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Investigation of tritium assimilation by sunflower crop at uptake through root

This study presents a quantitative assessment of tritium assimilation by sunflower *(Helianthus annuus* L.) crop at HTO uptake by root in vitro. It is found that at chronic uptake of tritium radionuclide as tritiated water (HTO) by sunflower through root system mean value of OBT/TFWT ratio during vegetation was 0.21. The conversation rate of tissue free water tritium (TFWT) to organic matter as organically bound tritium (OBT) varied from 0.08 to 0.044 % h^{-1}, with a maximum in the leafage phase. Translocation index (TLI) for sunflower crop at root uptake of tritium was 65 %. The distribution nature of tritium in sunflower parts at lasting root uptake of tritium for tissue free water tritium and organically bound tritium decreases in the next rows consequently: "roots>inflorescences>stems>leaves" and "inflorescences>stems>leaves>roots". At root uptake of tritium by sunflower as HTO form values of TFWT activity closely correlate with the activity of the isotope in water using for plant irrigation (r = 0.89). Established regularities of tritium assimilation and distribution in sunflower parts and OBT/TFWT ratio are of practical importance to biomonitoring of environmental contamination with tritium at impact territories of nuclear fuel cycle enterprises.

Keywords: sunflower, tritium, tritiated water, root uptake, assimilation, tissue free water tritium, organically bound tritium, conversion rate, ratio, translocation index.

Introduction

Tritium is produced during the operation of nuclear reactors of all types [1]. Currently, there are no efficient ways of capturing tritium, therefore the radionuclide together with discharges and releases from nuclear fuel cycle enterprises enters aquatic and terrestrial ecosystems and thus food chains, in which man can be the end link. The main depot of naturally occurring tritium isotope is water; therefore tritium together with a water flow and water vapors from places of primary contamination easily travels long distances [2–4].

A significant stage of tritium transfer is plants that are able to absorb man-made tritium incorporated in water through roots [5]. Next, as a result of identical chemical properties of ordinary water molecule (H_2O) and tritiated water (HTO), tritium is readily involved in photosynthesis processes, which result in its transfer to organically bound tritium (OBT) [6, 7]. Organically bound form of tritium is capable of being retained in cell structures for a long time, and its dose coefficients are roughly three times as high as tritiated water (HTO) ones [5, 8]. In this context, the study of problems of radiological and hygienic hazard of man-made tritium from the perspective of its uptake, distribution and incorporation in crop products, is a relevant task for developing strategic measures and predicting radioecological consequences in case of radiation accidents. However, risk assessment in case of contamination with tritium has remained a challenge up to now with many unknown quantities. Reasons are insufficiency, inconsistency of most in vitro and in vivo quantitative data describing processes of uptake, distribution and incorporation of man-made tritium. The paper is aimed at a quantitative assessment of the process of root uptake of HTO by plants in vitro the case of sunflower crop.

Experimental

Sunflower (*Helianthus annuus* L.) was chosen as an experimental plant because it is actively cultivated in the Republic of Kazakhstan as an oil crop. Research into quantitative indices of incorporation of tritium at HTO root uptake by plants was undertaken *in vitro*.

Drainage system, then background light-chestnut loamy soil, in which sunflower was germinated in vitro, were placed in plastic vegetation vessels (V–35 l). A total of 10 vessels were prepared. Seeds similar in size and weight were used for the experiment. 3 plants at most were cultivated in the same vessel. For tritium root uptake simulation, plants were watered with water with HTO high activity concentration for the duration of vegetation. Water with a high activity concentration of tritium was sampled in the territory of Semipalatinsk Test Site every 10 days to simulate natural dynamics of HTO in the soil solution of vegetation vessels. Experimental plants were watered with a regular portion of tritiated water 3 to 5 times for 10 days. In morning hours — with water at a pre-calculated rate maintaining optimal moisture of soil at the level of 60 % of the total moisture capacity [9].

Rather similar growth conditions of experimental plants were ensured by a phyto-lighting and thermoregulation system. The ventilation system in the laboratory premises was employed to exclude aerial uptake of tritium during the experiment owing to evaporation of HTO by soil after watering and transpiration activity of plants. The activity concentration of tritium in water and in plant samples was controlled simultaneously every 10 days.

