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CYTOTOXICITY STUDY OF WATER SAMPLES COLLECTED FROM LOCAL TRIBUTARIES BY *ALLIUM CEPA* TEST

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ABSTRACT

Urban effluents may contain several cytotoxic contaminants that are dumped into rivers, causing environmental degradation in downstream reaches. Rivers in Pune (Maharashtra) are the most polluted water bodies because of their function in transporting waste from municipalities and industries as well as run-off from agricultural fields in their basin. Despite different restrictions and regulations, a variety of small-scale companies discharge their trash directly into the river. The current work employed an *Allium cepa* bioassay to assess cell toxicity by water samples collected from polluted bodies of water in Pune as a result of industrial effluent. The study was carried out by collecting wastewater samples from three sampling sites (Site A - *Bhima River*, Site B - *Pavna River*, and Site C - *Indrayani River*) and investigating their physicochemical properties. Tap water was used as a control in every case. The water quality of the samples was evaluated using a TDS meter (Total Dissolved Solids). Water cytotoxicity was assessed using the *Allium cepa* bioassay. The study's findings revealed that *A. cepa* bulbs exposed to *Indrayani River* water had a considerable reduction in MI, followed by *Pavna* and *Bhima River* water. The *Indrayani* and *Pavna Rivers* frequently get a higher volume of domestic and industrial sewage from several municipalities than the *Bhima River*. The findings of this investigation reveal that the river water pollutants comprised several toxic compounds that are harmful to the aquatic environment and human health.

Keyword: *Allium cepa*, Cytotoxic, Mitotic index, Pollution, TDS meter, Water

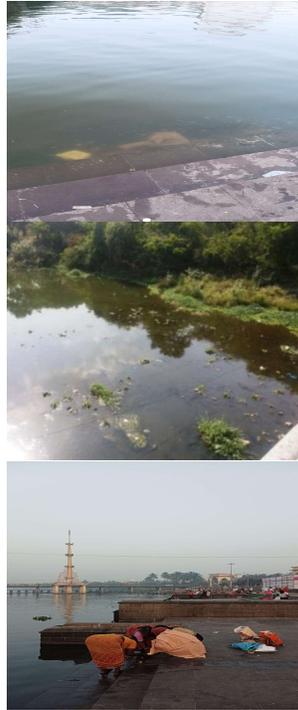
INTRODUCTION

Municipal effluents may include an assortment of genotoxic contaminants, which are released into rivers and degrade the aquatic ecology in downstream reaches [1]. It contributes to water pollution, which has recently received a lot of attention, because a wide range of substances capable of causing genetic damage are produced by various human activities and discharged into the environment daily, impacting the living things and human populations interacting with this ecosystem [2]. Demographics and industrialization have exacerbated a global issue and an existential threat of submerged environmental pollution caused by the discharge of industrial, agricultural, and/or home effluents containing numerous types of chemical compounds. When these chemical substances integrate into the aquatic environment, they promote the production of complex combinations that can harm human health, the ecosystem, and the species that dwell or use it [3]. Organisms living in locations affected by wastewater discharges may encounter DNA damage, while humans who consume polluted fluids are at a higher risk of acquiring cancer [3-5]. Contaminants' damage to organisms, genotoxic and mutagenic effects are concerning, due to their ability to create genetic damage, which can lead to a variety of ailments and also affect future generations, because these

mutations are inheritable. Thus, mutagenic assays must be used for ecological surveillance and risk assessment to determine the impacts of contaminants on aquatic life and human health, in addition to physical, chemical, and microbiological investigations [6-7]. Because direct risk assessment on humans is impractical for logistical, ethical, and practical reasons, a range of experimental models have been developed for evaluating the genotoxic impacts of environmental pollutants. Many of international organizations, notably the World Health Organization, have suggested that higher plants can be used as a model for measuring Genotoxicity and monitoring various chemical pollutants in situ. Because plant chromosomes are great biomarkers for mutagenesis research, it is beneficial, dependable, and cost-effective for environmental pollutant biomonitoring [7]. The *Allium cepa* test is a quick and accurate method for detecting ecological Genotoxins and mutagens [7-9]. It enables the assessment of numerous outcomes, including root development inhibition, mitosis, chromosomal aberration, and micronuclei production, allowing to assess the mechanism and impact of contaminants on the DNA of exposed animals [10-14]. Municipal wastewater quality discharged into water bodies should be scrutinized in particular, to reduce water

pollution and guarantee a safe environment for all. Periodic and frequent surveillance of the physicochemical qualities of wastewater effluent is recommended.

