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Toxicity assessment of natural waters from the Obninsk radioactive waste repository

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Abstract. The assessment of cyto- and genotoxicity of water samples from a regional radioactive waste repository using the *Allium*-test was carry out. Biotesting of natural waters from the site has demonstrated that negative biological effects are generated under the influence of water from well, located near the source of contamination. The percentage of aberrant cells in water samples from this well 4 is 2 times higher than in the control or other sources. Effects of water composition at the genome level are season independent. The *Allium*-test was shown to be highly sensitive and effective in testing a combined effect from radiation and chemical factors in field conditions.

1. INTRODUCTION

Currently, various ecosystems are exposed to a combined action of radiation/non-radiation factors. Assessment of the state and hazard prediction of the combined pollution of ecosystems is based on results from the ecological monitoring. In addition to traditional physico-chemical methods of environmental assessment, biological methods are also increasingly incorporated into the monitoring system. In the biological monitoring, test-reactions of living organisms to combined effects are used. In this case, tests at the cellular and genetic levels make it possible to assess sensitivity of organisms and danger of a given exposure at the earliest monitoring stages [1, 2].

Among the plant test-systems, *Allium*-test is characterized as highly informative at the cellular and genetic levels [1]. This type of biotesting has proved to be effective in estimating toxicity of natural and sewage waters, model solutions of various chemicals. Data on the use of this test for the environmental assessment of areas affected by radioactive contamination are scarce. The onion test-system was employed to evaluate soil toxicity from the Chernobyl affected region [3]. Results from biotesting of water bodies at the site of underground nuclear detonation in the northern part of the Perm region have demonstrated that combined action of chemical and radiation factors causes triggering of significant biological effects in the test-object [4]. The application of biotesting methods in conditions of combined radiation and chemical pollution of a territory is the basis for the development of new principles for radiation protection of biota [5].

The objective of the present paper was assessment of cyto- and genotoxicity of water samples from a regional radioactive waste repository using the *Allium*-test.

2. MATERIALS AND METHODS

The investigation of natural waters for toxicity using the *Allium*-test was carried out in the vicinity of an old regional radioactive waste repository located within the town of Obninsk. From the moment of its establishment up to the present date, the Radiation Safety Department of the RF State Scientific Center the "Leipunsky Institute of Physics and Power Engineering" has been performing permanent radiation monitoring of this area. In October 1998, after partial destruction of one of the concrete storage tanks, a fraction of its contents deposited in the soil and groundwaters. According to the data of the radiological

monitoring, the main contributor (90%) to radioactive contamination of the adjacent area was Sr^{90}, its human hazard outside this area was negligible.

To assess potential biological hazard, water samples were collected from two control wells within the storage area: well 4 near the accidental tank and well 7 located at a rather large distance from the local contamination source. Water samples were also taken from a small rivulet heading in the storage area and a boggy plot (swamp) 50 m from the border of the guarded area. As the control, settled tap water samples were used. Three replicates of natural waters were employed. The water samples were collected in May, July and October.

The radiochemical analysis of water at the above points has indicated the presence of β-radionuclide, ^{90}Sr. The highest strontium content is reported in water of well 4 (22 to 40 Bq). The radioactivity level in water from well 7 was insignificant (<0,15 Bq/l). The radionuclide content in natural waters outside the repository varies in accordance with the season (from 3 to 16 Bq/l). The radionuclide contamination of the given territory is added by the presence in water of heavy metal ions. The chemical analysis has revealed an increased ion contents of Fe (0,15 mg/l), Mn (1,18 mg/l), Ni (0,32 mg/l), Cu (0,002 mg/l), Zn (1,36 mg/l) in well 4. In the water from well 7, high ion concentration is reported of Ni (0,18 mg/l). For spring and bog water also typical is the considerable presence of Cu ions (0,003 mg/l).

To study biological effects induced by the sampled water composition, cells of the root meristem of onion (*Allium cepa, variety Stuttgarter Rizen*) were taken as a test-system. Bulbs with about the same mass (2,5–3 g) and size (1,8–2,2 cm) were placed in plastic pans with tapwater for 48 h for advance germination. Then onions were selected in which the root length was 1 cm or more and placed in the tested samples for 24 hours. Three replicates had 15 bulbs each. Upon completion of testing the roots 1,5–2,0 cm in length were fixed in acetoacetic alcohol. A conventional procedure of squash preparations and aceto-orcein staining was used.

The analyzed parameters were: a tissue mitotic index and cells with chromosome aberrations in the ana-telophase stage [1, 2, 7].

3. RESULTS AND CONCLUSIONS

Based on the biotesting results, toxicity of natural waters has been estimated. Water cytotoxicity (Fig. 1A) was described by the parameter such as mitotic activity of root meristem cells, genotoxicity – by the frequency of aberrant cells (Fig. 1B).

