Original Article

LIMNOLOGY FRESHWATER BIOLOGY

Biotesting of a freshwater (river) ecosystem water quality by genetic markers of *Tradescantia* model test system

Avalyan R.E.[1]*, Asatryan V.L.[2], Aroutiounian R.M.[1], Atoyants A.L.[1]

[1] *Research Institute of Biology, Yerevan State University, 8, Charents Str., 0025, Yerevan, Armenia*
[2] *Scientific Center of Zoology and Hydroecology, National Academy of Sciences of the Republic of Armenia, 7, P.Sevak Str., Yerevan 0014, Armenia*

ABSTRACT. In the field of assessing the quality of the aquatic environment, the use of biotesting methods is a mandatory element of environmental monitoring. Plant model test systems, such as *Tradescantia* clones, are used for biotesting of water quality in freshwater river ecosystems. To study the level of clastogenicity of the water samples of the Akhuryan River (Armenia), collected at five sampling points along the river in the summer, a test for micronuclei in the pollen mother cells of *Tradescantia* clone 02 (micronucleus assay – Trad-MCN) is used. The following two marker test-criteria were taken into account: the frequency of tetrads with micronuclei (Tetr/MCN) and the frequency of micronuclei in tetrads (MCN/tetr). The study of clastogenic effects in the sporogenic cells of Tradescantia showed increase in the frequency of occurrence of both test criteria in all studied water samples compared to the background with maximum manifestation in the Akhurik sample ($p < 0,01$). A reliable positive correlation was shown between the marker criteria of the micronucleus assay and the chemical elements: HCO_3, CO_3, B, Mg, Ca, As, Se ($p < 0.01$).

Keywords: biotesting, freshwater ecosystem (river), mutagenicity, *Tradescantia*

For citation: Avalyan R.E., Asatryan V.L., Aroutiounian R.M., Atoyants A.L. Biotesting of a freshwater (river) ecosystem water quality by genetic markers of *Tradescantia* model test system // Limnology and Freshwater Biology. 2025. - № 4. - P. 409-413. DOI: 10.31951/2658-3518-2025-A-4-409

1. Introduction

River ecosystems, as natural objects and main sources of fresh water, play a major role in nature and human life. As a rule, the quality of the aquatic environment depends on both natural and anthropogenic factors. The study of river ecosystems, which are under constant anthropogenic pressure, is of particular relevance for Armenia due to the increasing need for quality control and improved water resource management (Dallakyan and Asatryan, 2021; Avalyan et al., 2021; Khosrovyan et al., 2022)

Due to the constantly changing chemical composition of river water and active the physical and chemical factors, mutagenic activity is not constant and may vary. In this regard, it becomes necessary to determine the total load on the river ecosystem, which is the effect of combinations of mutagens and toxicants. To study the genetic effects of the consequences of the complex action of pollutants on river ecosystems and living organisms, it is very important to conduct genetic monitoring using sensitive test objects (Duan et al., 1999; Ma et al., 2005; Kwasniewska et al., 2013; Vergolyas and Goncharuk, 2016).

To study the genetic effects of the complex impact of pollutants on freshwater ecosystems genetic monitoring using sensitive test objects is highly relevant.

Among bioindicator plants, a special place is occupied by *Tradescantia* (clone 02), which is widely used in the genetic monitoring system to detect the mutagenic potential of xenobiotics in the aquatic environment. *Tradescantia* (clone 02) is a convenient test object for studying the effect of not only low doses of physical factors, but also chemical mutagens and environmental toxicants. The main advantages of *Tradescantia* as a test system are high sensitivity to external factors with a low spontaneous mutation rate, accuracy and ease of registration of biomarkers reflecting the external impact of xenobiotics (Majer et al., 2005; Scalon et al., 2013; Campos et al., 2024). *Tradescantia* (clones 02 and 4430) performs well in dose-response studies and as a biodetector of chemical mutagens in different environmental compartments even at low chemical levels (Crispim et al., 2012; Rocha et al., 2018). To study the impact of

