

Radiostrontium uptake by lichen *Hypogymnia physodes*

Martin Pipiška,
Miroslav Horník,
Martina Kočiová,
Jozef Augustín,
Juraj Lesný

Abstract Radiostrontium ^{85}Sr sorption experiments were carried out at 4°C and 20°C using natural samples of the epiphytic foliose lichen *Hypogymnia physodes*. Thalli were incubated in water solutions containing 10^{-5} to 10^{-1} mol·l $^{-1}$ SrCl_2 for up to 24 h at the initial pH 5.5. Sorption equilibrium at 4°C and 20°C was observed within 1 hour and did not change within the next 24 hours. Sorption process can be described well by Freundlich adsorption isotherm in both linearised and non-linearised form, not by Langmuir adsorption isotherm. Inactivation of lichen biomass by formaldehyde or temperature pretreatment did not cause loss of biosorption activity. ^{85}Sr biosorption was strongly pH dependent increased from negligible values at pH 2 up to nearly 100% uptake at pH 5.5. Bivalent cations Me^{2+} act as competitors for ^{85}Sr biosorption with the competition effect increasing in the order $\text{Co} < \text{Mg} < \text{Ca} < \text{Cd} < \text{Ni} < \text{Ba} < \text{Zn} < \text{Cu}$ for Me^{2+} concentration 0.01 mol·l $^{-1}$. A number of displacing agents have been tested for their ability to release extracellular bound $^{85}\text{Sr}^{2+}$ ions from *Hypogymnia physodes*. Displacing efficiency increases in the order: water \lll EDTA (acid) $<$ $\text{NaHCO}_3 < \text{Na}_2\text{EDTA} < \text{oxalic acid} < \text{BaCl}_2 < \text{MgCl}_2 < \text{CaCl}_2 < \text{SrCl}_2$. Efficiency of strontium biosorption by different lichen taxa will reveal their role in fixation of radiostrontium contamination in biosphere.

Key words ^{85}Sr • *Hypogymnia physodes* • biosorption • kinetics • desorption

Introduction

Radioactive pollution has been studied mainly from the point of view of potential nuclear disasters and nuclear weapons testing. In such situations radioactive fallout is the main route of surface contamination [18]. Radioactive caesium and strontium in the environment are a matter of serious concern and they are a significant component of nuclear spread-out whose effects are governed by their chemical homology with potassium and calcium, respectively.

Lichens have been widely used as monitors of heavy metal [7, 30] and radioactive pollution [1, 14]. Lichens lack roots, a protective outer cuticle, are long-lived and depend on sorption of nutrient elements often over their entire surface. Lichenized fungi accumulate a range of both essential and non-essential elements through a combination of mechanism including surface complexation, biomineralization, and physical trapping of dust and soil particles [24, 27, 31].

Mechanisms by which lichenized and non-lichenized fungi accumulate trace metal cations from aqueous solutions have been investigated by several authors (see reviews [5, 24, 27]). Experimental studies by Puckett *et al.* [22] revealed that uptake of Cu by *Cladonia mitis* and *Umbilicaria muhlenbergii* was rapid and monotonic, reaching equilibrium concentrations in about 1 h. The authors modeled these results in terms of rapid

M. Pipiška[✉], M. Horník, M. Kočiová, J. Augustín,
J. Lesný
Department of Biotechnology,
Faculty of Natural Sciences,
University of SS. Cyril and Methodius,
2 Nam. J. Herdu Str., 917 01 Trnava, Slovak Republic,
Tel.: +421335565384, Fax: +421335565185,
E-mail: pipiskam@ucm.sk

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saturation of active surface-complexation sites on the lichen biomass. Nieboer *et al.* [19] concluded, based on experimental studies of Ni uptake by *Umbilicaria muhlenbergii*, that short term accumulation of metals by lichens could be described as an “ion exchange” process involving carboxyl or hydrocarboxyl sites on external cell walls or structural macromolecules. Gadd [10] proposed that other functional groups may also influence uptake, including amine, hydroxyl and phosphate groups. However, there is limited experimental data elucidating quantitatively the biogeochemical properties of fungal or lichen biomass or the identities of functional groups active in uptake. Tuominen [28] from short-term experiments concluded that strontium uptake by lichen *Cladonia alpestris* is purely a process of physical chemistry without any indications of metabolic activity.

