Clastogenecity evaluation of water of Lake Sevan (Armenia) using *Tradescantia* micronucleus assay


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**HIGHLIGHTS**
- We tested clastogenecity of the water of a natural lake using *Tradescantia* assay.
- Clastogenic effects in pollen microspores might be linked to Co and Ni ions.
- A comparison was made with the results of another *Tradescantia*-based test.
- Occurrence of micronuclei in microspores coincided with that of dwarf stamen hair.

**ABSTRACT**

The clastogenic effects of water samples in seven locations of Lake Sevan (Armenia) with the application of Trad-MCN (micronuclei) bioassay using *Tradescantia* (clone 02) were investigated. A significant increase in the frequency of micronuclei in tetrads of pollen microspores and tetrads with micronuclei exposed to the test samples compared to the control has been revealed. A multivariate analysis indicated linkage between the frequencies of occurrence of micronuclei in the cells and Ni and Co ions. The results were compared with the endpoints of another *Tradescantia*-based test system (stamen hair mutation test) performed on the same water samples and generation of the plant: occurrences of micronuclei in sporogenic cells coincided with that of non-surviving stamen hair.

**1. Introduction**

The aquatic environment is the recipient of different contaminants, a large proportion of which could have genotoxic/carcinogenic potential (Claxton et al., 1998). Many hazardous substances could occur in concentrations beyond their detection limit, despite substantial improvements in methodologies and techniques for determination of low concentrations. Therefore, in parallel with the chemical analysis of water it is essential to evaluate its toxicity using appropriate biological responses. It was shown that plant bioassays are sensitive systems to detect the genotoxic and clastogenic effects of environmental pollutants (Majer et al., 2005). Among them *Tradescantia* bioassays *Tradescantia* micronucleus (Trad-MCN) and *Tradescantia* stamen hair mutation (Trad-SHM) are widely used for evaluation of genotoxicity and clastogenicity of natural reservoirs contaminated with heavy metals (Ma, 1999; Majer et al., 2002; Steinkellner et al., 1998), organic compounds (Kim et al., 2003), agrochemicals (Mohammed and Ma, 1999; Rodrigues et al., 1998) and also for mixtures of pollutants (Duan et al., 1999). Both assays are considered as valuable systems for revealing and monitoring a wide range of toxic agents in soil, air and water due to the simplicity of their procedure and high...
sensitivity (Aroutiounian, 2006; Batalha, 1999; Cesniene et al., 2010; García et al., 2011; Misik et al., 2011, 2016; Simonyan et al., 2016; Thewes et al., 2011). An increase in the level of micronuclei frequency in Trad-MCN test was found in microspore tetrads of *Tradescantia* treated with municipal wastewater, as well as groundwaters contaminated with industrial wastewater (Monarca et al., 2000; Haider et al., 2002; Biscardi et al., 2003).

Lake Sevan is one the largest freshwaters in the world, playing a unique role in the economic development of Armenia. The lake is fed by 28 rivers that drain residential, agricultural and industrial areas. Despite this, only hydrochemical (nutrients, metals, metalloids) and hydrobiological (quantitative and qualitative analysis of the food web) monitoring is regularly conducted in the lake’s basin. The aim of this study was to assess the clastogenic potential of water from Lake Sevan by Trad-MCN bioassay. A comparison was also conducted with the results of *Tradescantia* stamen hair mutagenesis test (Trad-SHM) performed in the earlier work (Avalyan et al., 2017) with the same water samples and the generation of *Tradescantia* (clone 02).

2. Material and methods

2.1. Study area and sampling

Lake Sevan, one of the largest and most exploited alpine limnosystems in the world. Anthropogenic pressures on the lake include water extraction since the 50s (which has resulted in the water level dropping by around 20 m and the drying out of the spawning grounds of two-subspecies of endemic trout), watershed development (which resulted in an increased organic input from domestic and industrial effluents), and overfishing (which resulted in the depletion of the commercial stock). The lake has turned from an oligotrophic “trout” water body into a mesotrophic “carp” reservoir. The problem of ineffective use and pollution of the ecosystem is urgent and an improvement of the ecological situation in the lake basin is extremely important (Hovhanissyan, 1994; Lake Sevan, 2016; Matishov et al., 2016).