*Corresponding author.
E-mail address: re avalyan@mail.ru (R.E. Avalyan)

Received: June 03, 2025; ***Accepted:*** July 27, 2025;
Available online: August 31, 2025

pollutants from reservoirs and rivers, industrial wastewater, or power plant discharges, genetic monitoring can be done either by growing plants in nearby fields or by collecting water samples for testing in laboratory and greenhouse (Zeng et al., 1999; Crebelli et al., 2005; Aghajanyan et al., 2020)

Micronucleus (MN) assays with early pollen tetrad cells of *Tradescantia* (Trad-MCN assay) are at present the most widely used bioassays with plants for the detection of genotoxins in the water environment. Using chromosome damage as an indicator of the carcinogenic properties of environmental xenobiotics, the Trad-MCN bioassay is a rapid and effective tool for screening aquatic pollutants (Ma, 2001; Garcia et al., 2011; Mizik et al., 2013).

Genotoxic components of the aquatic environment can cause primary DNA damage. Mutations occur on both, the gene level (gene mutations) and on the chromosomal level (structural aberrations). The pollutants can promote breaks in the genetic material are known as clastogenic and those who undertake to chromosome disjunction during division are aneugenic. Micronuclei and bridges are examples of results clastogenic effects (Majer et al., 2005).

The Akhuryan River is the longest river in Armenia (186 m), originating at an altitude of 2023 m, flows out of the Arpilich Reservoir and flows into the Araks River. The Akhuryan River flowing in the north of the republic near the city of Gyumri. The presence of dams along the river, large-scale water releases from the Arpilich reservoir, and active economic activity in the catchment area create a rather complex ecological situation in the water area of the river ecosystem.

The aim of this work was biotesting the potential mutagenicity of water samples from the Akhuryan River in its upper reaches in the summer using bioassay Trad-MCN of the model test object *Tradescantia* (clone 02).

2. Material and methods

Sampling sites

Water samples were collected from five water sampling taken points along the Akhuryan River in the summer. The water samples site were labeled: A1 – source of the Akhuryan River; A2 – Mets Sepasar; A3 – Amasya Dzor; A4 – Jradzor; A5 – Akhurik (Fig.1). Three water samples were taken from each water intake point. The samples were collected at a depth of approximately 30-50 cm in three replicates per sampling site in 1 L pre-cleaned plastic bottles. Samples were stored at + 4 °C until the assay was carried out. Purified tap water was used as a conditional background water sample (control).

Trad-MCN bioassay

To determine the mutagenicity level of the studied water samples, a micronucleus test (Trad/MCN) of the clone 02 of *Tradescantia* was used. The bioassay was carried out according to the standard protocol (Ma et al., 1994). The application of this assay allows recording the occurrence of chromosomal aberrations (acentric fragments or lagging chromosomes), which are registered as micronuclei (MCN) at the stage of early microspore tetrads of pollen mother cells at disturbances in the process of microsporogenesis (Mizik et al., 2011; 2013) (Fig.2). Young inflorescences of Tradescantia loaded into the studied water samples for 24 hours. Then young buds were removed and fixed in Carnoy’s solution (3 ethyl alcohol: 1 acetic acid). The micronuclei appearance was fixed by observing 300 tetrads per slide, using an optical microscope (Motic Images Swift M10L) at 400x magnification. For each sample 3000 tetrads were analyzed. The following two marker test-criteria were taken into account: the frequency of tetrads with micronuclei (Tetr/MCN) and the frequency of micronuclei in tetrads (MCN/tetr). The micronuclei frequency was calculated per 100 tetrads.