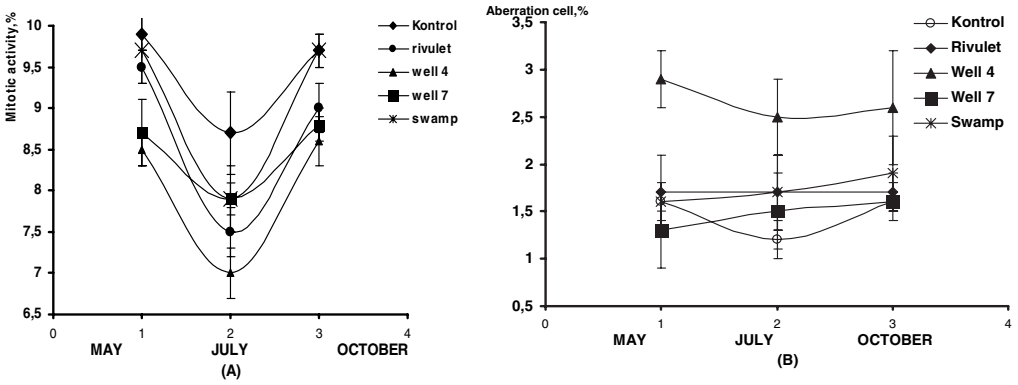

Figure 1. Assessment of cytotoxicity (A) and genotoxicyty (B) of water samples. Ordinate axis – Mitotic index, % (A), Frequency of aberrant cells, % (B). Abscissa axis – the date of testing of water samples.

It is seen from Fig. 1A that a significant reduction in the mitotic index of onion roots is observed for water from well 4, with the result being repeated independent of the date of sampling. Consequently, the water composition from this source is characterized by a cytotoxic effect. These data are in agreement with the results from the radiological and chemical monitoring.

On the contrary, effects of water composition at the genome level (Fig. 1B) are season independent. The percentage of aberrant cells in water samples from well 4 is 2 times higher than in the control or other sources. Hence, the genotoxic effect induced by water from well 4 is also confirmed.

It should be noted when comparing results from different sampling dates that cellular division activity in the summer period was low in all variants of testing. The observed seasonal dynamics of the mitotic activity can be explained by the presence of the internal biological rhythm of a test-organism. This implies that in experimental using of living organisms attention needs to be paid to the rhythmic pattern of natural processes [6].

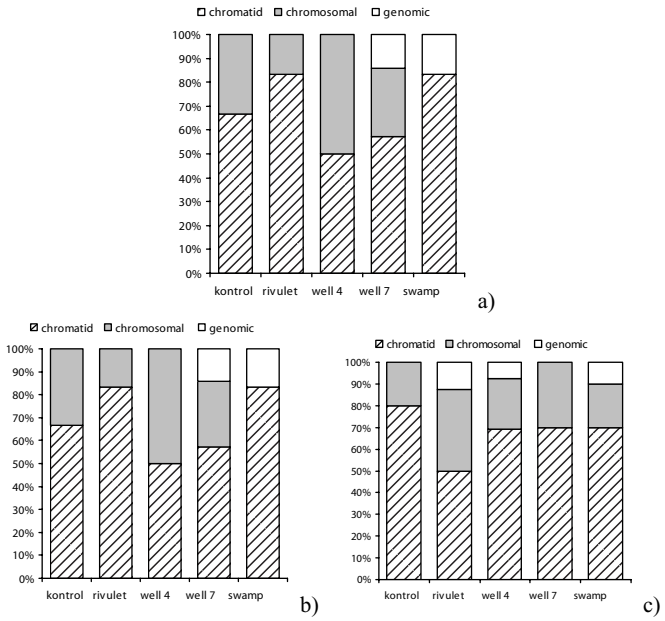

Figure 2. Spectrum of chromosome disturbance: a) – in May; b) – in July; c) – in October.

The spectrum of the observed chromosome aberrations is mainly presented by forms of chromosome disturbances (bridges and fragments). The occurrence of genome disturbances (chromosome lagging) is insignificant. For this spectrum, the absence of severe (lethal) alterations in cell (multi-polar mitoses, κ-mitoses, changes in the nucleus form, chromosome adhesion) is reported. Such genetic alterations in cells are caused by a combined action of radionuclides and chemicals found in water in relatively small concentrations [4, 7, 8].

The present findings lead to the following conclusions:

- Biotesting of natural waters from the site of radioactive waste repository has demonstrated that negative biological effects are generated under the influence of water from well 4 located near the source of contamination.
- The *Allium*-test was shown to be highly sensitive and effective in testing a combined effect from radiation and chemical factors in field conditions.
- In our tests, the parameter such as water genotoxicity was found to be the most stable and informative one.

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