Assessment of mutagenicity of water from Lake Sevan, Armenia with application of *Tradescantia* (clone 02)

R.E. Avalyan\(^a\), E.A. Aghajanyan\(^a\), A. Khosrovy\(^b\),\(\ast\), A.L. Atoyants \(^a\), A.E. Simonyan \(^a\), R.M. Aroutiounian \(^a\)

\(^a\)Laboratory of General and Molecular Genetics, RI “Biology”, Faculty of Biology Yerevan State University, 8, Charents Str., 0025, Yerevan, Armenia
\(^b\)UNESCO UNITWIN/WCap. Physical Chemistry Department, Faculty of Marine and Environmental Sciences, University of Cadiz. Polígono Río San Pedro s/n, Puerto Real 11510, Cádiz, Spain

**A R T I C L E   I N F O**

Article history:
Received 17 October 2016
Received in revised form 19 February 2017
Accepted 28 March 2017
Available online 31 March 2017

**Keywords:**
Water pollution
*Tradescantia* (clone 02)
Genotoxicity
Mutations
Environmental pollution

**A B S T R A C T**

For many decades water resources in Armenia have been affected by anthropogenic activity, therefore, a regular bioindication of genotoxic effects of the water bodies is desirable. The genotoxicity of water samples collected from different parts of Lake Sevan were assessed by means of Trad-SHM (stamen hair mutation) assay using *Tradescantia* (clone 02). Here we report a significant increase in the frequency of somatic mutations and morphological changes in the *Tradescantia* inflorescences exposed to the water samples compared to the control. The somatic mutations (recessive mutation and white mutation events) were mostly linked to the concentration of Al, Ni, As, Co and Pb in Artanish, Tsapatak and Karchaghbyur, Noradus, Martuni and Litchik, while morphological changes (non-surviving hairs) were related to Co level in Tsapatak and Karchaghbyur. The results obtained show that Lake Sevan contains substances which may cause genotoxicity and teratogenicity in *Tradescantia* and probably also in aquatic animals. The results also show that Trad-SHM assay can be used for monitoring natural resources.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Current levels of anthropogenic stress on freshwater resources make it practically necessary to periodically evaluate water quality and the health of hydroecosystems. Lake Sevan, one of the largest Alpine lakes in the world, plays a unique and important role in the economic development of Armenia. However, the basin of the lake drains 28 tributaries that flow through densely populated agricultural and industrial areas. Contaminant inflow from these tributaries carrying industrial, agricultural and domestic waste is an important cause of change in the lake’s ecosystem. In spite of this, monitoring of the state of the lake’s ecosystem has been restricted to monitoring the chemical content of the water and to biological monitoring. For the first time, genotoxicity of the lake’s water was assessed by a combination of the water chemical composition and three bioassays: comet assay in erythrocytes of gibel carp (*Carassius auratus gibelio*) and plant assays: *Tradescantia* micronucleus (Trad-MCN) and *Tradescantia* stamen hair mutation (Trad-SHM) assays [16]. The results revealed genetic alterations in the fish and plant, statistically attributed to Al, Fe, Cu and Mn levels in the water, which were collected from the sites other than those in this study. The results for fish and *Tradescantia* were in high agreement.

Plants represent important organisms for use in environmental monitoring as they are sensitive and reliable indicators of contamination of the biosphere. Test-systems based on plant use have shown high sensitivity and are recommended for monitoring genotoxicity and assessment of carcinogenicity of xenobiotics in the air, water and soil medium [8,12]. Among plants tested, a special place is occupied by the heterozygote clones of *Tradescantia* (clones 02 and 4430). They were shown to perform successfully in dose-response studies and as biodestructors of chemical mutagens [14,9,5,7,22]. *Tradescantia* was used in air monitoring programs [4], for evaluating toxicity of sewage sludge and liquid samples [10,15,2] and soil samples [1]. Trad-SHM test is a reliable and sensitive system for monitoring gaseous and liquid mutagens in the laboratory [12] and in situ [4].

