

Research Article

Estimation of toxicity and genotoxicity of water, bottom sediments and submerged macrophyte *Elodea canadensis* of the Yenisei River in the presence or absence of americium-241

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Abstract

Toxicity and genotoxicity of one of the most widely spread species of aqueous plants of the Yenisei River – *Elodea canadensis* and bottom sediments of the Yenisei River containing ²⁴¹Am have been evaluated for the first time. It is shown that the control suspensions and extracts of plants or bottom sediments which do not contain ²⁴¹Am, inherently decrease the survival of bacterial cells of specific strains as compared to the control without these samples. In the experiments the increased cell death of *E. coli* in the studied aqueous plants accumulating ²⁴¹Am was observed to be higher, which is consistent with the toxicity of the control samples of aqueous solutions of ²⁴¹Am salts. Non-radioactive suspensions of the control samples of the plants and bottom sediments also slightly influenced the survival of the *S. typhimurium* TA98 cells, whereas their survival abruptly decreased in the presence of ²⁴¹Am. The samples of the bottom sediments without ²⁴¹Am hardly influenced the «frequency of His reversions» of the indicator *S. typhimurium*. In the suspensions of the ²⁴¹Am-containing bottom sediments a dose-dependent effect was observed both in the «frequency of reversions» and in the quantity of the detected His revertants in the *S. typhimurium* TA98 strain.

Introduction

It is known that the radiation factor can play a dominant role in the negative impact of the environment on human health [1-8]. The entrance of long-lived radionuclides into a human organism usually results in the radionuclide deposition, and their effect on people can have the character of low dose chronicle inner irradiation. Under the ionizing radiation the destruction of water molecules occurs in a human organism, giving rise to the formation of reactive oxygen intermediates (ROI) including hydroxyl- and superoxide-radicals [1-7]. Under the action of ROI there occurs the oxidation of lipids, proteins, nucleic acids and other components of the human cells and blood [1-5,9] which can result in various diseases connected with somatic, genetic, immune and other disturbances.

The oxidation damages of DNA are the most significant sources of mutations and they are considered to play a crucial role in the processes of aging, development of cancer diseases and a number of other pathological [1-4,9]. For example, as a result of large-scale nuclear tests performed at the test site «Novaya Zemlya» in 1955-1962 the population of Tundra Nenets living in the Yamalo-Nenets Autonomous Okrug turned out to be under the continuous prolonged effect of incorporated radionuclides (¹³⁷Cs and ⁹⁰Sr) [7,10]. So far in the population of indigenous people an abrupt increase has been detected in cancerous diseases, secondary immunodeficiency states, allergies, complications of pregnancy and birth defects, ophthalmologic diseases, growth of the number of children with mental retardation, Down syndrome, optic atrophy, limb reduction, anencephaly and thoracopathy [8,9].

Recently the biological health state of the Tundra Nenets population of the Yamalo-Nenets Autonomous Okrug has been estimated based on the analysis data for auto-antibodies and lipoproteids of blood donors [8,11]. It has been found that the characteristics of lipoproteins in ~90% of donors under study deviate towards various pathologies (3-8% for the control population according to epidemiological studies). Moreover, ~41% of Caucasians and ~56% Tundra Nenets are shown to have high auto-antibody titers against DNA and cardiolipin which are comparable with those for patients with various autoimmune diseases. Thus, it is obvious that the prolonged influence of technogenic radioactive isotopes can have very harmful effects on human health. Taking this into account, the clarification of the danger level of technogenic pollution of the environment and its effect on population health is an urgent task of the global ecology and medicine.

The River Yenisei is one of the largest in the world. In the Krasnoyarsk Region (town of Zheleznogorsk) there is a Mining and Chemical Combine of the State Company ROSATOM including reactor facility and radiochemical production. The multi-year operation of the Combine has resulted in the radioactive contamination of the Yenisei

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River flood plain [7,12-15] including that with transuranium elements. The most significant among the transuranium elements are plutonium, americium and neptunium isotopes [7,15]. So far no attention has been paid to the behavior of transuranium radionuclides in the ecosystem of the River Yenisei. The evidence on the scale of the radioactive contamination of phototrophic organisms in the River Yenisei by transuranium radionuclides is also lacking. At present the scale of the Yenisei River pollution with transuranium radionuclides is being estimated based on gamma-spectrometry and radiochemical studies. The entrance ways of transuranium radionuclides into the biomass of aqueous plants are being determined based on the estimation of the transuranium elements content in different structures (leaves, stem, roots) of aqueous plants and bottom sediments. According to the evidence obtained the samples of the plants, bottom sediments and water of the River Yenisei contain radionuclides, including ^{241}Am . Here, using special bacterial strains the analysis has been made of the toxicity and genotoxicity of highly-dispersed aqueous mixtures and extracts of bottom sediments and plants of the Yenisei River which contain ^{241}Am .

