

Influence of time, temperature, pH and inhibitors on bioaccumulation of radiocaesium - ^{137}Cs by lichen *Hypogymnia physodes*

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Abstract Caesium bioaccumulation experiments were carried out at 4 to 60°C using natural samples of the lichen *Hypogymnia physodes*. Thalli were incubated in 2.5 $\mu\text{mol}\cdot\text{l}^{-1}$ CsCl solutions labelled with $^{137}\text{CsCl}$ for up to 24 h at pH values from 2 to 10. Bioaccumulation of Cs^+ ions in the first phase of the lichen-CsCl solution interaction is rapid, neither pH, nor temperature dependent within the range 4 to 60°C and observed also with the lichen biomass thermally inactivated at 60°C or chemically by formaldehyde. The second phase of ^{137}Cs bioaccumulation is time, temperature and pH dependent and is inhibited by formaldehyde and thermal inactivation. The process at the initial concentration $C_0 = 2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl and 20°C reached equilibrium within 12 hours. It can be described by the first order reaction kinetics equation: $\log [C_t] = 1.89 - 0.00153 t$, $R = -0.950$. Maximal values of Cs-bioaccumulation were observed at 20°C with minimum at 4°C and 40°C and at pH 4–5 with minimum at pH 2 and pH 6. Low caesium efflux values from lichen thalli by water and 0.1 $\text{mol}\cdot\text{l}^{-1}$ neutral salts at 20°C and 24 h equilibrium were observed. Efflux characterized by distribution coefficients $D = [\text{Cs}]_{\text{solution}}/[\text{Cs}]_{\text{biomass}}$ at biomass/solution ratio 1:25 (w/v, wet wt.), decreases in the order: $\text{Li}^+ - 78 \times 10^{-3} > \text{NH}_4^+ = \text{K}^+ - 15 \times 10^{-3} > \text{Cs}^+ = \text{Na}^+ - 11 \times 10^{-3}$. Low extractability of caesium from lichen by water and salt solutions can explain long persistent times of radiocaesium contamination sorbed by lichens, observed by many authors in caesium-contaminated forest and mountain regions. Hypothesis of the role of the lichen secondary metabolites as caesium binders is discussed.

Key words radiocaesium • lichen • *Hypogymnia physodes* • bioaccumulation • kinetics • efflux

Introduction

Environmental pollution resulting from the release of radionuclides into soils and natural waters is a serious threat to human and ecological health in many parts of the world. Radionuclides, originating from nuclear fission, such as ^{137}Cs and ^{90}Sr , are released into the environment by nuclear weapon testing, discharge of nuclear waste and accidental release from nuclear facilities and become more concentrated as they move up the food chain, often exceeding human health standards [33]. The environmental mobility of radiocaesium is influenced by many physical, chemical and biological factors. The effect of organisms within the soil horizon, including plants, microorganisms, lichens and chelating compounds secreted by living organisms, can exert a profound influence on the behavior of cations in aqueous solutions.

Although many studies have screened lichens for *in vivo* ^{137}Cs levels, mechanism of Cs^+ uptake in these organisms is not fully understood and these areas require further attention [6]. It has been established that, like fungi, lichens retain accumulated ^{137}Cs for long periods of time [14, 24, 25, 30]. Ellis and Smith [11]

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estimated that the biological half-life in lichen, i.e. time required for a 50% reduction in Cs⁺ levels in lichens was approximately 5–8 years. The remarkable ability of the lichens to accumulate many elements has been established unequivocally in the last 25 years. Richardson [28] published a review of the metal uptake in lichens and fungi. Three mechanisms have been proposed: (1) an intracellular uptake via an exchange process, (2) intracellular accumulation, (3) trapping of metal rich particulates. Various researchers have attempted to understand the binding processes by using techniques such as nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR) luminescence and microcalorimetric technique [3].

The mechanism of short-term cation-uptake by lichens is generally regarded as an abiotic process governed by surface complexation of aqueous cations with exposed functional groups on lichen biomass surface, or by the precipitation of solid phases on the exteriors of cell walls [15]. However, intracellular exchange mechanisms involving metabolic processes may also facilitate cation uptake, especially over longer incubation times [28]. The importance of intracellular uptake at longer incubation times remains largely unquantified.

The aim of this work was to investigate radiocaesium bioaccumulation in laboratory experiments using widely distributed epiphytic lichen *H. physodes*, under controlled conditions as a function of time, temperature and pH. Effects of metabolic inhibitor and thermal inactivation on bioaccumulation of ¹³⁷Cs by lichen biomass were studied as well. Efflux of ¹³⁷Cs from lichen biomass by water and salt solutions was investigated in order to assess the reversibility of caesium binding.

Material and methods

Biomass collection

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 (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. 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. In principle, the method by Kirchner and Daillant was used [18].

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), respectively, were used for bioaccumulation experiments.

Bioaccumulation of Cs⁺ ions

Bioaccumulation experiments were carried out in triplicate series in 100 ml Erlenmeyer flasks containing 19 ml distilled water and 1 ml ¹³⁷CsCl. If not otherwise stated, presented data are arithmetic mean values. Lichen biomass 0.2 or 0.5 g (wet weight) 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), 5, 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 ¹³⁷Cs solution capillary retained on biomass surface and water droplets were wiped off by gentle pressing between layers of cotton wool. ¹³⁷Cs activity in lichen biomass was measured. The pH values were recorded at the beginning and at the end of experiments.

