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Biological Monitoring of Estero De Paco Using the Allium Test

Asim Fathimath Layan[1], Hassan Fathmath Haima[1], Hussain Jenny Sivad[1], Joel Unday[2]

[1]Biology Students, School of Arts and Sciences, Emilio Aguinaldo College

[2]Faculty, School of Arts and Sciences, Emilio Aguinaldo College

ABSTRACT: The Estero de Paco is a major tributary of the Pasig River in Metro Manila, Philippines, that has undergone severe environmental degradation due to urbanization, domestic sewage, and industrial waste. Because communities living near the creek continue to rely on its water, establishing the biological impact of this pollution is critical.

This study evaluated the cytotoxicity and genotoxicity status of Estero de Paco using the *Allium cepa* (common onion) root tip chromosomal aberration assay, an established, cost-effective bioindicator for environmental screening.

Onions were exposed for five days to surface water samples collected from five distinct stations along the estero: Unilever Philippines, United Nations Avenue, San Gregorio, Paco Market, and Pedro Gil. Tap water served as the control group. Root tips were fixed, hydrolyzed, and stained with aceto-orcein for microscopic evaluation at 400× magnification. One thousand cells per slide were counted to determine the Mitotic Index (MI). Data was analyzed using SPSS via Analysis of Variance (ANOVA) and Scheffé post hoc tests at a 0.05 significance level.

The MI of *Allium cepa* root cells exposed to all Estero de Paco water samples was significantly lower than the control group (), confirming the presence of cytotoxic substances that inhibit cell division. San Gregorio (S3) exhibited the highest cytotoxicity with the lowest recorded MI (), likely due to elevated industrial and anthropogenic pressures. Interestingly, low cell division rates did not correlate with high genetic damage; only sticky chromosomes were observed, indicating minimal genotoxicity.

The water of Estero de Paco possesses prominent cytotoxic traits, heavily driven by deficient community wastewater management. Immediate environmental monitoring, chemical substance tracing, and community-integrated ecological rehabilitation are highly recommended to restore the tributary.

KEYWORDS: Estero de Paco, Pollution, Allium cepa, microscopic parameters, root tip cells, mitotic index

INTRODUCTION

Water is essential for life, yet only 0.5% of the planet's water is available for human use. The Pasig River, a lifeline for Metro Manila, has been a hub for fishing, farming, and trade. Estero de Paco, one of its tributaries, once thrived as a commercial and cultural center, especially after a market opened during WWII. However, over time, it becomes a dumping ground for sewage and garbage, leading to severe pollution. Despite various rehabilitation efforts, the river remains biologically compromised, affecting the livelihood of those who depend on it.

Because of its low cost, short test duration, ease of use, excellent cell division characteristics, and straightforward and affordable techniques for examining the induction of chromosomal aberrations, the Allium Test has been suggested for routine screening. The Allium Test was developed and used by Fiskesjo (1988) as a component of a battery of tests for environmental monitoring. Standard factors like macroscopic and microscopic characteristics are part of this technique and were employed in this study.

This study aimed to evaluate the extend of Estero de Paco pollution status using Allium cepa based on microscopic parameters.

METHODS

Study Site and Sample Collection

Surface-level water samples (10–15 cm deep) were collected from five sites in Estero de Paco using 3000 ml sterile plastic bottles. Onion bulbs (Allium cepa), averaging 4 cm in diameter, were obtained from Paco Market from the same batch harvested in February. Both the water and onion samples were collected during March.

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Root Growth of *Allium cepa*

Onion bulbs were sun-dried from February to March 2024 to prevent molding and rotting and to promote healthy root growth. Before testing, the outer layers were removed to expose the root primordia, enhancing root development. Ten bulbs per station were placed in plastic cups containing the respective water samples, with EAC tap water as the control. The lower halves of the bulbs were kept submerged, and the setup was conducted between 8:00 and 11:00 a.m., during peak mitotic activity. Water levels were checked and replenished twice daily, and the bulbs were allowed to grow for five days.

Slide Preparation

After five days of exposure, 5–10 mm root tips were measured, cut, and placed in 10 ml of acetic alcohol fixative (3 parts methanol, 1 part glacial acetic acid) to preserve chromosomal DNA. The beakers were sealed with aluminum foil and refrigerated at 2–5°C for four days, with daily monitoring to prevent evaporation.

