EFFECT OF SEWAGE WASTEWATER ON THE MITOTIC INDEX OF THE ROOT TIPS OF ALLIUM CEPA IN KOYA CITY /KURDISTAN REGION OF IRAQ

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Abstract:

The purpose of the experiment was to investigate onion (*Allium cepa*) root growth, cell division, and mitotic index. The onions were placed into four different water samples (Control water, Azadi bridge, Sar Razan, and Sar Baskan) for 72 hours in beakers. The results showed that the number of divided cells decreased significantly in the sewage water compared with the control water. The control water had a high percentage of divided cells (18%), whereas the Sar Razan sewage water had the least percentage of divided cells (1.3%). The percentage of divided cells in Sar Baskan sewage water was 2%, and the percentage of divided cells in Azadi Bridge was 2.6%. It was found that the mitotic index and the number of divided cells increased in the control water compared to the sewage water. The results showed that sewage water has a greater impact on root growth, cell division, and mitotic index of root tips of *A. cepa.*
Keywords: *Allium cepa*, sewage water, Root growth ,divided cells, mitotic index.

Introduction:

In most developing nations, the risk of drinking contaminated water and dealing with sanitation issues is growing daily as a result of urbanization and industrialization (Rajasulochana & Preethy, 2016). When untreated sewage water is utilized for irrigation in agricultural areas, the contaminated water—which contains hazardous chemical compounds—is immediately dumped into the fields. The contaminated water contains heavy metals, sewage, and other urban garbage. Using water contaminated with microorganisms poses serious health risks, particularly when individuals consume raw vegetables that have been irrigated with the contaminated water (Darlington, 1942). Wastes carried by water, in suspension or solution, that drain from a community are referred to as sewage. It is often referred to as the community's used water supply or wastewater flows. Its volume or flow rate, physical state, chemical composition, and the bacteriological organisms it includes all indicate that it is more than 99.9% pure water (Igwenyi, 2012).

The onion, or *Allium cepa* L., is a member of the Alliaceae family and genus Allium (Hanelt 2018). The Amaryllidaceae family contains 887 species of Allium that are distributed worldwide, encompassing both cultivated and wild plants. Allium species are highly significant herbaceous plants that are utilized as vegetables and seasonings around the world. Early taxonomy of angiosperms included Allium and related genera under the Liliaceae family. The monocotyledons are acknowledged as belonging to the unique family Alliaceae, which is closely related to the Amaryllidaceae, in the more modern and competent taxonomic approach

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(Dahlgren *et al.,* 1985). Since ancient times, it has been prized as a culinary and medicinal plant. It is a vegetable bulb crop that is known to most cultures and consumed all over the world, produced to a degree second only to tomatoes (FAO, 2012). According to Brewster (2018), it is a short-duration horticultural crop.

Because of certain chemicals in the water, using wastewater to irrigate agricultural land damages plants' ability to divide mitotically, which ultimately leads to plant extinction (Caritá & Marin-Morales, 2008). Eating these plants could have a negative impact on people's health. According to Sik et al. (2009), the chemical composition of plants cultivated in these fields may cause major side effects such as allergies at a young age, respiratory issues, heart problems, and cancer in middle age. Sewage wastes have been shown to be a dangerous factor since they have the potential to be genotoxic agents and can harm chromosomes, resulting in structural defects. The primary goal of this study is to evaluate how *A. cepa* roots develop in wastewater as opposed to tap water.

Material and methods

Onion preparation

For a period of 72 hours, little onion (*A. cepa*) bulbs of a similar size and weight (approximately 15-20g) were placed in beakers containing three sewage water samples, control water, and other water samples. The samples were gathered in from various parts of Koya City.

Experimental procedure

The onion bulbs were exposed for 72 hours in each experiment. They were placed in beakers with different water samples and stabilized with a wooden applicator. The experiment was completed after 72 hours, at which point the onion roots had grown.

Macroscopic parameters

The length of the root and additional characteristics, such as the typical shape and quantity of roots after 72 hours, are examples of macroscopic metrics.

Root and slide preparation

Three root tips from the actively growing plant were cut to be 1cm long using a scalpel or knives. Only the tapered end of the root tip was used. 2-3 roots were placed on a glass microscopic slide using forceps, and then covered with 2-3 drops of 1M hydrochloric acid. This helps to separate the tissue to improve visibility of the cells. After 5 minutes, any excess hydrochloric acid was blotted away using a paper towel. 2-3 drops of deionized water were then added to the root tips using a graduated pipet, and any excess water was blotted away with a paper towel. Next, 2% acetocarmine stain was added to the root tips using a pipette, and the root tips were soaked for approximately 3 minutes. Any excess acetocarmine was blotted away with a paper towel. One drop of deionized water was added to the root tips. Using forceps, one root tip was moved to a clean microscopic slide and a cover slip was placed on the root tissue. Gently applying pressure with a pencil, the root tissue was squashed to facilitate observation under the microscope and to observe the cell cycle phases.