Influence of pH, temperature and metabolic inhibition on ¹³⁷Cs bioaccumulation

Stock solutions were adjusted by adding 0.05 M HCl or 0.1 M NaOH to obtain values ranging from pH 2 to 10. Water bath was used to provide required temperature (4, 20, 25, 30, 40 and 60°C) during bioaccumulation experiments. In principle, the same bioaccumulation procedure as the above mentioned was used. Wet lichen biomass was immersed in ¹³⁷Cs solution of desired pH and temperature and agitated on a reciprocal shaker at 120 Hz for 24 h.

Formaldehyde as metabolic inhibitor was used. Lichen biomass (0.5 g wet weight) was pretreated in formaldehyde (0.2 w/v) for 0.5 hour. Pretreated biomass and native biomass (0.5 g wet weight, without pretreatment) were added into Erlenmeyer flasks containing 19.5 ml 0.2% formaldehyde solution and 0.5 ml ¹³⁷CsCl. The content was agitated on a reciprocal shaker at 120 Hz for 24 h at 20°C. Thermally inactivated biomass (15 min at 60°C) was also used in bioaccumulation experiments.

Efflux analysis

Lichen biomass after bioaccumulation phase was thoroughly rinsed in distilled water and water droplets were wiped off by gentle pressing between layers of cotton wool and ¹³⁷Cs activity was measured. Caesium efflux was measured under given experimental conditions described in Table 2.

Radiometric analysis

For radiometric determination of caesium, 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 $^{137}\text{CsCl}$ solution ($1000 \text{ kBq}\cdot\text{l}^{-1}$ in 0.1 M HCl , $50 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl) obtained from Research Institute of Nuclear Energy, Trnava, Slovakia was used in all experiments.

Statistical analysis

Correlation between variables was assessed by determining the linear correlation coefficient, using Microcal Origin 7.0 program.

Results and discussion

Time dependence of Cs uptake

Uptake of ^{137}Cs from water solution by native biomass *H. physodes* shown in Fig. 1 is time dependent process. Under given experimental conditions, i.e. the initial CsCl concentration $C_0 = 2.5 \mu\text{mol}\cdot\text{l}^{-1}$ and biomass $31 \text{ g}\cdot\text{l}^{-1}$ (wet wt.) at 20°C , concentration equilibrium is reached within approx. 12 h, where 98% uptake value was obtained. Uptake of Cs by *H. physodes* ($\mu\text{mol}\cdot\text{g}^{-1}$) in dependence on the initial Cs concentration ($\text{mol}\cdot\text{l}^{-1}$) was described in our previous paper [19].

Caesium uptake by *H. physodes* is two phase process, characterized by the first rapid phase lasting several minutes followed by the second slow phase, reaching equilibrium within several hours. The second phase of Cs uptake can be described by the 1st order reaction kinetics, typical for many chemical and enzymatic catalyzed reaction (Eq. (1)). Experimental data in semilogarithmic plot are shown in Fig. 2.

$$(1) \quad C_t = C_0 \cdot e^{-kt}$$

where C_t = equilibrium Cs concentration in solution at time t , C_0 = Cs concentration at time t_0 and k = 1st order rate constant.

Equation (1) in semilogarithmic form can be expressed by the eq. (2),

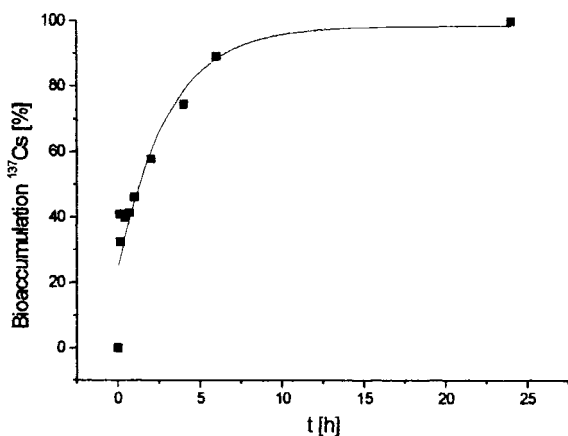


Fig. 1. Bioaccumulation kinetics of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl , $50 \text{ kBq}\cdot\text{l}^{-1}$) by *H. physodes* ($31 \text{ g}\cdot\text{l}^{-1}$, wet wt.) in $1 \text{ mmol}\cdot\text{l}^{-1}$ NaCl at 20°C under aeration. Initial pH 5.5, pH 4.7 after 24 h.