Materials and methods

Here, use was made of the samples of an ^{241}Am -containing aqueous plant *Elodea canadensis* and bottom sediments and those without the radionuclide which had been taken from the River Yenisei. In the control experiments, $^{241}\text{Am}^{3+}$ salt dissolved in nitric acid (2 M) was used. The initial activity of ^{241}Am was increased by evaporating water; the solution was neutralized with alkali (NaOH) and, if necessary, treated with catalase (in order to remove hydrogen peroxide which was present in the initial solution). As a control for the ^{241}Am reagent, use was made both of the dry biomass of an aqueous plant *Elodea canadensis* which had accumulated the ^{241}Am activity under the laboratory conditions [16] and of the extracts of the plant biomass obtained by physico-chemical fractionation methods [15].

Preparation of suspensions and extracts of the bottom sediments and *Elodea canadensis*

The aqueous suspensions of the samples were obtained by keeping a dry thoroughly-mashed mass of the control and test plants or bottom sediments (0.2-1.0 g), containing ^{241}Am or without it, dissolved in 10 ml of distilled sterile water at room temperature during 16-18 h, with stirring. Then, the mixtures were boiled for 20 min for additional extraction. In the experiments described below unfiltered aqueous suspensions of the plants and bottom sediments were used.

When successively extracting from the plants their components and ^{241}Am in order to obtain Extract-1, 35 ml of ammonium acetate was added to 40 mg of the dry mass of the radioactive or control plants, and then, the extraction was performed during 24 h (37°C) with constant stirring. The extract was filtered and the pH of the solutions was brought to 6.7-6.8 (Fraction 1). 30 ml of the solution with the activity of $^{241}\text{Am}=0.525$ Bq/ml was obtained. At this fractionation stage ~35% of radioactivity was extracted from that in the initial dry sample, from the total radioactivity in the dried sample.

To obtain Extract-2, 13 ml of 0.1 M H_2SO_4 was added to the plant mass which remained after obtaining the Extract1 and then, incubated on a shaker for 20 min at 37°C. The extract was filtered and neutralized with a concentrated solution of KOH to pH 7.0. 13 ml of the solution with the activity of $^{241}\text{Am}=1.275$ Bq/ml (~34% of radioactivity from the total initial activity in the dry sample) was obtained.

To obtain Extract-3, 12 ml of 30% H_2O_2 and 2 drops of 13 M HNO_3 were added to the plant mass which remained after obtaining the second extract. With the mixture heated on the stove, the plant biomass dissolved completely. The liquid was evaporated to the state of wet salts. Then, the residue was dissolved in 20 ml of water and evaporated again. Then, 12 ml of 30% H_2O_2 and 2 drops of 13 M HNO_3 were again added to the mixture, followed by complete evaporation of the liquid until the solution was dry. To remove H_2O_2 and the acid the dry residue was twice boiled off with 20 ml of water (each time 20 ml of water was added to the mixture and the mixture was completely boiled off.). At the last stage the dry residue was dissolved in 12 ml of water. The obtained solution was neutralized with KOH and treated with catalase to decompose the residues of hydrogen peroxide. As a result, 12 ml of the solution with the activity $^{241}\text{Am}=0.94$ Bq/ml (~25% of the initial radioactivity in the dry sample) was obtained.

Research methods

Estimation of the effect of the plant extracts on the survival of bacterial cells

The toxicity of the ^{241}Am -containing samples was estimated as related to the decrease of the number of living cells of the *E.coli* strains (PQ37, and BH910 (*mutT*; *fpg::Knr*; *uvrA::Tc^R*) after their cultivation during 2-24 h in a rich nutrient solution, to which the analyzed samples of the thoroughly mashed plants or their aqueous extracts were added. The viability of the cells (3 repeats) incubated with the control and radioactive samples of the plants was estimated based on the number of cells capable of further colony formation on a solid medium 24 hours after inoculation [19].

