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ORIGINAL PAPER

Effects of ^{137}Cs low level exposure (internal and external) doses on plants

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Abstract Effects are described of internal exposure doses of ionizing radiation in above- and underground parts of test plants under natural conditions and in roots of *Lepidium sativum* L. from accumulated ^{137}Cs under laboratory conditions. In the region most contaminated after the Chernobyl accident, in the tested 10 plant species for ^{137}Cs ionizing radiation the internal exposure doses 3.5 times exceeded those in the Ignalina Nuclear Power Plant environment. Under laboratory conditions the effect of low internal (0.6–600 μSv) and external (40–5500 μSv) exposure doses from ^{137}Cs on garden cress, *Lepidium sativum* L. roots was, practically, the same. Both internal and external exposure doses stimulated the plant root growth by 12 and 33%, respectively. Different effect of external and internal exposure on the developing plant cells was observed by analyzing the results of morphometric investigations of the primary root cap cells of *Lepidium sativum* L.

Key words radioecology • garden cress • *Lepidium sativum* L. cells • roots • seeds • morphometric analysis • radiocaesium

Introduction

Initially, investigations related to the effect of ionizing radiation on plants were carried out using external radiation sources, i.e. X-rays, gamma rays, mono-energetic neutrons of various energies, heavy particles, such as nitrogen ions, etc. [20, 21, 23]. The investigations have shown, that as compared to animals, some plants are more sensitive to ionizing radiation. Broad scale investigations of radionuclide effects on plants were initiated after the Chernobyl accident, when large areas of ariable soil and woods were contaminated with radionuclides. The radionuclides were accumulated mainly in soil and biota and were the source of potentially hazardous radiation doses. Particularly wide investigations of this problem under natural conditions were carried out in Ukraine, Belarus and Russia.

Ionizing radiation, as mutagenic factor, is not selective, because it inflicts damage to genes, coding for enzymes of various metabolic cycles. Besides, mutations are randomly distributed in the whole genome [6]. After the Chernobyl Nuclear Power Plant (NPP) accident it was determined that in the case of acute ionizing radiation doses, the effect of radionuclides, incorporated into the organism, can be 2–4 times higher than that of external irradiation because of the atom decay in the cell [9]. Biological effect of radionuclides, accumulated in the organism, differently from the external irradiation, is conditioned by the radionuclide

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accumulation level and their localization in the organism and cells; it can be influenced by various environmental factors and anthropogenic pollutants [8, 11]. In the cell, when radionuclides enter the inner cell compartments and particularly, when they are close to DNA molecules, the genetic effects can be induced not only because of the ionizing radiation due to the radionuclide decay but also due to transmutation, defined as changes of chemical nature of the decaying atom taking place directly at the site of radioactive decay [13, 15].

Internal exposure doses in plants can increase because of large amounts of radionuclides accumulated in their tissues, especially those with actively dividing cells [16, 19]. For example, radiocaesium like its chemical analogue, potassium, relatively strongly accumulates in young as well as in meristemic tissues [22]. The plant response to the incorporated ^{137}Cs, particularly to low ionizing radiation doses, is not sufficiently investigated [4].

The aim of this study includes:

- determination of internal exposure from accumulated ^{137}Cs in above- and underground parts of test-plants, taken in two different regions of Lithuania;
- evaluation and comparison of effects of internal and external exposure (at similar exposure doses) from ^{137}Cs on garden cress, *Lepidium sativum* L. seed germination and root growth under laboratory conditions.

