Genotoxicity and cytotoxicity induced by water and sediment samples from river systems in *Vicia faba* root cells

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INTRODUCTION

In the context of the Anthropocene, river ecosystems have been profoundly altered by the intensification of anthropogenic activities that modify their physical, chemical, and biological characteristics (Covarrubias-López et al., 2023). The objective of this study was to evaluate the genotoxic and cytotoxic potential of water and sediment samples from the Briones and Negros rivers (Tlaxcala, Mexico) through the application of the micronucleus (MN) test and the comet assay in *Vicia faba* cells during the rainy and dry seasons.

MATERIALS AND METHODS

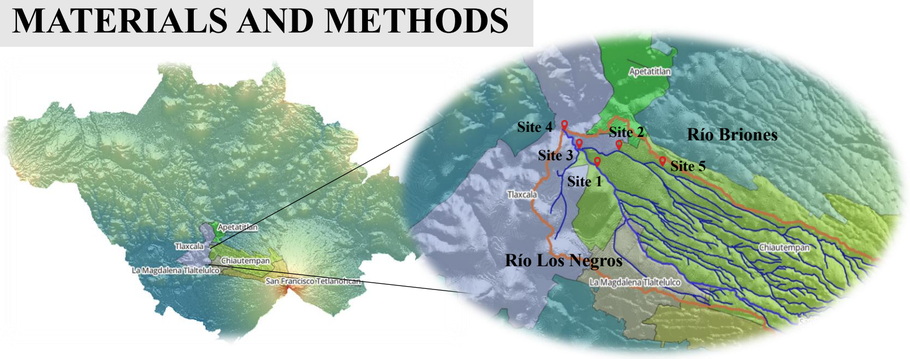
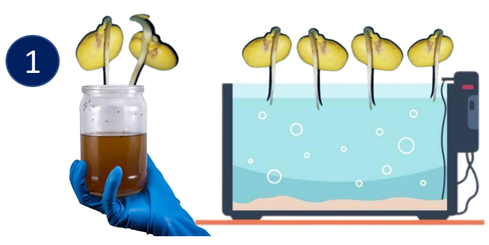


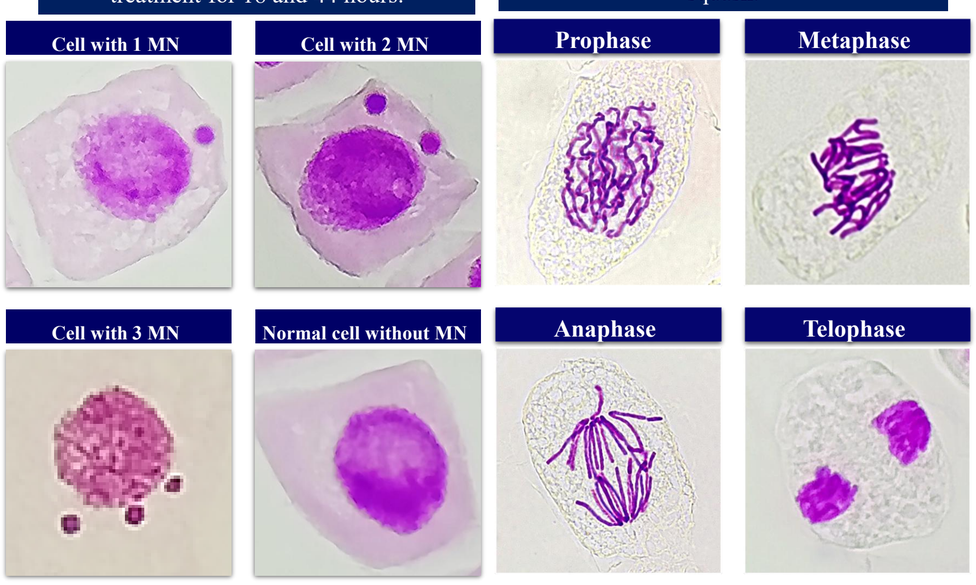
Figure 1. Sampling sites

Micronucleus assay in meristematic cells of the root of *Vicia faba*



Exposure for 4 hours and recovery treatment for 18 and 44 hours.

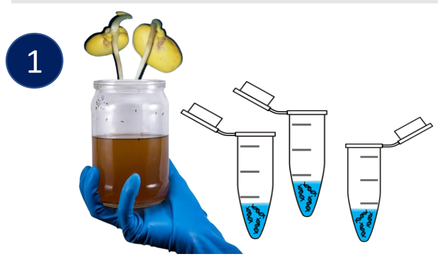
Feulgen staining and monolayer tissue squash.