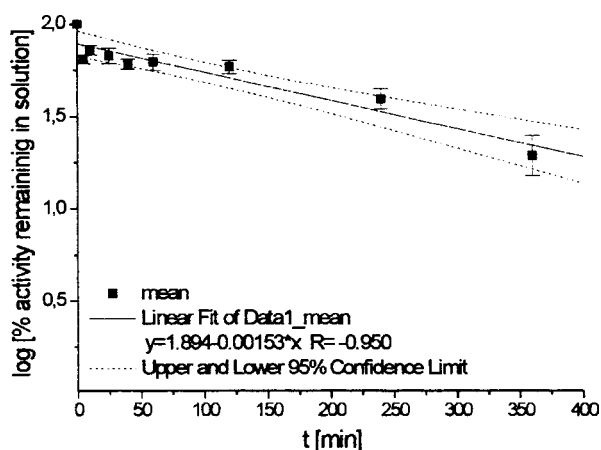


Fig. 2. First order kinetics of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl , $50 \text{ kBq}\cdot\text{l}^{-1}$) bioaccumulation by *H. physodes* ($25 \text{ g}\cdot\text{l}^{-1}$ wet wt., average value) in distilled water at 20°C under aeration. Experimental data represents mean values of 6 independent experiments. Error bars reflect standard error of the mean. Initial pH 5.5, pH 4.7 after 24 h.

$$(2) \quad \log [C_t] = q - a \cdot t$$

where q = y -intercept at time t_0 . It represents caesium uptake obtained immediately after contact of biomass with Cs solution. This process takes part as consequence of ionic interactions of Cs^+ ions with anion groups on the thallus surface. The second, time dependent phase of caesium uptake is given by expression ($k \cdot a$), where ' a ' is the slope of linear part of the kinetics in Fig. 2. The eq. (3) obtained from experimental data can be written in the form (3):

$$(3) \quad \log [C_t] = 1.89 - 0.00153 t$$

with $q = 1.89$, $k = 0.0035 \text{ min}^{-1}$, i.e. the 1st order rate constant ($-2.303 \cdot a$), and correlation coefficient $R = -0.950$, calculated from 6 independent experiments.

The first order rate constant under constant conditions depends on biomass concentration as can be seen in Fig. 3. Observed maximal (initial) rate constant of

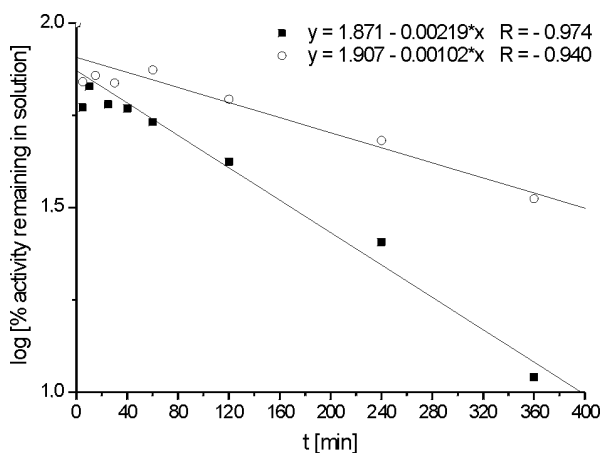


Fig. 3. First order kinetics of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl , $50 \text{ kBq}\cdot\text{l}^{-1}$) bioaccumulation by *H. physodes* at different biomass concentration (wet wt.): $31.5 \text{ g}\cdot\text{l}^{-1}$ (■-■-■) or $21.4 \text{ g}\cdot\text{l}^{-1}$ (○-○-○), respectively, in distilled water at 20°C under aeration. Initial pH 5.5, pH 4.7 after 24 h.

Cs uptake, calculated from the tangent line at t_0 time of the fitted bilinear uptake/time curve (Fig. 1) is $v_{\max} = 13.7 \text{ nmol Cs h}^{-1}$ per 1 g lichen biomass (wet wt.). The first phase of Cs uptake ($\sim 20\%$ of the total) can be attributed to ionic or sorption processes of Cs^+ ions on the thallus surface, the second phase by transport processes from the surface into the interior of the thallus.

Temperature dependence of caesium uptake

Uptake of Cs^+ ions by *H. physodes* is a strongly temperature dependent process (Fig. 4). At 4°C only the first, rapid sorption phase was observed on kinetics curves. The amount of bioaccumulated caesium did not exceed approximately 20% of the maximal values obtained in equilibrium at 20°C after 24 hours. This lack of the second phase can be explained by low permeability of biological membranes below certain temperature limits given by the transition of membrane lipids from permeable liquid state to solid gel state, what negatively influences permeability of biological membranes. Low rate constants values of Cs uptake at lowered temperatures can be explained also on the basis of the influence of temperature on enzyme catalyzed processes in terms of Arrhenius equation.

Maximal values of the Cs uptake were observed at 20°C , with significant decreases at 25 and 30°C , where uptake reaches 40 and 10% of the maximal values, respectively. The increase of reaction temperature to 40°C or higher caused drastic decrease of Cs uptake, with values comparable with those obtained at 4°C (Table 1). Caesium uptake at 60°C , after 6 h of interaction reached values comparable with those obtained at 4°C , however after 24 hours decreased on the 1 to 2% level.