The experiment lasted as long as the growth phase of sunflower seeds (when ripening begins) was 65 days. During the entire experiment, sampling of sunflower leaves was randomized every 10 days in three replications. At the end of the vegetation period, at the ripening stage, sunflower parts were additionally sampled (stems, leaves, inflorescences, roots) to establish the nature of tritium distribution in tissue-free water (TFWT — tissue-free water tritium) and in the organic matter (OBT — organically bound tritium). The weight of each plant sample averaged 100 to 150 g.

The activity concentration of tritium was measured in tissue-free water and the organic matter of plants. Free water from plants was separated through a special facility [10]. The facility represents a pressurized transparent vessel for feeding plant samples, connected with a cool metal surface and a receiver to collect condensate extracted from a sample. A 10–15 ml condensate collected was prepared for β-spectrometric measurement of the activity concentration of tritium with a liquid scintillation spectrometer.

Plant samples for measuring the activity concentration of OBT were prepared using a technique of combusting a dry plant sample with a “Sample Oxidizer 307” facility (Perkin Elmer, USA) followed by preparation of resulting water for β-spectrometric measurement of the activity concentration of ^3H with a liquid scintillation spectrometer.

The activity concentration of tritium in test samples was determined by liquid scintillation spectrometry using a “QUANTULUS 1220” spectrometer (Perkin Elmer, USA) [11]. Just before the analysis, all samplese passed through ha filtration stage to remove mechanical impurities. Then a 3 ml aliquot was collected from a measured sample and placed in a 20 ml plastic vial adding a scintillation cocktail in the ratio of 1 to 4. A scintillation cocktail Ultima Gold LLT specifically developed for measuring tritium in natural samples (tritium registration efficiency was in the order of 60 % ranging from 0 to 18 keV) was used to analyze samples. The measurement time of each sample was about 120 minutes. A beta-spectrum was processed, and tritium activity concentration was calculated with “Quanta Smart” software. The minimum detectable activity of tritium for the radiometric instrumentation used ranged as follows: for OBT — 4.5 to 7, for HTO — 2.7 to 5 Bq kg^{-1}.

Activity concentration ratio was determined as the ratio of activity concentrations of OBT and TFWT [12, 13].

The rate at which OBT is produced was calculated as per the formula [14]:

$$v \times C_{\mathrm{TFWT}} = \frac{dC_{\mathrm{OBT}}}{dt}, \qquad (1)$$

where C_{OBT} — activity of OBT in sunflower leaves, Bqkg^{-1}; C_{TFWT} — activity of TFWT in leaves, Bqkg^{-1}; t — observation time (exposure time), h; v — the rate at which TFWT turns into OBT, % h^{-1}.

For a quantitative assessment of OBT transfer to the plant, the translocation index was employed, which was determined by the following ratio [15]:

$$TLI = \frac{C_{\mathrm{OBT}}}{C_{\mathrm{TFWT}}} \times 100\ \%, \qquad (2)$$

where C_{OBT} — activity of OBT in plant parts, Bq kg^{-1}; C_{TFWT} — activity of TFWT in leaves.

Results and Discussion

During the experiment with sunflower crop, climatic changes affecting biological productivity of plants were measured (temperature and relative humidity). Air temperature varied from 23 to 32 °C, relative air humidity — 35 to 55 % and as a whole corresponded to normal growth conditions of sunflower. The duration of the experiment was 65 days before ripening began.

Values of the activity concentration of tritium in water (for watering) during the experiment varied from 95 to 540 kBq kg^{-1} (Fig. 1). As a whole, the variation range was 1 order of magnitude. Thus, natural dynamics of HTO was simulated *in vitro*.