After fixation, root tips were hydrolyzed sequentially in 50% alcohol, 30% alcohol, and 1N HCl for ten minutes each. They were then stained and macerated on slides using Aceto-Orcein under a coverslip. Orcein dye in acetic acid was used to clearly stain chromosomes, providing a quick and effective method suitable for preparing multiple slides.

Microscopic Examination of Root Cells

Before microscopic observation, slides were gently tapped with a pencil eraser to remove air bubbles, and excess Aceto-Orcein was wiped away. Each slide was examined at 400× magnification, and 1,000 cells were counted to determine how many were undergoing mitosis.

The mitotic index (MI) was calculated by dividing the number of dividing cells by the total number of cells and multiplying by 100. The MI values of water samples from different areas of Estero de Paco were then compared with the control (tap water).

Statistical Analysis

Quantitative data analysis was conducted to biologically monitor Estero de Paco using the Allium cepa test. The mitotic index (MI) was calculated across multiple samples, and results were organized in tables using SPSS software to facilitate comparison and analysis.

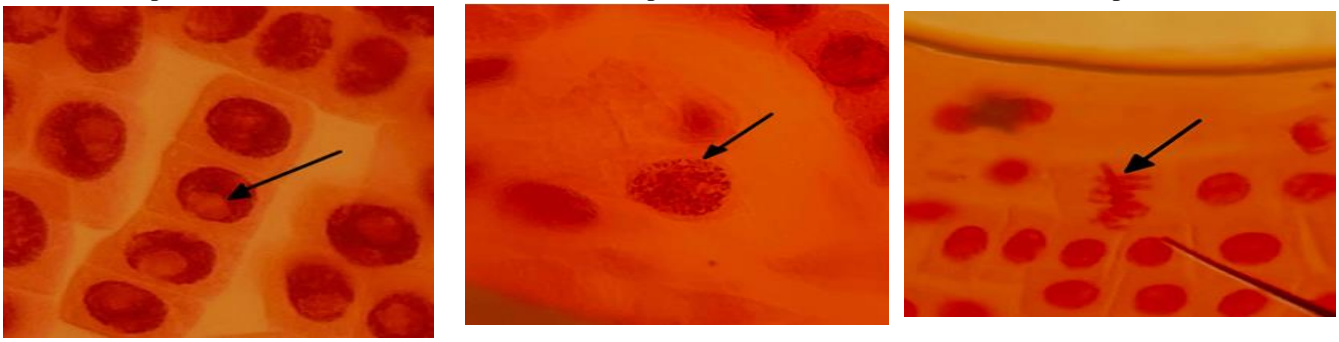
Analysis of Variance (ANOVA) was applied to determine significant differences between onions exposed to five test water samples and the control tap water, using a 0.05 significance level. A Scheffé post hoc test was also performed to identify which specific groups differed from each other.

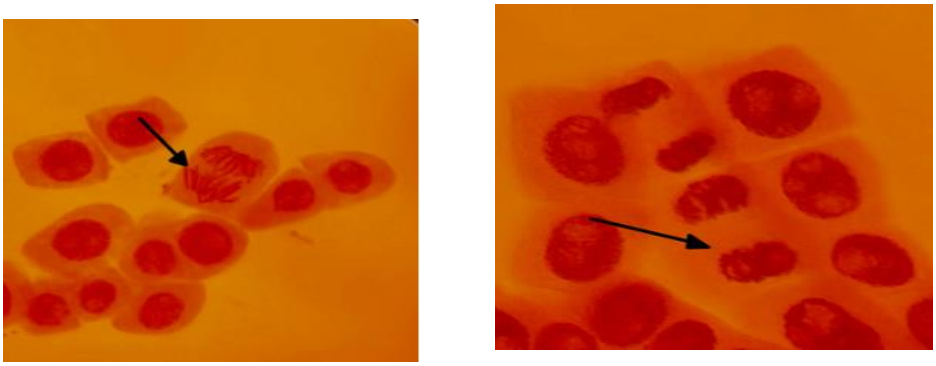
RESULTS AND DISCUSSION

Microscopic Observation

The following stages of mitosis observed from the root tips of *Allium cepa* on tap water, control. Photomicrographs of the different stages of mitosis were presented in Figure 1.