With the aim of understanding the role of lichen in radiostrontium cycling in natural conditions we realized a series of experiments designed to measure $^{85}\text{Sr}^{2+}$ sorption capacity of a widely distributed epiphytic foliose lichen *Hypogymnia physodes* growing in the Slovak territory, under controlled conditions (as a function of time, temperature and fluid pH) and to determine the effect of bivalent cations on biosorption process. ^{85}Sr as a model radionuclide for behavior of ^{90}Sr , one of the most important contaminant of radioactive fallout was used. *H. physodes* is one of the most widely employed lichens in spatial and temporal patterns of metal contamination due to its widespread, virtually cosmopolitan distribution and because it is one of the most tolerant macrolichens to SO_2 and metals (e.g. [11]).

Material and methods

Biomass collection

Lichen was collected from the territory classified by the Resolution of Ministry of Agriculture of SR as the locality with the soil with very low contamination risk of unlimited use [17]. Biomass of epiphytic lichen *H. physodes* was taken from the northern side of the oak (*Quercus* sp.) grown in the forest of the southeast hills of Strážovské vrchy (48,95°N, 18,42°E), Slovak Republic. Samples were taken in winter (February–March 2004, November–December 2004) and in spring (April–May 2004). Bark covered by confluent layer of lichens was scrapped from the oak trunks 0.7 to 2.0 m above the ground level. Great care was taken to minimize damage during collection. The method of collection was successfully used by Kirchner and Daillant [14]. Lichen biomass was maintained in Petri dishes at 20°C illuminated by daylight. Humidity was maintained by periodical spraying of distilled water on the lichen surface for no more than 2 weeks. Lichen samples on bark were pre-incubated in distilled water for 0.5 hour before laboratory experiments. Wet lichen biomass was then removed from bark by scalpel blade. Impurities and debris were removed by repeated washing in distilled water and water droplets were wiped off by gentle pressing between layers of cotton wool. Biomass 0.2 or 0.5 g wet weight (corresponding to 86 ±

8 or 215 ± 13 mg air dried at 20°C for 24 hours), respectively, was used for bioaccumulation experiments.

Biosorption kinetics

Biosorption experiments were carried out in triplicate series in 100 ml Erlenmeyer flasks containing 20 ml distilled water and $^{85}\text{Sr}^{2+}$ (126 nmol·l⁻¹ SrCl_2 , 59 kBq·l⁻¹). If not otherwise stated, presented data are arithmetic mean values. Lichen biomass 0.5 g wet weight (corresponding to 215 ± 13 mg air dried at 20°C for 24 hours) was added, the content was agitated on a reciprocal shaker at 120 Hz for 24 h at 20°C, illuminated with daylight exposure. In time intervals 0 min (before the adding of lichen biomass), 10, 25, 40 min, 1, 2, 4, 6 and 24 h, 2 ml clear liquid samples were taken, the radioactivity was measured and the samples were returned back to reaction vessels. At the end of the experiments, lichen samples were removed from reaction solution, rinsed in distilled water to remove $^{85}\text{Sr}^{2+}$ solution capillary retained on biomass surface and water droplets were wiped off by gentle pressing between layers of cotton wool. $^{85}\text{Sr}^{2+}$ radioactivity in lichen biomass was measured. The pH values were recorded at the beginning and at the end of experiments.

Equilibrium studies

The two widely accepted and easily linearized adsorption isotherm models (Langmuir and Freundlich) for single metal systems [29] were used to describe strontium biosorption by lichen biomass. Isotherms were obtained for concentration range 10⁻¹ to 10⁻⁵ mol·l⁻¹ SrCl_2 . A general form of the Langmuir model equation is: $Q_{\text{eq}} = b \cdot C_{\text{eq}} \cdot Q_{\text{max}} / (1 + b \cdot C_{\text{eq}})$ where Q_{eq} = the uptake of the metal by biomass; Q_{max} = maximum uptake; C_{eq} = equilibrium concentration of metal in solution and b = constant related to energy of adsorption. A general form of the Freundlich model equation is: $Q_{\text{eq}} = k \cdot C_{\text{eq}}^{1/n}$. k and $1/n$ values are the Freundlich constants referring to adsorption capacity and intensity of adsorption, respectively.