The aim of our study was to assess the genotoxicity of water from different parts of Lake Sevan, Armenia, Trad-SHM assay with *Tradescantia* (clone 02).
2. Material and methods

2.1. Sampling sites

Water samples were collected by hand during Spring 2016 at three different times from seven locations in Lake Sevan, Armenia (near river discharges and in the bay area) at an approximate depth of 30 cm depth in 1 L clean plastic bottles from three nearby locations per sampling site (Fig. 1). Samples were stored at +4 °C pending delivery to the laboratory and the initiation of tests which were done the next day. According to the long-term data of the Environmental Impact Monitoring Center of the Ministry of Nature Protection of Armenia, Litchk, Karchaghyur, Artanish and Tsapatakh sites are relatively less contaminated compared to Noradus, Masrik and Martuni sites. Litchk (40°11′11.391″N and 45°14′56.327″E) and Karchaghyur (40°10′54.225″N and 45°34′53.418″E) sites are located near spawning-rivers of endemic Sevan trout. Both rivers are spring-fed, relatively short and away from sources of industrial contamination. The Artanish (40°27′19.303″N and 45°25′12.123″E) site is located in the Artanish bay, which is a protected area and a nursing ground for Varicorhinus capoeta and Coregonus lavaretus fish species. Tsapatakh (40°24′34.465″N and 45°27′49.216″E) is another low contaminated site. Noradus (40°24′00.386″N and 45°10′1706″E), Masrik (40°13′25′447″N and 45°38′21.822″E) and Martuni (40°9′33.283″N and 45°30′21.649″E) sites are located near rivers which discharge untreated industrial or domestic waste to the lake. Near Masrik, the river is affected by mining activities in its drainage area, and Martuni and Noradus sites are affected by rivers carrying industrial and domestic effluents.

2.2. Tradescantia

Tradescantia (clone 02) is an interspecific hybrid T. occidentales and T. ehiensis [13], having a multitude of sprouts where inflorescences develop. Each inflorescence contains a large number of buds. Normally, the flower emerges every three days, has three blue petals that are surrounded with three sepalas, a pistil and six stamens. A characteristic feature of the flower is the presence of 30–50 and more hairs (occasionally fewer) on a stamen, and each hair, in turn, consists of 12–35 somatic cells. The plant is heterozygous for blue/pink alleles in the floral parts [13]. Blue flower pigment is dominant while mutant cells are pink. The plant is successfully acclimated to the conditions of Armenia, has strong root systems, can grow in a variety of soils and is very sensitive to environmental pollutants.

Tradescantia (clone 02) used for this study was taken from the greenhouse of Varevan State University.

2.3. Trad-SHM assay

Water samples were investigated for genotoxicity assessment by means of Trad-SHM assay. Three independent experiments were carried out with five replicates per sampling site. The contents of all bottles from each site (totaling 3 L) were mixed together and 150 mL samples taken for testing. The assay has been described in detail by Pogosyan et al. [15] and Agjahanyan et al. [2]. In brief, young inflorescences of the plant were submerged in water samples (3 inflorescences per vessel) for 18 h and after a 7-day recovery period, the flower blossoms were examined. Stamens of a flower were placed on a slide and stamen hair viewed under a magnifying glass at 10 x for counting blue-to-pink mutations in stamen hairs and morphological changes. Tap water was used as control in the bioassays.

Stamen hairs in Tradescantia flowers, where active cell division takes place, are the most sensitive place for mutations to occur. During the biotest Trad-SHM the following changes have been considered as indicator test-criteria: change of the cell color from blue to pink (recessive mutation events – RME) and appearance of white cells (unidentified white mutation events – WME). Besides somatic mutations, morphological changes are also taken into account: under-developed hairs of less than 12 cells (non-surviving hairs – NS) and branching hairs (BH). Lastly, changes in the flower structure were taken into account: change in the stamen numbers (normally, 6), fused petals, change in the flower color from blue to pink/white, other possible changes.

Flowers were examined daily for 21 days as they appeared. From 8000–14,000 stamen hairs were obtained per sample. The number of mutations per sample and per mutation were calculated per 1000 hairs, according to standard protocols [13].

2.4. Chemical and statistical analysis

Water samples were analyzed for chemical composition by a certified laboratory, according to standard methods [3]. Several elements were measured (total concentrations): Al, Ni, Zn, As, Cu, Fe, Cr, Co, Mo, Cd, Sn, Pb, Mg, Mn, Na, Ca, K.