MATERIALS AND METHODS



a) *Bhima River* water sample from Rajgurunagar, Pune 18.84 latitude and 73.88 longitude

b) *Pavna River* water sample from Ravet, Pune 18.64 latitude and 73.75 longitude

c) *Indrayani River* water sample from Alandi, Pune 18.67 latitude and 73.89 longitude

Figure 1: Water sampling Sites

Physicochemical properties of water samples:

Water samples were collected in three liters from each collecting point and placed in a suitable container for physicochemical analysis. pH, total dissolved solids (TDS), and other physicochemical variables were considered. A conductivity meter was used to measure the electrical conductivity of water samples. TDS meters are used to determine the quality of each water sample. The TDS level indicates whether the water

Sampling Sites: Water samples were taken at the following locations. These sampling sites collect industrial waste from a variety of point and nonpoint sources. The contaminated wastewater generated in town is also discharged into the river.

is safe to drink, needs filtering, or is heavily contaminated. Parts per million (PPM) is the unit of measurement used to determine TDS levels in water [16].

Macroscopic parameters

After three days of growing at room temperature, the number of roots of onion bulbs in each sample of effluent was measured and recorded for each treatment. The total root length of each treatment was measured with a calibrated ruler.

Microscopic parameters

In the Allium test, equal-sized bulbs were used. The outer scales of the bulbs were carefully removed just before usage, and the brownish bottom plates were scraped away without damaging the root primordia [15]. Small *Allium cepa* bulbs were purchased from a local vegetable market. The outer scales of the bulbs and the dry bottom plate were removed before starting the test without harming the root primordia [16-17]. For 48 hours, scarred onion bulbs germinated in conical flasks containing water samples. To avoid direct sunlight, bulbs with exposure medium were kept in the dark. The bulbs with satisfactory root lengths (2-2.5 cm) after 48 hours of exposure were used in the study. Microscopic examinations were conducted on root tips ranging in length from 1-2 mm.

For 1 hour, root tips were fixed in ethanol: glacial acetic acid (3:1, v/v) solution. The root tips were hydrolyzed in a 1N hydrochloric acid solution for 5 minutes at 60°C before being rinsed with distilled water. For 5 minutes, root tips were placed in watch glasses with Safranin to allow the stain to penetrate the primordial cells. After staining, root tips were placed on glass slides and the coverslip was gently pressed to crush the tip cells over the slide. To score mitotic phases, prepared slides for each exposure medium were examined under a light microscope at 400x magnification [17]. The mitotic index was calculated as the

$$MI = \frac{NDC}{TDC} \times 100$$

Where, NDC= Number of dividing cells,
TDC= Total number of cells observed

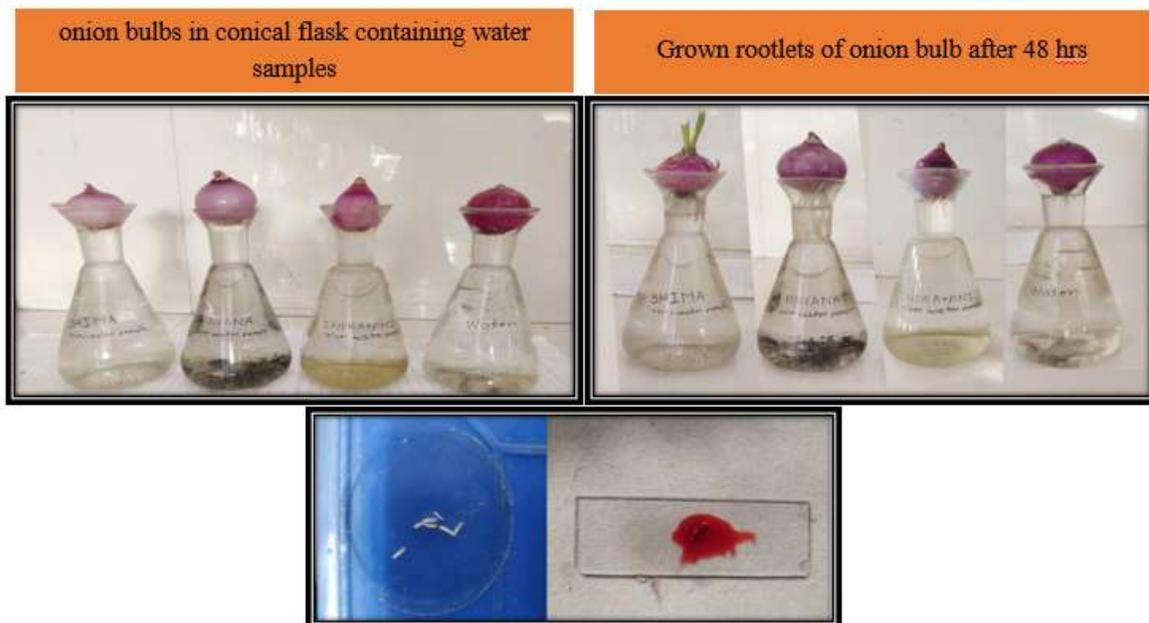


Figure 2: Mitotic division practical process

Data analysis

Graph pad prism 9.3.1 was used for statistical analysis. Using two-way analysis of variance (ANOVA), the effects of water from different sample sites on the mitotic index of *A. cepa* root cells were studied. At the time of analysis, differences between matched controls and exposure treatments were deemed statistically significant at ($p < 0.05$).

