^d	
	120	86.00 \pm 23.83 ^e	3.23 \pm 2.31 ^e	

Values are expressed as mean \pm standard deviation. Data having different superscripts across the column are significantly different ($p < 0.05$).

Key:TEA- Treated Effluents Afrprint

U – Death roots

Table 5: Average Length (cm) and Total Number of Roots of *A. cepa* Measured at Different Intervals of Treated Effluent from Sunflag (TESF)

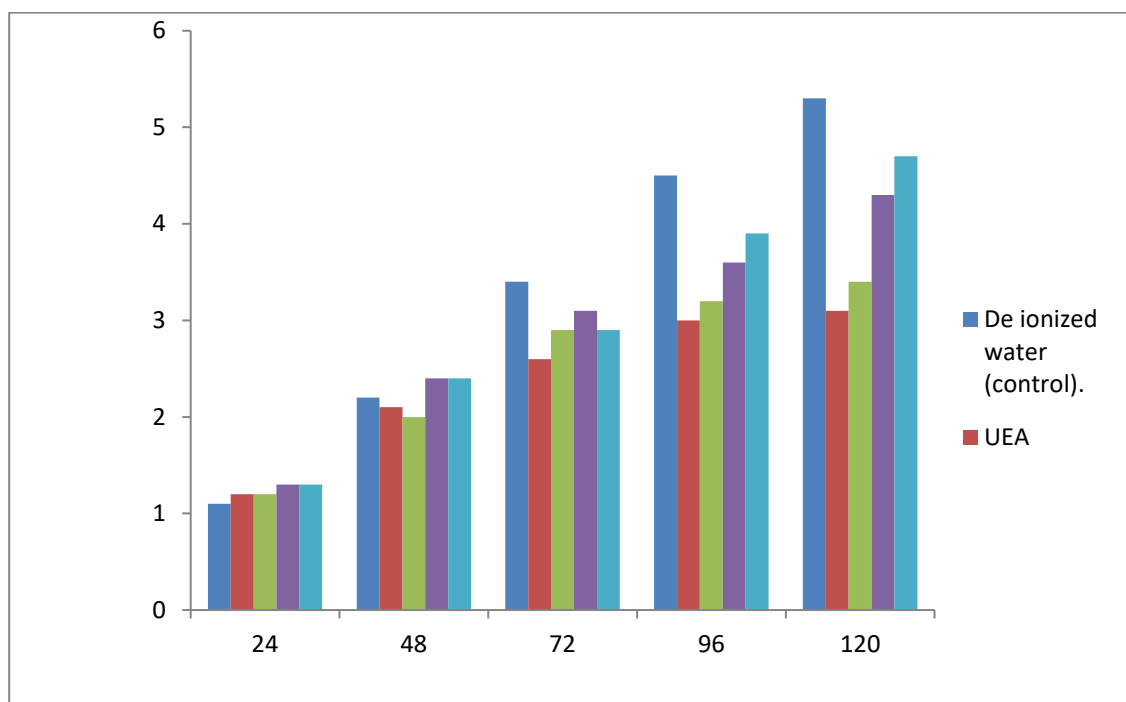
Water Source	Number of Hours	Average Number of Roots	Total Average Length of Marked Roots in all Containers Per Period (cm)	Remarks
TESF	24	57.25 \pm 12.82 ^a	1.30 \pm 0.36 ^a	
	48	56.75 \pm 17.58 ^b	2.43 \pm 0.59 ^b	
	72	69.00 \pm 20.41 ^c	2.90 \pm 0.52 ^c	
	96	77.75 \pm 23.73 ^d	2.93 \pm 1.96 ^d	U6
	120	86.00 \pm 23.83 ^e	3.53 \pm 2.37 ^e	

Values are expressed as mean \pm standard deviation. Data having different superscripts across the column are significantly different ($p < 0.05$).