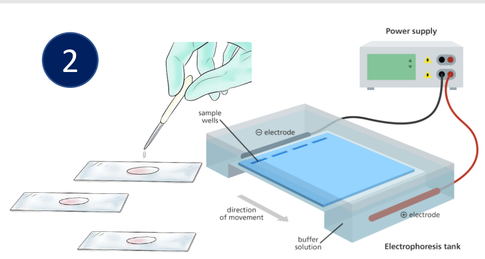


$$MN = \frac{Total\ number\ of\ MN}{Total\ number\ of\ cells\ analyzed} \text{ X } 100 \qquad IM = \frac{Total\ number\ of\ dividing\ cells}{Total\ number\ of\ cells\ analyzed} \text{ X } 100$$

The slides are analyzed with a Carl Zeiss brightfield microscope to visualize MN and IM, for which 1000 cells are counted per slide at 400x magnification.

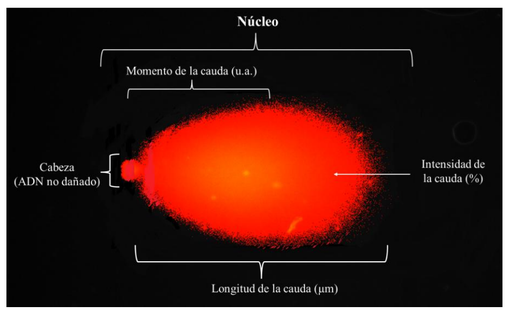
Single-cell alkaline electrophoresis technique (comet assay) in *Vicia faba* root cells





Exposure for 2 hours and obtaining a 300 µL suspension of nuclei (PBS, pH 7.4).

Lysis solution for 60 minutes at 4°C and alkaline electrophoresis at 25 V and 300 mA in dark conditions.



Staining with 70 µL of ethidium bromide and analysis with epifluorescence microscope and Comet Assay IV software.

RESULTS

Statistical analysis of the results was performed using GraphPad Prism 10.4.1 software. For both techniques, the micronucleus assay and the comet assay, a one-way ANOVA was applied, followed by Dunn's multiple comparison test with a 95% confidence interval. Differences were considered statistically significant when $P < 0.05$.

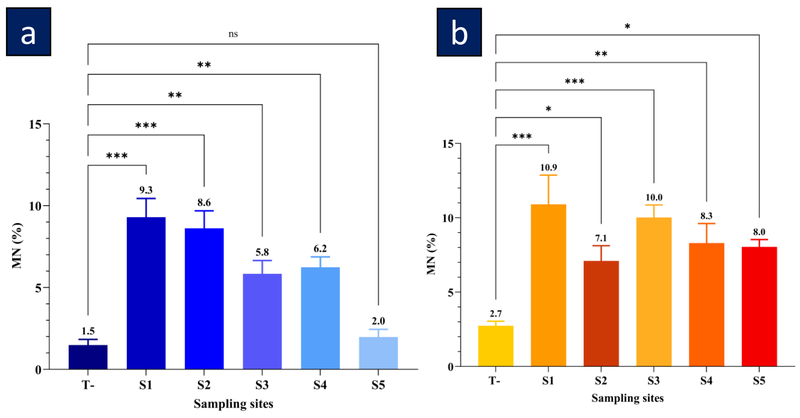


Figure 2. Frequency of MN in meristematic root cells of *Vicia faba*, induced by water samples collected during the rainy season (a) and the dry season (b).

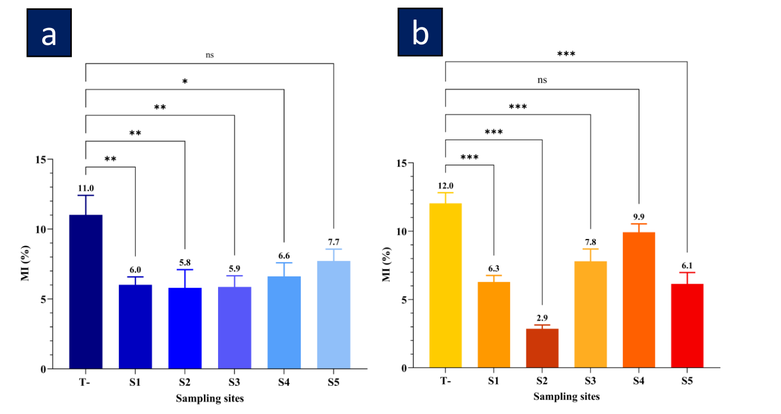


Figure 4. Mitotic index in meristematic root cells of *Vicia faba*, induced by water samples collected during the rainy season (a) and the dry season (b).

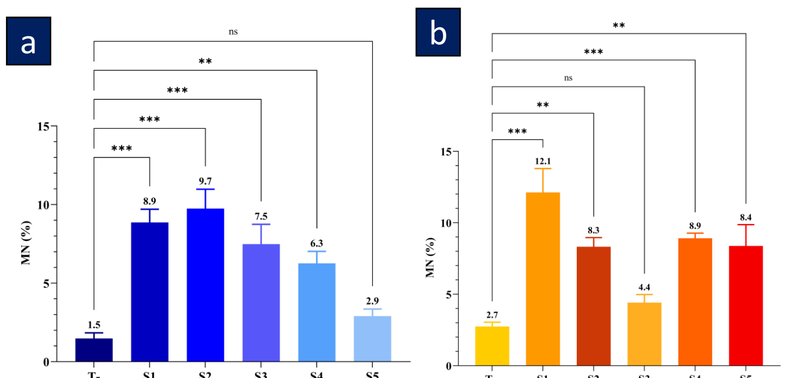


Figure 3. Frequency of MN in meristematic cells of the root of *Vicia faba*, induced by sediment samples collected during the rainy season (a) and the dry season (b).