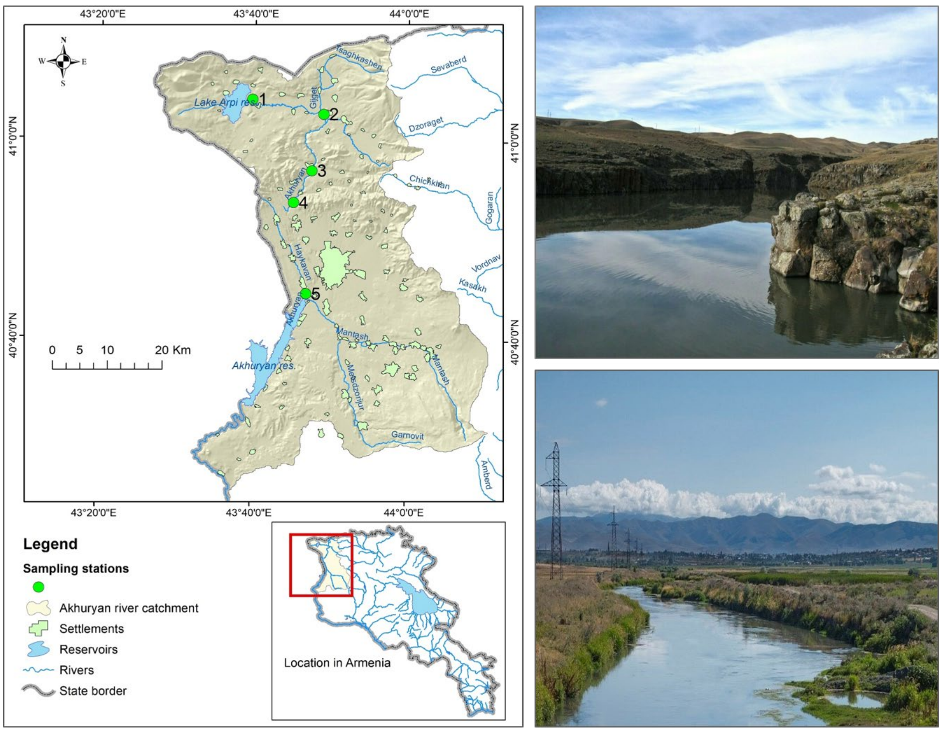

Fig.1. Map of water sampling sites and the Akhuryan River.

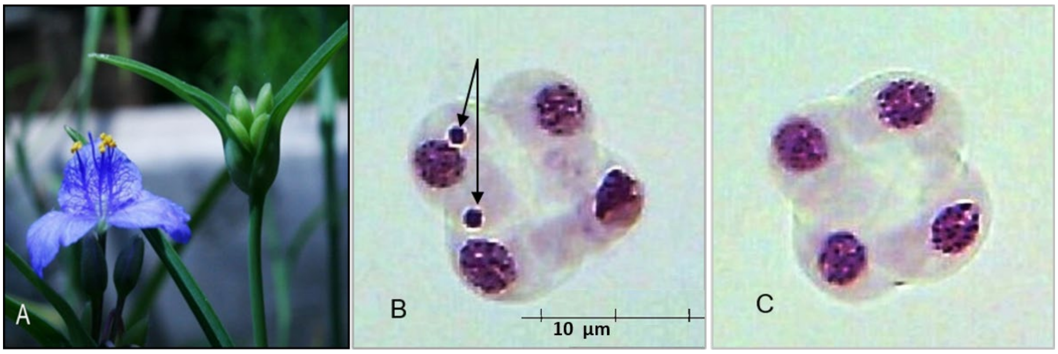

Fig.2. Flower and bud of *Tradescantia* (clone 02) (A), early pollen tetrads with MCN (arrow) (B) and without MCN (C).