Key:TESF - Treated Effluents Sunflag

U – Death roots

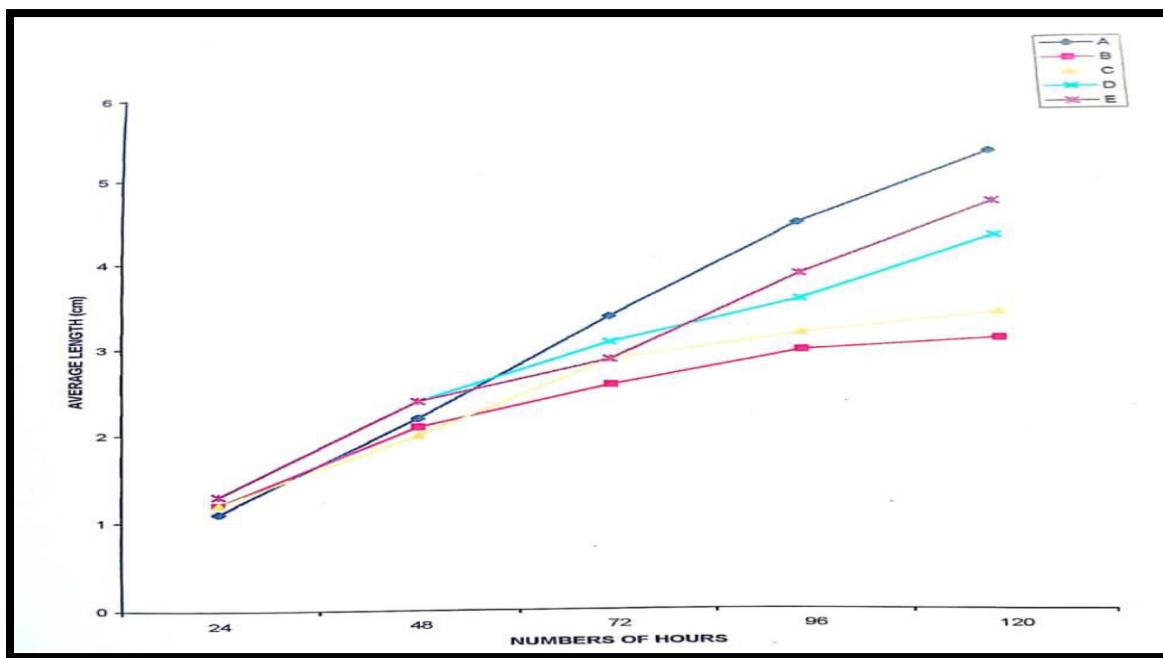
Fig. 2: Average Total Roots Lengths of *A. cepa* Measured at Different Intervals of Experimental Samples and Control.



Mean Root Lengths of the Test Organisms and Control

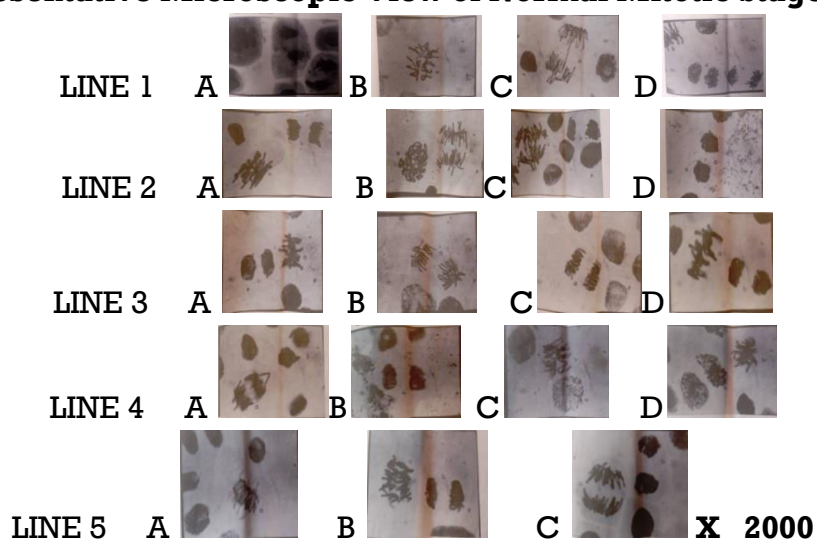
The roots of bulbs treated with tap water (de-ionized) showed continuous growth throughout the duration of treatment (Table 1, Fig. 2 and 3). New roots also continued to appear until the end period of the treatment (Table 1). The untreated effluents were deep blue with some brown particles. The roots appeared normal for the first 24 hours. At 48 hours, some of the roots changed color to blue. Tables 1 to 5 and fig. 2 and 3 above, showed the total mean root lengths of bulbs in control and experimental samples, each sample which showed continuous growth throughout the duration of the treatment just like that of control but a clear distinction between the control and the test organisms was noticed as the bulbs grown in the control had longer average root lengths than the ones grown with experimental samples, also, death roots were recorded in experimental samples. The statistical analysis showed that data having different superscripts across the row are significantly different ($p < 0.05$).

