

Bioavailability of ^{99}Tc to a macrophyte of the Yenisei River

Lydia Bondareva, Alexander Bolsunovsky

Institute of Biophysics, Siberian Branch of the Russian Academy of Sciences, Krasnoyarsk 660036, Russia

INTRODUCTION

Technetium-99 is a long-lived artificial radionuclide with a half-life of 2.13×10^5 years, which poses a considerable environmental hazard. The major sources of ^{99}Tc in the environment are fallouts from atmospheric nuclear weapon tests and releases from nuclear fuel reprocessing operations and accidents at nuclear plants. Another source is nuclear medicine, which uses $^{99\text{m}}\text{Tc}$ (decaying into ^{99}Tc) although its contribution is much less important than the other sources. The Mining-and-Chemical Combine (MCC) situated in the Krasnoyarskii Krai (Bolsunovsky, 2004) includes a radiochemical plant for reprocessing nuclear fuel and, thus, one can assume that large amounts of ^{99}Tc are contained in radioactive waste and are partly released into the Yenisei River ecosystem with MCC effluents. Investigation of ^{99}Tc behavior in model ecosystems can yield data for predicting its behavior in the Yenisei River.

The purpose of our study was to obtain data on accumulation and release of ^{99}Tc by biomass of *Elodea canadensis*, one of the abundant species of submerged plants in the Yenisei River, in laboratory experiments.

MATERIALS AND METHODS

Experiments were conducted in laboratory, using *Elodea canadensis* Mich. (waterweed) – a submerged plant occurring in the Yenisei River. Plant and water samples were collected from the Yenisei River upstream of the MCC discharge point. Plant samples were taken from the population growing in one of the river inlets. In our experiments we used 3.2-3.5 cm apical shoots. The plants were pre-washed with the river and tap water. The Yenisei River water was aseptically filtered through 0.2- μm -pore-size cellulose nitrate membranes ($d=47$ cm, Schleicher&Shuell, Germany) to remove suspended particles and microflora. The plants were maintained in 0.2 L of water in 0.25-L cylindrical glass vessels, at a temperature of 17-19°C. Half of the vessels were illuminated by luminescent lamps during 12 h a day and the side irradiance of a vessel was 4.5 klx; then they were used in the “day/night” experiment. The remaining vessels were enveloped in foil and kept in near darkness; they were used in the “night” experiment. The parameters of radionuclide accumulation by plants were calculated per unit of dry weight. ^{99}Tc was added to the water as a solution of TcO_4^- (in the presence of a 0.1M HNO_3 solution). The added solution of ^{99}Tc was neutralized with a NaOH solution (0.1 M) to pH 7.0. Then, plants were placed into the water. Experiments on accumulation of ^{99}Tc by *Elodea* shoots lasted 6 days each. To estimate ^{99}Tc release from *Elodea*, we took the plants out of the experimental vessels at a stage of maximal ^{99}Tc concentration in the biomass and placed them into vessels filled with the clean Yenisei water (0.25 L). The experiment on ^{99}Tc release lasted 21 days. During the ^{99}Tc accumulation and release experiments, at set intervals, aliquots of water and plant shoots were analyzed for the ^{99}Tc concentration. The concentration factor (CF) of ^{99}Tc was calculated as the ratio of the radionuclide concentration in the plant (Bq/kg dry wt) to the radionuclide concentration in the water (Bq/L). To calculate parameters of ^{99}Tc accumulation by *Elodea*, we used the averaged data for three experimental vessels with *Elodea* shoots. To estimate the mobility of ^{99}Tc contained in the plant, we used the method of sequential chemical extraction (Bolsunovsky et al., 2005). Technetium of the exchangeable fraction was separated by exposing the plant biomass to the action of a $\text{CH}_3\text{COONH}_4$ solution (1M) for 24 h. To separate ^{99}Tc of the adsorbed fraction, the plant biomass was treated with a H_2SO_4 solution (0.2 M) for 20 min. The ^{99}Tc that was still retained