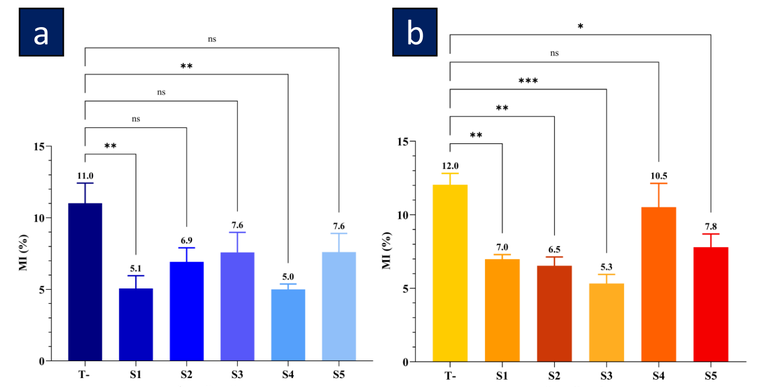


Figure 5. Mitotic index in meristematic root cells of *Vicia faba*, induced by sediment samples collected during the rainy season (a) and the dry season (b).

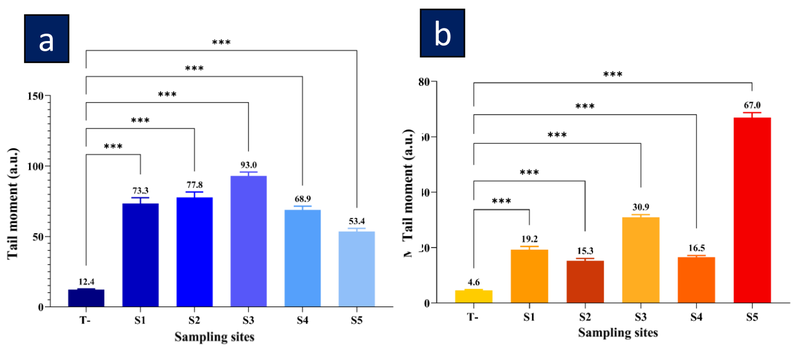


Figure 6. Tail fragmentation in root cells of *Vicia faba* induced by water samples collected during the rainy season (a) and the dry season (b).

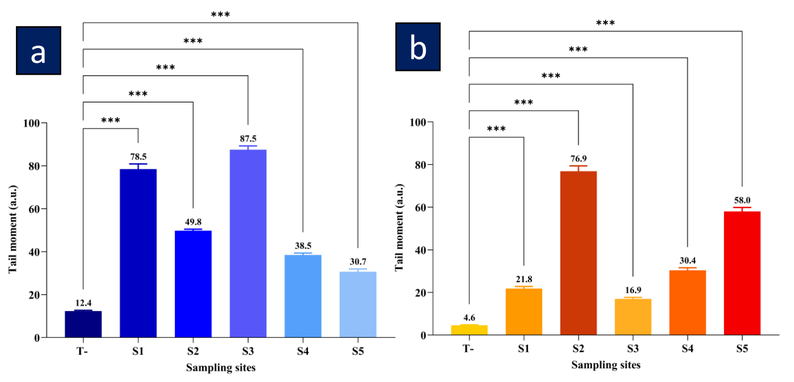


Figure 7. Tail fragmentation in root cells of *Vicia faba* induced by sediment samples collected during the rainy season (a) and the dry season (b).

DISCUSSION OF RESULTS

Water and sediment samples from the Briones and Negros rivers caused a significant increase in MN frequency and a reduction in the MI compared to the negative control. During the rainy season, the effect is attributed to surface runoff of contaminants; in the dry season, to their concentration and persistence within the fluvial system, as reported by studies such as Beltrán & González (2017) and Gomes et al. (2019).

Water and sediment samples induced significant DNA fragmentation in *Vicia faba* root cells, demonstrating genotoxic damage in both environmental matrices and across seasons. This confirms the accumulation and persistence of contaminants with genotoxic potential within the fluvial system. Similar findings have been reported in fish (Torres et al., 2014) and in sediment-exposed cell lines (García et al., 2017).

CONCLUSIONS

The results demonstrate the presence of contaminants with genotoxic and cytotoxic potential in water and sediment from the Briones and Negros rivers across both seasons. This highlights the need for continuous monitoring and restoration efforts in fluvial systems.

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