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Transfer of ^{137}Cs into onion (*Allium cepa* L.) under conditions of hydroponic model experiment

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Abstract. Short-term hydroponic experiment was conducted to evaluate intensity of the ^{137}Cs transfer from a solution with extremely high activity of the radionuclide into onion, considering different plant's compartments (green leaves, bulbs, roots). It has been revealed that the root uptake of ^{137}Cs was significantly discriminated, and not exceeded 0.01% of the total amount of radionuclide contained in the solution. At the same time, biometric indices of the plants – shoot height, roots elongation, phytomass inventory and structure – under conditions of ^{137}Cs stress exposure were not significantly changed in comparison with the control. The main accumulation of ^{137}Cs , which incorporated into onion, occurred in roots, especially in root tips, which are most actively involved in the processes of mass exchange between the plant and the external environment. The translocation of ^{137}Cs into the shoot part, including the onion bulb and green leaves, was significantly suppressed, which allows considering the root system of onion as a biological barrier providing the rhizofiltration of the toxicant.

1. Introduction

Global contamination of terrestrial ecosystems of the Northern Hemisphere by ^{137}Cs started from the 50s years of the 20th century as a result of nuclear weapons tests in the atmosphere, and after limiting and prohibiting such tests by the international pact of 1963 and 1996, introduced into the biosphere anthropogenic radioactive elements was associated with major accidents at nuclear power facilities. For example, the release of technogenic radionuclides into the atmosphere at Kyshtym accident (1957, USSR) was estimated as $7.4 \cdot 10^{16}$ Bq, Windscale/Sellafield accident (1957, UK) – $7.7 \cdot 10^{14}$ Bq, Chernobyl accident (1986, USSR) – $5.3 \cdot 10^{18}$ Bq, Goiania accident (1987, Brazil) – $5.1 \cdot 10^{13}$ Bq [1]. Emissions into the atmosphere as a result of the accident at NPP Fukushima Daiichi are estimated to be approximately one tenth of the emissions from the Chernobyl accident [2]. The ecological consequence of the distribution of long-lived radionuclides in the environment is their inevitable introduction into the biological cycle of elements, total, with their root consumption by plants from contaminated soils, and in the future - when spreading through food chains, the end user of which is possibly a human. The physiological role in living organisms of the natural analog of ^{137}Cs , stable ^{133}Cs , is currently unclear. However, it is presumably capable of causing metal toxicity stress, since it belongs to a group of heavy, and according to some ideas, even superheavy metals [3]. In turn to the incorporation of ^{137}Cs into living organisms can complement the negative effects of chemical nature



by the physical impact of ionizing radiation on them. Passing to a stable state, ^{137}Cs undergoes beta decay, which produces a barium isotope ^{137}Ba ($T_{1/2}$ 30.17), with 94.4% of the decay occurring with the intermediate formation of the ^{137}Ba nuclear isomer ($T_{1/2}$ 2.55 min), which in turn goes to the ground state with the emission of a γ -quantum with an energy of 661.7 keV. The total energy released during the decay of one ^{137}Cs nucleus is 1175.63 ± 0.17 keV. Value of ^{137}Cs as the main dose-forming radionuclide of bomb-derived and accidental fallout is amplified by the duration of its half-life and the ability to be firmly and irreversibly fixed by clay minerals of soils in the uppermost layer, while at the same time the main part of plant roots is concentrated [4, 5]. Thus, the greater ^{137}Cs entering terrestrial ecosystems is still in the space of intensive mass exchange between soil and plants, which facilitates the reproduction of the biogeochemical cycle of the radionuclide [4, 6, 7]. Hence, radioactive contamination of terrestrial ecosystems by ^{137}Cs is a matter of the recent environmental concern. Numerous studies on the intensity of ^{137}Cs root consumption by different plant cultures have shown that the specific activity of ^{137}Cs in plants is, as a rule, much less than in soils, i.e. bioavailability of the radionuclide in the field conditions is rather low [8]. However, in the mitigation of the ^{137}Cs root consumption process, it is difficult to differentiate the role of soil characteristics (namely, immobilization of the radionuclide due to irreversible sorption, as well as the configurational inaccessibility of the element localized inside the cloddy waterproof aggregates, etc.) and the actual biological properties of plants. The last one can be detected under conditions of hydroponic experiment, when the radionuclide is in ionic form and is completely accessible to plants. Besides, in comparison with field conditions, hydroponic experiments are easy to control and require less time to get general conclusions. Thus, they are used to investigate the bioaccumulation processes of potential phytotoxicants [9, 10]. Based on this, the purpose of this study was to analyze the intensity of the ^{137}Cs transition to onion plants (*Allium cepa* L.) and to identify the features of translocation efficiency from root to shoot in conditions of soilless hydroponic culture cultivation. In turn, the understanding of sensitivity or resistance of the crop to potentially phytotoxic radionuclide, as well as the revealing of mechanisms of biological adaptation to environmental stress are the vital factors in choosing suitable plants to grow at radioactively contaminated areas.