Fig. 3: Growth Curves of *Allium cepa* Average Root Lengths in Control and Textile Industries' Effluents



Microscopic Examination

Plate 1: Representative Microscopic View of Normal Mitotic Stages



Line 1 represents, **A** normal anaphase and **B** normal metaphase, **C** normal prophase, metaphase, anaphase and normal telophase and **D** normal metaphase for control.

Line 2 represents, **A** normal metaphase, **B** telophase and normal early anaphase, **C** normal telophase and **D** normal late telophase for untreated Afriprint effluent.

Line 3 represents, **A** normal metaphase and normal late telophase, **B** normal late anaphase and **C** normal telophase and **D** normal metaphase and normal telophase for untreated effluent from Sunflag.

Line 4 represents, **A** anaphase and telophase, **B** normal telophase, **C** normal prophase and metaphase, **D** normal prophase and metaphase.

Line 5 represents, **A** normal early anaphase, **B** normal early anaphase and normal telophase and **C** normal anaphase and telophase

The various stages of mitosis (prophase, metaphase, anaphase, and telophase) were observed in the bulbs treated with both tap water, untreated and treated effluents as shown above.

In bulbs treated with tap water, during prophase, chromosomes appeared as thin threads clustered together (Line 1 plate A). During metaphase stage, chromosomes aligned along the cell's equator (Plate Line 1 B). Anaphase revealed the separation of daughter chromosomes (Line 1 plates C and D). Telophase depicted daughter chromosomes at opposite poles (Line 1 plate D).

Cells from root tips of untreated effluents from Afriprint (Line 2) also exhibited normal mitotic stages. The treatment showed normal prophase (plate B), metaphase (Plates A), anaphase (plates B and C) and normal telophase (Plate D).

Line 3 contains plates of untreated effluent from Sunflag, plate **A** normal metaphase and normal late telophase, plate **B** normal late anaphase, plate **C** normal telophase and plate **D** normal metaphase and normal telophase.

Roots treated with effluent from treated Afriprint as shown in Line 4, plate **A** anaphase and telophase, plate **B** normal telophase, plate **C** normal prophase and metaphase and plate **D** normal prophase and metaphase.

Plate **A** in Line 5 is normal early anaphase, plate **B** normal early anaphase and normal telophase and plate **C** normal anaphase and telophase.

mitotic indices for both control and liquid effluents (untreated and treated) ranged between 23.08 ± 0.01^a to 46.00 ± 0.10^a for control, untreated effluent from Afriprint and Sunflag were 06.25 ± 0.61^b to 18.18 ± 0.01^b and 08.93 ± 0.09^c to 18.42 ± 0.01^c respectively, while those of the treated effluents were 07.69 ± 0.08^d to 30.00 ± 0.01^d and 17.79 ± 0.16^e to 22.86 ± 0.01^e , see Table 6 below. The data indicate that the mitotic index of untreated effluents was lower than that of treated effluents, while tap water had the highest index. According to Table 6, as the duration of treatment increased, the mitotic index decreased in both tap water (control) and experimental effluent.

Table 6: Mean Mitotic Index in the Root Tip Cells

Control				UEA				UESF				TEA				TESF			
Time (hrs)	I	II	III	I	II	III		I	II	III		I	II	III		I	II	III	
24	30	14	46.00± 0.10 ^a	22	4	18.18± 0.01 ^b		38	7	18.42± 0.01 ^c		20	6	30.00± 0.01 ^d		35	8	22.86± 0.01 ^e	
48	39	12	30.77± 0.02 ^a	40	7	17.50± 0.01 ^b		45	7	15.56± 0.01 ^c		71	16	22.54± 0.01 ^d		70	15	21.43± 0.10 ^e	
72	53	14	26.4± 0.01 ^a	58	9	15.52± 0.01 ^b		55	8	14.55± 0.01 ^c		35	7	20.00± 0.01 ^d		62	11	17.74± 0.01 ^e	
96	62	15	24.19± 0.01 ^a	20	2	10.00± 0.46 ^b		42	4	09.52± 0.46 ^c		62	12	19.35± 0.01 ^d		52	9	17.31± 0.01 ^e	
120	26	6	23.08± 0.01 ^a	64	4	06.25± 0.61 ^b		56	5	08.93± 0.09 ^c		52	4	07.69± 0.08 ^d		19	3	17.79± 0.16 ^e	

Results are expressed as mean \pm standard deviation. Data having different superscripts across the row are significantly different (p 0.05).