With the *S. typhimurium* TA98 and TA102 strains being used for the same purpose, the cell suspensions with the analyzed samples were kept without aeration for several days at room temperature. The number of viable cells was estimated at specific time intervals based on their ability to form colonies on a histidine-containing solid medium using the earlier described method [20].

Estimation of genotoxicity of the studied samples using the SOS-chromotest

Genotoxicity of the plant samples under study was estimated by means of the SOS-chromotest based on their ability to damage DNA and induce a SOS-response. For this purpose a special strain of *E.coli* was used, being deficient in a number of genes: *E.coli* PQ37 (F, *thr*, *leu*, *his-4*, *pyrD*, *thi*, *galE*, *galK* or *galT*, *srl300::Tn10*, *rpoB*, *rpsL*, *urvA*, *rfa*, *trp::Muc*, *sfiA::Mud(Ap, lac)*, *cts*, *lacβU169*, PhoC), which had been provided by Quillardet (France). The analysis was performed using a standard technique (Quillardet, Hofnung, 1985) with some modifications described in (Mersch-Sundermann, Kevekordes, Mochayed, 1991).

The samples of night culture (0.1 ml) of the strain used were diluted with 5 ml of the LA medium and incubated at 37°C, with their shaking on a shaker during 2-3 h until 2×10^8 bacteria/ml were obtained. Then, 1 ml of the culture was diluted with 9 ml of the fresh LA medium. 10-50 μl of the test solution of the extract of the control or analyzed samples containing ^{241}Am was added to 600 μl of the bacterial cell suspension, to be incubated for 2 h at 37°C. Then, 30 μl was taken each time for the analysis of the activity of β -galactosidase or alkaline phosphatase.

Estimation of the β -galactosidase activity

270 μl of the buffer B (0.2 M Na-phosphate buffer, pH 7.75, 0.1 M

KCl, 10 mM MgSO₄, 0.3 mM dithiothreitol and 0.1% SDS) was added to 30 µl of the suspension and the mixture was incubated at 37°C (10-15 min) for cytolysis. β-galactosidase reaction was initiated by adding ortho-nitrophenyl-β-galactoside (60 µl, 4 mg/ml) in accordance to the technique described earlier (Quillardet, Hofnung, 1985). After 30 min 0.2 ml of 1 M Na₂CO₃ was added to the mixture and optical density (A₄₂₀) was measured as compared to the control. The analysis was carried out under optimal conditions, A₄₂₀=0.1-0.4. The samples containing the LA medium substituted for the bacterial culture were used as a negative control. H₂O₂ (0.2-0.5 µmole per sample) rather than the extract was used as a positive control.

Analysis of alkaline phosphatase

The analysis of alkaline phosphatase was carried out using the earlier described technique [21]. The controls and conditions for the analysis of alkaline phosphatase were similar to those for β-galactosidase, except for 1 M tris-HCl buffer being used with pH 8.0, containing 0.1% SDS and p-nitrophenylphosphate as a substrate (60 µl, 4 mg/ml). The reaction was ceased by adding 200 µl of 1.5 M NaOH and the optical density (A₄₂₀) was measured.

Analysis of mutagenic activity of the extracts using indicator-strains salmonella typhimurium (ames test)

In the Ames test, histidine-dependent strains *S. typhimurium* TA98 and TA102 were used which carried mutation in histidine operon (Maron and Ames, 1983), these being provided by Ames. The analysis of mutagenic activity of the studied samples was carried out using a standard technique (Maron and Ames, 1983) without metabolic activation.

The sample extracts were obtained by keeping a dry thoroughly mashed mass of the control and test plants or bottom sediments (0.2-1.0 g), containing ²⁴¹Am or without it. The obtained unfiltered aqueous suspensions of the plants and bottom sediments were used in the experiments described below.