Water samples were collected during Spring 2016 from seven sites of Lake Sevan, Armenia: Artanish, Karchaghbyur, Tsapatakh, Noratus, Litchk, Martuni and Masrik (Fig. 1). The samples were collected by hand from approximately 30 cm depth from three nearby locations per sampling site in 1 L pre-cleaned plastic bottles per location. Samples were stored at +4 °C until delivery to the laboratory; tests were initiated next day. More detailed description of the sites can be found in Avalyan et al. (2017).

2.2. Trad-MCN bioassay

*Tradescantia* (clone 02) used for this study was obtained from the greenhouse of Yerevan State University. It is an interspecific hybrid *T. occidentales* and *T. ohiensis* (Ma et al., 1994), having a multitude of sprouts where inflorescences develop. The test is based on the scoring of MCN frequency in pollen mother cells at disturbances in the process of microsporogenesis (Ma et al., 1994; Misik et al., 2011). Formation of MCN is related to chromosomal instability and is indicative of genomic damage.

The contents of all bottles with sampled water (totally, 3 L) per site were mixed together and the sub-samples of 150 mL were taken for testing. The tests were conducted at room temperature, according to Klumpp et al. (2004). Five-seven young inflorescences of *Tradescantia* were dipped into the sub-samples. The duration of treatment was 24 h (18/6 day/night cycle). The inflorescences were removed and fixed in Carnoy’s solution (3:1 ethanol-glacial acetic acid). Preparation of the tetrads and the scoring of the micronuclei were carried out as described by Ma et al. (1994). The following test criteria were used: the number of tetrads with micronuclei (Tetr/MCN) and the number of micronuclei per 100 tetrads (MCN/tetr). For each water sample 3000 tetrads were analyzed. Tap water was used as a control and test vessels were set in triplicate.

2.3. Chemical and statistical analysis

Water samples were analyzed for chemical composition by a certified laboratory, following standard methods (APHA, 1998).
Several elements were measured (total concentrations): Al, Ni, Zn, As, Cu, Fe, Cr, Co, Mo, Cd, Sn, Pb, Mg, Mn, Na, Ca, K. pH level at all sites varied from 8.6 to 8.7. Electrical conductivity varied from 650 to 846 μS/cm: Artanish – 650, Karchaghbyur – 690, Noradus – 846, Masrik – 733, Tsapatakh – 699, Litchk – 752, Martuni – 840. Electrical conductivity of the tap water control was less than 200 μS/cm, pH 8.3.

Chemical data were compared to the legislated water quality standards for aquaculture (from the Environmental Impact Monitoring Center of the Ministry of Nature Protection of Armenia, www.armmonitoring.am), USEPA aquatic life guidelines and WHO drinking-water quality standards (Table 1). The details of chemical analysis were published by Avalyan et al. (2017).

Significant differences between the endpoints, observed in the samples and the control were revealed by the independent t-test with the help of STATGRAPHICS Centurion16.2 statistical program (StatPoint Technologies, Inc. USA; Warrenton, VA). The data are expressed as mean ± standard error of mean (SEM). Normality and homogeneity of variances were checked prior to the analysis to conform with the assumptions of the test.

Principal component analysis (PCA) with Varimax as the extraction method was selected as a multivariate analysis method to reveal linkages among the endpoints of the bioassay and contaminant levels. PCA test also allowed to identify associations between data and sampling locations. For interpreting linkages, loadings more than 0.4 (by absolute value) were considered and for the associations of parameters with the sampling locations positive factor scores were used.