Influence of pH, temperature and metabolic activity on $^{85}\text{Sr}^{2+}$ biosorption

Stock solutions were adjusted by adding 0.05 M HCl to obtain values ranging from pH 2 to 5.5. Water bath was used to provide required temperature (4 and 20°C) during biosorption experiments. In principle, the same procedure as in the biosorption kinetics study was used. Lichen biomass was shaken in $^{85}\text{Sr}^{2+}$ solution of desired pH and temperature for 24 h on a reciprocal shaker at 120 Hz. In order to eliminate interference of buffer components in strontium biosorption, distilled non-buffered solutions were used throughout all experiments. This approach was successfully used also by other authors [16] and in our previous paper [15]. Thermally inactivated biomass (15 min in water at 60°C) was also used in biosorption experiments.

Competition effect of Me^{2+} ions on $^{85}\text{Sr}^{2+}$ biosorption

Competition effect of Me^{2+} ions on strontium biosorption in concentration range 10^{-1} to 10^{-4} mol·l $^{-1}$ Me^{2+} (Ca^{2+} , Mg^{2+} , Ba^{2+} , Zn^{2+} , Cu^{2+} , Co^{2+} , Cd^{2+} , Ni^{2+}) was studied. Experiments were carried out in triplicate series in 100 ml Erlenmeyer flasks containing 20 ml required solution of Me^{2+} ions and $^{85}\text{Sr}^{2+}$ (126 nmol·l $^{-1}$ SrCl_2 , 59 kBq·l $^{-1}$). If not otherwise stated, presented data are arithmetic mean values. Lichen biomass 0.2 g wet weight (86 ± 8 mg, air dried at 20°C for 24 hours) was added, the content was agitated on a reciprocal shaker (120 Hz) at 20°C . Radioactivity in solution was measured after 5 hours and concentration equilibrium was calculated.

$^{85}\text{Sr}^{2+}$ desorption from lichen biomass

Lichen biomass, after biosorption was thoroughly rinsed in distilled water, water droplets were wiped off by gentle pressing between layers of cotton wool, and $^{85}\text{Sr}^{2+}$ activity was measured. Lichen biomass was repeatedly extracted by salt solutions, chelating agents and hydrochloric acid, respectively. Extractions were carried out by mixing on a reciprocal shaker (120 Hz) at 20, 40, 50°C for 15 min. Both radioactivity in liquid phase and in biomass was measured and concentration equilibrium of $^{85}\text{Sr}^{2+}$ was calculated.

Radiometric analysis

For radiometric determination of strontium, gamma spectrometric scintillation detector 54BP54/2-X with well type crystal NaI(Tl) (Scionix, The Netherlands) and data processing software Scintivision32 (Ortec, USA) were used. The counting time 400 s was sufficient for obtaining data with measurement error $<2\%$. Standardized $^{85}\text{SrCl}_2$ solution (97 kBq·ml $^{-1}$, SrCl_2 20 mg·l $^{-1}$ in 3 g·l $^{-1}$ HCl) obtained from Alldeco Inc., Slovakia was used in all experiments.

Results and discussion

Biosorption kinetics

In contrast to the results obtained with bioaccumulation of radiocaesium ^{137}Cs published in our previous paper [15], biosorption of radiostrontium by the same lichen *Hypogymnia physodes* differs practically in all parameters studied. The process of biosorption is very rapid and is not influenced by temperature within the range 4 to 20°C . Maximum biosorption was reached within one hour and did not changed within the next 24 hours (Fig. 1). The process of biosorption was not dependent on metabolic activity. Lichen biomass inactivated by thermal pretreatment in water for 15 min at 60°C or by formaldehyde solution (2 g·l $^{-1}$) showed the same biosorption kinetics and maximal $^{85}\text{Sr}^{2+}$ uptake binding capacity as lichen in native form. The above-mentioned characteristics are typical for interactions of cations with