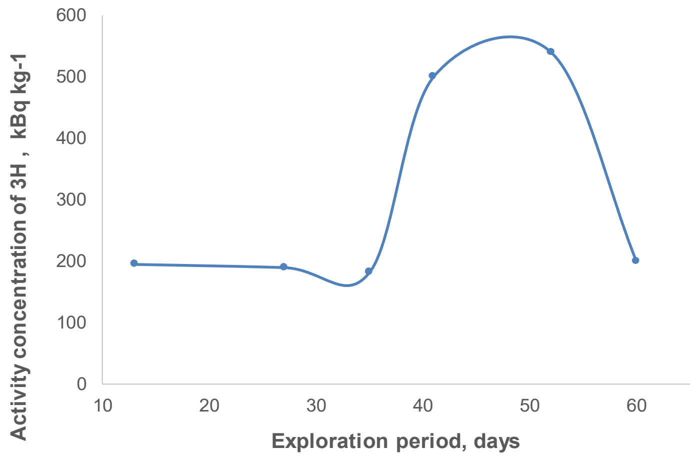

Figure 1. Measurement of the activity concentration of tritium in water (for watering)

Figure 2 illustrates the measurement results of TFWT and OBT activity concentration into sunflower leaves throughout the experiment.

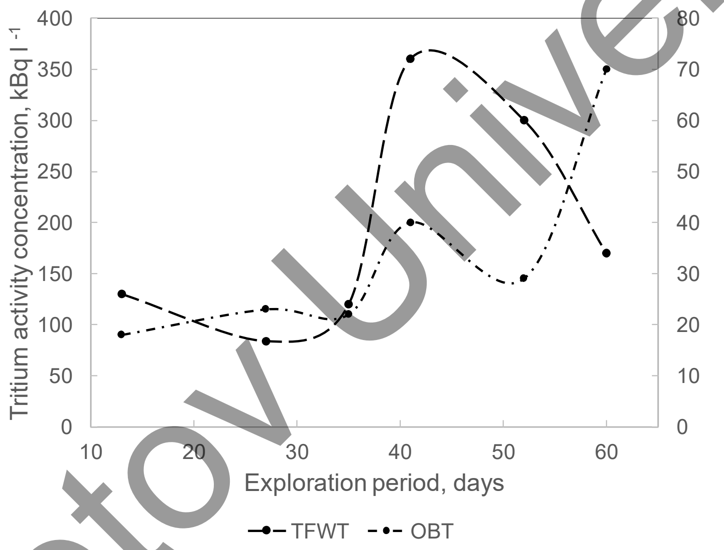

Figure 2. TFWT and OBT activity concentrations in sunflower leaves

According to provided data, values of OBT activity concentration ($n\cdot 10^4$) are one order of magnitude lower compared to TFWT values ($n\cdot 10^5$) for sunflower leaves during vegetation.

Based on derived quantitative parameters of TFWT and OBT, the conversation rate of TFWT to OBT in sunflower leaves was estimated (Fig. 3). During vegetation, rate values were varying between 0.080 and 0.044 % h^{-1}. The maximum rate at which OBT is produced was found in the active growth phase of sunflower, and at the end of vegetation (stage of seeds growth), the index of the rate at which OBT is produced decreased almost twice. A similar regularity was found when studying quantitative parameters of aerial uptake of tritium in vivo [16]. High rate of OBT formation at the early stages of plant development is attributed to intensive biosynthesis and consequently photosynthesis [5, 17]. A drop in the rate of tritium incorporation into organic structures of plants at the end of vegetation was probably caused by ageing of laminae and, accordingly, green plastids in which photosynthesis occurs [17].

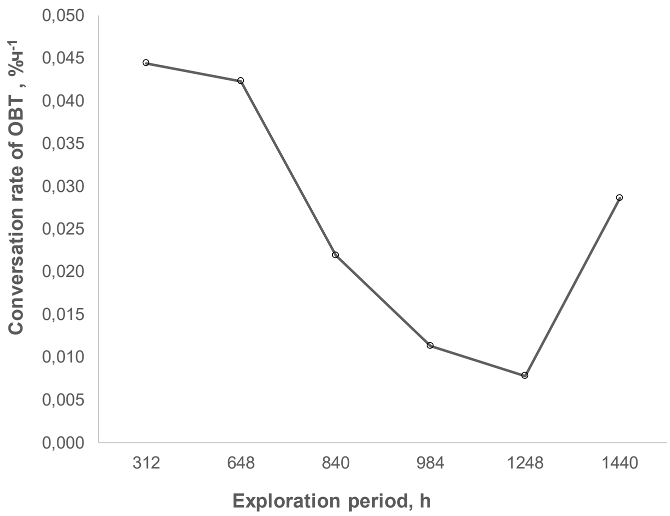

Figure 3. The conversion rate of OBT in sunflower leaves

Dependence derived from the conversion rate of OBT points to the fact that the process of ^3H incorporation is largely attributed to specific nature of the entire complex of internal processes physiological processes in the course of vegetation development of the plant.