RESULTS AND DISCUSSION

Physicochemical characteristics of raw municipal wastewater

Table 2 shows the amounts of physicochemical parameters obtained from water samples collected at various locations. These parameters differed significantly between sampling sites ($p < 0.05$). According to the physicochemical study of the wastewater samples, the middle site was much higher than the upper and lower downstream sites of the stream. This demonstrated that there is a statistically significant difference between sites due to varying levels of pollution at collection.

Table 1: Physicochemical characteristics

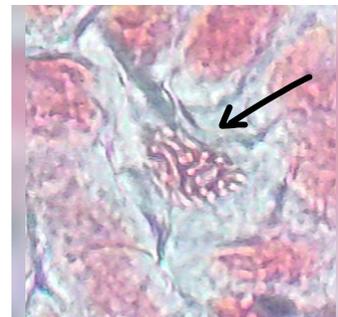
Parameters	Bhima River Water	Pavna River Water	Indrayani River Water	Control
pH	7.23±0.43	7.56±0.25	8.03±0.31	7.15±0.45
TDS measured value in ppm	550.23±0.85	668.28±0.59	706.85±0.49	164.65±0.52

All values are means of three replicates



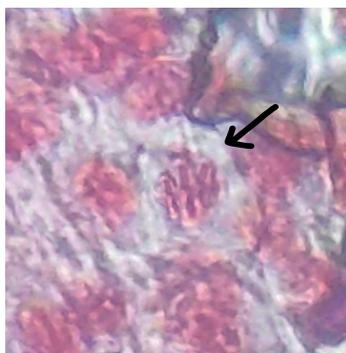
1. Interphase

The stage in the life cycle of a cell where the cell grows and DNA is replicated



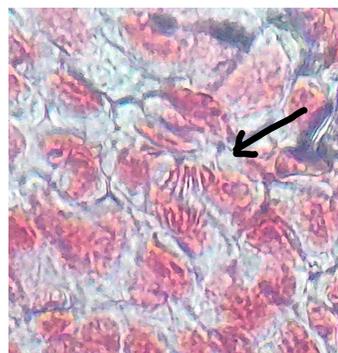
2. Prophase

The chromosomes are visible and tangled



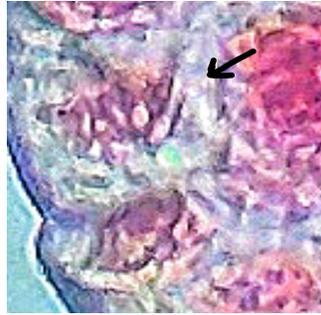
3. Metaphase

The chromosomes are arranged in the equatorial plate



4. Anaphase

The sister chromatids separate moving towards the spindle poles.



5. Telophase

Each daughter chromosome has arrived at the spindle pole and following occurs cytokinesis

Figure 3: The microscopic appearance of Mitotic phases

Table 2: Mitotic Index calculation in *A. cepa* (root tip cells) exposed to water samples

	Prophase	Metaphase	Anaphase	Telophase	No. of dividing cells	Total Cells Observed	Mitotic Index (Mean±SD)
	(Mean±SD)						
Tap Water (control)	35.67±1.2	4±0.81	1.33±0.47	15±2.44	56±1.632	100	56±1.63
<i>Bhima River Water</i>	25±0.81	2.67±0.47	0.66±0.471	8.33±2.054	36.66±0.57	100	36.6±0.57
<i>Indrayani River Water</i>	21.67±1.6	2.33±0.47	0.67±0.47	6.67±1.24	31.33±2.4	100	31.33±2.4
<i>Pavna River Water</i>	21.67±2.0	2±0.81	1.33±0.47	7.67±2.05	32.67±1.8	100	32.67±1.8

Mean±SD (Standard Deviation), One-way ANOVA** $p < 0.0048$

By analyzing mitotic depressions, the cytotoxic potentials of effluent on *A. cepa* root tip cells were validated under microscopic examination. In this experiment, river water affected cell division after being subjected to water specimens collected from three separate sampling sites. Microscopic parameter data demonstrated clear impacts of water on mitotic index (MI) values. The MI of root tip cells differed substantially from the control sample ($p < 0.0048$). The effect of water collected from three different rivers' streams (*Bhima*, *Indrayani*, and *Pavna River*) on MI (%) after water exposure was recorded as (36.6±0.57, 31.33±2.4 and 32.67±1.8) compared to the negative control, which was determined to have the

greatest (56±1.63) (Table 2). This implies a significant decrease in MI as well as an increase in wastewater concentrations when compared to the control.