Material

Environmental investigations

^{137}Cs activity concentration in test-plants for calculation of internal exposure doses were investigated in biotops of terrestrial ecosystem in the vicinity of Ignalina NPP: pine forest (*Hylocomium splendens* (Hedw.) Schimp., *Lichenies sp*., *Vaccinium myrtillus* L., *Calluna vulgaris* (L.) Hull, *Pteridium aquilinum* (L.) Kuhn), bog (*Sphagnum sp.*, *Calla palustris* L.), meadow (*Artemisia vulgaris* L., *Lipinus polyphyllus* Lindl., *Dactylis glomerata* L.) in 1996, 2000 and 2002. In the regions mostly contaminated after the Chernobyl NPP accident, the investigations of the above-mentioned biotops were carried out in 1994–1998 and in 2002. The choice of the test-plants species was based on the following criteria: high accumulation rate of radionuclides, wide spreading, occupation of large areas, large biomass and easy sampling.

Model experiments under laboratory conditions

The garden cress, *Lepidium sativum* L. was used in model experiments. This test-organism is widely applied in toxicological investigations [7, 24]. The biological effect was evaluated by *Lepidium sativum* L. seed germination and root growth, because the plant root meristem (a tissue with active cell divisions) due to the intensive metabolic processes is the most radiosensitive tissue [16, 19].

Methods

Internal exposure experiments

Experiments with *Lepidium sativum* L. were conducted according to the modified Magone method [12]. Experiments were performed with 10 ml of a treating solution in Petri dishes where 25 *Lepidium sativum* L. seeds were evenly distributed on a filter paper. Dishes were placed for 2 days in a thermostat at 24 ± 1°C in the dark. ^{137}Cs activity concentration in the studied lake water solution was 0.4, 4.0, 40, 440 kBq/l at pH 7.5. Model system was prepared using 0.1 ml ^{137}Cs aqueous hydrochloric acid solution, diluted 10^5–10^8 times; therefore, Cl^- ions had no influence on the plant seed germination and plant development. For control samples, lake water with pH 7.5 was used. The presented data are the arithmetical means of 2–3 experimental series with calculated standard errors.

^{137}Cs activity concentration measurements

Samples of test-plants which grew in natural conditions were dried and incinerated at a temperature of 400–450°C. ^{137}Cs activity concentration in these samples was measured using high resolution γ-spectrometers with various semiconductor detectors.

In model experiments, ^{137}Cs activity concentration in lake water solution and in *Lepidium sativum* L. biomass was determined by spectrometric analysis. For γ-spectrometric measurement, samples of plants were rinsed with lake water, dried at room temperature and pulverized. ^{137}Cs activity was measured using a γ-spectrometer with a well-type high purity germanium (HPGe) detector. This detector has a sensitive volume of 170 cm^3, the well inside the germanium crystal is 16 mm in diameter and 40 mm in depth; it can accommodate small samples with an effective volume of up to 4 cm^3. The resolution at full-width at half the peak maximum (FWHM) is 2.05 keV at 1333 keV. The overall uncertainty is around 6% if the uncertainty due to counting statistics does not exceed 1% [10].

Internal exposure dose calculations

Assuming that the radionuclide accumulated in the plant (or in its part) is distributed homogeneously, internal exposure (absorbed) dose to the whole plant (or its part) was calculated according to the methodology suggested by Blaylock *et al*. [3]:

$$\text{DEFF} = 1.6022 \cdot 10^{-13} \sum_i \sum_j \Phi_{ij} \cdot f_{ij} \cdot E_{ij} \cdot w_j \int_0^{\tau} C_{0i}(t) \cdot dt \quad (1)$$

where: DEFF (dose equivalent flora and fauna) is the internal exposure β- and γ-radiation dose, Sv; Φ_{ij} is the absorbed fraction of energy E_{ij}; f_{ij} is the yield, Bq·s^{-1}, of radiation j per disintegration of the nuclide i; E_{ij} is

the energy, MeV, of radiation j for the nuclide i; $C_{0,i}$ is the concentration of the radionuclide i in the organism, Bq·kg^{-1} wet weight.