2. Materials and methods

2.1. Plant material and hydroponic experiment design

Short-term indoor hydroponic experiment was carried out with onion bulbs of commercially obtained Stuttgart Riesen cultivar. Onion has been chosen due to big supplies of reserve nutrients that create the possibility of growing plants without physiological nutrient solution to avoid competition effects of monovalent beneficent ions (K^+ , NH_4^+). Besides, onion is known as a plant with sensitivity to toxicants, and by the opinion of A.A. Oudalova et al. [11] special-purpose *Allium*-test could be recommended as an effective tool for mutagenic, mitosis-modifying and toxic testing in case of combined contamination of radioactive and chemical origin.

7 onion bulbs were placed in a plastic box with $^{137}\text{CsCl}$ solution with extremely high activity of 2.8 MBq/l and were grown for 14 days at day and night temperatures of 23 °C and 18 °C, respectively, with 45–60% relative humidity, and 250 $\mu\text{mol}/\text{m}^2/\text{s}$ light intensity. At the same time, a control experiment was conducted on the cultivation of onion on deionized water.

The vital state of plants was assessed; the growth of shoots and the length of leaves of onion were recorded daily.

2.2. Sampling and laboratory analysis

After 14 days of vegetation the onion plants were removed from the box, and were carefully rinsed with deionized water. The root, bulb and leaf parts (the last – at the base of a stem) were separated using a scalpel. Fresh weight of the biomass compartments was evaluated.

Then 2 copies of the onion plants grown in the ^{137}Cs -containing solution were herbicated (have been pressed and dried) for further autoradiography studies. Remaining plants were dried at a

temperature of 80 °C in an oven until completely dried, were ground using a mortar and pestle for homogenization of plant material, and prepared for the further γ -spectrometric analysis.

The prepared plant samples were studied using digital autoradiography on a Perkin Elmer Cyclone device (USA). The samples were isolated from the external γ -background using a shelter made of lead plates. Since the ^{137}Cs activity was different, the exposure times were individually selected; on average, it was 24 hour.

Spectrometric measurements were performed using a GR 3818 semiconductor γ -spectrometer with a Canberra high-purity (HPGe) detector (United States). Before the start of the measurements, three calibration of the γ spectrometer with a plant standard of IAEA No. 372 with a mass of 0.5, 1 and 1.5 g were carried out. The registration efficiency coefficients were 0.06, 0.05 and 0.05, respectively. The specific activity of ^{137}Cs in prepared onion samples was measured in the standard geometry of the Petri dish. Energy spectra measurements were accounted for 1200 sec, and ^{137}Cs activities were calculated from the net full energy peak 661.7 keV using the “Progress 5.1” spectrometric analysis software package. The analytical error did not exceed 2%.

2.3. Calculations and statistical analysis

The tolerance index (TI) to estimate generally the process of plant growth and biomass yield was calculated by the equation:

$$TI = \frac{M_{\text{treatment}}}{M_{\text{control}}},$$

where $M_{\text{treatment}}$ is total dry mass of onion or the mass of the plant compartments under ^{137}Cs -containing hydroponic solution, and M_{control} is dry mass of the plants and their parts in control.