Observed relative narrow temperature optimum of the caesium uptake by *H. physodes*, around 20°C is below temperature optima of many bacteria (37°C), fungi and actinomycetes (28°C). This fact is in agreement with psychrophile character of the lichens growing in the shadow of the forests in mountains of Slovakia. Temperature optimum 28°C for caesium uptake by

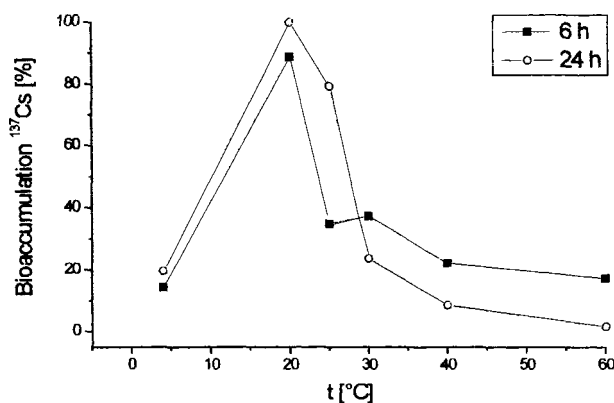


Fig. 4. Bioaccumulation of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl, $50 \text{ kBq}\cdot\text{l}^{-1}$) by *H. physodes* ($25 \text{ g}\cdot\text{l}^{-1}$, wet wt.) after 6 h (■-■-■) and 24 h (○-○-○) incubation in distilled water under aeration at 4, 20, 25, 30, 40 and 60°C . Initial pH 5.5, pH 4.7 after 24 h.

Table 1. Comparison of the effect of temperature, pH, thermal inactivation and inhibitors on ^{137}Cs bioaccumulation by *H. physodes*

Influence		Bioaccumulation [%]	
		6 h	24 h
Temperature ¹	4°C	14.2	19.8
	20°C	80.4	100.0
	40°C	22.2	8.7
	60°C	17.3	1.7 ⁴
Inactivation	Formaldehyde ^{2a}	27.2	24.4
	Formaldehyde ^{2b}	14.5	12.6
	Thermal ³	9.3	9.3
pH ⁵	pH 2	3.8	4.0
	pH 3	13.3	16.1
	pH 4	62.6	85.4
	pH 6	23.4	9.6

¹ Reaction condition see the legend to Fig. 4.

^{2a} Bioaccumulation in the presence of 0.2% formaldehyde at 20°C . Reaction conditions see the legend to Fig. 6.

^{2b} As in the case 2a, with lichen biomass pretreatment in 0.2% formaldehyde for 0.5 h before adding to the ^{137}Cs solution.

³ Bioaccumulation of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl, $50 \text{ kBq}\cdot\text{l}^{-1}$) by *H. physodes* ($25 \text{ g}\cdot\text{l}^{-1}$, wet wt.) in distilled water at 20°C under aeration with lichen biomass pretreated by thermal inactivation at 60°C for 15 min before adding to the reaction solution.

⁴ Leakage of intracellular lichen components, colored and turbid supernatant fluid observed after 24 hours.

⁵ Reaction condition see the legend to Fig. 5.

actinomycete *Rhodococcus ruber* was published by Ivshina *et al.* [16]. Temperature dependence of dark respiration, resembling the temperature dependence of Cs uptake observed in our paper described Lange and Green [20] for five lichen species. They observed maximal CO_2 production rates up to $3.25 \text{ nmol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$ in laboratory instrumentation around 20°C , decreasing with decreasing temperature. Zero values of respiration activity they observed within approx. 5°C to approx. -5°C , depending on season (lower for winter, higher for summer) and on the properties of the strain.

pH dependence of Cs uptake

Significant pH dependence of Cs uptake is depicted in Fig. 5. The curves in Fig. 5 display similar overall shapes, exhibiting uptake maximum for all incubation times within the pH range 4 to 5. Minimum uptake is observed at pH 2 and pH 6. The increase of the initial pH values to neutral pH 7 causes two-fold increase of the caesium uptake in 24 h equilibrium at 20°C and the next weaker increase at pH 10. Mild increase of Cs uptake with pH values in the interval pH 6–10 can reflect dissociation processes of the second or third -COOH groups of aliphatic di- and tricarboxylic acids (such as succinic and citric acid), substituted benzoic acids (such as phthalic acid) or -OH groups of carboxy- or aldehyde-p-substituted phenols as the next potential Cs^+ binders.

Observed pH optimum of Cs accumulation, shifted toward mild acid region, is in good agreement with acidophytic character of many epiphytic members of

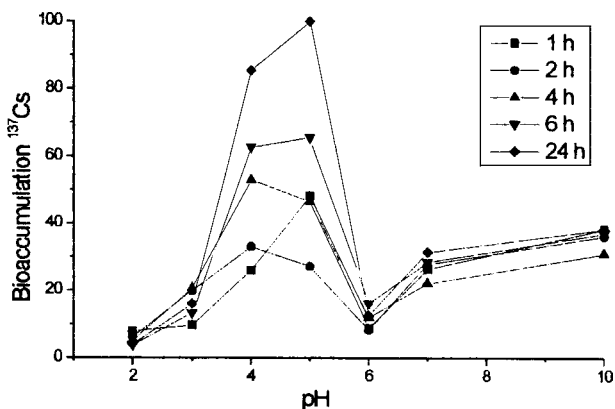


Fig. 5. Bioaccumulation of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl, $50 \text{ kBq}\cdot\text{l}^{-1}$) by *H. physodes* ($10 \text{ g}\cdot\text{l}^{-1}$, wet wt.) in dependence of the initial pH values. Data expressed as percentage of accumulated radioactivity in biomass after 1 h (■-■-■), 2 h (●-●-●), 4 h (▲-▲-▲), 6 h (▼-▼-▼) and 24 h (◆-◆-◆) incubation under aeration at 20°C . Lichen biomass added to the CsCl solution in distilled water after adjusting to desired pH values by adding 0.5 M HCl or 0.1 M NaOH . The shifts of pH values after 24 h reaction time were: pH 2.0, 3.0 and 4.0 without changes, pH 5.0 to 4.7; pH 6.0 to pH 5.0; pH 7.0 to pH 5.3; pH 10.0 to pH 8.5.

