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Effects of ^{137}Cs and ^{90}Sr on the plant *Lepidium sativum* L. growth peculiarities

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The paper presents an estimation of ^{137}Cs and ^{90}Sr effects on the plant test-organism *Lepidium sativum* L. seed germination, root and shoot growth as well as on the change of parenchyma cell parameters under experimental laboratory conditions.

All the ^{137}Cs activity concentrations studied ($4\cdot10^2$–$4\cdot10^5$ Bq/l) have been found to induce a statistically significant slight stimulation of *L. sativum* root growth. From all ^{90}Sr activity concentrations studied ($1\cdot10^3$ – $2\cdot10^5$ Bq/l), only the highest activity concentration statistically significantly increased *L. sativum* root growth. All the other ^{90}Sr activity concentrations statistically significantly slightly inhibited root growth. The effect of $4\cdot10^4$ Bq/l was most pronounced.

All ^{137}Cs activity concentrations studied demonstrated an insignificant stimulation of *L. sativum* shoot growth. The highest statistically significant stimulation level was observed at the ^{137}Cs activity concentration of $4\cdot10^4$ Bq/l.

Aqueous solutions containing separate ^{90}Sr activity concentrations ($1\cdot10^3$ – $3\cdot10^4$ Bq/l) also statistically significantly stimulated shoot growth, the stimulation being much more significant than in ^{137}Cs aqueous solutions. The different effect of ^{137}Cs and ^{90}Sr on the growth of plant vegetative organs (roots and shoots) can be explained by the different metabolism of ^{137}Cs and ^{90}Sr as stable chemical analogues of K and Ca, respectively, in plant cells. This predetermines their variable accumulation in separate plant organs and their different distribution in plant cells and tissues.

Key words: technogenic radionuclides, concentration activity, plant test-organism, seeds, roots, seedlings, parenchyma cells

INTRODUCTION

Many of the highest plant species, especially those of aquatic plants, are recognized as having a high accumulation capacity for the radionuclides. The amount of radionuclides accumulated in plants can be hundred times higher as compared with radionuclide content in their habitat medium (Марчюленене и др., 1992; Гудков и др., 2002; Нифонтов, 2003). Plant meristem (the tissues with actively dividing cells), due to intensive metabolic processes in it, can accumulate particularly large amounts of radionuclides (Sokolov et al., 2001; Shershunova et al., 2001). There has been determined that radiocaesium accumulates chiefly in the meristem while the radiostrontium enters the tissues of elongation growth and the differentiating tissues (Гудков, 2001). Plant-immobilised radionuclides can induce various biological effects. These effects are predominantly associated with suppression of seed germination, stimulation or inhibition of synthesis of metabolites, as well as an increase or a decrease of cell division and growth (Underbring et al. 1970; Нестеров и др., 2001; Гончаренко и др., 2003). Although such biological effects have effects on plant growth, plant response to the impact of plant-immobilised radionuclides is not sufficiently investigated yet (Евсеева и др., 2001; Butkus ir kt., 2003; Bystrejewska-Piotrowska et al., 2005).

Focusing on the radioecological state of the ecosystems, preferably in the environment of nuclear power plants, investigations on the biological effects caused by incorporated radionuclides are of important concern. Results of such kind of scientific investigations acquire a particular importance in case the ecotoxicological consequences of accidental radionuclide release into the environment are to be foreseen or standardization of radioecological patterns is considered.

The main objective of the present work was estimation of ^{137}Cs and ^{90}Sr effects on the plant test-organism *Lepidium sativum* L. seed germination, root (meristem cells) and shoot (parenchyma cells) growth as well as on the change of parenchymal cell parameters under experimental laboratory conditions.

MATERIALS AND METHODS

A study of the biological effects of ^{137}Cs and ^{90}Sr was performed applying the garden cress *Lepidium sativum* L. (*Brassicaceae*). This test-organism is widely employed particularly in toxicological studies (Deware, Bahadir, 1994; Wundram et al., 1997; Wong et al., 1999; Montvydienė, Marčiulionienė, 2004). The germination and root growth indices of this plant have been recommended to include into the biotest complex used for assessment of the toxic effects of energetic industrial effluents and heavy metals and their mixtures on living organisms (Montvydienė, 2002).