To assess intensity of ^{137}Cs transfer from hydroponic solution into onion plants the values of transfer factor (TF) were calculated:

$$TF = \frac{A^{Cs-137}_{\text{plant}}}{A^{Cs-137}_{\text{solution}}},$$

where $A^{Cs-137}_{\text{plant}}$ is specific activity of ^{137}Cs in dry mass of plant, $A^{Cs-137}_{\text{solution}}$ is specific activity of ^{137}Cs in hydroponic solution.

The specific bioaccumulation coefficient (BC) for the onion compartments was calculated as:

$$BC = \frac{A^{Cs-137}_{\text{plant compartment}}}{A^{Cs-137}_{\text{solution}}},$$

where $A^{Cs-137}_{\text{plant compartment}}$ (roots, bulbs, leaves) is specific activity of ^{137}Cs in dry mass of the once.

To quantify the translocation of ^{137}Cs from roots to aerial parts the translocation coefficient (TC) was used as:

$$TC = \frac{A^{Cs-137}_{\text{bulbs or leaves}}}{A^{Cs-137}_{\text{roots}}},$$

And the last, but not the least, potential phytoremediation factor (PPF) was defined as:

$$PPF = \frac{A^{Cs-137}_{\text{plant}} \cdot M_{\text{plant}}}{A^{Cs-137}_{\text{solution}} \cdot V_{\text{solution}}} \cdot 100 (\%),$$

where M_{plant} is total dry mass of plant, V_{solution} is total volume of hydroponic solution.

To assess mean values and variability of the obtained data Microsoft Excel 2010[®] (Microsoft Cooperation, USA) was performed.

3. Results and Discussion

3.1. Plant growth and biomass yield

After 14 days of the onion plants exposure to ^{137}Cs influence, there were no obvious impact on the aboveground parts and roots. The most significant toxic symptoms, such as chlorosis and senescence of leaves, wilted leaf tips, and changes in color (blackening) of roots were not noted in comparison with the control.

Changes in growth variables throughout plant development under ^{137}Cs -stress conditions were not significant comparing to control for the first week, but slightly raised during the second week of the experiment (figure 1). As in the experiment and in the control on the 12th-13th day of hydroponic cultivation of onion, the growth of leaves in height slowed down and passed to the stage of the plateau. By the end of the experiment, plants grown in the ^{137}Cs -containing solution had a slightly less developed habit than in the control variant, but these differences were not statistically significant. One of the 7 bulbs used in the experiment began to germinate only after the 10th day of the experiment, and to its completion the height of the shoot was only 2.9 cm, which was a > 5 time less than in the control. In the control, the activation of plants from the dry bulb condition to the vegetative growth phase was 100%.

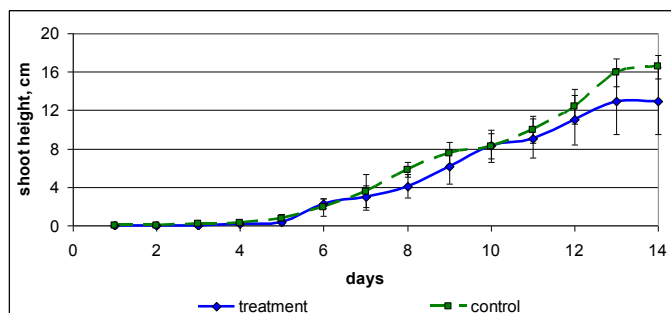


Figure 1. Dynamics in onion shoot growth during hydroponic experiment under ^{137}Cs treatment and in control conditions. Error bars represent $\pm t_{0.95} \cdot m$ at $n = 7$.

During the experiment, the raw weight of plants increased by $\approx 40\%$. At the same time, the height of shoots and root length, total biomass yield and the structure of its stocks by plant compartments when growing onion in the ^{137}Cs -containing solution were close to the control parameters (table 1), i.e. the significant phytotoxic effect of the radionuclide on biometric indices of onion in experimental and control conditions has not been established. Moreover, the total biomass of onion in the experimental version was even slightly higher than in the control, mainly due to the more juicy and dense leaves.