Chemical and statistical analysis

When determining water quality, a combination of physical and chemical indicators and other properties are taken into account and therefore it is extremely necessary to chemical analyze the composition of the main components of the aquatic environment, especially in freshwater ecosystems. (OSPAR Commission, 2002; Current issues of water toxicology, 2004)

Water sampling was carried out by the Hydrobiology laboratory of the Scientific Center of Zoology and Hydroecology of the National Academy of Sciences of Armenia. Chemical analysis was carried out in the analytical laboratory of Hydrometeorology and Monitoring Center of the Ministry of Environment of Armenia. The results of the center are published in the form of seasonal and annual reports (http://mnp.am/en/environment/environmental-monitoring).

Some elements total concentration were measured: HCO_3, SO_4, NO_3, NO_2, NH_4, PO_4, F, N, Na, K, Mg, P, Ca, Mn, V, Fe, Co, Cr, Cu, Ni, Zn, Ti, As, Mo, Cd, Se.

Pearson correlation analysis was performed between the frequency of micronuclei and the concentration of chemical elements in the studied water samples. All obtained results were statistically processed using the Student *t*-test by the *Statgraphics Centurion 16.2* computer program (StatPoint Technologies, Inc. USA; Warrenton, VA).

3. Results and discussion

Tradescantia micronucleus bioassay is considered to be the most sensitive, as meiotic chromosomes are more susceptible to mutagens and toxins than mitotic ones. For study the level of clastogenicity of the aquatic environment, a test for micronuclei in the pollen mother cells of *Tradescantia* clone 02 (micronucleus assay – Trad-MCN) is used.

The study of clastogenic effects in sporogenous cells of *Tradescantia* showed a significant increase of the occurrence frequency of both test criteria (Tetr/MCN and MCN/tetr) in all studied water samples compared to the background level. (Fig.3).

The highest frequency of these test markers was observed in sample A5 (Akhurik), and exceeded the background level by 4–5 times ($p < 0.01$) respectively. A relatively low level of clastogenic effects was observed in water sample A1 (source of the Akhuryan River) and exceeded the background level by 2 times ($p < 0.05$).

In genetic monitoring, correlation analysis is an important statistical tool for determining the dependence of the frequency of genetic markers on the content of chemical components. A reliable positive correlation was shown between the marker criteria of the micronucleus test and the chemical components: HCO_3, CO_3, B, Mg, Ca, As, Se ($p < 0.01$) (Table 1).

The increase in the level of observed genetic effects, especially in the lower reaches of the studied section of the Akhuryan River trajectory, may be due to the unevenness of technogenic loads containing toxic components on the water area of the river ecosystem, as well as the seasonal specificity of pollution. Sampling point A5 (Akhurik) is located along the Akhuryan River after the large city of Gyumri. At the same time, this section is possibly subject to multifactorial anthropogenic impact under the influence of backflow from nearby irrigated lands and industrial and domestic wastewater entering the river network in this section of the river. All these factors make a significant contribution to the anthropogenic load on the quality of the Akhuryan River water.

Among the chemical elements with which a positive correlation was found, only the metalloid arsenic (As) has pronounced carcinogenic properties. Arsenic is a very strong biological xenobiotic, which has a toxic effect not only on plants, but also on other living organisms. (ATSDR, 2019). Also, according to the chemical composition of water samples, the concentration of arsenic at water sampling points A3, A4 and A5 exceeded the maximum permissible concentrations (MPC).

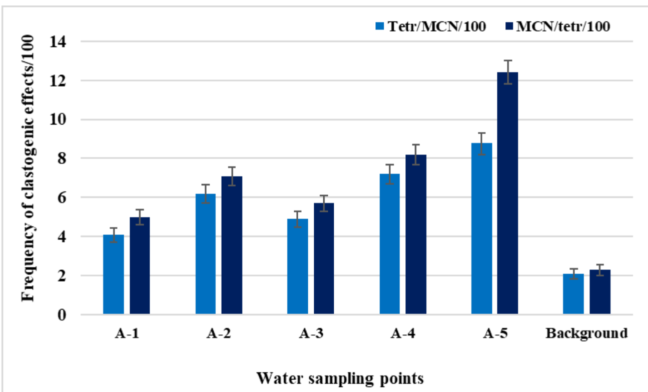

Fig.3. The level of clastogenicity of the Akhuryan River water samples Statistically significant at: $p < 0,01$.

