THE ACCUMULATION PROCESS OF ^{137}Cs AND ^{90}Sr IN THE CELL OF *NITELLOPSIS OBTUSA* ALGAE*

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Abstract. *In the present study, we investigated the accumulation of ^{137}Cs and ^{90}Sr in compartments of the Nitellopsis obtusa cells. The effect of Sr^{2+}, Cs^{+} and Ca^{2+}, K^{+}, which are chemical analogues of ^{90}Sr and ^{137}Cs, to the bioelectric parameters of these algae were studied simultaneously. The aim of this work was studying the penetration of ^{137}Cs and ^{90}Sr through regulating membrane barriers in the cells of starry stonewort (Nitellopsis obtusa). ^{137}Cs and ^{90}Sr are accumulated mainly in the cell membrane (75% to 92%) of these algae. The cell membrane as a cation exchanger regulates ion flow through the first cells diffusion barrier – its thick outer cytomembrane (the complex consisting of the cell wall and plasmalemma). Significantly, smaller amounts of ^{137}Cs and ^{90}Sr enter into the cytoplasm than in the cytomembrane, 10-20% and 3-10%, respectively. Analysis of the accumulation levels of ^{137}Cs and ^{90}Sr in the compartments of the Nitellopsis obtusa cells show their accumulation in the cell membrane as well as their active transport through outer and inner cytoplasmic membranes. Membrane potentials determined mainly by the gradient of the K^{+} ions are doing an important regulatory function in this process. From the obtained data it results that cells of Nitellopsis obtusa algae can be a convenient radioecological model for the study of the accumulation of radionuclides in plants at the cellular level.*

***Key words*:** *Accumuliation, ^{137}Cs, ^{90}Sr, Nitellopsis obtusa, cell compartments*

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1. INTRODUCTION

It is known that pecto-cellulosis cell wall affects the process of the entering of mineral substances into a plant especially from low concentration solutions [1]. The cell membrane is a subacid cation-exchanger whose matrix has a certain amount of carboxyl groups linking cations [2]. The highest cation specificity of cell membrane is signified for Ca^{2+} [3].

The properties of the cell membrane as a barrier may occur when radionuclides are entering into the plants' cells. In order to ascertain these "barrier" properties, the data about the accumulation of radionuclides in the cell membrane is needed. A series of radionuclides $^{65}Zn \geq {}^{144}Ce > {}^{210}Pb > {}^{90}Sr > {}^{137}Cs$ established by the levels of accumulation in the membrane represents the ability to adsorb mainly 3- and 2-valent cations $La^{3+} > Ca^{2+} > Na^{+} > K^{+}$ [4]. The faster saturation of 2-valent cations than 1-valent is probably caused by Ca^{2+} binding and its chemical and biological analogue Sr^{2+} in membrane with negative electrical charges of pectins. This process for ^{144}Ce, ^{65}Zn и ^{210}Pb was longer because there is a gradual displacement of other cations (Ca^{2+} and Mg^{2+}) from fixed sites and formation of stronger bonds [5].

It should be noted that the accumulation of ^{65}Zn decreased 3 times in the cell membrane treated with detergent Triton X-100, while the accumulation of ^{144}Ce, on the contrary, increased 2 times in comparison with the variants of untreated membrane [6]. This indicates that the accumulation processes of these radionuclides are different. Apparently ^{65}Zn is being preferentially bound by a polysaccharide-protein matrix in the membranes and ^{144}Ce is accumulated mainly in the free space between membrane's phospholipidic layers [7].

Nowadays a topical radioecological problem is determining the effect of radionuclides on biological objects, especially at low specific activity level, as well as in both separately and in conjunction with various environmental factors, including the anthropogenic origin pollution. It is common knowledge that the cells of algae *Nitellopsis obtusa* (*Charophyta* division) have a quick and sensitive response to the impact of various chemical agents by modulation of its membrane bioelectrical parameters. The cells of these algae are widely used in studying the toxicity of heavy metals as well as determining water quality [8, 9]. Here emerges the question if it is possible to apply electrophysiological methods for studying the processes of radionuclides transference into plant cells *N. obtusa* as well as the biological effect on these cells of low specific activity level. However, this matter has not been sufficiently studied.