by the biomass was considered to be strongly bound to plant components. ^{99}Tc bound by organic compounds and mineral residue of the plant biomass was separated by “wet combustion”, using H_2O_2 (30%) and a HNO_3 solution (0.1 M). ^{99}Tc in water samples of the “*Elodea* – Yenisei River water” model system and in the biomass fractions was measured using a Tri-Carb-2800 liquid scintillation analyzer (USA), with $\sim 100\%$ registration efficiency for ^{99}Tc . Aliquots of water and liquid fractions were mixed with a Hi-Safe-2 scintillation cocktail in 20-ml plastic vials at a ratio of 8 to 12. Prior to measurements, the resulting mixtures were left to stabilize in the dark at $t=8-9^\circ\text{C}$ for 48 h. The longest measurement time was 420 min.

RESULTS AND CONCLUSIONS

Typical dynamics of ^{99}Tc activity concentration in the water environment are shown in Figure 1. The dynamics of water depletion of ^{99}Tc in the “day/night” and “night” experiments are similar. During the first 24 h *Elodea* rapidly took up ^{99}Tc (accumulating about 20% of the added activity) and then, for 4 days, the water was gradually depleted of the radionuclide. At day 6 differences between ^{99}Tc activities in the water of experimental vessels were not significant: 44 ± 6 Bq/L in the “day/night” experiment and 60 ± 8 Bq/L in the “night” experiment. ^{99}Tc activities accumulated in plants differed depending on the experimental conditions (Table 1). In our opinion, this could be accounted for by different contributions of accumulation processes that occurred in experimental vessels. For instance, in the “night” experiment, physical and chemical sorption of ^{99}Tc in plants could prevail over radionuclide binding due to photochemical reactions (characteristic of the “day/night” experiment)

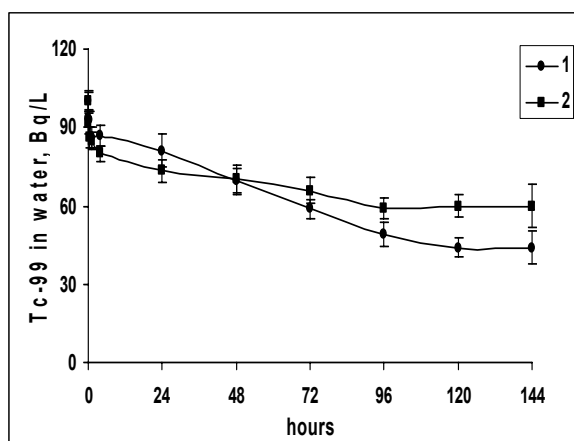


Fig. 1. Dynamics of the added ^{99}Tc activity in the in the water of experimental vessels (Bq/L): 1 – “day/night” experiment, 2 – “night” experiment

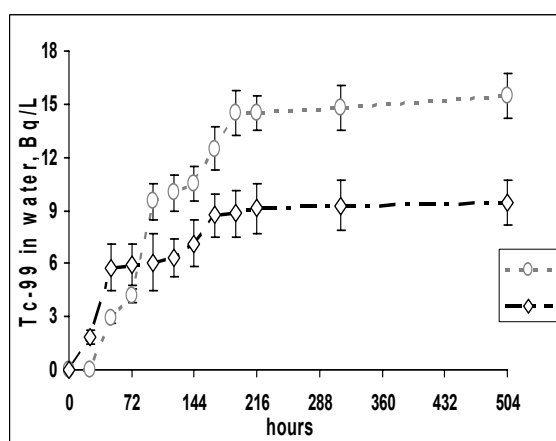


Fig. 2. Dynamics of ^{99}Tc release from the *Elodea* shoots to the water (Bq/L) of experimental vessels: 1 – “day/night” experiment, 2 – “night” experiment.