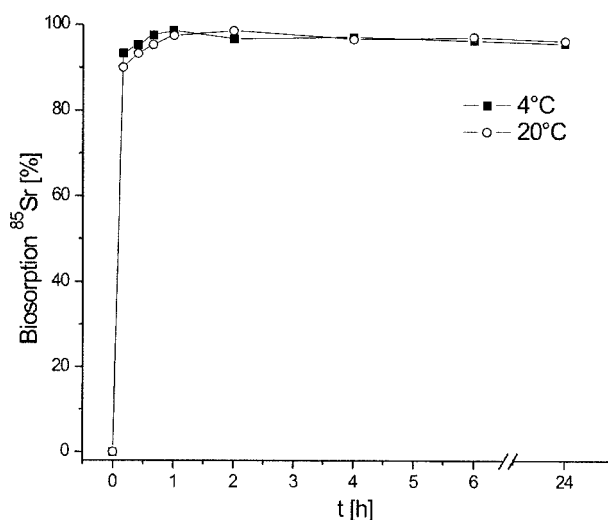


Fig. 1. Biosorption kinetics of ^{85}Sr (0.126 $\mu\text{mol}\cdot\text{l}^{-1}$ SrCl_2 , 59 kBq·l $^{-1}$) by *H. physodes* (215 ± 13 mg/20 ml air dried at 20°C) in water at 4°C (-■-■-) and 20°C (-o-o-) under aeration. Initial pH 5.0; pH 4.7 after 24 h.

anionic groups of many synthetic ion-exchangers. It is clear that strontium will be distributed within both algal and fungal parts of the thallus after primary ionic interactions with anionic groups of biomass surface. However, more detailed study will be necessary for obtaining quantitative data. Important role of CO_3^{2-} anions as well as oxalic acid and other lichen secondary metabolites of acidic character can be expected in strontium uptake and redistribution within biomass. Kasama *et al.* [13] in lichen *Trapelia involuta* observed significant fixation of Ca^{2+} ions as Ca-oxalate in outside of apothecium. Generally extracellular organic metal-rich crystals (oxalates and metal-lichen acid complexes) thalli of crustose lichens were observed by Purvis *et al.* [25].

Equilibrium studies

The concentration dependence of biosorption processes is usually described by adsorption isotherms. We tried to describe strontium biosorption by *Hypogymnia physodes* both by Langmuir and Freundlich isotherm. We found that the relationship between reciprocal values for Q_{eq} and C_{eq} was nonlinear and did not fit the Langmuir model (data not shown). Sr^{2+} adsorption to lichen biomass did not conform to the Langmuir adsorption isotherm. Nonlinear relationships between reciprocal values for Q_{eq} and C_{eq} are generally considered to indicate multilayer adsorption, although it has been suggested that metal adsorption data that do not conform to the Langmuir model are also indicative of complex metal-ligand interaction that would not be accounted for solely by electrostatic attraction [3]. Data presented in Fig. 2a,b showed that the process of strontium biosorption by *H. physodes* can be described well by the Freundlich isotherm in both linearized and non-linearized forms with Freundlich constants values $k = 1.27337$ and $1/n = 0.44797$, $R^2 = 0.966$. The magnitude of k and n showed easy uptake of strontium

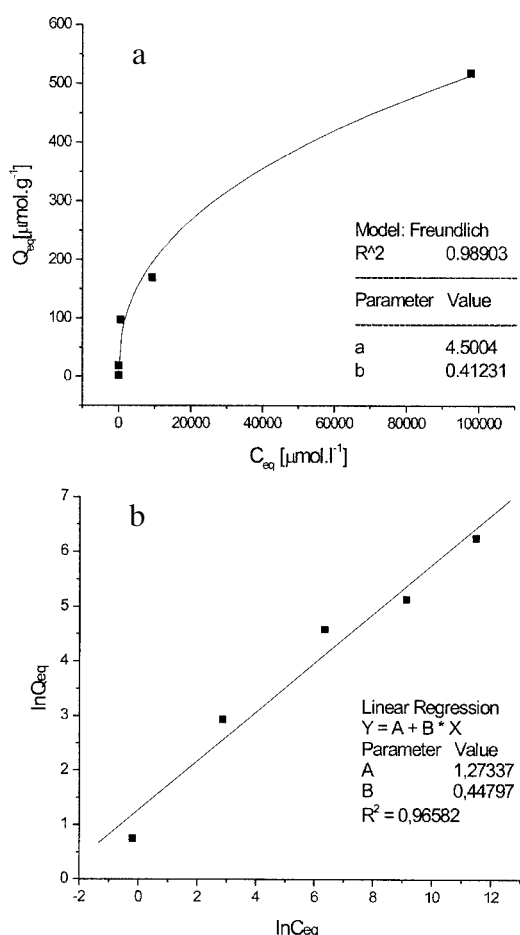


Fig. 2. Experimental data (■) and the fitted nonlinear (a) and linear (b) forms of Freundlich isotherm for the biosorption of strontium by *H. physodes* in concentration range 10^{-1} to 10^{-5} mol·l⁻¹ SrCl₂. C_{eq} represents equilibrium concentration of SrCl₂ in solution after 24 hours, Q_{eq} represents strontium sorbed by lichen biomass (86 ± 8 mg/20 ml, air dried at 20°C). Temperature 20°C; initial pH 5.0.