The aim of this study was to research the entering process of ^{137}Cs and ^{90}Sr into the cells of *N. obtusa* and

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to determine the role of plant cell membrane as a barrier involved in penetration of radionuclides into cellular compartments.

2. Object and methods

Algae *Nitellopsis obtusa* (Desv.) J. Groves collected in two Lithuanian (Žuvintas and Dusia) lakes was used for this research. Both lakes are located in the south part of Lithuania. The area of Žuvintas lake is 9.65 km². It is the shallowest lake in Lithuania with the greatest depth being 3 m and average only 0.6 m. Water in lake Žuvintas is 6.5-7.0 pH, cations concentration in water: $4*10^{-5}$ mol/L for K^+, $3*10^{-4}$ mol/L for Na^+, and $1*10^{-3}$ mol/L for Ca^{2+}. The area of Dusia lake is 23.17 km². Its greatest depth is 32 m and average depth is 15 m. Water in lake Dusia is 8.4 pH, cations concentration in water: $5*10^{-5}$ mol/L for K^+, $2*10^{-4}$ mol/L for Na^+, and $1*10^{-3}$ mol/L for Ca^{2+}.

The experiments were performed in aquariums containing 1.5 L of filtered Žuvintas lakes water adding the cesium and strontium solutions, which were turned into the basic radionuclides chlorides form of the 10^5 Bq/L activity concentration. The water temperature was 24-25 ^{0}C. The duration of the experiments was 8 days. Algal cells which length was up to 21 cm were dissevered into the cell membrane, the protoplasm and the vacuolar sap by the Hampson's method [10].

These algae cells contain big central vacuole surrounded by a cytoplasmic membrane – the tonoplast. The cytoplasm surrounding the vacuole is separated from external environment with the plasmalemma and the cell wall. The accumulation coefficient (*AC*) comprises the ability to accumulate radionuclides in cells compartments. *AC* is determined as the ratio of radionuclide concentration in compartments of cells (wet weight) and in the water.

The cells of *N. obtusa* were separated mechanically, because the cells are large (about 21 cm in length and 1.5 mm in diameter) have regular cylindrical shape and pronounced differentiation of compartments. Vacuolar sap was removed by cutting off one end of the cell. The vacuolar sap usually flows out itself or by a light push of tweezers along the cell. It is important to watch at this moment that the cytoplasm does not flow out together with vacuolar sap. This can be seen in color and consistency of the preparation. The vacuolar sap is clear and watery; and general fraction of cytoplasm is green and viscous. Further, the cytoplasm was extruded out with tweezers. The admixture of the cytoplasm in the vacuolar sap is not more than 15 per cent; and the admixture of the residue of the vacuolar sap in the cytoplasm preparation is insignificant. The cell wall was obtained by cutting off both ends of the cell and removing the cytoplasm by strong pressure of the tweezers along the entire cell.

The method used for separation of cellular compartments is not very accurate, but has several advantages. It is simple, allows quickly preparing several preparations for measurement, and allows measuring the radioactivity in each compartment of the plant cell.

Preparations of the cell wall, the cytoplasm, the vacuolar sap, and the water were sampled at the same time after 3 h, 2 and 8 days. 6 parallel samples of cell wall, 4 parallel samples of the cytoplasm, 4 parallel samples of the vacuolarsap, and 4 parallel samples of the water (each 1 ml) were collected for each experimental point. Measurement of ^{137}Cs and ^{90}Sr content were performed in accordance with conventional radioecological methods [6, 11, and 12].

Conventional intracellular methods of microelectrodes ant voltage clamp technique were applied to investigate the impact of ^{137}Cs and ^{90}Sr on the plasma membrane bioelectrical properties of *N. obtusa* cell. During the experiments *N. obtusa* internodal cells of about 10 cm length and 0.6 mm diameter was placed into a plexiglass chamber and continuously bathed in artificial pound water or experimental solution. The reference electrode was immersed into the experimental solution near the cell and the microelectrode was inserted into the cell to measure electrical potential differences between outside and inside the intermodal cell. The details of experimental procedures, parameters and specifications of the experimental techniques were thoroughly described in previous studies [13-15].