The value of the integral TI index was 1.08, which indicated the comparative tolerance of onion plants to the presence in the ^{137}Cs ion environment, at least in the range of those short-term experiment conditions that were created in the current investigation.

Table 1. Average values and variability of biometric characteristics of onion under ^{137}Cs treatment and in control conditions.

Index	^{137}Cs treatment	Control
Shoot height (cm)	13.0 ± 3.5^a	16.6 ± 1.2
Root length (cm)	7.9 ± 2.6	6.8 ± 2.3
Fresh weight per 1 plant (g)	4.5 ± 0.30	—
Leaves	0.9 ± 0.09 (20%)	—
Bulbs	3.2 ± 0.22 (71%)	—
Roots	0.4 ± 0.07 (9%)	—
Dry weight per 1 plant (g)	1.42 ± 0.07	1.16 ± 0.06
Leaves	0.34 ± 0.03 (24%)	0.22 ± 0.03 (19%)
Bulbs	1.02 ± 0.07 (72%)	0.90 ± 0.05 (78%)
Roots	0.06 ± 0.02 (4%)	0.04 ± 0.01 (3%)

^a hereafter confidence limits of the mean was calculated as $\pm t_{0.95} \cdot m$ at $n = 7$

3.2. Visualization of ^{137}Cs root uptake and distribution in onion by digital autoradiography

A digital autoradiographic study of the fractions of onion biomass grown on a ^{137}Cs -containing medium showed the presence of a radionuclide in all organs of the plant (figures 2-4). Similar results for the transition of ^{137}Cs into crops of sunflower, reed and poplar under conditions of hydroponic experience were described by P. Soudek et al. [12, 13], who, based on a relatively uniform tonality of the image on autoradiographic prints, concluded that isotope was transported together with nutrients via the transpiration stream and distributed through this process across all tissues. This sharply distinguishes the behavior of ^{137}Cs in the “soil-plant” system from the behavior of a heavy natural radionuclide ^{238}U , which is stored mainly in roots of a wide number of plant species cultivated under hydroponic conditions [14, 15, etc.].

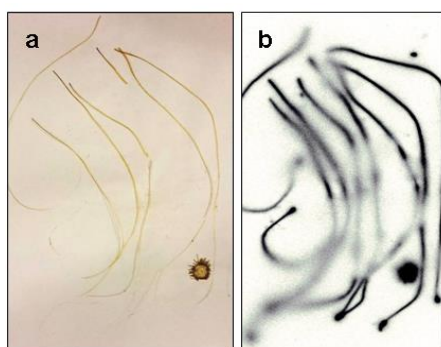


Figure 2. Accumulation of ^{137}Cs by the roots of onion: (a) photograph, (b) autoradiography (expose time 48 hours).

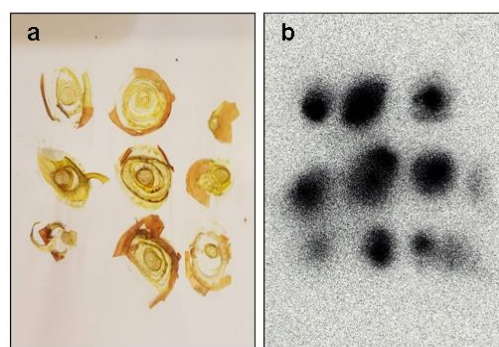


Figure 3. Accumulation of ^{137}Cs by the bulbs of onion: (a) photograph, (b) autoradiography (expose time 48 hours).

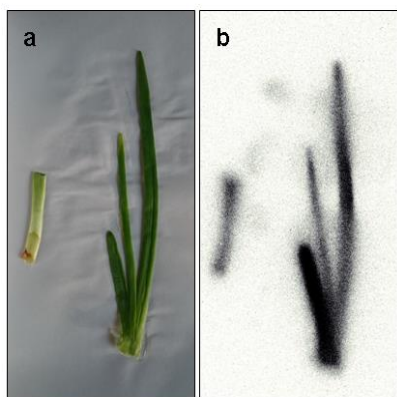


Figure 4. Accumulation of ^{137}Cs by the green leaves of onion: (a) photograph, (b) autoradiography (expose time 48 hours).