Microscopic parameters

The scoring of dividing cells, the mitotic phases, and the mitotic index were all included in the cytogenetic study. The mitotic index, or MI, is the percentage ratio of the total number of scored cells to the number of dividing cells. The mitotic index drops as one gets farther away from the root tip. This indicates that as cells pass from the zone of cell division to the zone of cell elongation, their rate of division will gradually decrease. The rate at which cells divide inside a tissue is determined by the mitotic index. It is especially useful for assessing the effects of xenobiotics like pollutants, germination agents, and poisonous compounds. Accurately recognizing dividing cells is significant because cell division is a crucial process in the growth and repair of tissues in organisms. It also serves as an indicator of overall organismal growth. Careful microscopic inspection, especially in the cortex, can do this. Dividing cells are easily visible under a microscope since they are tiny and usually round in form. For a final conclusion in situations where their division is not readily apparent, microscopic investigation is essential. Using a microscope guarantees accurate cell division identification. (Smail 2020 and Ibrahim *et al.,* 2021). The following calculations were used to compute the mitotic index and determine the proportion of each type of divided cell phase, such as prophase, metaphase, anaphase, and telophase, using slides observed under a compound microscope with an oil-immersed 100X objective lens.

$$\text{Mitotic index (\%)} = \frac{\text{Total number of dividing cell}}{\text{Total number of cell examined}} \times 100$$

2.6 Statistical analysis:

A one-way analysis of variance (ANOVA) test was used to examine the impact of sewage wastewater status on the phase index (%) of different wastewater sites of mitotic distributions in *A. cepa*. A statistically significant result is defined as a p-value less than 0.05. Additionally, the analysis was conducted using SPSS version 20.

Results

Table 1 shows how the mitotic index of *A. cepa* root tip cells is affected by sewage effluent from various places. In comparison to the groups exposed to wastewater, the control group, which was not exposed to wastewater, has a higher mitotic index (18%), indicating more active cell division. Significantly lower mitotic indices of 2.6%, 1.3%, and 2% at Azadi Bridge, Sar Razan, and Sar Baskan, respectively, imply that sewage wastewater has a cytotoxic effect that inhibits cell division.

Table 1: Effect of sewage wastewater on mitotic index on the root tip of *A. cepa*

Waster waste locations	Number of cell counted	Total number cell divided	Total number cell non divided
Control	150	27(18%)	123(82%)
Azadi Bridge	150	4(2.6%)	146(97.3%)
Sar Razan	150	2(1.3%)	148(98.6%)
Sar Baskan	150	3(2%)	147(98%)

The distribution of mitotic phases in *A. cepa* root cells exposed to sewage effluent from various places is displayed in the table. In the control group, the telophase has the highest frequency (44.4%), followed by prophase, metaphase and anaphase. On the other hand, groups exposed to wastewater have skewed distributions, particularly in cases where telophase percentages are elevated (e.g., Sar Razan at 50% and Azadi Bridge at 75%). The large F value (7.963) and P value (0.0035) support the idea that sewage effluent disturbs normal cell cycle development, resulting in notable variations in mitotic phase distribution(Table 2)

Table 2: Effect of sewage wastewater Status of phase index (%) of different wastewater locations of mitosis distributions *A. cepa*

Waste water locations	Prophase	Metaphase	Anaphase	Telophase	Total	F value	P value

Control	7(25.9%)	5(18.5%)	3(11.1%)	12(44.4%)	27(100%)	**7.963**	**0.0035**
Azadi Bridge	1(25%)	0(0%)	0(0%)	3(75%)	4(100%)		
Sar Razan	1(50%)	0(0%)	0(0%)	1(50%)	2(100%)		
Sar Baskan	2(66.6%)	0(0%)	0(0%)	1(33.3%)	3(100%)		

Significant = (P≤0.05), using One-Way ANOVA

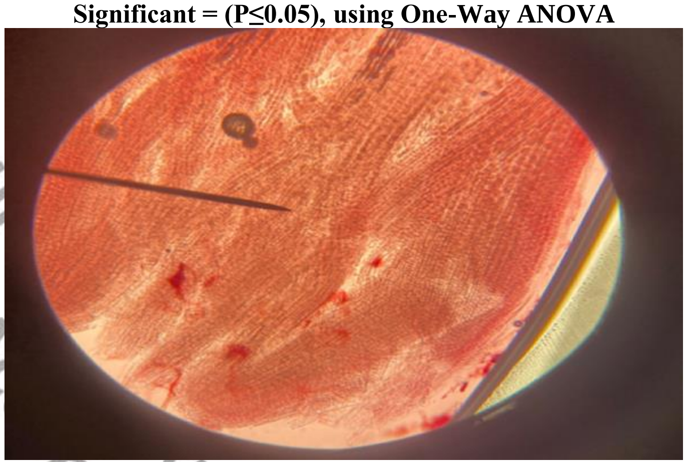

Figure 1: General aspect of the root meristem cells of *A. cepa* (40X magnification).