3. Results and discussion

The obtained data indicated the two-phase kinetics of the ^{137}Cs and ^{90}Sr accumulation in the cell membrane of *Nitellopsis obtusa* algae. The first is a fast nonlinear lasting for hours. The second phase lasting several days is slow and linear. Particularly rapid saturation was noticed for ^{90}Sr (< 1.5 h) during the nonlinear phase of accumulation. The kinetics of accumulation of the studied radionuclides was mostly linear in the cytoplasm and the vacuolar sap.

The highest *AC* of ^{137}Cs and ^{90}Sr were established at the cell membrane (124±27 and 256±40 respectively) (Fig. 1 and Fig. 2). *AC* values of these radionuclides were significantly lower and differed in lesser units in the cytoplasm (14.0±4.1 and 7.4±1.9 respectively). *AC* in vacuolar sap were 3.0±0.4 and 0.7±0.1 respectively. The obtained data demonstrates that *AC* of ^{137}Cs and ^{90}Sr were 9 and 35 times respectively higher in the cells membrane than in the cytoplasm.

High accumulation level of these radionuclides in the cells membrane can apparently be explained by its cation-exchange characteristics and the ability to adsorb bivalent cations Ca^{2+} more intensively than monovalent cations K^+. Negative potential of the cell membrane (about 50-70mV) recorded by microelectrodes equally proves the existence the fixed negative charges whose amount determines cation exchange of the membrane and potential. The cell membrane acting as a cations-exchanger regulates the ions flow through the first diffusion barrier of the cell – its thick membrane (complex of cell wall and plasmalemma).

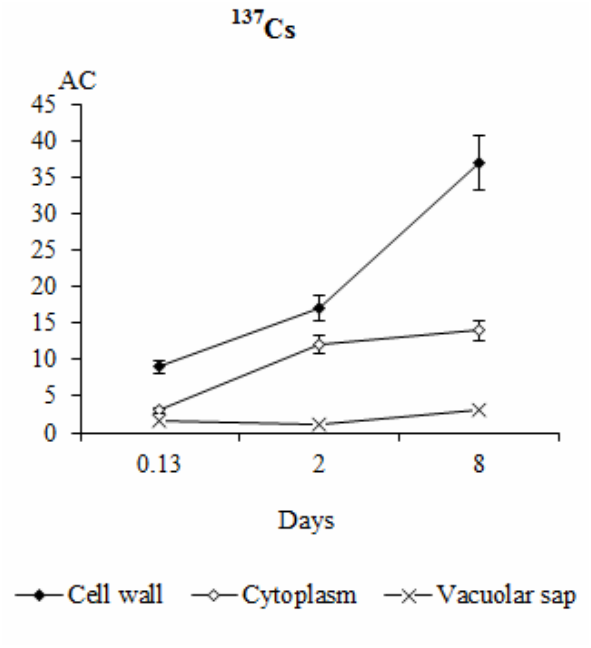

Figure 1. The kinetics of ^{137}Cs accumulation in compartments of Nitellopsis obtusa cell.

Higher *AC* of ^{90}Sr than *AC* of ^{137}Cs in the cell membrane may be explained by the preferred absorption of Ca^{2+} (analogue of Sr^{2+}) compared to K^+ (analogue of Cs^+). The decrease of the membrane potential dependence K^+ from ingressed Ca^{2+} also proves the selective absorbtion of Ca^{2+} ions. It is known, that Chara algae are inclined to accumulate ions (metals and, especially, Ca^{2+}) in alkaline pH and availability of carbonates in solution [16-18]. However, the pH of solution used in experiment were lower than 8. The cells of *N. obtusa* were not calcium encrusted initially. The cell wall of cells taken initially was optically transparent and flexible.

11% of ^{137}Cs and only 3% of ^{90}Sr accesses the cytoplasm of the cells of *N. obtusa* from the cell membrane. This points the various cytoplasm's permeability for ^{137}Cs and ^{90}Sr. Greater intake of ^{137}Cs than ^{90}Sr from the cell membrane into the cytoplasm may be due to selective permeability of plasmalemma for K^+ and the ability of protoplasm to concentrate potassium ions whose amount in plasmalemma is 1000 times higher than in the lake water [19]. Apparently, the relatively weak penetration of ^{90}Sr into the cytoplasm can be associated with a slow and very low level of Ca^{2+} accumulation in the cytoplasm of algae and other Embryophytes [20].

