Allium-test as a tool for toxicity testing of environmental radioactive-chemical mixtures

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Abstract. Bioassay-based approaches have been propagated to assess toxicity of unknown mixtures of environmental contaminants, but it was rarely applied in cases of chemicals with radionuclides combinations. Two Allium-test studies were performed to assess environmental impact from potential sources of combined radioactive-chemical pollution. Study sites were located at nuclear waste storage facilities in European and in Far-Eastern parts of Russia. As environmental media under impact, waters from monitor wells and nearby water bodies were tested. Concentrations of some chemicals and radionuclides in the samples collected enhanced the permitted limits. Cytogenetic and cytotoxic effects were used as biological endpoints, namely, frequency and spectrum of chromosome aberrations and mitotic abnormalities in anaphase cells as well as mitotic activity in Allium root tips. Sample points were revealed where waters have an enhanced mutagenic potential. The findings obtained could be used to optimize monitoring system and advance decision making on management and rehabilitation of industrial sites. The Allium-test could be recommended and applied as an effective tool for toxicity testing in case of combined contamination of environmental compartments with radionuclides and chemical compounds.

1. Introduction

Environmental exposure levels could be substantially increased through anthropogenic nuclear and industrial activities. Many surveys have been carried out to specify and justify constraints for radionuclide discharges and chemical substances releases into environment compartments, but a development of strategies for regulatory decision-making is still a challenge for specialists in radiation and chemical protection of the environment and the public. In real-time cases when territories appear contaminated through human civil and military activities such as nuclear facilities operation, mining sites, nuclear weapon testing, there is an essentially complex impact so that a mixture of radionuclides is often supplemented by other potentially hazardous substances (e.g. heavy or alkali metals, organic compounds, etc.). An actual hazard of such combined contaminations is difficult to estimate and predict correctly because of a lack of knowledge about relationships between various types and combinations of environmental pollutants and consequences for biota and human health. To mitigate the problem, bioassay-based approaches have been propagated to assess toxicity of unknown mixtures of environmental contaminants [1-3], but it was rarely applied in cases of chemicals with radionuclides combination. One of the standardized bioassays is the Allium-test that has been developed as a laboratory-based, single-species method for rapid screening of chemicals and environmental samples to assess their toxicity and genotoxicity [4-6].
In this paper findings are presented from the Allium-test application at an assessment of environmental impact from potential sources of combined radioactive-chemical pollution at two different study-sites.

2. Materials and methods

2.1. RWSF study-site

The first study was implemented in a vicinity of the radioactive waste storage facility (RWSF) in the European part of Russia [7]. The storage was established in the beginning of 1960th, radioactive wastes disposal had continued 8 years. Since 1998, there had been detected a leakage of $^{90}$Sr from one of the storage tanks, and there were found a significant increase of $^{90}$Sr specific activity (up to 40 Bq/l) in one of the monitor wells [8]. Maximum concentration of radioactivity occurred in a vicinity of the leaking storage tank (up to 180-210 Bq/l). To estimate consequences for the environment and population, a comprehensive radioecological survey was completed, and a bioassay-based study was one of its components. In a few years, the leakage was fixed, and a full-scale countermeasure program was implemented. By now, the RWSF has been decommissioned.

Here bioassay data are presented from testing water samples collected at and around the RWSF in 2004. Samples were taken from 7 monitor wells (Samples 5-7) and nearby waterbodies (Samples 3, 4, 8, 9). Water from a forest brook was considered as control (Sample 1), also distilled water was tested (Sample 2). In the most contaminated waters, $^{90}$Sr specific activities were about 10 times of the intervention level (IL = 5.0 Bk/kg) adopted in Russia for drinking water. In all samples except control heavy metals concentrations exceeded the Russian guideline values for maximum permissible content (MPC) in waterbodies, especially for Zn and Mn (70-250 times of MPCs), Ni (18-30 times of MPC).

2.2. STS study-site

The second study was carried out within the site for temporary storage (STS) of radioactive wastes and spent nuclear fuel in the Far-East of Russia where a military coastal technical base of the nuclear fleet of the former USSR used to operate. The STS is now a branch of the regional RW management center under supervision of the Federal State Unitary Enterprise ‘DalRAO’. From previous studies [9], gamma dose rate within the STS amounted from 0.1 to 65 μSv/h, at regional natural background level of 0.10-0.15 μSv/h. The main source of exposure is radiation penetrating walls of specialized containers and buildings where radioactive materials are stored. Radioactive contamination of territory is mainly formed by $^{137}$Cs and $^{90}$Sr; their specific activities in soil vary significantly within the site.

