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UPTAKE OF TIGHTLY BOUND TRITIUM BY PLANTS

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A study of the bioavailability of a tightly bound tritium in the soil *in situ* under the conditions of the Semipalatinsk test site (STS) and in a laboratory vegetation experiment was conducted. Significant concentrations of organically bound tritium (OBT) were recorded in *Stipa capillata* plants growing on the territory of the STS. At the same time, tritium in the free water of plant tissues (TFWT) was not detected. In a model experiment using *Cucumis sativus* culture, the specific activity of tightly bound tritium in the composition of the solid phase of the soil and tritiated water (HTO) in the composition of the soil solution at the beginning and at the end of the experiment differed significantly. At the same time, TFWT activity in *Cucumis sativus* was 2 orders of magnitude less compared to the activity of tightly bound tritium in the soil and slightly differed from the activity of tritiated water in the soil solution, which is accessible to the root system of plants. The results of vegetation studies have shown that the direct source of tritium for experimental plants was a soil solution. However, changes in the tritium activity in the soil liquid and solid phases in vegetation vessels indicate that the source of tritiated water in the soil solution was a tightly bound form of radionuclide as a result of leaching processes. It is assumed that the possible causes of leaching of tightly bound radionuclide from the solid phase into the soil solution are the moisture regime, as well as the influence of the rhizosphere of plants.

Keywords: *Semipalatinsk test site, tightly bound tritium, bioavailability, Stipa capillata, Cucumis sativus.*

Introduction

Tritium is a radioactive form of hydrogen that is produced either naturally in the environment as a result of cosmic ray interactions in the atmosphere, or during normal operation of nuclear facilities and in large quantities in certain types of accidents [1 The most common forms of tritium in the environment are gaseous (HT, HTO_{steam}, CH_3T), liquid (HTO, T_2O) or organically bound (tritiated organic compounds - carbohydrates, proteins, amino acids, DNA, etc.) [1–3]. These forms of tritium are characterized by ultra-high migration ability both in the trophic structure of the ecosystem and in general in all its compartments. In this connection, the ecological significance of these radionuclide compounds is obvious. However, a special strongly bound form of tritium has been found in the soil of the Semipalatinsk Test Site (STS) near the epicentres of nuclear tests [4, 5]. Moreover, the isotope content in this form in soil reaches significant levels from 5 to 240 kBq/kg [4]. It should be noted that the soil on the migration path of any radionuclides in the terrestrial ecosystem is the main depositing link from which the vegetation cover is able to extract them and, thus, to involve them in further biogeochemical cycles, in particular through the trophic structure. As for the tightly bound tritium compounds, their ecological significance in this aspect is unclear, so the aim of the work was to investigate the bioavailability of this form of the isotope in soil, in order to understand the mechanisms of its migration in the ecosystem.

Objects and Methods Research

The bioavailability of tightly bound tritium was studied *in situ* (directly at nuclear test sites) and in a laboratory vegetation experiment In the STS territory, where areas with high specific activity of tightly bound tritium have been previously established in the soil cover [4], 16 sites near 5 epicenters of above-ground nuclear tests have been laid down (P-1, P-2, P-3, P-5, P-7). The dominant species of needlegrass was used as an object of study under natural conditions (*Stipa capillata*). Plants were selected from 1 to 2 m^2 depending on the projective cover of the plots. The mass of the plant samples averaged between 100 and 150 grams.

Soil from technical site P-1 with a known high specific activity of tightly bound tritium was used for the vegetation experiment in laboratory conditions. Soil samples were taken to a depth of 5 cm, then pre-dried, sieved through a sieve (d – 5 mm) and mixed. The total mass of the model soil sample was about 50 kg.