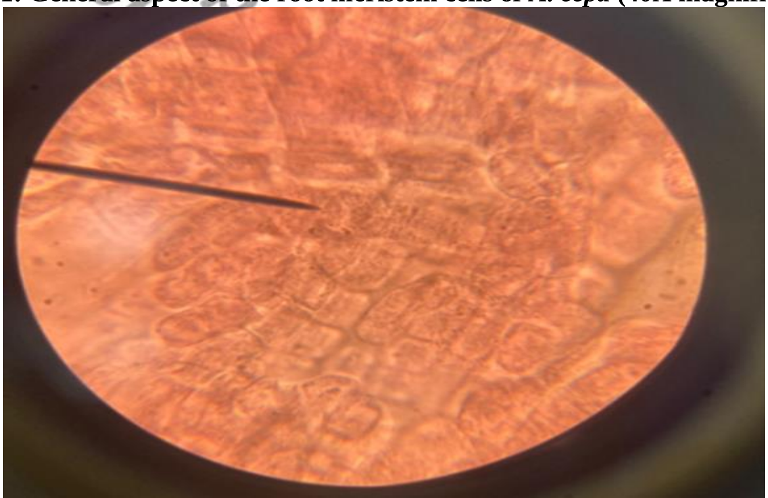

Figure 2: At a 100X magnification, meristematic cells of *Allium cepa* root tips in various phases are detected.

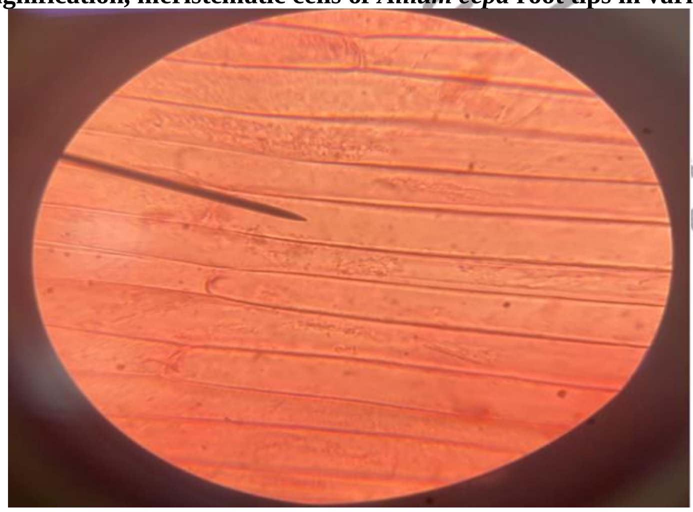

Figure 3: Meristematic cells at 100X magnification in the root tips of *A. cepa* are non-dividing cells in sewage effluent.

Discussions

In this experiment, the roots of *A. cepa* were grown in different water samples (control water, Azadi bridge, Sar Razan, and Sar Baskan) for a period of 72 hours. The growth rate differed in each sewage water sample compared to the control water sample. In each slide, 150 cells were counted. The growth rate and number of divided cells of the onion roots grown in the control water was 27 cells (18%). However, the number of undivided cells was 123 cells (82%). The number of divided cells in Azadi bridge water was 4 cells (2.6%), while the number of non-divided cells was 146 cells (97.3%). In Sar Razan's water, 2 cells (1.3%) were divided and 148 cells (98.6%) were undivided. The other sample, Sar Baskan, had 3 cells (2%) that divided, while the number of non-divided cells was 147 cells (98%) at 72 hours (**Table 1**).

Heavy metals are known as environmental pollutants that present a significant peril to living organisms. These substances are resistant to decomposition and tend to accumulate within organisms, leading to numerous detrimental effects. Many of the heavy metals, including mercury, lead, cadmium, chromium, zinc, copper, and nickel, are known to cause toxic effects (Alengebawy *et al.*, 2021). The primary site for the uptake of these metals in plants is their root system. As heavy metals become adsorbed into the soil, they easily accumulate in plant roots (Shokri *et al.*, 2022).