ions from reaction solution with a high adsorptive capacity of lichen biomass. The Freundlich model physically provides a more realistic description of strontium adsorption by lichen biomass because it accounts for different bindings sites, which resembles the situation in chromium and iron ion uptake by algal biomass, described by Aksu *et al.* [2].

pH dependence of ⁸⁵Sr²⁺ uptake

The strong pH dependence of radiostrontium biosorption by *H. physodes* is depicted in Fig. 3. Biosorption increases from negligible values at pH 2 up to nearly 100% uptake at pH 5.5. Low ion sorption for alkali metal earth and alkali metal ions at low pH values and high in mild acidic solutions are known for all cation exchangers used in analytical chemistry (see e.g. [8]). Lichens produce very broad spectrum of secondary metabolites with acidic character. Some of them can form metal-lichen acid complex such as Cu-norstic acid and Cu-psoromic acid complexes [26]. Calcium oxalates are formed by the primary metabolism of fungal hyphae

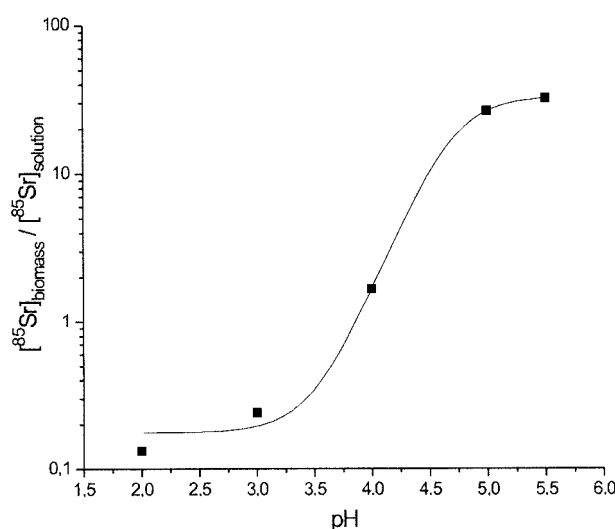


Fig. 3. Biosorption of ⁸⁵Sr ($0.126 \mu\text{mol}\cdot\text{l}^{-1}$ SrCl₂, $59 \text{ kBq}\cdot\text{l}^{-1}$) by *H. physodes* (86 ± 8 mg/20 ml, air dried at 20°C) in dependence on the initial pH values. Data expressed as $[\text{Sr}^{85}]_{\text{biomass}} / [\text{Sr}^{85}]_{\text{solution}}$ ratio after 24 h incubation under aeration at 20°C. Lichen biomass added to the SrCl₂ solution in water after adjusting to desired pH values by adding 0.5 M HCl.

[13, 23]. It is generally accepted that apothecium and thallus of some lichens contain polysaccharides because many lichenized fungi have neutral to acidic polysaccharides [13, 21]. Polysaccharides are negatively charged, so that positively charged metal ions can be adsorbed onto their surfaces. On the basis of titration studies of two lichen species by Tuominen [28] we can expect the existence of acidic lichen compounds with acidic dissociation constants in the range pK_a 2.8–4.4 which fit well with our strontium biosorption data. Increasing uranium biosorption by lichen *Peltigera membranacea* within pH range from pH 2 to pH 5 was observed by Haas *et al.* [12]. Strontium biosorption by native biomass of *H. physodes* at neutral pH values can be expected in carbonate form during CO₂ production of respiring biomass.