The night culture of the strains TA98 or TA102 was obtained using a standard technique of incubation (16 h at 37°C) in the ampicillin-containing LB medium. The Ames test was performed according to (Maron and Ames, 1983) using the double-layer agar technique. The bottom layer with 2% of agar, minimal medium, 20% of glucose and 50 µg/ml ampicillin also contained 50 tetracycline in the case of the TA102 strain. To obtain the upper layer, 0.2 ml of the 0.5 mM solution of histidine/biotin (for the TA 102 strain biotin is not necessary) was added to 2 ml of the 0.7% melted agar (0.5% NaCl), and, then, after cooling the mixtures down to 45°C, 100 µl of the night culture, containing the analyzed aqueous suspensions or without them, was added to these mixtures. The cells of the TA98 and TA102 strains incubated without the analyzed samples or with aqueous suspensions of the non-radioactive samples were used as negative controls. The mutagens: 4-nitroquinoline-1-oxide and H₂O₂ were used as positive controls.

The number of the surviving cells was estimated by the number of colonies grown in the presence of histidine taking into account the corresponding dilution before inoculation. The reversion frequency was estimated by the ratio of all the revertants detected in the test towards the total number of the surviving cells. The average data on the three independent experiments (9 dishes in each independent experiment) are presented.

Results and discussion

At the initial stage we investigated the influence of the control samples of neutral aqueous solutions of ²⁴¹Am nitrate on the cell growth of *E.coli* PQ37 in the rich nutrient medium. The increase of the radioactivity amount of ²⁴¹Am in the control samples was shown to result in the strong dose-dependent decrease in the survival rate of the bacterial cells in the exponential growth phase as compared to the controls without radioactivity. The amount of the viable cells as compared to the control without Am was 1.2×10^9 . In the case of their cultivation with the control solution of ²⁴¹Am depending on the radionuclide content in the stationary phase their decrease was observed to be 2.6×10^8 at 0.045 Bq/ml, 1.8×10^8 at 0.090 Bq/ml and 1.0×10^6 at 0.225 Bq/ml. The time increase of the *E.coli* PQ37 cells incubation in the presence of ²⁴¹Am (0.225 Bq/ml) resulted in the decreased amount of the surviving cells: after two hours the amount of the surviving cells was 2.5%, after six hours - 1.6% and 0.75% after 24 hours.

When analyzing the *Elodea canadensis* samples, «the portion» of the surviving *E.coli* PQ37 cells was calculated as a ratio of the number of the viable cells after 24 hour pre-incubation in the medium containing various amounts of the suspension of the thoroughly mashed plants to the number of viable cells grown in the medium without them.

In these experiments a strong decrease of the amount of the viable cells was observed in the stationary phase (24 hours) in the presence both of the plants containing ²⁴¹Am, and non-radioactive plants. For example, when adding into the system 14 mg of the dry plant biomass of the control sample the amount of the viable cells decreased by ~23 times, from 5.5×10^9 to 2.0×10^8 . When introducing ²⁴¹Am (3 Bq), with a lower amount of the biomass introduced, the cell survival was observed to decrease as follows: the biomass containing 8 mg/sample – by 83 times (6.6×10^7); 10 mg/sample – by 138 times (4.0×10^7). The increase of the cell death percentage in the case of ²⁴¹Am-containing plants was in agreement with the decrease of their survival after adding the control samples of ²⁴¹Am.

A relatively small difference in the influence of the experimental (+²⁴¹Am) and control (-²⁴¹Am) plant samples could be attributed to the fact that the bacteria cells are partially adsorbed on the plant surface, resulting in their accelerated inactivation. Besides, some compounds (for example, salts of heavy metals) could be extracted from the plants, these compounds being toxic to the bacterial strain used. With regard to this toxicity, the effect of ²⁴¹Am itself or of its complexes with the plant components could partly have been disguised. Taking this into account, for the further toxicity analysis the plant extracts were used, with the plant biomass being removed.

As is known, free radicals and strong oxidizers – the products of water radiolysis which appear in the presence of radioactive substances can greatly influence the cell sustainability [1-6]. Thus, to estimate the toxicity of the plant extracts a strain of *E.coli* BH910 (*mutT*, *fpg::Cn^R*, *uvrA::Tc^R*) was used which had mutations in the genes responsible for the repair process of the DNA oxidative damage [22]. This strain is highly sensitive to ionizing radiation and substances leading to the formation of reactive oxygen intermediates, in particular, hydroxyl radical. Moreover, the toxicity of the extracts analyzed in the case of this strain could be indicative of their genotoxicity (due to the DNA damage by free radicals and ROI) [23].