The escalating presence of heavy metal pollutants in water, soil, and air has emerged as a pressing issue in recent times. Even at minimal concentrations, heavy metal pollutants pose a considerable threat. Moreover, their non-biodegradability prevents them from breaking down into harmless forms. Consequently, heavy metals can accumulate in water sources and soil, subsequently being absorbed by plants. This poses a risk to plant life (Sharma *et al.*, 2023). The presence of heavy metals and other contaminants in soil can cause similar detrimental effects, which can further result in the contamination of plants. The primary objective of this study is to gain a more comprehensive understanding of the consequences of sewage water waste and its impact on plants in terms of patterns, probability, and the extent of pollution when the *A. cepa* plant comes into contact with sewage-contaminated water (Goyal *et al.*, 2020). *A. cepa* root growth is a fundamental endpoint for the investigation of heavy metals in contaminated water because root growth in *A. cepa* is believed to be suppressed by the presence of heavy metals, such as lead, in contaminated water. It can be used as an indicator of water toxicity (Lyu *et al.*, 2020). Furthermore, *A. cepa* cells grow at a great rate due to their large size. This characteristic makes the species extremely susceptible to the impacts of heavy metals. Additionally, because *A. cepa* roots are large and have a much greater specific surface area than other plant roots of similar masses, the roots and cells of onions are extremely susceptible to heavy metals. In order to develop efficient water treatment techniques (da Cunha Neto *et al.*, 2023), meristematic cells of root tips of *A. cepa* were examined at 40X magnification, as shown in **Fig. 1.**

The effect of sewage wastewater on the mitotic index of the examined root tips and the total number of examined cells (150 cells), classified into interphase and dividing cells (prophase, metaphase, anaphase, telophase), was shown in **Table 2.**

The percentage of mitotic index and number of divided cells in the control water (18%) are higher than in the other water samples. Lower mitotic index and divided cells (1.3%) were observed in the Sar Razans sewage water. The mitotic index and divided cells of other sewage water samples are significantly different in comparison with the control. The percentage of divided cells and mitotic index in Azadi bridges sewage water is 2.6% and in Sar Baskans sewage water is 2%, respectively. In the growth of *A. cepa* in control and different sewage water samples, there is a significant difference between the phases (prophase, metaphase, anaphase, telophase) (Table 2). Among the dividing cells, telophase has the highest number, followed by prophase, metaphase, and anaphase. The Azadi bridges sewage water has the highest percentage of telophase (75%), while Sar Baskans sewage water has the lowest percentage (33.3%). The other three stages dividing cells (prophase, metaphase, and anaphase) also differ. The control water and Azadi bridge sample have the same and lowest percentage of prophase (25.9%) and (25%) respectively, while Sar Baskans sample has the highest percentage of prophase (66.6%), followed by Sar Razans sample (50%). The lowest number of metaphase is seen in Azadi bridge, Sar Razan, and Sar Baskans samples, which are all 0%, followed by the control sample, which has the highest percentage (18.5%). The highest percentage of anaphase is seen in the control water sample (11.1%), but the lowest percentage of anaphase is seen in Azadi bridge, Sar Razan, and Sar Baskans samples, which are all 0% (Table 2).The cell division process is essentially a parent cell dividing into daughter cells. Apart from accomplishing simple multiplication of cells, it is also crucial for development and growth in multicellular organisms. Lastly, it is integral in regeneration and repair in multicellular organisms (Elchaninov *et al.*, 2021).

A. cepa, a freshwater plant, holds great significance for multiple reasons. Firstly, it possesses such a productive biomass that it covers large areas of the substrate where it grows. Secondly, *Allium cepa* produces an abundant quantity of leaves, which it discards as a result of competition with other large aquatic plants or when environmental conditions are stable and suitable. The leaves of *A. cepa* are thin, broad, and short-lived, allowing them to efficiently capture light when it becomes available. Lastly, under favorable conditions, this plant can achieve a substantial biomass, only to begin decomposing swiftly, thereby adding a significant amount of organic matter to sediment and water (Paramonova *et al.*, 2021).

Sewage water is a result of a combination of domestic, commercial, and industrial wastes. The water is produced by activities such as cleaning, through the use of detergents, bleaches, and soaps, cooking, through the boiling of water, and carrying out experiments in the laboratories that require the use of water. The major contributors to the production of sewage water are the commercial and industrial sectors (Gaur *et al.*, 2020). Regardless, the water has been utilized and is no longer required, thus it is classified as either sewage water or wastewater.

Conclusions

To sum up, sewage-contaminated water may hinder the growth and division of *A. cepa* roots due to harmful heavy metals such as lead and mercury. The small size and surface area make *A. cepa* roots susceptible to toxicity caused by these metals. When compared with controls, mitotic index analysis shows that cell division is reduced in wastewater samples, while changes in distinct phases during cell division emphasize diverse unfavorable impacts on root development triggered by contamination from sewage water.

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