I. Mean number of cells in the fields, II. Mean number of dividing cells in the fields, III. Mitotic Index \pm SD.

Discussion

The increase in root length observed in both tap water (control) and liquid effluents may not solely be due to mitotic division but also to cell elongation. However, the rate of increase in average root length in all effluents was uneven until the end of treatment compared to the control (tap water) (Tables 1 to 5). Additionally, the tables show no proportional growth rate increase with duration in the effluents as observed in the control (tap water). A reduction in total average root length and inconsistency in the number of new roots developed with increasing treatment duration suggest the presence of chemicals in the effluents that hinder mitosis. This aligns with observations made by^{16,26} while investigating effluents from various industries. The clear distinction observed between the mean root lengths in effluents of experimental samples when compared with the control indicates that there were probably some chemicals in the effluents (may be obtained toxic substances from the samples) which could cause blockage of active division of the cells. A similar result was obtained by^{10,27} when they worked on potassium nutrition in plants and its interactions with other nutrients in hydroponic culture.

Changes in color observed during the experiment, both in the root tips and the entire plant, may indicate exposure to specific salts (e.g., blue-green from copper sulfate) or toxic effects leading to brownish root tips and cell death¹⁸. Changes in root tips of untreated and treated effluents confirmed the presence of chemicals in the effluents. The result obtained is in accordance with result when Ana Gabriela worked on Pharmaceutical pollutants²⁸.

The presence of dead roots observed in the effluents after some hours of treatment suggests that chemicals in the effluents may have caused root death and decay. Such

effects were absent in the control experiment (tap water), indicating the toxicity of the effluents also in line with result obtained by²⁸.

Chemicals that inhibit mitotic division do so by blocking active division during interphase, possibly due to a prolonged G2 period or inhibition of DNA synthesis when Betül Çalışkan and Ali Cengiz Çalışkan worked on potassium nutrition in plants and its interactions with other nutrients in hydroponic culture²⁷. This observation is in line with the result obtained in this present study.

Chromosomal aberration (CA) analysis of the root tip cells of *A. cepa* is considered as an effective test in determining cytotoxic potential of chemical agents and industrial solid effluents. CA has been characterized by alterations in either of chromosomes structure, which can occur both spontaneously and as well as Single nucleotide polymorphism (SNP), its frequency, distance of occurrence and the functional parts of DNA genome result of the exposure to physical or chemical agents^{29, 30}.

Various types of chromosomal aberrations were considered over the four stages of the cell cycle (prophase, metaphase, anaphase, and telophase) as showed in plates above. Throughout the treatment period, all observed mitotic stages (prophase, metaphase, anaphase, and telophase) in the effluents were normal, similar to those observed in the control (tap water). This suggests that the effluents likely did not contain chemicals that could cause chromosomal aberrations. Similar findings were reported by¹⁴ when testing unknown chemicals against drinking water as a control.

In Table 6, an increase in treatment duration corresponded to a decrease in the mitotic index for control, treated and untreated effluents. A decrease in the mitotic index indicates fewer dividing cells, suggesting that chemicals may inhibit cell division by preventing mitosis. Ashutosh Yadav reported similar result when they worked on phytotoxicity, cytotoxicity and genotoxicity evaluation of organic and inorganic pollutants rich tannery wastewater from a common effluent treatment plant and Mercykutty and Stephen also reported similar results when studying antibiotics^{31, 32}.

While no chromosomal aberrations were observed in any of the water samples tested, there was a noticeable reduction in the mitotic index, growth rate, and number of new roots developed as the treatment duration increased. Additionally, changes in the color of root tips and the presence of dead roots further underscored the adverse effects of untreated effluents. Although treated effluents showed a mitotic index somewhat closer to that of water, the aforementioned observations suggest that such effluents are unsuitable for discharge into public sewage. Therefore, both untreated and treated effluents should not be discharged into public sewage without proper treatment to mitigate their potentially harmful effects.