It is worth noting that in the plant extraction using water or using the finely ground plant suspensions a great amount of the radionuclide is not extracted from their solid mass. This can be due to the fact that ²⁴¹Am is rather tightly bound with the intracellular structures of the biomass [16]. For a more complete extraction of all the forms of ²⁴¹Am

from the plants the technique of the sequential chemical fractionation of plant mass was used which resulted into obtaining three radioactivity-containing sub-fractions [15]. Using ammonium acetate the first exchange fraction was obtained corresponding to the readily extracted («easily soluble») ²⁴¹Am. The adsorptive part of ²⁴¹Am was extracted from the residue using the solution of sulfuric acid (fraction 2). And, finally, the tightly bound ²⁴¹Am was extracted from the plant residue formed when being decomposed with H₂O₂ and HNO₃ (Fraction 3). In the experiments with the strain of *E.coli* BH910 all three different fractions were used corresponding to the plant extracts containing ²⁴¹Am and without it.

The cells of the strain of *E.coli* BH910 were grown for 2, 4, 8 and 24 h in the LB- rich medium at 37°C, and then, their survival rate was analyzed taking into account the formation of colonies on the solid medium. An almost similar decrease of the bacteria growth rate was observed during 2-8 hours in the presence of the Extracts 1 and 2 both containing ²⁴¹Am and without it. However, in the stationary phase, after 24 hours the number of the surviving cells in the presence of the control extracts was higher as compared to the radioactive plants, especially in the case of Fraction 1. A more pronounced decrease of the cell growth rate was observed in the presence of Fraction 3 obtained from the plant containing ²⁴¹Am after 2 hours of cultivation, and then, the evidence of the survival rate became comparable.

Different effects of Extracts 1-3 from the plants containing ²⁴¹Am or without it on the bacteria culture in the logarithmic and stationary growth phases is indicative of the fact that the extracts are different in the total of the content of the non-radioactive components + radionuclide. They also differ in their toxicity as related to the bacterial cells. It is worth noting that, in general, the toxicity of the plant extracts containing ²⁴¹Am is considerably higher than that for the control plants, which is, in principle, rather natural. It cannot be excluded that some difference in the effect of Extracts 1-3 is due to the fact that some of them could contain not only toxic plant compounds but a more toxic mixture formed with ²⁴¹Am. As a result, in this case one deals with the effect of synergism in the reaction of the radionuclide with other components formed in the dissolved biomass.

To study the toxicity of the plants and bottom sediments and plants containing ²⁴¹Am, using the strains of *S. typhimurium* TA98 and TA102, the aqueous suspensions of the extracts of these samples were employed. It was found that keeping the bacterial cells TA98 with the control preparation of ²⁴¹Am nitrate (0.240 Bq/ml) without aeration resulted in a considerable cell death as early as on the third day, while in the control sample (H₂O) the number of the cells had considerably increased by that time (Table 1). Similar results were also obtained for the strain TA102.

The samples of the control aqueous suspensions of the bottom sediments and plants without radioactivity considerably decreased the survival rate of the cells TA98 (as in the case of the control with H₂O) only on the 30th day. In the case of the bottom sediments containing ²⁴¹Am from 0.360 to 1.100 Bq/ml the number of the living cells decreased by ≈10⁵-10⁷ times as early as on the third-fourth day (Table 1).

The plant suspensions containing ²⁴¹Am from 0.360 to 1.100 Bq/ml decreased the cell survival by approximately two times on the tenth incubation day (Table 1). The higher toxicity of the bottom sediments containing ²⁴¹Am as compared with the radioactive plant samples could be due to a great number of elements, including heavy metals which were extracted together with ²⁴¹Am, thus increasing the negative effect on the TA98 cells.