External irradiation experiments

In external irradiation experiments, Petri dishes with 10 ml of lake water and 25 seeds of *Lepidium sativum* L. evenly distributed on a filter paper were placed for 2 days in an irradiation chamber of UPD-INTER equipment where ^{137}Cs ionizing radiation source was installed. In the irradiation chamber exposure dose rate could be changed from 2.4 to 115 μSv/h (± 15%) at a constant temperature of 21 ± 2°C in the dark.

Cell morphometric analysis

For morphometric analysis after 48 h of growth, seedlings were fixed in 4% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2). Later, the seedlings were postfixed in 1% O_sO_4, dehydrated and embedded in Epon by standard procedures. Measurements of statocytes of the 3rd to 7th columella storeys were performed employing light microscopy on median longitudinal sections of the root cap with a digitizer of the IBAS-1 system (Germany).

Statistical analysis

Statistically significant difference between experimental and control samples was assessed by t-test (at $p < 0.05$) using Statgraphics plus version 2.1 program.

Results and discussion

Analysing the results of ^{137}Cs accumulation in test plant species of the land ecosystem, it was found that in one of the most contaminated regions after the Chernobyl Nuclear Power Plant accident, internal exposure dose from ^{137}Cs (0.5–32 μSv) during plant vegetation period (190 days) was up to 3.5 times higher than that in the Ignalina NPP vicinity where it reached 0.1–9.0 μSv (Fig. 1).

Internal exposure doses from accumulated ^{137}Cs in above-ground and underground parts of the tested plants (eight species) differed from 0.2 to 20 times (Fig. 2). Internal exposure doses from accumulated ^{137}Cs in five tested plant species were higher in the above-ground part. The underground part of three species received a higher internal exposure dose comparable to the above-ground part. Uneven radionuclide distribution could depend on the plant species, the type and form of roots. Also, it could depend on the level of soil contamination with ^{137}Cs and on the amount of potassium and ^{137}Cs/K ratio in the above-ground and underground parts of plants and soil [2].

Earlier, our investigations which were carried out with *Tradescantia*, showed that 0.5 μSv internal exposure dose from accumulated ^{137}Cs in this plant induced

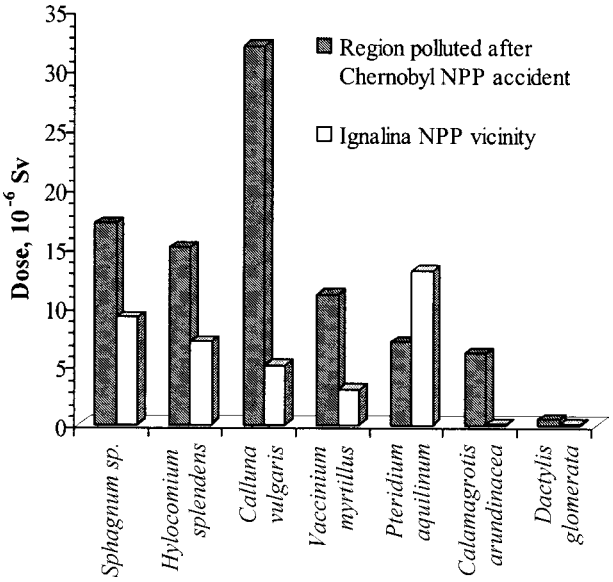

Fig. 1. Maximum internal exposure doses from accumulated ^{137}Cs in plant above-ground parts in two Lithuanian regions.

lethality in 25% of *Tradescantia* stamen hair cells and 1.3% of somatic mutations. According to Shevchenko and Pomeranceva [17], 1% of somatic mutations induced in *Tradescantia* stamen hair cells point to genotoxic changes which can cause disappearance of radiosensitive species as well as changes in the whole ecosystem.