By specifying the distribution of ^{137}Cs in plant organs by the tone of the image, the authors suggested that in plants the radionuclide was localized especially in young leaves, leaf veins and tips, in the nodes, young shoots, and in the root system as well. The instrumental resolution of the used in the current study autoradiograph device did not allow confirming or disproving such assumptions, it can only be asserted that the contrast of the plant's organ and its thickness and / or plant tissue density is obvious.

In particular, it is characteristic that the bottom of the bulb (flattened in the plate between the root and shoot parts of the plant) has a high contrast autoradiographic image, apparently not because of increased accumulation in this part of the radionuclide, but because of the increased thickness and density of the tissue. The same is seemingly valid for thickened green onion leaves or areas of their superposition on each other in a herbarium specimen. An important detail that goes beyond the observations is visualization of the process of preferential accumulation of ^{137}Cs on onion root tips, while over the rest of the length of the uniformly cylindrical root cross section, the radionuclide is distributed evenly. Since it is at the ends of the roots that the fission, stretching and absorption zones

are concentrated, which are most actively involved in the growth and root nutrition of plants, then the increase in the accumulation of ^{137}Cs in them can have further cytogenetic and cytotoxic effects, leading to further chromosome aberrations. Thus, even in the absence of pronounced general symptoms of ^{137}Cs phytotoxicity in the hydroponic study, the possible negative damaging effect of radionuclide presence in the nutrient medium remains valid.

3.3. Assessment of ^{137}Cs root uptake and distribution in onion by γ -spectrometry

Quantitative determination of ^{137}Cs in the biomass of onion and its fractions showed that the process of root consumption of the radionuclide was characterized by an average intensity: the specific activity of the radionuclide in plants was 3-4 orders of magnitude lower than in the hydroponic solution (table 2). Characteristically, despite the extremely high activity of the model solution on which onion was grown, as well as the availability of the ionic form of the radionuclide to plants, the intensity of ^{137}Cs transition into plants was less than the averaged predicted values of TF from soil to plant group of non-leafy vegetables: $3.5 \cdot 10^{-2}$ (sandy soils) – $9.1 \cdot 10^{-3}$ (clay soils) [8]. Thus, the ability to eliminate the transition of ^{137}Cs from nutrient media to plants is an inherent property of the latter, indicating the presence in plants of a number of metabolic and physiological adaptation mechanisms to cope with stress by decreasing toxic ions accumulation.

Table 2. Average values and variability of biometric characteristics of onion under ^{137}Cs treatment and in control conditions.

Plant compartment	^{137}Cs activity (Bq/g)	^{137}Cs inventory per 1 plant (Bq)	% of ^{137}Cs inventory	TF / BC ^a	TC
Leaves	424.2	144.2	24.5	$0.15 \cdot 10^{-3}$	0.09
Bulbs	156.9	160.0	27.2	$0.05 \cdot 10^{-3}$	0.03
Roots	4732.1	283.9	48.3	$1.69 \cdot 10^{-3}$	–
Total biomass	414.2	588.2	–	$0.24 \cdot 10^{-3}$	–

^a TF value was calculated for total biomass, and BC was calculated for the biomass compartment, correspondingly.

At the same time, the highest values of ^{137}Cs activity, 10-30 times more than in the victorious part, were eliminated in the roots of onion. Significantly higher concentrations of ^{137}Cs in the roots suggest that the roots play in the plants in the cells. Taking into account the biomass structure of the plant, it can be argued that about ^{137}Cs deep, which passed from solution to plants, accumulated in the roots of onion, while the proportion of the roots themselves in the total biomass is less than 5% of the dry weight.