3. Results and discussion

One can see that concentrations of many ions (Al, Mg, Cr, Cu, V) exceeded standard levels in almost every station, except for Masrik and Martini where only V and Cr levels were higher than the standards (Table 1). Clastogenic effects in the generative sphere of Tradescantia showed a significant increase in the frequency of the occurrence of MCN in tetrads and the number of tetrads with micronuclei in all studied samples compared to the control (Fig. 2 a,b). The maximum manifestation of the clastogenic effects by both criteria was observed in water samples Masrik, Martuni, Tsapatakh, Lichk. These results are in good agreement with the gene mutations in the somatic cells of Tradescantia detected by the Trad-SHM test (Avalyan et al., 2017). However, higher clastogenic responses were revealed in Masrik site by Trad-MCN, while the Trad-SHM test detected a higher number of somatic mutations in Noradus and Litchk (recessive mutation events, RME), Litchk and Tsapatakh (white mutation events, WME) and morphological changes (dwarf or non-surviving stamen hair, NS) in Masrik. On the contrary, both tests similarly identified the samples with a smaller number of clastogenic and genotoxic responses - Artanish and Karchaghbyur.

It should be noted that both criteria of the Trad-MCN test characterized the samples in a similar manner: higher number of clastogenic effects in Masrik, followed by almost same-level responses in other stations and with the lowest in Artanish (Fig. 2a b). The previous Trad-SHM test revealed a similar distribution of NS responses across the stations (Avalyan et al., 2017).

The multivariate analysis indicated a possible linkage between NS, WME, Tet/MCN and Ni and Co with relevance to Masrik and Noradus (Fig. 3a and b). Co ions were also associated with all the biological responses in Tsapatakh, Noradus, Litchk, Masrik. However, in Tsapatakh and Litchk, none of the other metal ions (Al, As, K, Mg, Ca, Cu, Cr and Fe) could be connected to the biological effects (Fig. 3c). Increasing levels of Ni from anthropogenic sources in different environments can cause deleterious effects in living organisms (Nickel Institute, 2011). In several studies with application of Trad-MCN (Misik et al., 2011; Pedro-Escher et al., 2014) and other plant objects (Kieling-Rubio et al., 2012) the toxicity of Ni among other metal contaminants was expected. Opposite results, where Ni did not increase the micronuclei frequency in the pollen tetrads of Tradescantia plants are also known (Fargasova et al., 2015). To the best of our knowledge, the toxicity of Co and Ni to aquatic organisms received little attention, possibly because of difficulties in revealing the mechanisms of toxicity especially in plants (e.g., Blaherao et al., 2015). However, in our earlier research these two metals were also related to the mutation events in the stamen hair

### Table 1

Total concentrations of chemicals in different parts of Lake Sevan and control, national water quality standards for aquaculture, USEPA aquatic life criteria for continuous concentrations and WHO drinking-water quality standards. Marked area show levels exceeding standards.

<table>
<thead>
<tr>
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<th>Karchaghbyur</th>
<th>Noradus</th>
<th>Masrik</th>
<th>Tsapatakh</th>
<th>Litchk</th>
<th>Martuni</th>
<th>Control (tap) water</th>
<th>National water quality standards for aquaculture (since 1990)</th>
<th>Aquatic life criteria for continuous concentration (USEPA)</th>
<th>Guidelines for drinking water quality (WHO)</th>
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- Standards not available.
- Cr(III)/Cr(VI).
- Depends on geographic region (typically, 1–6 µg L⁻¹) (WHO, 2000).
of *Tradescantia* in Tsapatakh, Artanish and Karchaghbyur samples (Avalyan et al., 2017), suggesting that the exposure pathway employed in our studies (inflorescences submerged into water) could provide important information for understanding the effects of dissolved metals. Further research on the uptake of ionic Ni and Co (including their combination) via different exposure pathways may elucidate their toxicity and mechanisms.