Hypogymnia genus. According to data published by Wirth [31] *H. physodes* is considered as mild acidophytic, able to grow within the pH range 4.9 to 5.6 and even rather acidophytic, able to grow within the pH range 3.4 to 4.0. Optimum pH for caesium uptake by bacteria *Rhodococcus ruber* in mild alkaline pH 7.8–8.6 was described by Ivshina *et al.* [16].

Inhibition of Cs^+ uptake by inhibitors and thermal inactivation

Formaldehyde ($2 \text{ g}\cdot\text{l}^{-1}$) was used as a metabolic inhibitor, in order to distinguish bioaccumulation processes dependent and not dependent on metabolic activity. Native lichen biomass was able to accumulate all caesium present in solution ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$). Lichen biomass in the presence of formaldehyde ($2 \text{ g}\cdot\text{l}^{-1}$) accumulated only 24% of Cs, comparing with non-inhibited lichen biomass (Fig. 6, Table 1).

Similar inhibitory effect was observed also in the case when lichen biomass was inactivated by pretreatment at 60°C for 15 min or pretreated with formaldehyde ($2 \text{ g}\cdot\text{l}^{-1}$) for 30 min. In both, thermal and formaldehyde pretreated biomass, the second, time dependent phase of bioaccumulation process obeying 1st order reaction kinetics was fully inhibited. Obtained data are presented in Table 1 and Fig. 6. Caesium uptake by formaldehyde pretreated and thermally pretreated at 60°C lichen biomass was approx. 13% and 9%, respectively of the control experiments (not pretreated biomass) and could be ascribed to the processes not dependent on metabolic activity, such as interaction of Cs^+ ions with anionic functional groups of the lichen surface.

Sensitivity of caesium uptake by *H. physodes* on inactivation is in agreement with the present knowledge of transport processes of alkali metal ions. Microbial Cs^+ uptake is generally mediated by monovalent cation

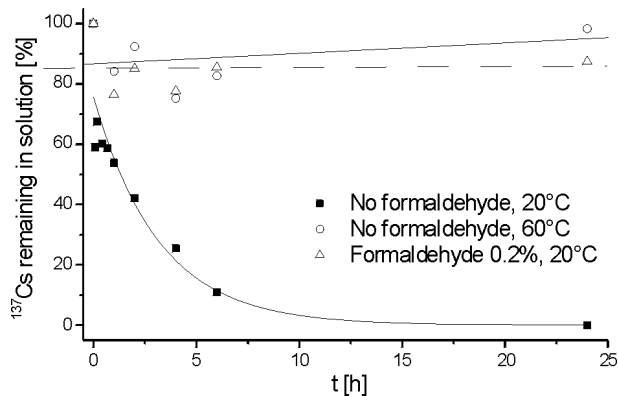


Fig. 6. Inactivation effect of formaldehyde ($2 \text{ g}\cdot\text{l}^{-1}$, $\Delta-\Delta-\Delta$) and temperature 60°C (o-o-o) on bioaccumulation of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl, $50 \text{ kBq}\cdot\text{l}^{-1}$) by *H. physodes* ($25 \text{ g}\cdot\text{l}^{-1}$, wet wt.). Lichen biomass added to the CsCl solution in distilled water with or without formaldehyde and incubated at 20 or 60°C . Initial pH 5.5, pH 4.7 after 24 h.

transport system located on the plasma membrane. These systems differ widely in specificity for alkali metal cations and consequently microorganisms display large differences in their ability to accumulate Cs^+ ions. Caesium appears to have an equal or greater affinity than K^+ for transport systems in certain microorganisms. Microbial Cs^+ accumulation is markedly influenced by the presence of external cations, e.g. K^+ , NH_4^+ , and H^+ , and is generally accompanied by an approximate stoichiometric exchange for intracellular K^+ . Microbial tolerance to Cs^+ may result from sequestration of Cs^+ in vacuoles or changes in the activity and specificity of transport systems mediating Cs^+ uptake [5, 7 (and references cited)].

Bacteria *Escherichia coli* contain a functional Kup (formerly TrkD) system able to take up Cs^+ with moderate rate and affinity. Kup is a separate K^+ uptake system with relatively little discrimination in the transport of cations K^+ , Rb^+ and Cs^+ [8].

Efflux analysis

Efflux of toxic metals accumulated by biological objects such as plants, used for remediation of contaminated environment will cause resolubilization, redistribution and circulation of pollutants. Data describing efflux processes of Cs^+ ions in *H. physodes* are shown in the next paragraph.