Meristemic cells of roots are very sensitive to the effect of ionizing radiation, but the internal exposure dose from ^{137}Cs can exceed that in the above-ground plant part; therefore, under laboratory conditions the effect of accumulated ^{137}Cs in *Lepidium sativum* L. on seed germination and root growth was investigated. It was determined that irrespective of the internal exposure dose (0.6–600 μSv) from this radionuclide, seed germination was similar to that in the control, i.e. 96–98%. Roots did not grow in 6–8% of the germinated seeds and in control samples this percentage amounted to 8%. The mentioned differences were not statistically signifi-

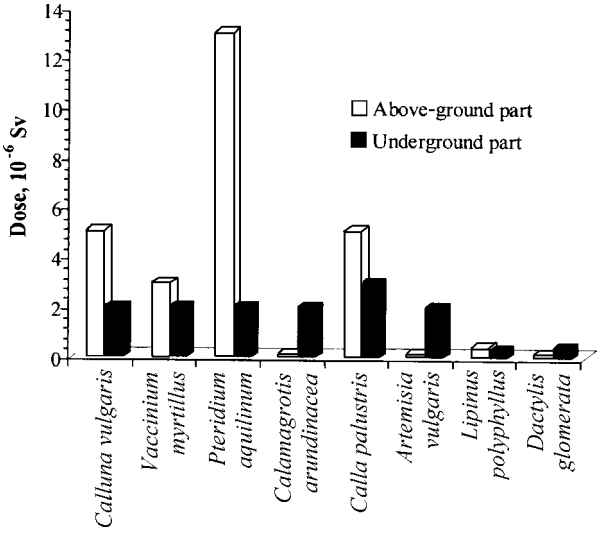

Fig. 2. Internal exposure doses from accumulated ^{137}Cs in plants above-ground and underground parts.

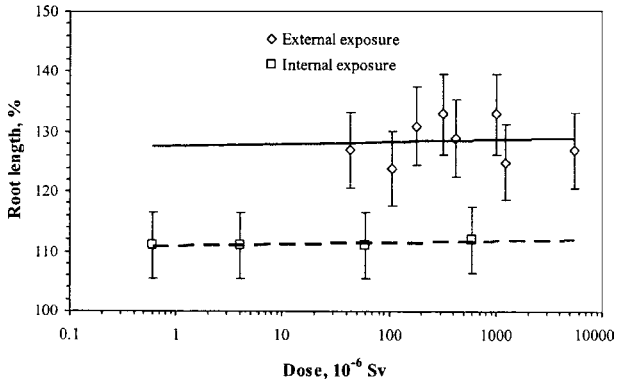

Fig. 3. Effect of internal and external irradiation (from ^{137}Cs) on root growth of *Lepidium sativum* L.

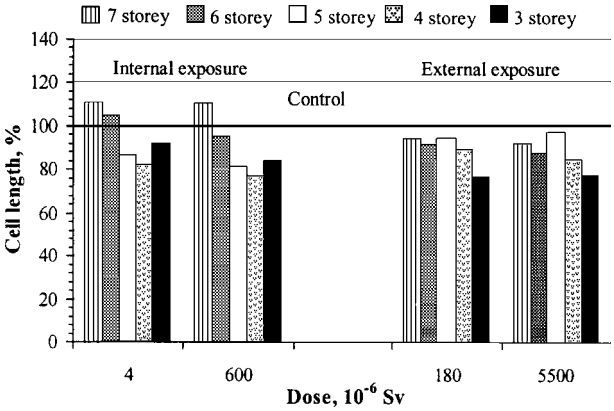

Fig. 4. Length of the primary columella cells of *Lepidium sativum* L. root at different internal and external exposure dose from ^{137}Cs.

cant. Hence, stimulation effect of the internal ^{137}Cs on the root growth was observed irrespective of the exposure dose. The experimental plant roots were statistically significantly (by 11–12%) longer comparing to the control plants (Fig. 3).

Analysis of the effect of external ^{137}Cs-γ irradiation on *Lepidium sativum* L. seed germination has shown that irrespective of external irradiation dose (40–5500 μSv), seed germination was similar to that of control samples and varied from 86 to 96%. Roots did not grow from 5–11% of germinated seeds and in control samples this index came up to 11%. The mentioned differences were not statistically significant. Irrespective of the ionizing radiation dose, the plant roots were statistically significantly longer than in the control (by 24–33%; Fig. 3).