Aside from treatment concentrations, it was obvious that different sampling locations had distinct impacts on the MI of *A. cepa* root tips. The water from the *Indrayani River* had a considerable drop in the number of proliferating cells in the root tip meristems, indicating that the *Indrayani River* water has a more cytotoxic effect than other places. The cytotoxic effects of the stream on *A. cepa* root tip cells continue to differ significantly between the *Bhima* and *Pavna River* sites. This reduction can provide information on the changes in

toxicity levels at different sampling sites due to the presence of various pollutants.

In general, the effects of pollutants in river water were directly correlated with all sampling sites; however, remarkable variations in both macroscopic and microscopic parameters were observed among the water sampling sites, and the effect was observed to decrease in the order of *Indrayani* > *Pavna* > *Bhima* [16-17].

Municipal effluents are complex combinations of diverse substances that have a significant impact on the physicochemical properties of water bodies. Some of these are frequently harmful and are still dumped directly into rivers and lakes with no waste treatment. This causes environmental contamination, which has a negative impact on living systems, including humans. The testing results showed that pollutants were moving directly into rivers. The water from the river is used as a raw source of water for the downstream communities' public water supply. The physicochemical data gathered in this investigation demonstrates the excessive level of pollution brought into the river. The observed results varied greatly among the sampling sites, indicating different levels of pollution pressures. The physicochemical data gathered offers a glimpse into the level of pollutants brought into river wastewater discharges and, as a result, its impact on river water quality. The observed results

varied greatly among the sampling sites, indicating different levels of pollution pressures. The physicochemical data gathered offers insights into the level of pollutants brought into river wastewater discharges and, as a result, its impact on river water quality. According to observations, wastewater discharged straight into the river poses considerable contamination threats to the aquatic ecology. However, this approach cannot provide a true picture of the effect of untreated wastewater flow on diverse aquatic life. As a result, the cytotoxic assay of water from rivers was evaluated using *A. cepa* root to supplement physicochemical monitoring. The toxicity potential for water sample locations on macroscopic and microscopic metrics was strongly related to the effluent physicochemical properties. Similarly, to macroscopic characteristics, the cytotoxic effects of river water samples are associated with a considerable drop in the MI of *A. cepa* bulbs as sampling sites' distance from the city area rises.

The mitotic index (MI) value was used to measure the cytotoxicity of wastewater. **Table 2** shows the impact of wastewater mixed in water from rivers on MI in *A. cepa* root tip cells. When compared to the control, the bulbs exposed had a significant influence on reducing MI. This finding clearly shows that the presence of contaminants may have a negative influence on *A. cepa* root cells

[14]. MI was significantly reduced in *A. cepa* bulbs when exposed to *Indrayani* river water, subsequently followed by *Pavna* and *Bhima* river water. This shows that the water composition varies across the stream's sampling sites. The *Indrayani* River stream absorbed wastes from various directions of sewers in the city, which contained a diverse range of compounds from a variety of sources, which could be the cause of MI reduction. The decline of the mitotic index is used to determine the cytotoxicity levels of environmental contaminants/pollutants [16]. The observed reduction in MI in this investigation suggests that pollutants contain cytotoxic agents. This is not surprising given that the *Indrayani* and *Pavna* rivers frequently receive a higher volume of domestic and industrial waste from different directions of the municipality than the *Bhima* River. The findings of this investigation reveal that the river water pollutants comprised a variety of harmful compounds that have a negative impact on the aquatic environment and human health.

CONCLUSION

Increasing Water contamination is a significant issue in all rivers. The most serious health risk is contaminated water, which continues to jeopardize both the quality of life and public health. The current study's findings suggested the presence of cytotoxic chemicals in the water of Pune rivers, which receive numerous pollutants

from both point and nonpoint sources. MI was significantly reduced in *A. cepa* bulbs exposed to *Indrayani* river water, followed by *Pavna* and *Bhima River* water. The *Indrayani* and *Pavna Rivers* frequently get a higher volume of domestic and industrial sewage from several municipalities than the *Bhima River*. The findings of this investigation confirm the presence of a variety of dangerous chemicals in river water contaminants.

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Conflict of interest

The authors declare that there is no conflict of interest.

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