The intensity of ^{137}Cs from the roots of onion to non-chlorophyll flasks, as well as to green assimilating leaves, is 1–2 orders of magnitude smaller than the transition from solution to roots. TC values did not exceed 0.1, for both onion bulbs and leaves. At the same time, the specific activity of ^{137}Cs and the TC value in bulbs were significantly less than in the leaves. Perhaps, including the regulation of the specific plasma membrane of ^{137}Cs (excluding contact surface contamination) transporters.

It can be assumed that under conditions of ^{137}Cs -stress, a strategy of toxic object rhizofiltration is implemented, in which the roots serve as a barrier that discriminates against the transition of the radionuclide to the escape. Radiocaesium fixed by roots, can protect more susceptible airborne parts from metal and radioactive toxicity [16] and oats [17], which, like the onion, belong to the class of monocotyledonous plants. An advantageous accumulation in the roots and a weak translocation into the aerial parts were also revealed with respect to a number of heavy metals known for their phytotoxic properties at extremely high concentrations of their mobile forms in soils – Cd, Pb, and Cu [10, 18–20]. Explaining the similar barrier function of the onion roots by the accumulation of the

organic toxicant TNT (2,4,6-trinitrotoluene) grown as a hydroponic culture, Ja. Kim et al. [21] noted as possible causes for the heterogeneous distribution of matter between roots and green leaves: (1) the toxicant transported from the roots can be greatly diluted in the leaf volume, and (2) the translocation rate to the leaves from the roots is relatively low. Taking into account the last consideration, it can be assumed that for a longer experiment the ^{137}Cs distribution by the onion organs could change quantitatively, but it seems unlikely that a fundamental change in the structure of the radionuclide stocks will be possible.

Another assumption arose on the basis of the data obtained in the experiment: the value of the specific activity of ^{137}Cs and, as a consequence, the value of BC in the onion organ showed a directly proportional relationship with the relative difference between the wet and dry weight of the plant tissue, i.e. with the content of cellular juice in it (figure 5). Indeed, belonging to the group of monovalent alkali metals, ^{137}Cs is not capable of creating chelates or sparingly soluble salts, so it is quite logical to assume its occurrence in the intracellular fluid, rather than fixing the cell wall in the substance.

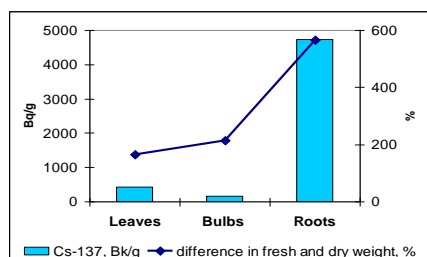


Figure 5. Relationship between ^{137}Cs activities in the onion compartment and the difference in fresh and dry weight of the correspondent compartment.

In general, during the model experience only a very small part of the radionuclide contained in the solution passed into plants. With the onion cultivation scheme used, the total PPF did not exceed 0.01%, and the total participation in the extraction of ^{137}Cs potentially easily removed from the soil of the shoot part of the plant did not exceed 0.006%. In this connection, the potential ability of onion plants to use them for phytoremediation of soils contaminated with ^{137}Cs should be considered to be of little promise.

4. Conclusions

Although hydroponic short-term studies do not reflect the real field of radioactively contaminated lands, it can be assumed that they are an efficient method for the express prediction of ^{137}Cs behaviour in the "soil-plant" system. Thus, in the present study it was shown that even under conditions of extremely high concentration of ^{137}Cs in the hydroponic solution, the transfer of the radionuclide to the onion was significantly limited due to the biological characteristics of the plant, which discriminate the process of root consumption of the toxicant. At the same time, biometric indices of plants – shoot height, roots elongation, biomass inventory and structure – under conditions of ^{137}Cs stress exposure were not significantly changed in comparison with the control. The preferential accumulation of ^{137}Cs occurred in the roots of the plant, and to the maximum extent, presumably at the root tips, most actively participating in the processes of mass exchange between the plant and the external environment. The translocation of ^{137}Cs from the roots to the shoot was significantly suppressed, which allows considering the root system of onion as a biological barrier that provides toxic filtration of the toxicant.

Acknowledgments

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