Genotoxicity of the samples under study in the SOS-chromotest

The genotoxicity of the studied samples in the SOS-chromotest is estimated based on their ability to damage DNA and induce a SOS-response [17]. Using the indicator strain of *E.coli* PQ37 (*sfmA::lacZ*) allows one to estimate the expression of one of the SOS-inducible genes (*sfmA*) based on the β-galactosidase activity. The effect of the substances being tested on the survival of the bacterial cells or on the protein synthesis is determined based on the level of the constitutive expression of alkaline phosphatase. The DNA-damaging activity (genotoxicity) is determined using the value of the factor of induction (FI). The factor of induction is an indicator of the relative activity of β-galactosidase which correlates with the induction of expression of the gene *lacZ* in the indicator strain *E.coli* in response to the DNA damage caused by the substances under study [17].

In the present study the FI value was shown to increase with the increasing amount of ²⁴¹Am salt in the control samples. The minimum activity of ²⁴¹Am inducing a reliable SOS response amounted to 5-6 Bq per sample (for comparison, the minimum inducing dose of alpha and gamma radiation is equal to 2.5 Gy [18]). Similar FI values were obtained when using H₂O₂ (0.2 μmole per sample) as a genotoxic substance.

The genotoxicity of the plant samples was analyzed using the samples containing small amounts of ²⁴¹Am. A relatively low genotoxicity level of these plant extracts was detected. Nevertheless, the observed FI values are comparable with the values for the control solutions containing the same amount of ²⁴¹Am.

Analysis of mutagenic activity in the ames test (*salmonella/ames* assay)

The test on the induction of mutations in *S. typhimurium* is a

Table 1. Time dependence of the influence of the bottom sediment and plant suspensions containing ²⁴¹Am and without it on the survival rate of the bacteria *S. typhimurium* TA98.

| Sample | Am ²⁴¹ Bq/ml | Number of colonies per dish x 10 ^{5*} | | | | |
|--|----------------------------|--|----------------------|-----------------------|----------------------|-----------------------|
| | | Days | | | | |
| | | 0 | 3 | 4 | 9 | 30 |
| Control (H ₂ O) | 0 | 77 ± 4 | 166 ± 15 | 157 ± 16 | 108 ± 11 | 10 ± 2 |
| Control (H ₂ O + ²⁴¹ Am) | 0.24 | 80 ± 20 | 3 ± 1 | 0 (<10 ⁵) | 0(<10 ⁵) | 0(<10 ⁵) |
| Bottom sediments - ²⁴¹ Am (control) | 0 | 87 ± 1 | 144 ± 7 | 182 ± 4 | 119 ± 6 | 4 ± 1 |
| Bottom sediments with ²⁴¹ Am | 0.36 | 69 ± 3 | 3 ± 1 | 0 (<10 ⁵) | 0(<10 ⁵) | 0 (<10 ⁵) |
| | 1.08 | 13 ± 3 | 0(<10 ⁵) | 0(<10 ⁵) | 0(<10 ⁵) | 0(<10 ⁵) |
| Plants - ²⁴¹ Am (control) | 0 | 76 ± 4 | 127 ± 3 | n.d.** | 116 ± 5 | 5 ± 1 |
| Plants (with ²⁴¹ Am) | 1.60 | 76 ± 4 | 78 ± 3 | n.d. | 50 ± 3 | 0(<10 ⁵) |

* Average data from 3 independent experiments, the initial number of the cells is the same in all the cases.

** n.d. - not determined

Table 2. Estimation of the mutagenic activity for the test solution of ²⁴¹Am nitrate and bottom sediment samples in the Ames test (strain *S. typhimurium* TA98)*.

| No | Sample | Activity ²⁴¹ Am, mBq/dish | Number of revertants per dish | Number of surviving cells ×10 ⁵ | Frequency of reversions |
|----|---|--------------------------------------|-------------------------------|--|-------------------------|
| 1. | H ₂ O (negative control) | 0 | 31 | 255 ± 9 | 1.2 × 10 ⁻⁶ |
| 2. | H ₂ O ₂ (positive control)** | 0 | 97 | 267 ± 4 | 3.6 × 10 ⁻⁶ |
| 3. | Control (H ₂ O+ ²⁴¹ Am) | 22.0 | 27 | 250 ± 9 | 1.1 × 10 ⁻⁶ |
| | | 81.0 | 30 | 248 ± 9 | 1.2 × 10 ⁻⁶ |
| 4. | Bottom sediments + ²⁴¹ Am | 17.0 | 28 | 257 ± 10.8 | 1.1 × 10 ⁻⁶ |
| 5. | Bottom sediments without Am ²⁴¹ (control for No 6) | 0 | 30 | 269 ± 11 | 1.1 × 10 ⁻⁶ |
| 6. | Bottom sediments + ²⁴¹ Am | 51.0 | 79 | 254 ± 10.3 | 3.1 × 10 ⁻⁶ |
| 7. | Bottom sediments without Am ²⁴¹ (control for No 8) | 0 | 32 | 255 ± 6.1 | 1.3 × 10 ⁻⁶ |
| 8. | Bottom sediments + ²⁴¹ Am | 102.0 | 62 | 233 ± 5.0 | 2.7 × 10 ⁻⁶ |