In this study, laboratory experiments were carried out following the modified Magone (1989) method. Briefly, 10 ml of lake water (as control) or test aqueous solution of ^{137}Cs or ^{90}Sr was pipetted onto three layers of filter paper fitted into a 9-cm glass Petri dish. Twenty-five healthy looking *L. sativum* seeds of similar size were distributed evenly on filter paper. The Petri dishes were kept in the dark at 24 ± 1 °C for two days. Afterwards seed germination and the root length of seedlings were measured. The experimental set of each testing scheme involved three control dishes and three replicates for each test concentration activity of radionuclides. The pH of lake water and test solutions of ^{137}Cs and ^{90}Sr was 7.5.

In experiments on ^{137}Cs and ^{90}Sr effect on plant physiological changes, *L. sativum* shoots grown in plastic boxes with lids were used. Each box contained 65 ml of water solution and 470 mg, i. e. about 160, seeds evenly distributed on a glass plate covered with filter paper. The seeds germinated at 24 ± 1 °C for 1 day in the dark, and the shoots were grown for 6 days under continuous light at a temperature of 23 ± 1 °C.

The morphological anatomical studies encompassed all *L. sativum* shoots. We measured the full length of a shoot (with straightened leaves) and of the hypocotyls, as well as the weight of all shoots of 50 shoot units. A light microscope was used to determine hypocotyl parenchymal cell length, width and area.

The hydroponic system for experiments was prepared from ^{137}CsCl and $^{90}SrCl_2$. The initial 0.1 ml volume of each chloride was diluted 10^3–10^6 times or even more in order to obtain a radionuclide activity concentration necessary for the study. In a separate variant of experimental series we used different radionuclide activity concentrations. ^{137}Cs and ^{90}Sr activity concentrations in aqueous solution and plants are presented in Table 1.

Plant samples were dried in a drying cabinet at 100 °C until constant dry weight. ^{137}Cs activity concentration in the aqueous solution and in dry plant biomass (after two days) was assessed after the gamma spectrometric measurement. To assess ^{137}Cs activity in the solution, 3 ml of different activity solutions was poured in each of the vials of standard geometry. ^{137}Cs activity concentration in the small-volume samples was measured with a gamma-spectrometer interfaced with a p type pure germanium (HPGe) detector equipped with a well 40 mm deep and 16 mm in diameter. The relative efficiency of the detector was 17% (for ^{137}Cs 661.7 keV radiation). The measurement uncertainty did not exceed 6%, with a statistic error not larger than 1% (Gudelis et al., 2000).

^{90}Sr activity in aqueous solution and in dry plant biomass (after two days) was measured using a low background UMF-1500 M device (detector BT-13, registration efficiency 23% – 0.06 cps).

The data presented below are the arithmetical means of 2–3 experiments for which the errors were calculated. Standard error did not exceed 5% for data presented in Figs. 1–3. A statistically significant difference between experimental and control samples was assessed by the test (at $p < 0.05$) using Statgraphics Plus Version 2.1 program.

RESULTS AND DISCUSSION

By investigating the toxic impact of ^{137}Cs of different level (activity concentration step from $4 \cdot 10^2$ to $4 \cdot 10^5$ Bq/l was one order of magnitude) on *L. sativum* seed germination and root growth, it was established that after two days the seed germination insignificantly differed from the controls. However, this radionuclide, statistically significantly as compared to controls, stimulated (11–12%) root growth (Fig. 1). Seed germination in the ^{90}Sr medium, like in the case of ^{137}Cs, statistically did not differ from controls. A different influence on the plant *L. sativum* root growth after two days was obtained because of activity concentration ($1 \cdot 10^3$–$2 \cdot 10^5$ Bq/l with an increase step of one order of magnitude) of ^{90}Sr. However, ^{90}Sr activity in the range $1 \cdot 10^3$–$2 \cdot 10^4$ Bq/l after two days induced a statistically significant (8–12%) inhi-

Table. **^{137}Cs and ^{90}Sr activity concentration in aqueous solution (initial) and plants *L. sativum* (after 2 days)**

^{137}Cs		^{90}Sr	
Aqueous solution activity concentration, Bq/l	Plant activity concentration, Bq/kg, dry weight	Aqueous solution activity concentration, Bq/l	Plant activity concentration, Bq/kg, dry weight
$4 \cdot 10^2$	$3.4 \cdot 10^3$	$1 \cdot 10^3$	$5 \cdot 10^3$
$4 \cdot 10^3$	$2.6 \cdot 10^4$	$3 \cdot 10^3$	$7 \cdot 10^4$
$4 \cdot 10^4$	$3.4 \cdot 10^5$	$3 \cdot 10^4$	$3.6 \cdot 10^5$
$4 \cdot 10^5$	$3.3 \cdot 10^6$	$2 \cdot 10^5$	$2 \cdot 10^6$