The prepared soil was placed in 20 litre plastic growing vessels. Cucumber culture (*Cucumis sativus*) was used as an experimental plant, which was grown to full maturity. Optimal lighting conditions were maintained using an artificial phyto-lighting system (up to 10 kilolux). Plants were watered with distilled water, maintaining soil moisture at 60% of full water capacity. In the laboratory where the vegetation experiment was conducted, a ventilation system was constantly operating to prevent secondary contamination of the plants with tritium in case the experimental plants transpired or the vegetative vessels evaporated from the soil surface. Therefore, the only possible source of tritium for plants in the experiment was the tightly bound form of the radionuclide in soil. The duration of the experiment was 50 days. Repetition was fivefold.

Soil samples for control of specific tritium activity were taken before planting and at the end of the growing season. The weight of each soil sample averaged 300 g. Plant samples were taken from the aboveground part of shoots by organs at the end of vegetation period. The weight of each plant sample averaged between 100 and 150 g. To prevent tritium loss, soil and plant samples are immediately packed in zip bags after sampling and stored in a freezer at −20 °C until laboratory tests are performed.

The specific activity of tritium in the free water of plant tissues (TFWT) was measured and in the organic component (organically bound tritium - OBT). In soil samples, the specific activity of the radionuclide was measured in soil solution (tritiated water – HTO) and in the solid phase (tightly bound tritium).

Preparation of soil samples for measurement of specific activity of tritium in soil solution was carried out by distillation at 105 °C in a desiccator. Then, to extract the tightly bound form of tritium from the soil solid phase, the samples were subjected to autoclave decomposition [5].

Free water extraction from plant tissues was carried out using a special unit [6]. Samples for OBT determination were prepared on a Sample Oxidizer 307 (Perkin Elmer, USA).

The specific activity of tritium was measured by liquid scintillation spectrometry using a spectrometer (Perkin Elmer, USA) [7]. The samples were first passed through a filtration stage to remove mechanical impurities, then a 3 mL aliquot was taken and placed in a 20 ml plastic vial. Then scintillation cocktail was added to the vial in the ratio of 1:4 (sample-to-scintillator ratio). Ultima Gold LLT scintillation cocktail, specially designed for measuring tritium in natural samples, was used to analyze the samples (tritium detection efficiency was about 60% in the range from 0 to 18 keV). The measurement time of each sample was at least 120 minutes. Beta spectra were processed and tritium activity was calculated using Quanta Smart software. The minimum detectable tritium activity for the radiometric instruments used ranged from 4.5 to 7 for OBT and from 2.7 to 5 Bq/kg for HTO.

The quantitative assessment of the bioavailability of strongly bound tritium was carried out indirectly using the OBT/TFWT ratio [3].

RESULTS AND DISCUSSION

Field research

In plant samples of *Stipa capillata* collected from nuclear test sites characterized by high levels of specific activity of tightly bound tritium, free tissue water could not be obtained due to arid growing conditions. At the same time, high values of specific activity of tritium incorporated into organic matter were found in the majority of *Stipa capillata* samples (Table 1).

In general, the values of variation-statistical parameters presented in Table 1 indicate a high variability of OBT concentration in *Stipa capillata*, which is possibly due to the different levels of tritium content in the soil on which the plants grew. This is confirmed by the previously obtained data on the distribution of tightly bound tritium in the soil of the investigated sites [4]. Since organically bound tritium (OBT) in plants is formed only from tritium in the free water of plant tissues (TFWT) [1–3, 8], its significant concentration in *Stipa capillata* samples indirectly indicates the presence of high TFWT concentrations during certain growth periods.

Table 1. Specific activity of tritium in Stipa capillata samples

Selection site	*n*	Specific activity of organically bound tritium, kBq/kg	
		Average±SD*	Range (мin-мax)
P-1	8	4,4±4,9	0,8–16
P-2	4	1,00±0,67	0,3–2
P-3	6	1,00±0,69	0,3–2,3
P-5	6	0,60±0,57	0,1–1,6
P-7	7	5,0±10,8	0,1–29

****Note to Tables 1–3:*** *SD – Standard Deviation*

Tritium is known to penetrate the free water of plant tissues by both aerial and root routes [3]. However, according to earlier research [9], tritium was not detected in the surface air of the study area, and there are no surface and underground watercourses that could become a source of the radionuclide. Thus, the source of tritium for *Stipa capillata* could only be the soil cover, which strongly bound tritium is present in high concentrations [4]. At the same time, it should be noted that from the point of view of soil chemistry, tightly bound tritium is not a mobile form of moving [5], respectively, it does not have a direct high bioavailability. Accepting this hypothesis, a modelling experiment using cucumber culture was carried out (*Cucumis sativus*).