Efflux values at 20°C into water after 24 h incubation under aeration reached only <5% of the total Cs uptake at the water:biomass = 40:1 ratio. Reaction time 24 hours was sufficient for obtaining new concentration equilibrium. This value was not changed even after the next 48 hours. Efflux values 65, 21, 29, 20 and 30% of the total Cs^+ uptake at solution:biomass = 25:1 into 0.1 M LiCl, NaCl, KCl, CsCl and NH_4Cl solution, respectively, were observed. When the next 24 h incubation with fresh salt solutions was done, the next portion of Cs^+ was released from biomass, reaching total efflux (as a sum of the first and second steps) 82% for LiCl, 32% for NaCl, 33% for KCl, 32% for CsCl and 50% for NH_4Cl

Table 2. Two step elution of ^{137}Cs from *H. physodes* with 0.1 M electrolyte solutions. Lichen, after 24 h accumulation of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl, $50 \text{ kBq}\cdot\text{l}^{-1}$, biomass $25 \text{ g}\cdot\text{l}^{-1}$, wet wt.) at 20°C under aeration was extracted 24 h at 20°C and with fresh salt solution next 24 h at the biomass:solution ratio 1:25 (w/v). Data expressed as the ratio of the total radioactivity eluted to the radioactivity of biomass $D = [\text{Cs}_{\text{solution}}]/[\text{Cs}_{\text{biomass}}]$

Solution [0.1 mol $\cdot\text{l}^{-1}$]	D [Cs $_{\text{solution}}$]/[Cs $_{\text{biomass}}$]	
	24 h	48 h
LiCl	77.6×10^{-3}	39.1×10^{-3}
NaCl	11.0×10^{-3}	7.3×10^{-3}
KCl	15.0×10^{-3}	2.1×10^{-3}
CsCl	10.6×10^{-3}	7.6×10^{-3}
NH $_4$ Cl	14.4×10^{-3}	14.2×10^{-3}
HCl	746.0×10^{-3} *	–

* Efflux >95% obtained with 0.1 M HCl in the first step. Swelling and gelling action of biomass was observed at 24 h. Efflux <5% obtained with distilled water under the same conditions.

of the total Cs $^+$ uptake, respectively, were obtained. Efflux process resembled concentration equilibrium of solutes in extraction processes. Efflux kinetics in lichen in our study therefore did not obey the 1st order reaction kinetics, which was observed in Cs $^+$ efflux in *Holomonas israelensis* [29]. Our efflux data expressed as the ratio of total radioactivity extracted to radioactivity in biomass are shown in Table 2.

Caesium efflux of *H. physodes*, expressed by distribution coefficient $D = [\text{Cs}]_{\text{solution}}/[\text{Cs}]_{\text{biomass}}$ at the [biomass]/[water] ratio 1:25 (w/v, wet wt.) after 24 h equilibrium at 20°C decreased in the order:

$$\text{Li}^+ - 78 \times 10^{-3} > \text{NH}_4^+ = \text{K}^+ - 15 \times 10^{-3} > \text{Cs}^+ = \text{Na}^+ - 11 \times 10^{-3} \text{ (see Table 2)}$$

Differences in Cs $^+$ efflux expressed as distribution coefficient could be partly explained by differences in ionic radii, increasing in the order (pm):

$$\text{Li} - 78 < \text{Na} - 98 < \text{K} - 133 < \text{Rb} - 149 < \text{Cs} - 165,$$

with the value for NH $_4^+$ – 142, therefore near to the K $^+$ ionic radius value.

Observed D values 0.003–0.05:1 in the first extraction step and 0.0005–0.01:1 in the second step with fresh salt solutions show, that significant part of bioaccumulated caesium is not easily solubilized at 20°C . Even under more drastic conditions such as 40 and 50°C , significant portion of caesium was retained in biomass (Figs. 7a and 7b). It indicates that caesium is bind into the impermeable cell structures or in compounds with low water solubility.

Caesium efflux values into water increased from <5% to 60% after pretreatment of lichen biomass with toluene (24 h at 20°C). Toluene and other non-polar organic solvents destroy cytoplasm membrane and other lipid structures of microorganisms. High efflux values after toluene pretreatment can be therefore attributed

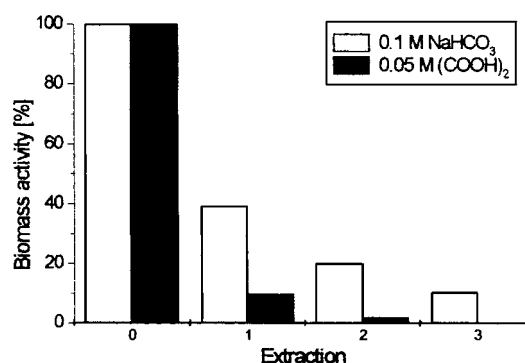


Fig. 7a. Sequential extraction of ^{137}Cs from *H. physodes*. Lichen after 24 h accumulation of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl, $50 \text{ kBq}\cdot\text{l}^{-1}$, biomass $25 \text{ g}\cdot\text{l}^{-1}$, wet wt.) at 20°C under aeration was extracted repeatedly 15 min at 50°C with 0.1 M NaHCO $_3$ or 0.05 M oxalic acid at biomass:solution ratio 1:15 (w/v). Y-axis represents percentage of radioactivity remaining in biomass after bioaccumulation (column 0) and after repeated extraction (column 1, 2, 3).