Further, in Masrik and Martuni samples, V and Cr concentrations exceeded the standards (Table 1). However, the results of PCA did not associate biological responses with V neither in these nor any other sample, although highest occurrences of micronuclei (Tetr/MCN and MCN/tetr) in microspores of *Tradescantia* were found in the above-mentioned samples. This may suggest the high sensitivity of the test to V and Cr in general or to their particular chemical forms. Knasmuller et al. (1998) reported clear dose-effect responses obtained from the *Tradescantia* pollen MCN test when exposed to Cr$^{6+}$ and Ni$^{2+}$ (in contrast to Cr$^{3+}$), although the concentrations tested were much higher than those in our study. Alternatively, a presence of other agents not measured in this work cannot be excluded.

Importantly, the levels of many metals in water (e.g., Ni, Co, Mn, Fe, As, Mo, Pb) did not exceed national regulatory standards. However, effects may be observed at concentrations far below those predicted to be safe by regulatory frameworks (Liess et al., 2016). Furthermore, *Tradescantia* test systems are known for their high performance at low concentrations and the present results have also shown this. Moreover, the toxicity of a mixture depends not only on the exposure concentration of each mixture constituent and its ratio but also on the means of the toxicants to act jointly (Altenburger et al., 2004). A chemical mixture of metals appears more toxic than individual metals if their action is synergistic. On the other hand, the antagonistic effects of some metals in a mixture diminish the uptake of individual metals (Barata et al., 2002; Panda and Panda, 2002) e.g., by competing for the same uptake sites at the cell surface (Bervoets and Blust, 2000; Qu et al., 2013).

**Fig. 2.** Frequency of clastogenic effects in the sporogenous cells of *Tradescantia* (clone 02) exposed to water samples from different zones of Lake Sevan basin. a – frequency of tetrads with micronuclei, b – micronuclei in tetrads. ***p < 0.001 - significant differences from the control.
The results of PCA in this study were not always connected to the higher occurrences of micronuclei in tetrads and tetrads with micronuclei. However, the genotoxicity of contaminants in an environmental sample should to be assessed as a whole, not for each component (Duan et al., 1999). The responses produced by the Trad-MCN test had the same pattern as NS responses of the Trad-SHM test across the samples. This implies that these responses (although targeting different cells) can be induced in Tradescantia inflorescences by the same agents in the water, assuming the collective effect of more than individual metals, even at low concentration.

4. Conclusion

In this work, we intended to identify toxicants in complex mixtures by linking exposure information to biological effects. According to the results of our studies, a marked increase in the level of clastogenicity in the water samples of Lake Sevan has been detected. This points out to the presence of toxic agents that are able to influence the processes occurring in the generative sphere of Tradescantia. It also indicates the effectiveness of the application of Trad-MCN bioassay for the assessment of clastogenicity effects of natural waters, especially at low level of metal contaminants. Tetr/MCN and MCN/tetr endpoints similarly identified the impact of the lake's water on microspores of the Tradescantia. When compared to the results of Trad-SHM test, it showed a similar pattern to NS occurrences across the sites. Both tests demonstrate high sensitivity to environmental agents in the lake that are able to cause mutations in somatic and clastogenicity in sporogenic cells of Tradescantia (clone 02).

References

Garcia, A.C.F.S., Marcon, A.E., Ferreira, D.M., Santos, E.A.B., Amaral, V.S., Medeiros, S.B.R., 2011. Micronucleus study of the quality and mutagenicity of natural waters, especially at low level of metal contaminants. Tetr/MCN and MCN/tetr endpoints similarly identified the impact of the lake's water on microspores of the Tradescantia. When compared to the results of Trad-SHM test, it showed a similar pattern to NS occurrences across the sites. Both tests demonstrate high sensitivity to environmental agents in the lake that are able to cause mutations in somatic and clastogenicity in sporogenic cells of Tradescantia (clone 02).
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