Water samples were collected in 2013 from 7 monitor wells (Samples 6, 12-16, 21) located within the STS [10]. Tap water was taken as control. Specific activities of $^{137}$Cs in some samples reached up to 30% of the IL; for $^{90}$Sr the corresponding IL was enhanced 2-3 times. Samples were of low mineralization, but for some chemicals the MPCs were broken in many cases.

2.3. Bioassay with Allium-test

To test samples cyto- and genotoxicity, the Allium-test was applied. General procedure is described in [4, 11], detailed description is also given in [7, 10]. Onion, Allium cepa L., was taken as a test-species. Bulbs (10-30 per every water sample) were germinated on water samples under testing. Onion roots of the 15 mm length were randomly sampled and fixed in an alcohol/acetic acid mixture (3:1). To test samples cyto- and geno-toxicity, squashed slides of A. cepa root tips were prepared with aceto-orcein as a dye and examined by optical microscopy. All slides were blindly and randomly coded before cytogenetic analysis. Sample cytotoxicity was estimated based on root proliferation that was quantified in parallel for all samples as mitotic index. Geno-toxicity was assessed with a frequency of aberrant cells in root meristem of A. cepa.

3. Results and discussion
3.1. Cyto- and genotoxicity of waters from the RWSF study-site

The bioassay results obtained at the onion bulbs germination in the water sampled from the RWSF and nearby territory are shown in figure 1. The mitotic activity in root meristem changed from 55 to 190% of control level (figure 1a). The inhibitory effect was observed for water collected from a small bog (Sample 3) and distilled water (Sample 2); both these samples were obviously unfavorable for higher plant ontogenesis. Sample 4 was taken from a brook flowing close to the RWSF fence; this sample contained high amounts of $^{90}$Sr, Zn and Cu. Comparing to Sample 1 (control), an increase of mitotic activity was found in Samples 8, 5 and 6. Interesting that these samples had rather high concentrations of chemicals, and it was difficult to relate increased mitotic index and chemical contamination.

For cytogenetic disturbances (figure 1b), a transparent effect of water contamination was shown for all water samples taken from and nearby the territory of the RWSF since the aberrant cells occurrence in root meristem of Allium cepa was significantly over the control, which indicated a potential adverse impact on the natural environment in a vicinity of the storage. The main contribution to the genotoxic impact was given by laggard chromosome occurrence, which frequency significantly enhanced the control level in many samples [7]. Remarkable role was also played by such severe disturbances as chromosome bridges and tripolar mitoses.

Thus, in water samples from the territory affected by the RW storage in European part of Russia, combined effect of different toxicants induced a wide spectrum of responses (from inhibition to stimulation) according to the bioassay of “proliferative activity in root meristem of Allium cepa L.”. Genotoxic effect was revealed for all water samples collected from the impacted territory which showed an efficacy and high sensitivity of the bioassay of “aberrant cells frequency in root meristem of Allium cepa L.” in a case of combined contamination of environment with $^{90}$Sr and heavy metals.

![Figure 1](image_url)

**Figure 1.** a - Mitotic index (%), b - aberrant cell frequency (%) in root tip cells of Allium cepa bulbs germinated in waters sampled from the RWSF and nearby territory, (mean ± se).

* Difference from the control (Sample 1), p < 0.05.

3.2. Cyto- and genotoxicity of waters from the STS study-site

At onion bulbs germination in waters sampled from the monitor wells at the STS territory there were found changes in mitotic activity of root meristem cells of both types: an inhibition and stimulation (table 1). Mitotic index decreased below the control in Samples 13, 16 and 21. These alterations were statistically significant, but in absolute value they amounted only to 10-13% of the control level; it could be considered as a small cytotoxic effect. In other water samples (6, 12, 14, 15) a significant increase of mitotic activity up to 50-60% of the control level was observed.

Mutagenicity estimated via the aberrant cells frequency in root meristem was high in the same samples (13, 16, 21) which showed the inhibition effect on the proliferative activity (table 1). Moreover, there was a close correlation between mitotic index and aberrant cell occurrence ($r = -0.88$, $p = 0.008$). In other samples, genotoxicity was not different from the control level, and in two samples...
(14 and 15) a significant, 3-fold decrease was detected. Chromatid aberrations recognized as damage of moderate severity were prevailing type in aberration spectrum in most samples [10]. However, in cyto- and genotoxic waters in Samples 13, 16 and 21, there were found an appreciable fraction of genomic disturbances such as laggings and conglutinations.

Thus, the Allium-test application revealed that onion bulbs germination in waters from the STS monitor wells (far-Eastern part of Russia) resulted in statistically significant alterations of both the mitotic activity in root meristems in all impacted samples and aberrant cells frequency in most water samples, and these two biological indices correlate to each other at high level of significance.