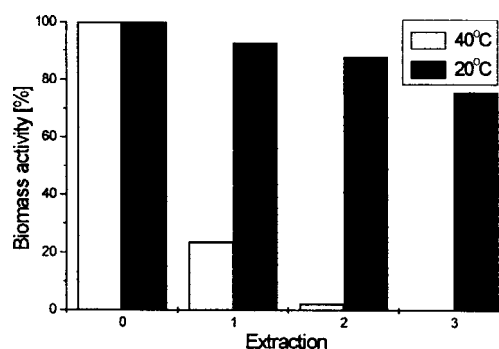


Fig. 7b. Sequential extraction of ^{137}Cs from *H. physodes*. Lichen after 24 h accumulation of ^{137}Cs ($2.5 \mu\text{mol}\cdot\text{l}^{-1}$ CsCl, $50 \text{ kBq}\cdot\text{l}^{-1}$, biomass $25 \text{ g}\cdot\text{l}^{-1}$, wet wt.) at 20°C under aeration was extracted repeatedly 15 min at 20 or 40°C with 0.05 M HCl at biomass:solution ratio 1:15 (w/v). Y-axis represents percentage of radioactivity remaining in biomass after bioaccumulation (column 0) and after repeated extraction (column 1, 2, 3).

to the removal of lipid components participating in immobilization of caesium binders or to destruction of permeability barriers preventing flux across the compartments of lichen cell structures. Relatively high proportion of caesium, nearly 40% of the total, not extractable with water even after toluene pretreatment supports the assumption, that this part of caesium is bound on cell constituents characterized by low water solubility.

Cs efflux of lichens shows quite different properties as Cs efflux of bacteria. Sakhnini and Gilboa [29] described Cs influx and efflux of halophile bacteria *Halomonas israelensis*. Cs $^+$ efflux into fresh cultivation media was quantitative in reaction times comparable with times requiring for completing Cs $^+$ influx under the same conditions. The 1st order rate constants within 2×10^{-4} to $24 \times 10^{-4} \text{ min}^{-1}$ for both influx and efflux of caesium ($25 \text{ mmol}\cdot\text{l}^{-1}$) in $0.2\text{--}4 \text{ mol}\cdot\text{l}^{-1}$ salt concentration were found. The curve of ^{133}Cs efflux obtained from ^{133}Cs NMR study was fitted to the equation: $I = I_0 \cdot \exp(-k' \cdot t) + C$, where k' is the 1st order efflux rate constants for caesium. Observed k' values in the presence of $0.2\text{--}4 \text{ mol}\cdot\text{l}^{-1}$ NaCl were within the range 0.0013 to 0.0022 min^{-1} .

Possible caesium binders in lichen biomass

Low efflux values observed in our work suggest the existence of strong caesium binder molecules in *Hypogymnia* lichens. It is generally known, that lichens produce and accumulate very broad spectrum of secondary metabolites. Many of these substances can be considered as potentially efficient caesium binders. Although lichens are well known as efficient metal binders including Cs^+ ions (see e.g. [28]), lichen secondary metabolites attracted attention mainly for their biological activity, and their caesium-binding properties has not been more extensively studied.

In the following paragraphs short review of high molecular (proteins, polysaccharides, membrane constituents) and low molecular naturally occurring and synthetic compounds potentially able to bind Cs^+ ions is given, summarized in Table 3.

Pulvinic acid and pulvinic acid derivatives produced by lichens such as calycin, leprapinic acid, leprapinic acid methyl ether, pinastric acid, pulvinic acid, pulvinamide and rhizocarpic acid can be considered as potential Cs binders in lichen biomass. This assumption can be supported by the ability of badione A and norbadione A to bind Cs ions isolated from *Xerocomus badius* described by Aumann *et al.* [4] and Garaudee *et al.* [12].

Norbadione A forms a 1:1 complex with caesium chloride. The complex undergoes decomposition upon exposure to the strongly acidic cation exchanger Dowex 50-W X4. Caesium-norbadione complexes can be isolated on Sephadex LH-20 columns. Pulvinic acids: atromentic acid and methyl atromenthate are weaker ligands for caesium compared with norbadione A.

Lin *et al.* [22] investigated the interaction of caesium ion with the anionic intracellular components of human red blood cells. $^{133}\text{Cs}^+$ bound more strongly to binding sites in 2,3-bisphosphoglycerate and in red blood cell membranes than in any other intracellular components in red blood cells at physiological concentrations. The

positively charged Cs^+ ions can interact with or bind to anionic metabolites ATP, ADP, 2,3-bisphosphoglycerate and inorganic phosphate. They obtained the Cs^+ binding constants per binding site in 2,3-bisphosphoglycerate: $66 \pm 8 \text{ M}^{-1}$, ADP $19 \pm 1 \text{ M}^{-1}$, ATP $25 \pm 3 \text{ M}^{-1}$ and red blood cell membranes $55 \pm 2 \text{ M}^{-1}$.

Chitosan can be used for the extraction of caesium and other cations from natural water systems at pH 7, Duolite Cs-100 for the extraction of caesium and strontium [17].

Polar [27] detected the association of ^{137}Cs with phenolic moiety of black tea (*Camellia sinensis*) phenylglucosides harvested after Chernobyl accident and in artificially ^{137}Cs labelled aqueous extracts. Radiocaesium ^{137}Cs in fermented tea infusate was reported to be chiefly in a cationic form [32].