A. Interphase **B. Prophase** **C. Metaphase**





D. Anaphase E. Telophase

Figure 1: Microscopic appearance [(a) Interphase, (b) Prophase, (c) Metaphase, (d) Anaphase, (e) Telophase]

Based on Cytotoxic Effects

Table 1 presents the descriptive statistics for each indicator in the study. The mean values for prophase, metaphase, and anaphase are moderate, while telophase, total dividing cells, and the mitotic index show low mean values. Prophase is the most frequently observed stage because it takes longer to complete processes such as chromatin separation and spindle formation. In contrast, telophase is the least observed stage due to its short duration as the final phase of mitosis before cytokinesis (Rehman et al., 2023). The low mean number of dividing cells is linked to data from the five sampling stations in Estero de Paco, which showed consistently lower values compared to the control group. Consequently, the mitotic index is also low since it is calculated based on the total number of dividing cells.

Table 1. Cytotoxic Effects in terms of Mitotic Index

Indicator	Weighted Mean	Standard Deviation	Verbal Interpretation
Prophase	289.30	35.074	Moderate
Metaphase	21.23	9.164	Moderate
Anaphase	16.43	8.033	Moderate
Telophase	11.47	7.722	Low
Total Dividing Cells	338.43	39.826	Low
Mitotic Index/%	33.843	3.982	Low

Based on Mitotic Index

The mitotic indices of *Allium cepa* root cells exposed to water from different stations along Estero de Paco were significantly lower than those grown in control tap water, indicating that pollutants in the samples inhibited cell division and caused cytotoxic effects. San Gregorio (S3) recorded the lowest mitotic index, consistent with findings that areas with higher anthropogenic or industrial activity show greater cytotoxicity. These results suggest the presence of harmful agents such as heavy metals, pesticides, or other chemical pollutants, similar to reports from urban streams in Santa Cruz do Sul, Brazil (Athanásio et al., 2014).

Table 2. Differences in the Mitotic Index in terms of Station

Indicator	Station	Mean	F Value	Sig	Decision	Interpretation
Mitotic Index/%	Control	40.440	22.089	0.000	Reject H_o	Significant
	Unilever Philippines	34.500				
	UN Avenue	31.740				
	San Gregorio	28.640				
	Paco Market	34.120				
	Pedro Gil	33.620				

Based on Chromosomal Aberrations

Only sticky chromosomes were observed (Fig. 2), suggesting minimal genetic damage (Leme & Marin-Morales, 2009). A micronucleus assay comparing Estero de Paco and Estero de Vetas found Estero de Paco to be less genotoxic, with improved physico-chemical parameters (e.g., DO, BOD, pH, and lower heavy metals) indicating partial restoration success (Mikaella et al., 2021).

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Although roots exposed to Estero de Paco water showed significantly lower mitotic indices than the control, this did not correspond to increased chromosomal aberrations. Cytotoxicity and genotoxicity are not always directly correlated. For example, Alam, Wangkay, et al. (2016) reported no mitotic aberrations in *Allium cepa* exposed to 100% Pasig River water due to extreme toxicity eliminating viable cells. Similarly, Radić et al. (2010) found no strict correlation between cytotoxicity and genotoxicity near an artificial fertilizer plant, possibly due to the stimulatory effects of nutrients such as nitrate, nitrite, ammonium, and phosphate.



Figure 2: Sticky Chromosome

CONCLUSION AND RECOMMENDATION

Conclusion

The study found a significant difference in mitotic indices between *Allium cepa* grown in Estero de Paco water and control tap water, indicating the presence of cytotoxic substances. Pollution from sewage and solid waste, likely due to informal settlements lacking proper septage systems, may have contributed to the water's deterioration and observed cytotoxic effects. As a major tributary of the Pasig River, Estero de Paco requires urgent pollution management, continuous monitoring, and identification of harmful substances to guide appropriate treatment measures.

Recommendation

Future research should focus on comprehensive chemical analyses to identify specific pollutants and clarify their links to genotoxic effects, enabling more targeted rehabilitation strategies. Expanding bioindicators to include fish, aquatic invertebrates, and microbial assays would provide a broader ecological assessment. Considering environmental factors such as temperature, pH, and nutrient levels can improve research accuracy, while long-term monitoring will help evaluate water quality trends and rehabilitation progress. Additionally, promoting community involvement in monitoring and pollution management is essential for sustainable restoration efforts.

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