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.

Principal component analysis (PCA) with Varimax as the extraction method was selected as a multivariate analysis method to observe linkages between mutation events and contaminant levels, and their association to the sampling sites. For interpreting variables within a principal component, loadings more than 0.4 (by absolute value) were considered.

Significant differences between the endpoints, observed in the samples and the control (p < 0.05, p < 0.01, p < 0.001), were analyzed with the Student t-test (equality of variances is not assumed) by the statistical program STATGRAPHICS Centurion 16.2 (StatPoint Technologies, Inc. USA; Warrenton, VA).

3. Results and discussion

Genotoxic and morphological effects of the water samples determined by the Trad-SHM assay are given in Fig. 2(a,b,c) and the chemical content of water samples (total concentrations, mg L⁻¹) in Table 1. The data represent the mean of three independent experiments. For comparison, national water quality standards for aquaculture, USEPA aquatic life criteria for continuous concentrations and WHO drinking-water quality standards are also given in Table 1. For metals where toxicity is hardness-dependent (Pb, Ni and Zn), a re-calculation was made as suggested by USEPA guidelines [18]. While the concentrations of most elements were below national water quality standards for aquaculture, the concentrations of Mg, Al, V, Cr, and Cu exceeded them in essentially all samples. V, Cr, Zn and Cu concentrations in tap water control also exceeded the national standards for aquaculture (Table 1). Cd and Sn levels were almost 100 times below their corresponding standards (5 and 1 µg L⁻¹, not shown). However, when compared to the USEPA aquatic life criteria for continuous effect concentrations, only Cr (VI) concentration in all sites but Litchk and Martuni might have exceeded the corresponding criteria, although in our study we measured total Cr concentrations. When compared to the available WHO guidelines for drinking-water quality [20], only Al concentration in Litchk sample exceeded the guideline, while chemical levels at most sites were far below the drinking-water guideline levels (Table 1). In the tap water control, the concentrations of all measured elements were below WHO’s guideline levels. 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.
The mean total number of stamen hairs examined per sampling site (depending on the number of flowers that blossomed during the 21-day count) were as follows: Artanish – 8155, Masrik – 8336, Karchaghybur – 8977, Noradus – 9237, Martuni – 13693, Litchk – 13738, Tsapatak – 14420, and control – 10752.

The study of genotoxicity of the water samples by the TradSHM assay has shown a significant increase in the frequency of somatic mutations (RME and WME) and morphological changes (NS) in all water samples compared to the control. In all samples, the level of RME exceeded that of the control by 3.5–9 times, depending on the sample. The highest frequency of RME occurred in the Noradus and Litchk samples (8–9 times) and in the Martuni and Tsapatak samples (6 times) (Fig. 2a). The frequency of WME in all samples also exceeded that of the control (3.5–4 times), with the highest rate in the Litchk and Tsapatak samples (Fig. 2b). Similarly, morphological changes (NS hairs, so called dwarf cells which are evidences of potential teratogen effects in Tradescantia ([22])) were also significantly more frequent in all samples, compared to the control (5–30 times), with highest frequency at Masrik (Fig. 2c).

Thus, from the results of disturbances in the stamen hairs of the plant (RME, WME, NS), it can be suggested that water samples collected from different parts of Lake Sevan contained substances that were able to cause genotoxic and possibly, teratogen effects.

The results of this study show that although the levels of elements measured did not exceed the drinking-water quality guidelines set by WHO, somatic mutations and morphological changes in the plant’s stamen hairs were frequent in all samples. Notably, the national water quality standards for aquaculture and USEPA aquatic life criteria were stricter for some elements, compared to the WHO standards for drinking-water, e.g., Al, Cr, Cu. At all sites, V and Cr levels exceeded the national water quality standards for aquaculture. At Litchk, Tsapatak, Karchaghybur and Artanish sites that have usually been considered less contaminated, at least three elements had concentration above the national water quality standards for aquaculture. For example, at Litchk, concentrations of Al, V, and Cr considerably exceeded the standards: Al by a factor of three, V and Cr by seven times. At Artanish and Karchaghybur, Na, Mg, Al, V, Cr, Ni and As levels also exceeded the standards, in particular V five times and Cr twenty. Hence the high frequency of somatic mutations occurred in the plant exposed to the waters from less contaminated sites does not seem unusual, given the high concentrations of several chemicals. Alternatively, this may suggest any of the following: the assay is highly sensitive to particular elements; the assay is highly sensitive to low concentrations of chemicals; there was an occasional supply of contaminants in those areas by river discharges or wave/current action at the time of sampling. In addition, effects may be observed at concentrations far below those predicted to be safe by regulatory frameworks [11].