Competitive effect of Me²⁺ ions in ⁸⁵Sr²⁺ biosorption

Percentage of ⁸⁵Sr²⁺ biosorption in dependence of concentration of cold SrCl₂ within concentration range from 0.1 to 0.0001 mol·l⁻¹ in semilogarithmic scale showed sigmoid curve, typical for sorption processes. Data in linearized form as $\ln Q_{eq} = f(\ln C_{eq})$ are presented in Fig. 2b. Similar sigmoidal curves were obtained when Me²⁺ salts were used in ⁸⁵Sr biosorption processes instead of cold SrCl₂. We found that alkali earth metal ions Mg²⁺, Ca²⁺, Ba²⁺ and other bivalent cations act as the efficient competitors for ⁸⁵Sr biosorption by *H. physodes*. Data are summarized in Fig. 4. Competitive effect at 0.01 mol·l⁻¹ increases in the order: Co < Mg < Ca < Cd < Ni < Ba < Zn < Cu, and Co < Mg < Ca < Cd ≅ Ni ≅ Ba ≅ Zn < Cu at 0.001 mol·l⁻¹. Data obtained in the presence of metals added in concentrations $\leq 0.1 \text{ mmol}\cdot\text{l}^{-1}$ are influenced by concentration of naturally occurring metals in biomass, mainly by Ca²⁺ and Mg²⁺ ions.

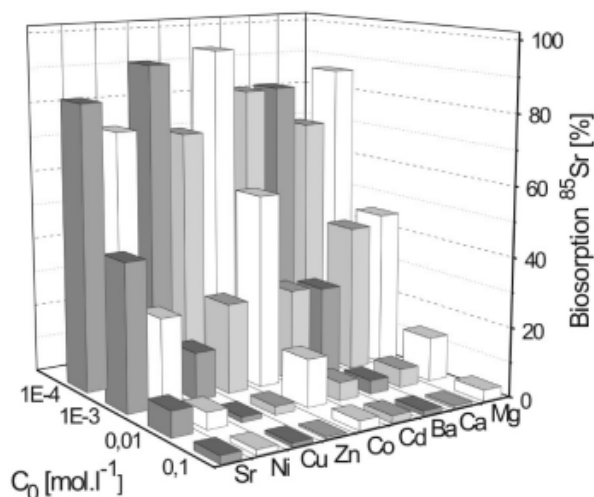


Fig. 4. Competitive effect of Me^{2+} ions at initial concentration range 10^{-1} to 10^{-4} $\text{mol}\cdot\text{l}^{-1}$ on ^{85}Sr biosorption ($0.126 \mu\text{mol}\cdot\text{l}^{-1}$ SrCl_2 , $59 \text{ kBq}\cdot\text{l}^{-1}$) by *H. physodes* ($86 \pm 8 \text{ mg}/20 \text{ ml}$, air dried). Reaction time 5 h at 20°C , initial pH 5.0.

There is tendency to use nonvascular plants (mosses biomass) as bioindicators of water contamination with metals and radionuclides [4, 9]. False low concentrations of contaminated metals or radionuclides due to the presence of higher salt concentrations in waters with variable pH values can be obtained when lichen biomass will be used for the same monitoring purposes. Temperature will not play more significant role.

$^{85}\text{Sr}^{2+}$ desorption from lichen biomass

Strontium ^{85}Sr sorbed by lichen biomass can be extracted with electrolytes such as acids and inorganic salts. As can be seen from data in Fig. 5B,C new concentration equilibrium is formed after repeated transfer of biomass to the fresh solution. Strontium can be efficiently desorbed by repeated extraction of lichen biomass with hydrochloric acid, oxalic acid and Na_2EDTA , with less efficiency with NaHCO_3 , with saturated solution of EDTA as a free acid but very poorly with distilled water. The role of oxalic acid in strontium cycle is known in the case of fungi producing oxalic acid. *Resinicium bicolor* growing in the presence of the naturally occurring mineral strontianite solubilized strontium from mineral phase, translocated the ions and reprecipitated the strontium into strontium-containing calcium oxalate crystals [6]. Radiostrontium is released from biomass also by repeated washing with $0.1 \text{ mol}\cdot\text{l}^{-1}$ SrCl_2 . It means that strontium is available for ion exchange reactions with cold SrCl_2 and with other bivalent alkali metal earth cations (Fig. 5A).

Strontium cannot be removed from lichen biomass by extraction with polar and non-polar organic solvents within the range of dielectric constants: toluene ($\epsilon = 2.4$), chloroform ($\epsilon = 4.8$) and methanol ($\epsilon = 32.6$) (data not shown). It means that strontium is not bind in lichen biomass into low molecular organic compounds.