Table 1. Correlation coefficient between micronucleus test criteria and concentration of chemical components

	HCO_3	CO_3	B	Se	Mg	Ca	As
MCN/tetrads	0.74*	0.74*	0.75*	0.58	0.64*	0.67*	0.74*
Tetrads/MCN	0.78*	0.81**	0.74*	0.63*	0.69*	0.71*	0.72*

Note: *– p < 0,05; **– p < 0,01

Dangerous concentrations of arsenic can accumulate in groundwater due to anthropogenic pollution and enter freshwater ecosystems. It is possible that in this case arsenic (along with other components) may have an indirect toxic effect by increasing the level of genetic effects in the sporogenous cells of Tradescantia.

The constant increase of pollutants in river waters, especially adjacent to regions with a high level of agricultural and industrial production, leads to the need and expediency of conducting constant eotoxicological monitoring of natural waters using reliable test systems and biomarkers, in this case, bioassays of *Tradescantia*, to assess the potential toxicological risk of pollutants present in water for the health of living organisms and humans.

4. Conclusion

According to the results of the study, an increase in the frequency of micronuclei formation, as a result of an increased degree of chromosome damage during microsporogenesis, indicates the presence of a clastogenic effect exerted by water samples of the river on sporogenous cells of *Tradescantia*. The increased clastogenic activity of the Akhuryan River water is apparently due to the unevenness of domestic and man-made loads, as well as the content of a complex of chemical and toxic components in the river water, especially outside the city of Gyumri.

The use of the Trad-MCN test of *Tradescantia* (clone 02) is appropriate for an adequate assessment of the mutagenicity level of freshwater (river) ecosystems.

Acknowledgements

This work was carried out jointly with the Hydrobiological laboratory of Scientific Center of Zoology and Hydroecology, in the framework of the research project by the Higher Education and Science Committee of RA (22RL-023).

Conflict of Interest

The authors declare no conflicts of interest.

References

Aghajanyan E., Avalyan R., Atoyants A. et al. 2020. Assessing freshwater ecosystem using *Tradescantia model* test object. Water Air Soil Pollution 231–244: 1-8. DOI: 10.1007/s11270-020-4407-3

ATSDR. 2019. Agency for Toxic Substances and Disease Registry. Substance Priority List. CERCLA. URL: https://www.atsdr.cdc.gov/toxicological-profiles/about/index.html

Avalyan R.E., Aghajanyan E.F., Atoyants A.L. et al. 2021. Bioassay of water from river systems of Armenia. Theoretical and Applied Ecology 1: 97–103. DOI: 10.25750/1995-4301-2021-1-097-103

Campos Júnior E.O., Campos J.M.S., Dias R.J.P. et al. 2024. Novelties on *Tradescantia*: Perspectives on water quality monitoring. Chemosphere 368: 143-732. DOI: 10.1016/j.chemosphere.2024.143732

Crebelli R., Conti L., Monarca S. et al. 2005. Genotoxicity of the disinfection by-products resulting from peracetic acid- or hypochloritedisinfected sewage wastewater. Water Research 39: 1105–1113. DOI: 10.1016/j.watres.2004.12.029

Crispim B.A., Vaini J.O., Grisolia A.B. et al. 2012. Biomonitoring the genotoxic effects of pollutants on Tradescantia pallida (rose) DR hunt in Dourados, Brazil. Environmental Science and Pollution Research 19 (3): 718-723.