The obtained data show that the tested internal and external exposure doses did not affect *Lepidium sativum* L. seed germination; however, they stimulated *Lepidium sativum* L. root growth. The latter did not depend on both internal and external exposure dose levels. The plant root lengthening could be caused by more intensive cell elongation. Under laboratory conditions the effect of low internal (0.6–600 μSv) and external (40–5500 μSv) exposure doses from ^{137}Cs on the garden cress *Lepidium sativum* L. roots was practically the same. The applied internal and external exposure doses stimulated the plant root growth by 12 and 33%, respectively. Statistically significant difference in root growth stimulation results was obtained. Effect from internal exposure was smaller in comparison with that from the external one; however, direct "dose-effect" relationship was not observed in the case of both internal and external exposure.

Morphometry of columella gravitropically sensitive cells of *Lepidium sativum* L. primary root has shown a reduction in the cell length in the columella 7th to 3rd storeys (i.e. towards meristem) in the case of both internal and external exposures. Internal exposure (4 and 600 μSv) resulted in the following alterations in cell length: cells of the 7th storey were statistically significantly longer, but the cells of 5th, 4th and 3rd storeys were statistically significantly shorter than those in the control. After external exposure (180 and 5500 μSv), the length of all cells was lower than that of the control (Fig. 4).

By comparing the total length of columella cells, it was observed that control cells are longer than the cells subjected to internal or external exposure. Besides, the total length of columella cells, which were subjected to external exposure, was less than that of cells affected by internal exposure, but the cell width did not significantly differ and the range was in the error limits.

Results of the investigations show that under exposure conditions the cell areas from the root tip to the quiescent center of the meristem (from 7th to 3rd storey) uniformly decrease. After internal exposure, the cell area in the 7th storey was statistically significantly larger but the area of cells in the 5th, 4th and 3rd storeys was less than that of the respective cells in the control. A similar tendency was determined for the cells affected by external exposure, but in this case, the differences were less appreciable. The area of cells, even in the 4th and 3rd storeys, was statistically significantly smaller than in the control (Fig. 5). Different effects of external and internal exposure were also observed by analyzing the amyloplast area. The amyloplast is an organ accumulating starch essential for plant growth and development. Area of amyloplasts subjected to internal exposure at most was smaller than in the control. However, as a result of external exposure the area of amyloplasts decreased from 7th to 3rd storey, but the effects at separate storeys were uneven. The area of

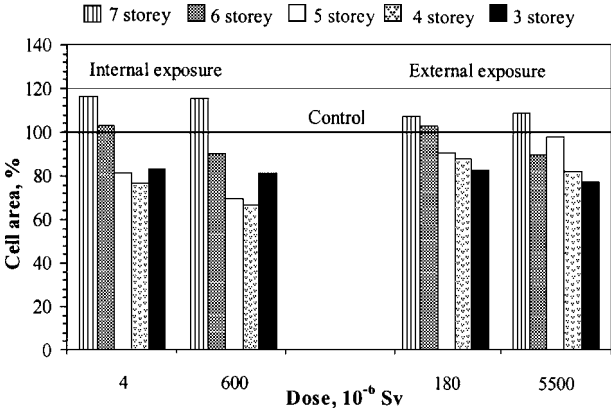

Fig. 5. Area of *Lepidium sativum* L. primary root columella cells at different exposure doses from internal and external ^{137}Cs.