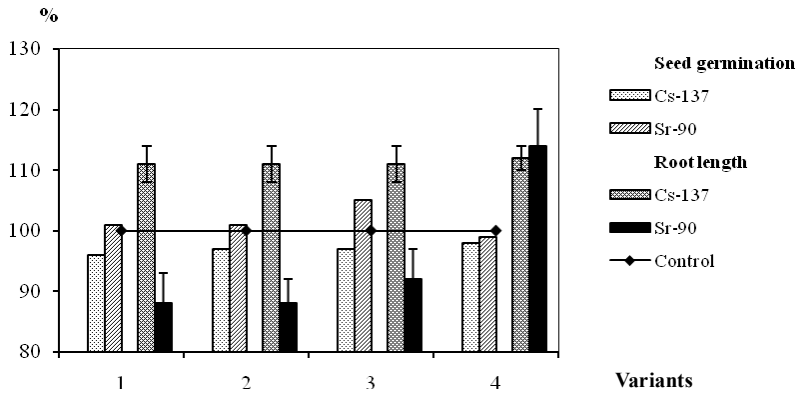

Fig. 1. Impact of incorporated ^{137}Cs and ^{90}Sr on seed germination and root growth of *L. sativum* (after 2 days) depending on radionuclide activity concentration in aqueous solution
^{137}Cs: *1* – $4\cdot10^2$; *2* – $4\cdot10^3$; *3* – $4\cdot10^4$; *4* – $4\cdot10^5$ Bq/l
^{90}Sr: *1* – $1\cdot10^3$; *2* – $3\cdot10^3$; *3* – $3\cdot10^4$; *4* – $2\cdot10^5$ Bq/l

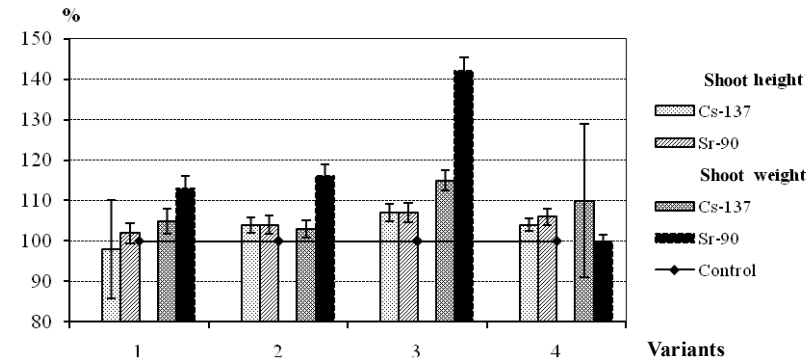

Fig. 2. Effect of ^{137}Cs and ^{90}Sr on shoot growth of *L. sativum* (after 7 days) depending on radionuclide activity concentration in aqueous solution
^{137}Cs: *1* – $4\cdot10^2$; *2* – $4\cdot10^3$; *3* – $4\cdot10^4$; *4* – $4\cdot10^5$ Bq/l
^{90}Sr: *1* – $1\cdot10^3$; *2* – $3\cdot10^3$; *3* – $3\cdot10^4$; *4* – $2\cdot10^5$ Bq/l

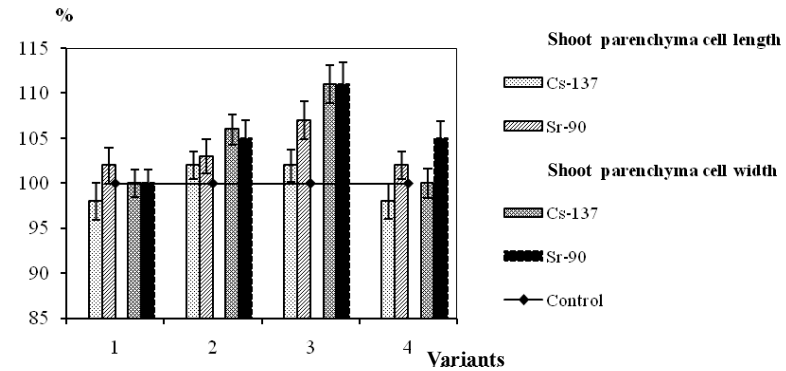

Fig. 3. Effect of ^{137}Cs and ^{90}Sr on parenchyma cell parameters *L. sativum* (after 7 days) depending on radionuclide activity concentration in aqueous solution
^{137}Cs: *1* – $4\cdot10^2$; *2* – $4\cdot10^3$; *3* – $4\cdot10^4$; *4* – $4\cdot10^5$ Bq/L
^{90}Sr: *1* – $1\cdot10^3$; *2* – $3\cdot10^3$; *3* – $3\cdot10^4$; *4* – $2\cdot10^5$ Bq/L

bition of root growth; however, the highest used ^{90}Sr activity concentration ($2\cdot10^5$ Bq/l), on the contrary, stimulated root growth (14%) compared to control (Fig. 1).