The results of principal component analysis are represented in two separate graphs to avoid data crowding in a single graph (Fig. 3a,b). Only statistical associations found between the disturbances observed and chemical levels are shown. The percentage of total variance explained by each component is shown near components. It can be seen that the concentration of Al, P, Ni and As ions were statistically linked to the WME events observed in stamen hair cells of Tradescantia (clone 2) at Artanish, Tsapatak and Karchaghybur, while both somatic mutation events (RME and WME) were linked to Al concentration at Noradus and Litchk (Fig. 3a). The ions of Co were also related to somatic and morphological disturbances at Tsapatak and Karchaghybur and Pb levels to somatic disturbances at Noradus, Litchk and Martuni (Fig. 3b). Given that both Pb and Co concentrations were far below regulatory values (Table 1), this may assume a likely effect of even low concentrations of multiple toxicants on plant cells. Toxicological effects may occur at concentrations far below regulatory levels and synergistic action of multiple stressors on an organism often exceeds the effects of individual stressors [6,11,19]. Morphological disturbances (NS) were mostly related to Tsapatak and Karchaghybur sites (Fig. 3b). Appearance of dwarf NS hairs in Tradescantia is associated with teratogenic effects [22] and since NS frequency in all water samples was significantly higher than in the control, it may be evidence of the presence of teratogens in the lake, especially at these sites. Somatic mutations (RME and WME) were closely correlated with each other, which seems to be usual for lower doses of exposure. Underbrink et al. [17] mentioned that at higher doses this relationship changed to one aberrant type predominance.

These results confirm that toxicological effects may be caused even at low levels of contaminants as it has been acknowledged by previous authors. As well, the results confirm the reported high sensitivity of Trad-SHM assay to low levels of chemicals.
Moreover, while in all samples the differences in the frequency of mutations and morphological changes were significantly higher than in the control, NS events were mostly connected to Noradus and Masrik sites and RME and WME with the other sites, according to PCA results. This may suggest that different parts of Lake Sevan basin are affected by a different contaminant mix, possibly caused by the type of the anthropogenic activity evolved in the area and their cumulative effect is variant. Nevertheless, based on the significantly higher frequency of disturbances observed in all samples (Fig. 2) one may assume that river discharges in Lake Sevan supply chemicals that are capable of inducing genotoxicity and teratogenicity in Tradescantia (clone 02) and probably in the resident aquatic organisms. Related to this, one of the important tasks of the biomonitoring of the natural water resources in Armenia is not only a systematic control over the chemical content of the waters but also ecotoxicological assessment of the impact of contamination. Our study confirms the usefulness of Trad-SHM assay for genetic monitoring of the natural water resources and its high sensitivity to even low concentrations of environmental pollutants. However, despite the results of Trad-SHM assay having indicated the occurrence of genotoxic effects, it is highly recommended to conduct simultaneous testing with indicator freshwater organisms to evaluate genotoxicity and other responses in these animals and find a relation between their and Tradescantia responses.

4. Conclusion

These results indicate that Trad-SHM bioassay of Tradescantia (clone 02) can be applied for biotesting of water quality of aquatic ecosystems, in particular, for genotoxicity testing. Genotoxic effects of various waters on Tradescantia (clone 02) suggest that the lake's basin is contaminated by xenobiotics with the genotoxicity and teratogenicity potential. Mainly, concentrations of Al, As, Ni, Pb and Co may be the cause of the disturbances observed. It is recommended to combine the results of Trad-SHM assay with that of other toxicity assays on aquatic animals to reveal a relationship between them.

Acknowledgments

This research program (Scientific Basis of the Integrated Management of Natural Resources of Lake Sevan, 15RF – 049) was funded by State committee of science (Ministry of Education and Science of Armenia).

References