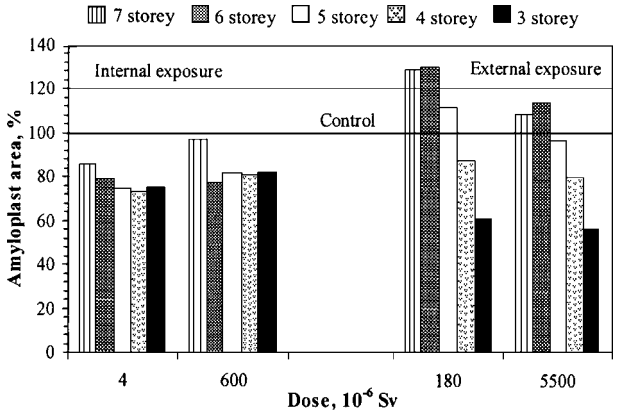

Fig. 6. Area of amyloplasts in *Lepidium sativum* L. primary root columella cells at different exposure doses from internal and external ^{137}Cs.

amyloplasts in 7th and 6th storeys was statistically significantly larger, but in the 4th and 3rd storeys the area of amyloplasts was statistically significantly smaller than in the control (Fig. 6).

According to the morphometric investigations of garden cress primary root cap cells after internal and external exposure from ^{137}Cs radiation, the neighbouring meristem cell length (as well as their area) decreased, but there was an increase in the area of cells which were located further from the meristem. Different effects of external and internal exposure on the plant developing cells were observed. As a result of internal exposure from ^{137}Cs, the difference in root cap cell length and area in the columella storeys from 7th toward 3rd was larger and the difference in the amyloplast area was smaller than resulting from external exposure. By generalizing morphometric investigations of the cells of primary root cap after internal (4 and 600 μSv) and external (180 and 5500 μSv) exposure from ^{137}Cs it could be asserted that both types of exposure affect the meristem cell length, however, the consequences of internal exposure were expressed more strongly.

The presented data show different effects of external and internal exposure on the developing plant cells. It is known that growth of the plant cells is the outcome of three different processes: cell division, protoplasm rise and cell elongation. The cell division and protoplasm rise take place in meristem (in the embryonic zone). The initial cell length at their development stage (when protoplasm growth is stopped) can increase by 10–50 or more times. The cell division can be slown down even due to the low ionizing radiation doses [18]. Therefore, it can be stated that an increase in plant root length after the effect of external and internal exposure is caused due to the more intensive process of cell growth into length.

The stimulating effect of radionuclides can cause morphogenetic changes in plant, which reveal themselves in the early development stages [13, 14]. Morphological changes in plants were observed after the Chernobyl Nuclear Power Plant accident, in a 30 km zone around the Plant [9]. Using the usual pine tree as a test-object by bioindication methods, it was determined that low and middle radioactivity waste storage and reprocessing were connected with an extra environmental contamination; this induced cytogenetic disturbances of both vegetative and reproductive pine organs [5].

The damage to plant reproductive organs can decrease germination of ripe seeds. Toxicants, at concentrations that do not exceed the toxic range, can stimulate plant metabolism as well as growth processes. Nevertheless, the enzyme activity can be disturbed by metabolic products in proportion to the metabolical intensity [1]. Therefore, the observed stimulating effect of internal (0.6–600 μSv) and external (40–5500 μSv) ^{137}Cs radiation on garden cress *Lepidium sativum* L. root growth can affect the further plant development.

Conclusions

Under natural environmental conditions, internal exposure doses from accumulated ^{137}Cs in underground parts (including roots, sensitive organ to ionizing radiation) of three (from the eight tested) plant species were (up to 20 times) higher than those in the above-ground part.

By investigating effects of internal and external exposure from ^{137}Cs on *Lepidium sativum* L. seed germination and root growth under laboratory conditions the following results were obtained:

- both internal (0.6–600 μSv) and external (40–5500 μSv) exposure did not exert an influence upon seed germination, however, a stimulation effect on root growth (12 and 33%, respectively) was determined;
- different effect on growing plant cells was observed: after internal exposure columella cell length, area and amyloplast area were statistically significantly lower compared with those after external exposure.

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