To analyze experimental quantitative parameters of tritium uptake by sunflower crop, a non-parametric statistical technique was applied — rank correlation analysis. According to obtained data, a significant trustworthy correlation (at $p < 0.05$) dependence was only revealed between values of activity concentration of tritium in the source of uptake (water that was used for watering) and TFWT (r — 0.89, r — 0,89, at $p < 0.05$).

Based on experimental data, values of OBT/TFWT ratio were derived for sunflower at root uptake (Fig. 4), which can be employed as tritium contamination markers.

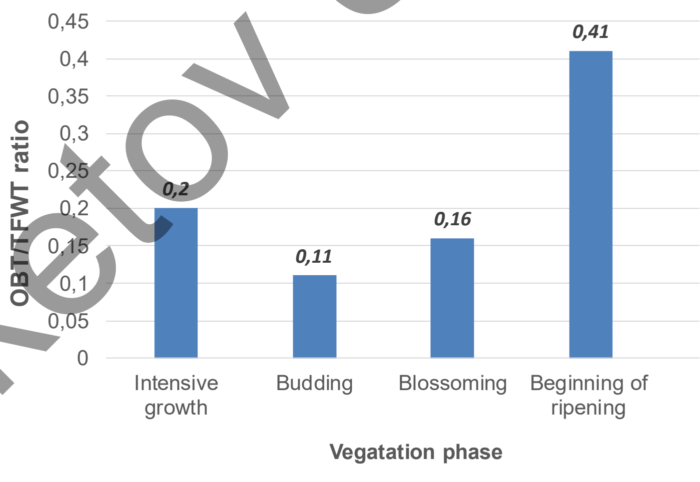

Figure 4. Values of OBT/TFWT ratio for sunflower at root uptake of HTO

The mean OBT/TFWT ratio over the vegetation period for sunflower was 0.21.

Figure 5 provides the nature of tritium distribution in parts of sunflower at root uptake at the end of the vegetation period.

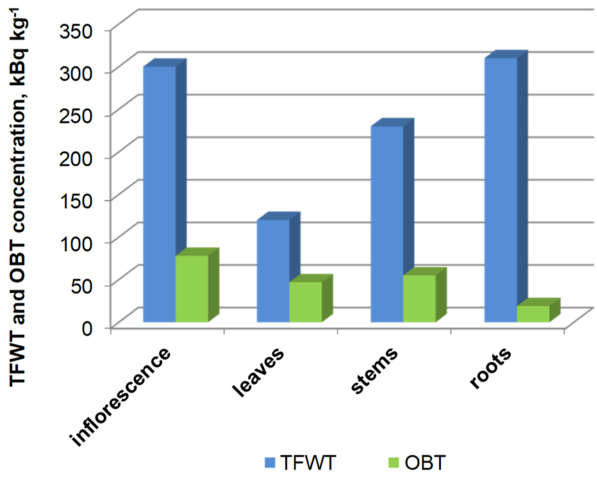

Figure 5. Tritium distribution in sunflower parts

As seen from Figure 4, at root uptake of tritium the distribution of TFWT and OBT in parts of sunflower is different. For example, the TFWT activity decreases in the series "roots>inflorescences>stems>leaves", and that of OBT — in "inflorescences>stems>leaves>roots". According to earlier studies [16], at aerial HTO uptake, tritium distribution is opposite, and maximum activity concentrations of TFWT and OBT are observed in aboveground plant parts. This fact indicates that tritium distribution in the plant is affected by radionuclide pathway.

The translocation index at root uptake of tritium was 65 %, which is almost 2 times higher than a similar value at aerial uptake of the radionuclide [16].

Conclusions

Experimental quantitative parameters of uptake and incorporation of tritium in the case of sunflower crop demonstrated that the TFWT activity depends on the activity level of tritium in the source whereas that of OBT is a quantity being dependent on plant vegetative growth.