In our experiments activity concentration of ^{99}Tc in *Elodea* samples was 120 ± 10 Bq/g dry wt in the “day/night” experiment and 170 ± 11 Bq/g dry wt in the “night” experiment (Table 1). The maximum technetium concentration factor for *Elodea* was calculated to be 2700 ± 500 L/kg dry wt (“day/night”), which was comparable to the concentration factor obtained in the “night” experiment (Table 1). Our values for ^{99}Tc concentration factors (Table 1) are considerably different from the values obtained in other studies for freshwater model systems: 25-300 L/kg dry wt. J. Hattink and his co-authors in their study (Hattink et al., 2001) used *Lemna minor* L. (common duckweed), a submerged plant whose morphology is different from that of *Elodea*. Russian specialists of the Mayak nuclear complex conducted experiments with *Elodea* (Mashkin et al., 2000), adding high ^{99}Tc activity (10^6-10^{10} Bq/L) for

spectrophotometric detection, with the plant mass being relatively small: 15 g fresh wt. However, in those experiments, *Elodea* plants, as well as plants of several other species, died at days 10-15 of the experiment. A reason for the death of the plants can be that ^{99}Tc is chemically toxic to living organisms, similarly to $\text{Cr(VI)} - \text{CrO}_4^-$ (Prévèral et al., 2006). The adverse effect of ^{99}Tc on plants was exhibited in their decreased growth and concentration of chlorophylls. Toxicity symptoms in the form of chlorosis and necrosis developed. In our experiments we added 100 Bq/L of ^{99}Tc , and plants developed well throughout the experiments – the shoots grew rapidly.

Table 1. Results of experiments on ^{99}Tc accumulation by *Elodea canadensis*

| Conditions of experiment | Dry mass, g Fresh mass, g (shoot length, cm) | | Equilibrium activity of ^{99}Tc | | | Concentration factor, L/kg |
|--------------------------|--|--|--|--------------------------|----------------|----------------------------|
| | Initial | Final | In plants, Bq | In plants, Bq/g dry mass | In water, Bq/L | |
| “day/night” | <u>0.082±0.003</u> 1.31 (3.4 cm) | <u>0.095±0.004</u> 2.33 (7.3 cm) | 11.4 | 120±10 | 44±6 | 2700±500 |
| “night” | <u>0.082±0.003</u> 1.31 (3.4 cm) | <u>0.081±0.002</u> 1.88 (5.1 cm) | 8 | 170±11 | 60±8 | 2850±450 |

Note: the table lists the averaged data for three experimental vessels with *Elodea* shoots.

To determine the strength of binding of ^{99}Tc in *Elodea*, we performed experiments on ^{99}Tc release. As mentioned above, to estimate ^{99}Tc release from the biomass, we took the plants out of the experimental vessels and placed them into vessels filled with the clean Yenisei water. The initial ^{99}Tc activity in *Elodea* in the vessels was 3.8 ± 0.2 Bq per vessel (the “day/night” experiment) and 2.6 ± 0.2 Bq per vessel (the “night” experiment). The typical dynamics of ^{99}Tc activity in the water of the vessels containing the plants are shown in Figure 2. In the “day/night” experiments, in 24 h after *Elodea* shoots were placed into the vessels with the filtered water, we were able to register some ^{99}Tc activity in the water environment (Fig. 2). During the following days of experiment, the ^{99}Tc activity in the water increased, reaching saturation at 192 h. Then, from 192 h to 504 h no increase in ^{99}Tc activity in the water was registered, i.e. no ^{99}Tc was released by plant biomass. ^{99}Tc activity concentration in *Elodea* samples decreased over the course of the experiment from 120 to 22 Bq/g dry wt; the dry weight of *Elodea* remained almost unchanged, although the shoots became twice longer. In the “night” experiments, ^{99}Tc was detected in the water in 24 h (Fig. 2). ^{99}Tc activity concentration in *Elodea* samples decreased over the course of the experiment from 170 to 61 Bq/g dry wt. In addition to ^{99}Tc natural release, rapid biomass destruction was also observed, leading to gradual separation of plant segments containing the ^{99}Tc that they had accumulated before. Our hypothesis that biomass-bound ^{99}Tc is mostly found in the surface layer is confirmed by the data on the distribution of ^{99}Tc in biomass fractions, which have been obtained using chemical fractionation (Fig. 3).