Current issues of water toxicology. 2004. In: Flerov B.A. (Ed.). Borok: Institute of Inland Water Biology, Russian Academy of Sciences. (in Russian)

Dallakyan M.R., Asatryan V.L. 2021. Studying macrobenthic community and assessing the ecological status of the Tandzut River for improving hydrobiological monitoring system in Armenia. Ecosystem Transformation 4 (4): 24-31. DOI: 10.23859/estr-210522

Duan C.Q., Bin Hu Z.H., Wang C.H. et al. 1999. Tradescantia bioassays for the determination of genotoxicity of waters in the Panlong River, Kunming, People´s Republic of China. Mutation Research 426:127-131. DOI: 10.1016/s0027-5107(99)00054-8

Garcia A.C., Marcon A.E., Ferreira D.M. et al. 2011. Micronucleus study of the quality and mutagenicity of surface water from a semi-arid region. Journal of Environmental Monitoring 13 (12): 3301–3488. DOI: 10.1039/c1em10582e

Khosrovyan A., Avalyan R., Atoyants A. et al. 2022. Assessment of the mutagenic potential of the water of an urban river by means of two Tradescantia – based test systems. Mutation Research–Genetic Toxicology and Environmental Mutagenesis 876–877: 1–7. DOI: 10.1016/j.mrgentox.2022.503449

Kwasniewska J., Jaskola R., Maluszynska J. 2013. Cytogenic tests in the assessment of the genotoxicity of river. Water, Int.J.Environ.Research 7: 869-876. DOI: 10.22059/ijer.2013.668

Ma T.H., Cabrera G.L., Chen R. et al. 1994. Tradescantia micronucleus bioassay. Mutation Research 310: 220–230. DOI: 10.1016/0027-5107(94)90115-5

Ma T.H. 2001. *Tradescantia* micronucleus bioassay for detection of carcinogens. Folia Histochemica et Cytobiologica 39(2): 54-55.

Ma T.H., Cabrera G.L., Owens E. 2005. Genotoxic agents detected by plant bioassays. Reviews on Environmental Health 20: 1–13. DOI: 10.1515/reveh.2005.20.1.1

Majer B.J., Grummi N., Uhl M. et al. 2005. Use of plant bioassay for the detection of genotoxins in the aquatic environment. Acta hydrochimica et hydrobiologica 33: 45–55. DOI: 10.1002/aheh.200300557

Misik M., Ma T.H., Nersesyan A. et al. 2011. Micronucleus assays with Tradescantia pollen tetrads: an update. Mutagenesis 26 (1): 215–221. DOI: 10.1093/mutage/geq080

Mizik M., Pichler C., Rainer B. et al. 2013. Micronucleus Assay with Tetrad Cells of Tradescantia. In: Dhawan A., Bajpayee M.(eds.), Genotoxicity Assessment: Methods and Protocols, Methods in Molecular Biology. New York: Springer Science, 1044: pp. 405–415. DOI: 10.1007/978-1-62703-529-3_22

Rocha A., Candido L.S., Pereira J. et al. 2018. Evaluation of vehicular pollution using the TRAD-MCN mutagenic bioassay with *Tradescantia pallida* (Commelinaceae) Environmental pollution 240: 440–447. DOI: 10.1016/j.envpol.2018.04.091

Scalon M.C., Rechenmacher C., Siebel A.M. et al. 2013. Genotoxic potential and physicochemical parameters of Sinos River, southern Brazil. The Scientific World Journal 2013(1): 1-6. DOI: 10.1155/2013/209737

Survey on Genotoxicity Test Methods for the Evaluation of Waste Water within Whole EffluentAssessment. 2002. In: OSPAR Commission. URL: https://www.ospar.org/documents?v=6932

Vergolyas M.R., Goncharuk V.V. 2016. Evaluation of water quality control by using test organisms and their cells. Journal of Water Chemistry and Technology 38(1): 62–66.

Zeng D.M., Li Y., Lin Q.Q. 1999. Pollution monitoring of three rivers passing through Fuzhou City, People’s Republic of China. Mutation Research 426:159–161. DOI: 10.1016/s0027-5107(99)00061-5