Conclusion

The industrial effluents studied had a significant impact on the root growth of *Allium cepa*. This was evidenced by a reduction in the mitotic index as the duration of treatment increased. Additionally, the physical condition of the roots, such as a decrease in growth rate, a reduction in the number of new roots, changes in root tip color, and the appearance of dead roots, worsened with prolonged exposure. Therefore, it is crucial that effluents are properly treated before being released into public sewage systems.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used [NAME TOOL / SERVICE] in order to [REASON]. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

References:

1. Walker, Kira (2022). Ancient Systems keep water flowing. Nature middle east.
2. PanelRakesh Shrestha, Sagar Ban, Sijan Devkota, Sudip Sharma, Rajendra Joshi i, Arjun Prasad Tiwari, Hak Yong Kim and Mahesh Kumar Joshi (2021).Technological trends in heavy metals removal from industrial wastewater: A review. Journal of environmental chemical engineering, 9(4).106014.
3. Coon, D. & Harvey, J. (1997). The ground pollution primer. Conservation council, Frederiction, N. B. 44PP.
4. Jong-Joo Kim, You-Sam Kim &Vijay Kumar (2019). Heavy metal toxicity: an update of chelating therapeutic strategies. J. Trace Elem. Med. Biol.54;226-231.
5. Ademoroti, C. M. A. (1996). Bioaccumulation of heavy metals in some mangrove fauna and flora. In: Environmental Chemistry and Toxicology. Ademoroti, C. M. A. (ed.). Foludex Press Ltd. 215.
6. Amusa, M. A. (2001). Toxicity of groundwater samples from well installation around waste dumpsites against *Allium cepa* and *Oryctolagus cuniculus*. B.Sc. Project University of Lagos. 86pp.
7. PanelPrabhat Kumar Rai, Sang Soo Lee, Ming Zhang, Yiu Fai Tsang & Ki-Hyun Kim (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. Journal ofEnvironment International. Vol. 125; 365-385
8. Olakunle, T. P., Bamkefa, B. A., Olowe, B. M., Oyesiku, O. O. & Ogunlaja, O. O. (2024).Determination of Cytotoxic Impact of Heavy Metals on Plant Cells Using

- Allium cepa* (Onion). Dutse Journal of Pure and Applied Sciences (DUJOPAS). 10 (1c), 140-149.
9. Elana Bonciu, Peter Firbas, Carnem S. Fontanetti, Jiang Wusheng, Mehmet Cengiz Karaismailoglu & Donghua Liu (2018). An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay, International Journal of Cytology, Cytosystematics and Cytogenetics, (71);3. 191-209.
 10. Maria Sabeen, Qaisar Mahmood, Zulfiqar Ahmad Bhatti, Faridullah, Muhammad Irshad, Muhammad Bilal, Malik Tahir Hayat, Usman Irshad, Tahir Ali Akbar, Muhammad Arslan, and Naeem Shahid (2020). *Allium cepa* assay based comparative study of selected vegetables and the chromosomal aberrations due to heavy metal accumulation. Saudi J Biol Sci. 27(5): 1368–1374. 12.
 11. Grant, W. F. (1982). Chromosome aberration assays in *Allium cepa*. A report of the U. S. Environmental protection agency. Gene – Toxicity programme. Mutation Research. 99: 235-241.
 12. Ojodu, A. A. (2000). Effects of heavy metals (Cadmium salt) on *Allium cepa* root tips M.Sc. Thesis submitted in the department of Cell Biology and Genetics, University of Lagos, Nigeria. 64pp.
 13. Panel Jessica Briffa, Emmanuel Sinagra, Renald Blundell (2020). Heavy metal pollution in the environment and their toxicological effects on humans, J. ScienceDirect, 6(9); 1-26.
 14. Fiskesjo, G. (1981a). *Allium cepa* test on Copper in drinking water. Vatten. 37: 232-240.
 15. Dimuthu, W. M., Nilmini Wijeyaratne, L. G. & Wadasinghe, Y. J. G. (2019). *Allium cepa* Bio Assay to Assess the Water and Sediment Cytogenotoxicity in a Tropical Stream Subjected to Multiple Point and Nonpoint Source Pollutants. Journal of Toxicology. 2019; 1-10
 16. Jibril Sani Mohammed, Yahaya Mustapha, Usman Abubakar, Eghobor Sunday, Bashir Mohammed Mayaki, Yahaya Sadiq Abdulrahman, Mohammed Isa Auyo & Aisha Wada Abubakar (2023). Evaluation of Cyto-Genotoxicity of Pharmaceutical Industrial Effluent in Kano Metropolis, Kano State, Nigeria, Using *Allium cepa* L. Assay, UMYU Scientifica, 2(1); 106 – 114.
 17. Fiskesjo, G. (1985a). The *Allium* test as standard in Environmental monitoring. Hereditas. 102:99-112.
 18. Pradillo M, Evans D. & Graumann K. (2019). The nuclear envelope in higher plant mitosis and meiosis. Nucleus. 10(1):55-66. [PMC free article] [PubMed]
 19. Wierzbicka, M. (1994). Resumption of mitotic activity in *Allium cepa* L. root tips during treatment with lead salts. Environmental and Experimental Botany. 34: 173-180.