Analysis of ^{137}Cs effect on *L. sativum* shoot growth showed that after seven days of exposure to aqueous solution containing ^{137}Cs, shoot height and particularly weight had been stimulated only by the highest ^{137}Cs concentrations studied ($4\cdot10^4$ ir $4.4\cdot10^5$ Bq/l) (Fig. 2). In many cases, shoot parenchymal cell length and width stimulation was observed, which was most pronounced when ^{137}Cs concentration in the aqueous solution was $4\cdot10^4$ Bq/l (Fig. 3).

Analysis of ^{90}Sr effect on *L. sativum* plant shoot growth revealed that after seven days of exposure to

^{90}Sr aqueous solution the stimulation of both shoot height and weight was most intensive at a ^{90}Sr activity concentration of $3 \cdot 10^4$ Bq/l (Fig. 2). The same ^{90}Sr activity concentration, much more significantly than all the other concentrations studied, stimulated shoot parenchymal cell length and width (Fig. 3).

The obtained data show that the effects of accumulated ^{137}Cs and ^{90}Sr on *L. sativum* shoot and parenchymal cell growth have been similar. Both ^{137}Cs and ^{90}Sr stimulated shoot height and weight as well as parenchymal cell length and width. The stimulation was most pronounced when the activity concentration of these radionuclides in aqueous solution was $4 \cdot 10^4$ and $3 \cdot 10^4$ Bq/l, respectively; however, the stimulating effect of ^{90}Sr was much stronger than that of ^{137}Cs. At an activity concentration of ^{137}Cs $2 \cdot 10^5$ Bq/l and of ^{90}Sr $4 \cdot 10^5$ Bq/l, the stimulation of shoot height and weight as well as of parechymal cell length and width was much weaker, and these parameters differed only insignificantly from the control.

It is known that growth of the plant cells is the outcome of three different processes: cell division, protoplasm rise and cell elongation. Cell division and protoplasm rise take place in the meristem (in the embryonic zone). The initial cell length at their development stage (when protoplasm growth is stopped) can increase 10–50 or more times. Cell division can be reduced even by low ionising radiation doses (Сидоров, 1990).

The stimulating effect of radionuclides can cause morphogenetic changes in a plant, which reveal themselves in early development stages (Mericle & Mericle, 1967; Марчюленене и др., 1992). Using usual pine tree as a test-object and bioindication methods, it was determined that low and intermediate radioactivity waste storage and reprocessing were connected with an extra environmental contamination and induced citogenetic disturbances of both vegetative and reproductive pine organs (Гераскин и др., 2000).

Plant changes because of damaged reproductive organs can decrease the germination of ripe seeds. It has been found that toxicants at concentrations not exceeding the levels producing a toxic effect can stimulate plant metabolism as well as growth processes in plants and their cells. Nevertheless, the plant enzyme activity can be disturbed by metabolic products, and such the higher the degree of injuries the more intensive the metabolism (Adelman et al., 1988; Britt, 1996).

The different impact of ^{137}Cs and ^{90}Sr activity concentrations the studied on *L. sativum* root and shoot cells can be explained by different metabolism of these radionuclides in plant. The transport pathway and the distribution of ^{137}Cs and ^{90}Sr in plants are different (Bauer et al., 1998; Bradford, 1976; Евсеева и др., 2001; White, Broadley, 2000) because their stable chemical analogues are macro elements K and Ca, respectively. The highest amounts of ^{90}Sr in the plant cell are localized in chloroplasts, while ^{137}Cs distributes evenly in cell protoplasm (Гродзинский и др.,1991; Марчюлёнене, 1994). Accumulation of ^{137}Cs in cell wall, depending on plant species, is 2 to 7 times lower than that of ^{90}Sr. However, the release of ^{137}Cs from cell wall to the protoplasm is higher (10–20%) than that of ^{90}Sr (3–10%) (Марчюлёнене, 1994). The distribution of these radionuclides in the organs and tissues of a plant is also different (Сидоров, 1990). ^{137}Cs in plants accumulates mostly in the zones of cell division and active metabolism (e. g., in plant meristem and young tissues) (Евсеева др., 2001), whereas ^{90}Sr is mostly accumulated in plant tissues with dominating elongation growth and in the young tissues of stem and leaves (Гудков, 2001).