Table 1. Mitotic index and aberrant cell frequency in root tip cells of Allium bulbs germinated in waters sampled from the STS territory

| Sample (No. monitor well) | Mitotic index (%) | Aberrant cells (%) |
|---------------------------|-------------------|--------------------|
| Tap water                 | 11.0±0.2          | 2.04±0.42          |
| 6                         | 16.2±0.3<sup>a</sup> | 1.08±0.24          |
| 12                        | 16.6±0.3<sup>a</sup> | 1.75±0.35          |
| 13                        | 10.1±0.2<sup>a</sup> | 3.72±0.58<sup>a</sup> |
| 14                        | 16.8±0.3<sup>a</sup> | 0.69±0.21<sup>a</sup> |
| 15                        | 16.7±0.3<sup>a</sup> | 0.78±0.23<sup>a</sup> |
| 16                        | 10.0±0.2<sup>a</sup> | 3.96±0.58<sup>a</sup> |
| 21                        | 9.8±0.2<sup>a</sup> | 5.01±0.66<sup>a</sup> |

<sup>a</sup> Difference from tap water (control), p < 0.05.

4. Conclusions

Biological monitoring data obtained with the plant bioassay – the Allium-test – at testing the environmental media from territories impacted by two different facilities for radioactive material management are presented in this paper. In both study-sites, an industrial intake of chemicals and radionuclides into the environment appeared during the facilities operation. As a rule, the enhanced concentrations of some contaminants were found in technical or nearby areas around the RWSF and the STS that could pose a risk both to human health and to biotic components of natural ecosystems.

The identification and quantification of single chemicals considered as harmful for man or the environment are routine procedures in a system of environmental monitoring. Measurements of doses or contaminant concentrations could give us a detailed description of pollution levels, but they hardly indicate or forecast potential biological consequences from mixed pollution of the environment. Bioassays, in turn, quantify biological impact of mixtures. To have a realistic estimation of risk to the environment caused by any contamination, the simultaneous application of pollutant chemical control methods followed by biological tests is necessary.

The Allium-based root tips assay of chromosome aberrations in anaphase-telophase has been used in many studies to test genotoxicity of wastewater, contaminated soils, remediation techniques, new substances, etc [4, 6, 11, 12]. It was not so extensively exploited at testing of media contaminated with radionuclides or radionuclide-chemical mixtures, and this gap has been partly fulfilled by the findings presented here. Indeed, in both studies carried out in different radioecological conditions, water samples bearing high genotoxicity potential were revealed, with the important contribution from severe types of cell damage. The positive results obtained in the Allium-test should be considered as a warning and also an indication that the tested environments may be of risk to both human health and biological components of natural ecosystems.

Thus, the Allium-test is shown to be an effective tool for toxicity testing in case of combined contamination of environmental compartments with radionuclides and chemical compounds.
Information gained from the bioassay can help in optimizing monitoring and rehabilitation activities. An effectively linking of bioassay-screening assays to the well-established environmental pollution monitoring approach is a way of improving and upgrading an existing system of the public and the environment protection.

Acknowledgements
This work was supported by Russian Foundation for Basic Research (№ 05-04-96721 and № 16-48-400837) and Federal Target Program “Industrial utilization of weapons and military equipment of the nuclear complex for 2011-2015 and for the period till 2020” (contracts № 10-3-14-2013 and № 31401098199).

References
[1] Sharma C B S R 1983 Current Science 52 1000–2
[2] Grant W F 1999 Mutat. Res. 426 107–12
[3] Geras’kin S A, Evseeva T I and Oudalova A A 2011 Encyclopedia of Environmental Health vol 4, ed Nriagu J O (Burlington: Elsevier) pp 571–9
[4] Grant W F 1982 Mutat. Res. 99 273–91
[5] Fiskesjo G 1985 Hereditas 102 99–112
[6] Rank J and Nielsen M H 1993 Hereditas 118 49–53
[7] Oudalova A A, Geras’kin S A, Dikarev V G and Dikareva N S 2014 Radiat. Biol. Radioecol. 54 97–106 (in Russian)
[8] Starkov O V, Vaizer V I, Bogdanovich N G and Kozmin G V 2003 Nuclear Energetics 2 67–73 (in Russian)
[9] Shandala N K, Kiselev S M, Lucyanec A I, Titov A V, Seregin V A, Isaev D V and Akhromeev S V 2011 Radiat. Prot. Dosimetry 146 129–32
[10] Oudalova A A, Pyatkova S V, Geras’kin S A, Kiselev S M and Akhromeev S V 2016 Radiat. Biol. Radioecol. 56 208–19 (in Russian)
[11] Fiskesjo G 1988 Mutat. Res. 197 243–60
[12] Leme D M and Marin-Moralis M A 2009 Mutat. Res. 682 71–81