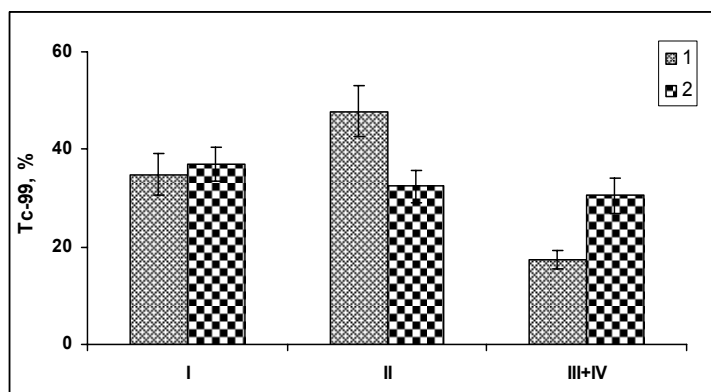


Fig. 3. Relative distribution of ⁹⁹Tc among fractions of *Elodea* biomass (1 – “day/night” experiment, 2 – “night” experiment): I - exchangeable fraction; II - adsorbed fraction; III+IV - fractions of organics and mineral residue.

⁹⁹Tc tightly bound to *Elodea* (III+IV – the fractions of organics and mineral residue) constituted just 17% (the “day/night” experiment) and 31% (the “night” experiment) of the total ⁹⁹Tc activity. These data suggest that ⁹⁹Tc may be incorporated into *Elodea*’s cellular structures, mostly into cell membranes.

The experiments on accumulation of ⁹⁹Tc by *Elodea* showed that ⁹⁹Tc activity concentration can reach 120 ± 6 (the “day/night” experiment) and 170 ± 7 (the “night” experiment) Bq/g dry wt, with the maximum concentration factor for ⁹⁹Tc 2700 ± 500 L/kg dry wt. In experiments on ⁹⁹Tc release, over 504 h more than 60 % of the total ⁹⁹Tc activity was released into the water from plant biomass; most of ⁹⁹Tc was released within the first 192 h. Results of chemical fractionation of the biomass show that ⁹⁹Tc contained in *Elodea* was mainly concentrated in the exchangeable and the adsorbed fractions (83% – the “day/night” experiment, 69% – the “night” experiment).

REFERENCES

- Bolsunovsky A., 2004. Artificial radionuclides in aquatic plants of the YENISEI River in the area affected by effluents of a Russia plutonium complex. *Aquatic Ecology*, 38: 57-62.
- Bolsunovsky A., T.Zotina, L. Bondareva, 2005. Accumulation and release of ²⁴¹Am by a macrophyte of the Yenisei River (*Elodea canadensis*). *J. Environ. Radioactivity*, 81: 33-46.
- Hattink J., H.T. Wolterbeek, J. de Goeij, 2001. Influence of salinity and eutrophication on bioaccumulation of ⁹⁹Tc in Duckweed. *Environ Toxicology Chemistry*, 20: 996-1002.
- Mashkin A.N., S.L. Shikov, 2000. Technetium assimilation by aquatic plants. *Radiokhimiya (Radiochemistry)*, 42: 268-272.
- Prévèral S., E. Ansoberlo, S. Mari, A. Vavasseur, C. Forestier, 2006. Metal(loid)s and radionuclides cytotoxicity in *Saccharomyces cerevisiae*. *Biochimie*, 88: 1651-1663.