Investigations under model laboratory conditions

The values of specific tritium activity in model soil samples before planting and at the end of the vegetation experiment with use are presented in Table 2.

Table 2. Specific activity of tritium in the soil of vegetation vessels

Selection time	*n*	Specific tritium activity, kBq/kg			
		Solid phase (“tightly bound form”)		Soil solution (tritiated water)	
		Average±SD	Range (мin–мax)	Average±SD	Range (мin–мax)
Before planting	5	58,0±9,4	56–59	0,04±0,02	0,02–0,05
The end of vegetation	5	40±9	31–54	0,7±0,2	0,6–0,9

According to Table 2, the specific activity of tightly bound tritium in the soil solid phase and tritiated water (HTO) in the soil solution at the beginning and at the end of the experiment are significantly different. So, at the end of the experiment, the specific activity of tritium in soil moisture available to plant roots increased by an order of magnitude, indicating the processes of radionuclide leaching from solid phase.

Table 3 presents the results of measuring the specific activity of tritium in the free water of tissues and organic component of the experimental culture of *Cucumis sativus.*

Table 3. Specific activity of tritium in plant samples of Cucumis sativus

Indication	n	Activity concentration of ^{3}H, kBq/kg					
		leaves		stems		fruits	
		TFWT	OBT	TFWT	OBT	TFWT	OBT
Average±SD	5	260±27	17±11	120±13	8,0±3,2	160±56	17±14
Range (мin–мax)	5	240–290	7–29	110–140	4–11	117–220	4–31

As can be seen in Table 3, TFWT activity is an order of magnitude higher than OBT activity. This pattern can be explained by the biochemical origins of tritium, which is bound organically and results from its inclusion in metabolic processes. It should also be noted that the activity of tritium in free water is usually slightly lower than that of the radionuclide in the source [1, 3, 8], due to the simultaneous influence of several factors: equilibrium on the one hand [3] and transpiration loss [1, 3, 10] on the other hand. According to the results of the experiment, TFWT activity in *Cucumis sativus* is 2 orders of magnitude lower than that of strongly bound tritium in soil, and is in the same range as that of tritiated water in soil moisture available to plant root systems. Thus, it is obvious that the direct source of tritium in experimental plants is soil moisture. At the same time, the change in the specific activity in the liquid and solid phases of the soil in the vessels for growing plants at the beginning and at the end of the vegetation experiment (Table 2) showed that the source of tritium for plant roots was a soil solution. Accordingly, this indicates that the strongly bound form of tritium is capable of leaching. It is likely that one of the reasons for these processes could be active moistening of soil during irrigation. Also, chemically active exometabolites of plant roots in the rhizosphere zone could contribute to leaching [11–13]. This assumption is confirmed by studies of the rate of movement and distribution of tritium in soil compartments, including the rhizosphere, the root zone of plants, especially towards the end of the growing season [14].

The value of the OBT/TFWT ratio for *Cucumis sativus* averaged 0.1, which indirectly indicates the weak bioavailability of the radionuclide in a strongly bound form. According to the literature data [1, 3], this range for terrestrial ecosystems with a constant supply of tritium varies from 0.6 to 1.0.

CONCLUSION

Taking into consideration the results of field and laboratory tests, it can be confidently stated that tightly bound tritium has no direct bioavailability to plant roots. Obviously, the bioavailability of tightly bound tritium is indirectly related to the influence of soil water conditions and, possibly, to the exometabolic activity of roots. However, this question is still open and requires further research.