Practical utility of research findings is that regularities established for tritium distribution in parts of sunflower and OBT/TFWT ratio values can be used in biomonitoring of contamination with tritium, in particular, to reveal the source of entry of this radionuclide into the environment. Values of translocation index derived for OBT to the yield can be used in assessing radiation exposure of the population living in nuclear facility impacted areas.

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Күнбағыс дақылының тамырымен сіңіру кезінде тритийді инкорпорациялауды зерттеу

Мақалада күнбағыс дақылының (*Helianthus annuus* L.) тамырымен HTO *in vitro* сіңіру кезінде тритийдің инкорпорациялануына мөлшерлік баға берілген. Күнбағыспен тритий суы (HTO) түріндегі тритий радионуклидінің созылмалы түрде тамырмен сіңірілуі кезінде вегетация кезеңінде ОСТ/ТСВ арақатынасының орташа мәні 0,21 құрағаны анықталды. Тіндердің бос суындағы тритийдің (ТСВ) органикалық байланысқан тритийге (ОВТ) айналу жылдамдығы жапырақтану фазасында максимум 0,08-ден 0,044 % сағ$^{-1}$-ге дейін өзгерді. Тритий тамыр арқылы сіңірілгенде күнбағыс өніміне арналған транслокация индексі (TLI) 65 % құрады. Тритийдің күнбағыс мүшелерінде тамырларымен ұзақ сіңуімен таралу сипаты ТСВ үшін — «тамырлар > гүлшоғыр > сабақтар > жапырақтар», ал ОСВ үшін — «гүлшоғыр > сабақтар > жапырақтар > тамырлар» қатарында азаяды. Тритийдің күнбағыс дақылының тамырымен сіңірілуі кезінде ТСВ меншікті белсенділігінің мәні тамырлармен сіңірілетін судағы радионуклидтің белсенділігімен тығыз байланысты (r — 0,89, $p < 0,05$). Күнбағыс мүшелерінде тритийдің таралуының белгіленген заңдылықтары және ОСТ/ТСВ арақатынасы ядролық отын циклі кәсіпорындарының импакт аумақтарындағы қоршаған ортаның тритиймен ластануының биомониторингі үшін қолданбалы мәнге ие.

Кілт сөздер: күнбағыс, тритий, тритийленген су, тамырдың сіңірілуі, ассимиляция, тритий тіндердің бос суындағы тритий, органикалық байланысқан тритий, конверсия коэффициенті, қатынас, транслокация индексі.

Е.Н. Поливкина, Л.Ф. Субботина, Ф.Ф. Жамалдинов

Исследование корневого поглощения трития культурой подсолнечника

В статье представлены результаты исследования корневого поглощения и ассимиляции трития культурой подсолнечника (*Helianthus annuus* L.) в условиях модельного эксперимента. Установлено, что при длительном корневом поглощении изотопа трития в растения значения соотношения ОСТ/ТСВ в течение вегетационного цикла изменяются в диапазоне 0,1–0,27, среднее значение составило 0,21. Скорость образования органически связанной формы трития изменялась от 0,08 до 0,044 % ч$^{-1}$, достигая максимума в фазе облиствления подсолнечника. Индекс транслокации для культуры подсолнечника при корневом поступлении трития составил 65 %, что значительно выше (почти в 2,5 раза) по сравнению с аэральным поглощением радионуклида. Характер распределения трития по органам подсолнечника при хроническом корневом поглощении трития для ТСВ и ОСТ уменьшается в следующих рядах соответственно: корни>соцветия>стебли>листья и соцветия>стебли>листья>корни. В условиях корневого поступления трития в подсолнечник значения удельной активности ТСВ тесно коррелируют с удельной активностью изотопа в составе тритированной воды, использованной для полива растений (r — 0,89, при $p < 0,05$). Выявленные закономерности поглощения, транслокации и распределения форм трития по органам подсолнечника имеют прикладное значение для биомониторинга тритиевого загрязнения окружающей среды на импактных территориях предприятий ЯТЦ.

Ключевые слова: подсолнечник, тритий, насыщенная тритием вода, корневое поглощение, ассимиляция, тритий свободной воды тканей, органически связанный тритий, коэффициент конверсии, соотношение ОСТ/ТСВ, индекс транслокации.

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