Analysis of the morphometric indices that characterize the vital power of plant shoots in the early stage of ontogenesis has been performed (Найдич, 1999). The analysis has shown that stimulation of plant shoots by ionizing radiation was observed on the background of enlarged cytogenetic damages.

Consequently, the determined stimulating effect on *Lepidium sativum* root and shoot growth by ^{137}Cs and ^{90}Sr could not be understood as useful or even harmless plant response to the impact of ionizing radiation. Cytogenetic injuries during the latest plant development can influence its genetic organs and reduce plant reproductive abilities. Also, they can cause changes of meristemic tissues because of biochemical derangements of cell metabolism (Евсеева, Гераскин, 2000; Шевченко и др., 1996).

All the ^{137}Cs activity concentrations studied (4.10^2 – 4.10^5 Bq/l) have been found to induce a insignificant stimulation of *L. sativum* root growth. Of all ^{90}Sr activity concentrations studied ($1 \cdot 10^3$–$2 \cdot 10^5$ Bq/l), only the highest activity concentration statistically significantly increased *L. sativum* root growth. All the other ^{90}Sr activity concentrations slightly inhibited root growth. The effect of $4 \cdot 10^4$ Bq/l was most pronounced. Aqueous solutions containing separate ^{90}Sr activity concentrations studied also stimulated shoot growth, the stimulation being much more significant than in ^{137}Cs aqueous solutions. The different effect of ^{137}Cs and ^{90}Sr on the growth of plant vegetative organs (roots and shoots) can be explained by a different metabolism of ^{137}Cs and ^{90}Sr as stable chemical analogues of K and Ca, respectively, in plant cells. This predetermines their variable accumulation in separate plant organs and different distribution in plant cells and tissues.

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^{137}Cs IR ^{90}Sr POVEIKIS AUGALO *LEPIDIUM SATIVUM* L. AUGIMO YPATUMAMS

Santrauka

Eksperimentinėmis sąlygomis ištirtas technogeninių radionuklidų ^{137}Cs ir ^{90}Sr poveikis augalo testorganizmo *Lepidium sativum* L. sėklų daigumui, šaknų (meristeminių ląstelių) augimui bei parenchiminių ląstelių parametrams. Nustatyta, kad visų tirtų aktyvumo koncentracijų ^{137}Cs (nuo $4{\cdot}10^2$ iki $4{\cdot}10^5$ Bq/l) nedaug, tačiau statistiškai patikimai stimuliavo *L. sativum* šaknų augimą. ^{90}Sr aktyvumo koncentracijos vandeniniame tirpale buvo nuo $1{\cdot}10^3$ iki $2{\cdot}10^5$ Bq/l, tačiau šio augalo šaknų augimą statistiškai patikimai stimuliavo tik didžiausios aktyvumo koncentracijos radionuklidas. Kitų tirtų aktyvumo koncentracijų šis radionuklidas statistiškai patikimai slopino šaknų augimą.

Visų tirtų aktyvumo koncentracijų ^{137}Cs stimuliavo *L. sativum* daigų augimą. Labiausiai daigų augimą stimuliavo (statistiškai patikimai) $4{\cdot}10^4$ Bq/l aktyvumo koncentracijos radionuklidas. Tirtų aktyvumo koncentracijų ^{90}Sr ($1{\cdot}10^3$–$3{\cdot}10^4$) statistiškai patikimai stimuliavo daigų augimą, tačiau ji buvo didesnė negu vandeniniame tirpale su ^{137}Cs.

Skirtingą ^{137}Cs ir ^{90}Sr poveikį augalo vegetatyvinių organų šaknų ir daigų augimui galima paaiškinti nevienodu šių radionuklidų metabolizmu augalo ląstelėse. Metabolizmas sąlygoja skirtingą radionuklidų akumuliaciją tam tikrose augalo dalyse bei nevienodą jų pasiskirstymą augalo audiniuose ir ląstelėse.

Raktažodžiai: technogeniniai radionuklidai, aktyvumo koncentracija, augalas testorganizmas, sėklos, šaknys, daigai, parenchiminės ląstelės