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ҚАТТЫ БАЙЛАНЫСҚАН ТРИТИЙДІ ӨСІМДІКТЕРДІҢ СІҢІРУІ

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Семей сынақ полигоны (ССП) жағдайында және зертханалық вегетациялық тәжірибе барасында in situ топырағында тритийдің тығыз байланысқан түрінің биологиялық қолжетімділігіне зерттеу жүргізілді. Ядролық сынақтар жүргізілетін орындарда ССП аумағында өсетін *Stipa capillata* өсімдіктерінде іріктеу кезінде тіндердің бос суында тритий (БСТ) болмаған жағдайда органикалық байланысқан тритийдің (ОБТ) елеулі шоғырлануы тіркелген. *Cucumis sativus* дақылдарын қолданатын модельдік тәжірибеде тәжірибенің басында және соңында топырақ ерітіндісінің құрамындағы топырақтың қатты фазасы мен тритийленген судың (НТО) құрамындағы тығыз байланысқан тритийдің меншікті белсенділігі айтарлықтай ерекшеленді. Сонымен қатар, *Cucumis sativus*-тегі БСТ-нің меншікті белсенділігі топырақтағы тығыз байланысқан тритийдің белсенділігімен салыстырғанда 2 есеге аз болды және өсімдіктердің тамыр жүйесі үшін қол жетімді топырақ ерітіндісіндегі тритийленген судың белсенділігінен сәл өзгеше болды. Вегетациялық зерттеулердің нәтижелері тәжірибелік өсімдіктер үшін тритийдің тікелей көзі топырақ ерітіндісі екенін көрсетті. Алайда, вегетациялық тамырлардағы топырақтың сұйық және тығыз фазасындағы тритийдің меншікті белсенділігінің өзгеруі сілтілеу процестерінің нәтижесінде радионуклидтің тығыз байланысқан түрі топырақ ерітіндісіндегі тритийленген судың көзі болғанын көрсетеді. Тығыз байланысқан радионуклидтің тығыз фазадан топырақ ерітіндісіне сілтіленуінің ықтимал себептері ылғалдандыру режимі, сондай-ақ өсімдік ризосферасының әсері болып табылады.

Түйін сөздер: *Семей сынақ полигоны, тығыз байланысты тритий, биологиялық қолжетімділік, Stipa capillata, Cucumis sativus.*

ПОГЛОЩЕНИЕ ПРОЧНОСВЯЗАННОГО ТРИТИЯ РАСТЕНИЯМИ

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Проведено исследование биологической доступности прочносвязанной формы трития в почве *in situ* в условиях Семипалатинского испытательного полигона (СИП) и в лабораторном вегетационном эксперименте. В растениях *Stipa capillata,* произрастающих на территории СИП в местах проведения ядерных испытаний, зафиксированы значимые концентрации органически связанного трития (ОВТ) при отсутствии трития в свободной воде тканей (ТСВ) на момент отбора. В модельном эксперименте с использованием культуры *Cucumis sativus* удельная активность прочно связанного трития в составе твердой фазы почвы и тритированной воды (НТО) в составе почвенного раствора в начале и в конце эксперимента существенно отличались. При этом удельная активность ТСВ в *Cucumis sativus* была на 2 порядка меньше по сравнению с активностью прочно связанного трития в почве и незначительно отличалась от активности тритированной воды в почвенном растворе, который является доступным для корневой системы растений. Результаты вегетационных исследований показали, что непосредственным источником трития для экспериментальных растений являлся почвенный раствор. Однако, изменения удельной активности трития в жидкой и твердой фазе почвы в вегетационных сосудах указывают на то, что источником тритированной воды в почвенном растворе являлась прочно связанная форма радионуклида в результате процессов выщелачивания. Сделано предположение, что возможными причинами выщелачивания прочно связанного радионуклида из твердой фазы в почвенный раствор является режим увлажнения, а также влияние ризосферы растений.

Ключевые слова: *Семипалатинский испытательный полигон, прочно связанный тритий, биологическая доступность, Stipa capillata, Cucumis sativus.*