16% of ^{137}Cs and 12% of ^{90}Sr are transferred into the vacuole of *N. obtusa* cells. Consequently, the inner cytoplasmic membrane appeared to be more permeable for the studied radionuclides than the outer cytoplasmic membrane. It is known that the main potential gradient at the cell of freshwater algae is created at the plasmalemma [21]. Its resistance is 80-85% of the total resistance of complex "plasmalemma+tonoplast". Our results featured that almost 2 times less amount of ^{137}Cs and ^{90}Sr is transferred through the tonoplast in the cells of *N. obtusa*, i.e. the tonoplast of these algae cells is 2 times less permeable than the plasmalemma.

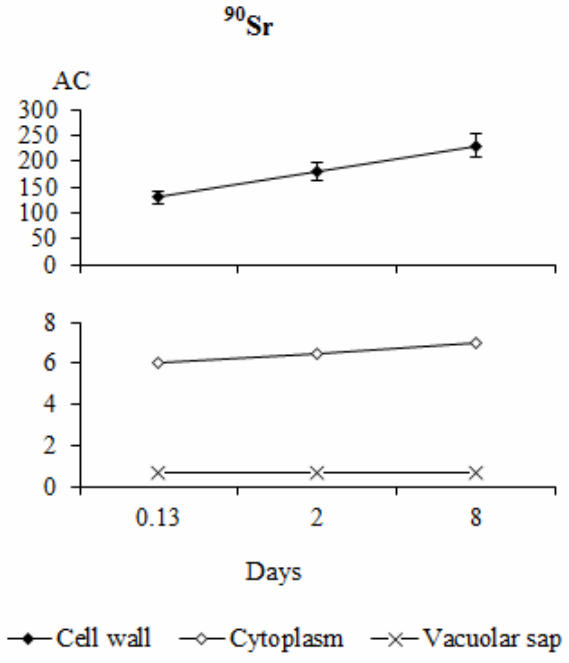

Figure 2. The kinetics of ^{90}Sr accumulation in compartments of *Nitellopsis obtusa* cell.

The analysis of the accumulation levels of ^{137}Cs and ^{90}Sr in the compartments of the *Nitellopsis obtusa* cells denote that the accumulation in the cell membrane is also being actively transported through outer and inner cytoplasmic membranes. Membrane potentials are mainly determined by electrical gradient across the plasma membrane for the K^+ ions performing a significant regulatory function in this process. It is known that membranes are the most labile and is initial part in the mechanism of active reaction of the plant organism to external influences [22, 23].

The study of comparatively low specific activity (7, 15, 30 and 65 kBq/L) of tritium effect on membrane bioelectrical parameters of algae *N. obtusa* cells shows that 15 kBq/L specific activity concentration increased the specific resistance of *N. obtusa* cells membrane, reduced current densities of Cl^- and Ca^{2+} in the depolarization phase of the action potential, and decelerated the recovery of the membrane action potential in the fast repolarization phase [24].

Tendencies in changes of pattern of bioelectrical response of *N. obtusa* as well as in transmembrane fluxes of chloride and potassium ions during the action potential were observed due to exposure of algae cell to ^{137}Cs of comparatively low specific activity. Estimated cesium-induced alterations in electrical activity of giant alga cell membrane could result in further impairment of the electrogenesis, signal transduction and finally cell dysfunction, therefore more extensive electrophysiologycal investigations of radionuclides' impact on plant cell are required.

4. Conclusions

The analysis of kinetics and accumulation levels of radionuclides in the cell compartments of algae *N. obtusa* indicated that the process of accumulation of ^{137}Cs and ^{90}Sr in the cellulose-pectin cell wall is unlimited in time and can last for days. The accumulation of these radionuclides in the cells of algae *N. obtusa* depends on their accumulation in the ion exchange complex (the cell membrane and the plasmalemma) regulating the entrance of the radionuclides into the cytoplasm. Membrane potentials are mainly determined by the gradient of the potassium ions and electronic active transport complete an important regulatory function in the transport of ^{137}Cs and ^{90}Sr through the outer and inner cytoplasmic membrane.

Using the obtained data it can be clearly seen that *N. obtusa* cells can be a convenient radioecological model for the investigations of radionuclides' accumulation and it's toxicity for plants' bioelectricity within the single cell. Therefore *N. obtusa* cells can be applied for the evaluation the effect of radionuclides to plants.

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