20. Srdjan Z. Marković, Ljubinka I. Nikolić, Jasmina Lj. Hamidović, Marija G Grubor, Milena M. Grubor & Dragana A. Kastratović, (2017). Chromosomes aberations and enviromental factors. Hospital Pharmacology International Multidisplinary Journanl, 4(1):486-490
21. Jos, A., Repetto, G., Hazen, M. J., Rios, J. C., Molero, M. L., del Peso, A., Salguero, M., Fernandez-Freire, P., Perez-Martin, J. M., Repetto, M. (2003). Ecotoxicological evaluation of carbamazepine using six different model systems with eighteen end point. Toxicology invitro. 7: 525-532.
22. Association of Official Analytical Chemists(AOAC), 21st Edition, (2019)
23. Adeyemi-Ale, O.A., Anifowoshe A. T. & Abdulkareem, S. I. (2018). Ectoxicological Assessment of Leachate from Amilegbe Dumpsite, Ilorin, Nigeria Using *Clarias gariepinum* (Burchell 1822) and *Allium cepa*. Agrosearch. 18 (2); 59-71
24. Abubacker, M. N and C. Sathya. Genotoxic Effect of Heavy Metals Cr, Cu, Pb and Zn. Using *Allium cepa* L.(2017) Bioscience Biothechnology Research Asia. 14(3), 1181-1186.
25. Odutayo F.O.I, Oyetade O.B, Esan E.B and Adaramola F.B. (2016). Effects of Industrial Effluent on the Environment Using *Allium cepa* and *Zea mays* as Bioindicators, International Journal of Environment and Pollution Research. (4)3, 28-39.
26. Betül Çalışkan and Ali Cengiz Çalışkan. Potassium Nutrition in Plants and Its Interactions with Other Nutrients in Hydroponic Culture. (2017).
27. Ana Gabriela Estrada-Almeida, María Luisa Castrejón-Godínez, Patricia Mussali-Galante, Efraín Tovar-Sánchez, Alexis Rodríguez(2024). Pharmaceutical Pollutants: Ecotoxicological Impacts and the Use of Agro-Industrial Waste for Their Removal from Aquatic Environments, J. Xenobiot. , 14(4), 1465-1518.
28. Handle Morgil, Yusuf Can Gercek and Isil Tulum (2020). Single Nucleotide Polymorphisms (SNPs) in plant Genetics and Breeding. 572(2020).
29. Michael Monday Onakpa, Anoka Ayembe Njan and Ogbureke Chidiebere Kalu (2018). A Review of Heavy Metal Contamination of Food Crops in Nigeria. Annals of Global Health. (2018):3, 488– 494.
30. Ashutosh Yadav, Abhay Raj , Diane Purchase, Luiz Fernando R. Ferreira, Ganesh Dattatraya Saratale , Ram Naresh Bharagava(2019). Phytotoxicity, cytotoxicity and genotoxicity evaluation of organic and inorganic pollutants rich tannery wastewater from a Common Effluent Treatment Plant (CETP) in Unnao district, India using *Vigna radiata* and *Allium cepa*. (2019): 224. 324 – 332.