bacterial test-system for evaluating reverse mutations from histidine auxotrophy to prototrophy under the action of different substances inducing the mutations, for instance, the substitution of the base pairs or a shift of the reading frame in the genome of these bacteria [21]. The presence of the mutagenic effect in the compounds under study is estimated based on the reverse mutations (reversions) to histidine prototrophy induced by these substances. The criteria of the positive result are either a statistically valid dose-dependent increase in the number of revertants, or a reproducible and statistically valid response, at least, for one concentration of the reagent. Thus, an area is normally revealed where the number of the revertants increases when increasing the dose of the mutagen under study.

When using a highly sensitive to oxidizers test strain *S. typhimurium* TA102 the frequency of the reversions in the case of the control aqueous solutions containing ²⁴¹Am was shown to be dose-dependent, which was observed to grow with the increase of radioactivity in the sample. On the contrary, in the TA98 strain the aqueous solutions containing ²⁴¹Am in the same dose range did not result in any considerable mutagenic effect. However, to study the control water and bottom sediment samples a less sensitive strain *S. typhimurium* TA98 proved to be more appropriate. The results of the mutagenicity analysis of the water and bottom sediment samples which either contain or do not contain ²⁴¹Am are presented in Table 2.

The number of His revertants in the test strain TA98, which appear upon adding the control aqueous solutions containing ²⁴¹Am (to 0.81 Bq per dish), does not exceed the number of revertants in the control experiments with the bottom sediment samples which do not contain ²⁴¹Am (Table 2). At the same time, upon adding the bottom sediments containing ²⁴¹Am in the amount of 0.051-0.102 Bq per dish, the number of revertants considerably exceeds that for the control solution of ²⁴¹Am with the comparable radioactivity.

Thus, we showed a considerable inhibiting effect of the control water samples containing ²⁴¹Am on the growth of the bacterial cells. At the same time, the toxic effect of the test water samples containing ²⁴¹Am is less expressed than the influence of the suspensions and extracts of the plants and bottom sediments. In spite of the fact that the control plants themselves decreased the survival of the cells of *E. coli* PQ37, the increase of the cell death in *E. coli* in the case of the plants containing ²⁴¹Am was higher, being in agreement with the toxicity of the control samples of the ²⁴¹Am salt aqueous solutions. The most vivid result as concerns the influence of the samples from the Yenisei on the cell survival was obtained in the case of the bacteria of the *S. typhimurium* TA98 strain. While the non-radioactive plant and bottom sediment samples slightly influenced the survival of these cells (the effect was observed only on the 30th day), the radioactive suspensions of the plants

and bottom sediments greatly decreased the cell survival and their toxic effect was considerably higher than that of the control water samples containing a comparable amount of ²⁴¹Am (Table 2).

The bottom sediment samples without ²⁴¹Am hardly influenced the «reversion frequency» of special strains. For the suspensions of the bottom sediments of the control systems containing ²⁴¹Am, a dose-dependent effect was found both in the «reversion frequency» and in the amount of His revertants (Table 2). Thus, it is evident that the accumulation of ²⁴¹Am in the plants and bottom sediments of the Yenisei river channel results in their transformation to genotoxic components of ecosystem. Moreover, the bottom sediments containing ²⁴¹Am, are more toxic as compared to the radioactive plant samples due to a considerable number of substances present in the bottom sediments. These assumptions are evidenced by our experimental data on different effects of Extracts 1-3 obtained by sequential extraction of the same ²⁴¹Am-containing plants on the cell growth.

The authors declare that they have no conflict of interest.

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