It can be concluded that *Hypogymnia physodes* shows basically Sr biosorption properties observed of lichens belonging to other genera such as *Cladonia*, *Umbilicaria*

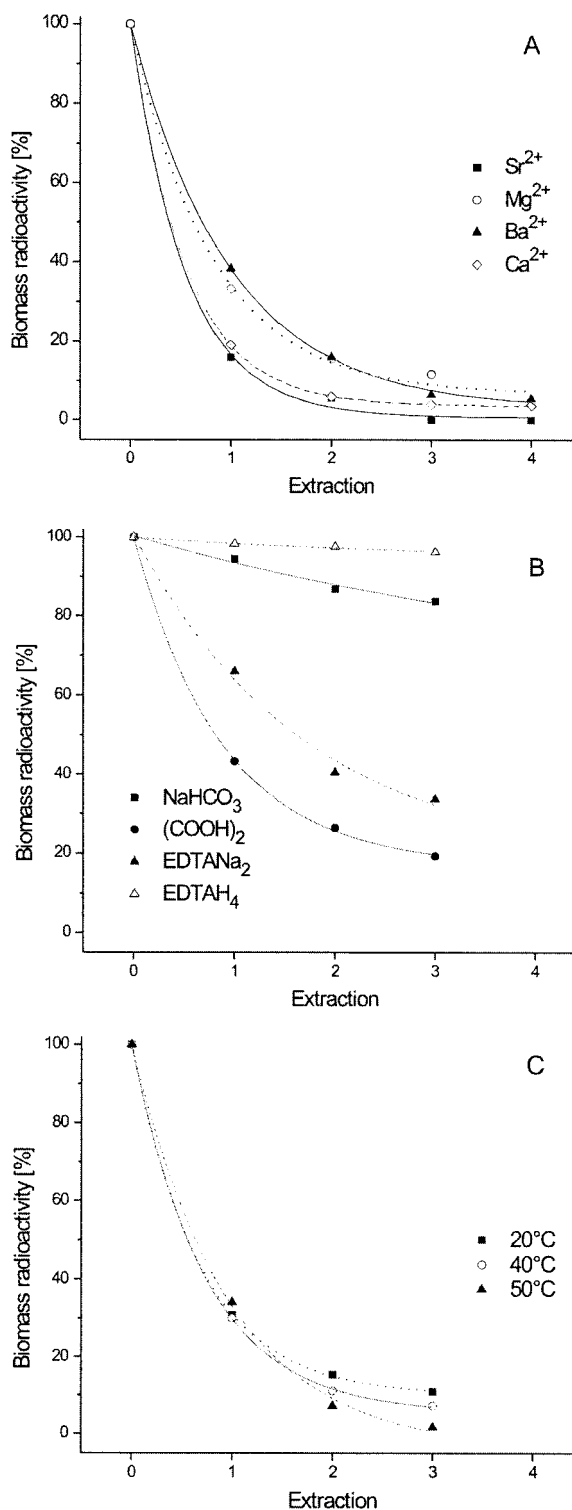


Fig. 5. Desorption of ^{85}Sr from *H. physodes* by repeated extraction (A) with 0.1 M SrCl_2 , MgCl_2 , BaCl_2 and CaCl_2 solutions (15 min each at 50°C), (B) with 0.1 M NaHCO_3 , 0.05 M (COOH)_2 , 0.05 M EDTANa_2 and 0.05 M EDTAH_4 solutions (15 min each at 50°C) and (C) with 0.05 M HCl solutions (15 min each at 20 , 40 and 50°C). Biomass was extracted (three and four times, respectively) with fresh solutions at biomass:solution ratio = 1:23 (A) and 1:36 (B, C) (w/v, dry biomass). Experimental data were fitted as exponential decay of first order. Y-axis represents percentage of radioactivity in biomass after biosorption (X-axis point 0) and after repeated extraction (X-axis points 1, 2, 3 and 4).

and *Peltigera* studied by Nifontova *et al.* [20]. They postulated that the mechanisms of ^{90}Sr adsorption from water solutions by the above mentioned lichens is primary based on physicochemical sorption and lichen retained radionuclide is in a relatively mobile ion-exchange form. *Cladonia amaurocracea*, very rare in Europe, is extreme acidophytic lichen. *Peltigera canina* is mild acidophytic to neutral lichen. *H. physodes* studied in our paper is classified as acidophytic lichen. The next study can explain the role of acidophilicity or basophilicity of lichens in the efficiency of strontium biosorption.

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