Numerous inorganic compounds can exchange alkali metal cations from water solutions, such as insoluble hexacyanoferrate(II) and (III), hydrous oxides, Na-substituted fluormicas, glassy chromium polyphosphate, crystalline gamma-zirconium phosphate salts of transition metals. Anion exchanger, ammonium dodecamolybdophosphate $[(\text{NH}_4)_3\text{Mo}_{12}\text{O}_{40}\cdot\text{H}_2\text{O}]$ contains exchangeable NH_4^+ ions which can be exchanged for Cs^+ and other alkali metal cations such as Rb^+ and K^+ . However, due to its very high affinity, caesium ions can be sorbed quantitatively from the solutions containing large concentrations of potassium and rubidium due to high selectivity for caesium. The distribution coefficient (K_d) values of alkali metals on molybdophosphate decrease in order: $\text{Cs}^+ = 6000$, $\text{Rb}^+ = 230$, $\text{K}^+ = 3.4$, $\text{Na}^+ < 1$ [13].

Ionophores are classified as either mobile carriers (e.g. valinomycin) which diffuse forward and backward across the membrane, or as channel formers (e.g. gramicidin), that form a tiny pores through the membrane. Both of these types operate by shielding the charge of the transported ion so that it can penetrate the hydrophobic interior of the lipid bilayers. The ionophore-ion

Table 3. Possible chemical caesium traps in environment

No.	Group	Compound	Reference
1	Proteins	Ribokinase, glutamin synthetase	[2, 21]
2	Polysaccharides	Chitosan	[17]
3	Membrane phospholipides		[22]
4	Phenylglycosides	Phenols	[27]
5	Metalloantibiotics, ionophore antibiotics	Alamethicin, monensin, nigericin, salinomycin, lasalocid, valinomycin	[23]
6	Intermediates	ATP, ADP, BPG	[22]
7	Inorganic cation exchangers	Chromium polyphosphates, hexacyanoferrates, hydrous oxides, molybdophosphates, Na-substituted fluoromicas	[13]
8	Synthetic organic compounds	Dicarbollides, crown ethers and calixarenes	[10]
9	Secondary metabolites		
	– fungi	Pulvinic acid deriv.: badione A, norbadione A	[4]
	– lichen	Pulvinic acid deriv.: calycin, leprapinic acid, leprapinic acid methyl ether, pinastric acid, pulvinic acid, pulvinamide, rhizocarpic acid	–

complex, in some cases (e.g. valinomycin – K^+), has a net electrical charge, whereas the unoccupied carrier is neutral. In other cases, only electrically neutral complexes are formed between the ionophore and the ion (e.g. nigericin). For more details see e.g. Ming [23] and 772 references cited. Alamethicin is channel-forming peptide antibiotics for monovalent cations with ion specificity: $H^+ > Cs^+ = Rb^+ = Na^+ = Li^+$. Monensin is polyether antibiotics acting as a Na^+ ionophore by forming stable complexes with monovalent cations enabling them to cross the plasma membranes and increases their solubility in organic solvents with the following ion-specificity: $Na^+ > K^+ > Rb^+ > Cs^+ > Li^+ > NH_4^+$. Salinomycin is polyether K^+ sensitive ionophore antibiotic with a unique tricyclic spiroketal ring system. Valinomycin is cyclododecadesepsipeptide ionophore antibiotics with ion specificity: $Rb^+ > K^+ > Cs^+ > Ag^+ > NH_4^+ > Na^+ > Li^+$. Hexadecaisoleucinomycin (HEXIL) is structural analogue of valinomycin. Forms complexes with with Cs^+ ions. Nigericin is polyether ionophore antibiotic with ion specificity: $K^+ > Rb^+ \geq Cs^+ \gg Na^+$. Nigericin forms highly stable Li^+ and Cs^+ complexes [1]. Lasalocid (antibiotic X-537A) forms in chloroform 2:2 complexes with K^+ , Rb^+ and Cs^+ and 1:1 complexes with Li^+ [26].

Synthetic caesium binders

Dicarbollides, crown ethers and functionalised calixarenes were used for extraction of Cs^+ and Sr^{2+} from highly active radioactive wastes. New calixarene derivatives blocked in 1,3-alternate conformation were used for selective extraction of caesium even from matrixes with high salt contents [10]. Quadruple-bridged calix [6] arenes were used for construction of Cs^+ specific selective electrodes with lowest detection limit $\log a_{Cs^+} = -6.3$ and with selectivity of potentiometric response in the order $Cs^+ > Rb^+ > K^+ = NH_4^+ \gg Na^+ = Li^+$ [9].

Proteins

Radiocaesium is accumulated in various body tissues, including brain and muscle tissues. On the basis of X-ray crystallography Liaw *et al.* [21] confirmed that caesium was incorporated in glutamine synthetase in the place of the binding site for monovalent cations. *Escherichia coli* ribokinase is activated by potassium with an apparent $K_d = 5$ mM. The enzyme can be fully activated under physiological conditions [2].

Conclusion

Many published papers accentuate the sensitivity of lichens as bioindicators of environmental pollution, including pollution with toxic metals. Our paper more accentuates high influx values of caesium to native lichen biomass, with pH optimum within the range pH 4.0–5.0, dependent on temperature with optimum at 20°C, inhibited thermally at 60°C and chemically by pretreatment with 0.2% formaldehyde as metabolic inhibitor. Caesium accumulated by lichen is charac-

terized by low efflux values into water and neutral salt solutions under physiological conditions i.e. neutral pH values and 20°C.

More detailed studies are needed in order to characterize topographic distribution of caesium in subcellular organization of the thallus as well as for specification of potential caesium binders responsible for rather high irreversibility of Cs^+ ions bound in lichen biomass under physiological conditions.

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