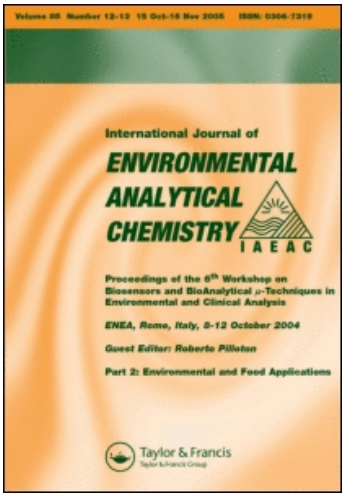

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Specificity of Lichen Species in Respect to ^{137}Cs Binding

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SPECIFICITY OF LICHEN SPECIES IN RESPECT TO ^{137}Cs BINDING

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The aim of this work was to investigate the significance of species specificity on the efficiency of ^{137}Cs isolation from lichens. It was shown that a 5% solution of both ammonium oxalate and phosphoric acid was able to solubilize 77.5% of ^{137}Cs from *Cetraria islandica*, 47.6% from *Cladonia fimbriata* and 46.4% from *Usnea barbata*. Since the tested lichen species had similar specific radioactivities (i.e. amount of ^{137}Cs) the difference could be explained by the existence of different types of bonds between radiocesium and the corresponding binding sites. Crystals precipitated from these extracts incorporated most of the soluble ^{137}Cs. The amount and specific radioactivity of the crystals varied between lichen species which could also be interpreted as the presence of specific ions in each lichen that either participated in crystal formation or inhibited the process. The potential of a tested solution to extract and "concentrate" ^{137}Cs in crystalline form may be a tool to correlate mass and radioactivity of ^{137}Cs.

Keywords: Lichens; ^{137}Cs; isolation

INTRODUCTION

Nuclear weapon testing and accidents involving nuclear reactors release radioactive elements into the environment. Radionuclides are sources of ionizing radiation energy that can initiate harmful reactions in tissues. ^{137}Cs is a significant component of nuclear spread-out whose effects are governed by its chemical homology with potassium and its long half-life (30.2 years). The absolute mass of radiocesium representing a rather high radiation effect is vanishingly small compared with the inactive mass of the element. The concentration of stable cesium in natural materials can be determined by atomic absorption spectrometry[1] or by cyclic voltametry,[2] while radiocesium is still quantified in radioactivity units.[3,4] Biomonitoring is an experimental method to measure the

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response of organisms to environmental pollution. Lichens have been chosen as suitable biomonitors because they accumulate large amounts of various pollutants, including radionuclides. They absorb mineral elements from atmospheric deposits, rain water and substrata. The accumulation of metals is due primarily to the slow growth rate and longevity of lichens, but specific lichen substances may contribute as donors of binding sites for metal ions. Also, lichens do not have any impermeable cuticular material that could limit free cation uptake.[5] Lichens are, thus, considered to be substantial reservoirs of ^{137}Cs for a long period of time.

Mechanisms of element uptake in lichens are particulate entrapment, ion exchange, passive diffusion and active intracellular transport. Rates of uptake and release processes depend on the chemical composition, size and characteristics (e.g. solubility) of the particles associated with the elements, the composition of substrata,[6,7] as well as on the elements present in lichens and their biochemical structure. The nature of cesium interactions with lichen biomolecules is still unknown. In previous work, we have shown that most of the radiocesium could be extracted (91.5% of the initial amount) without complete destruction of the lichen itself (69.2% of the initial weight remained).[8] Different lichen species of the same type (in respect to the support they live on) collected at the same time and location had significantly different specific radioactivities.[9] This indicates that radiocesium accumulation and, maybe, release is most probably dependent on the specific biochemical structure of each lichen. In order to validate this assumption it was decided to investigate isolation of ^{137}Cs from three different lichen species which had similar specific radioactivities, i.e. amount of ^{137}Cs. To develop the most efficient extraction system several chemicals were tested, the choice being made according to previous results.[10]

EXPERIMENTAL

Samples

The following lichens were investigated: *Cetraria islandica* (specific activity was 1.15 Bq/g, grows as a shrub), *Cladonia fimbriata* (1.74 Bq/g, spreads on the soil) and *Usnea barbata* (1.14 Bq/g, grows on trees). Lichens were air-dried to constant weight.

^{137}Cs extraction

In the first experiment samples of the lichen, *C. islandica* (10.0 g) were treated with 1%, 2% and 5% solutions (3 × 200 mL) of ammonium chloride, ammonium

oxalate, hydrochloric acid, sulfuric acid and phosphoric acid. After each extraction step (20°C, 24 h) lichen samples were dried and their masses and specific radioactivities were measured. In the second experiment different lichen species (10.0 g) were treated with solutions (3 × 200 mL) that were 1%, 2% and 5% concentrated in respect to both ammonium oxalate and phosphoric acid. In the third experiment lichens (10.0 g) were extracted with solutions (3 × 200 mL) that contained both 5% ammonium oxalate and 5% phosphoric acid, employing mixing on a magnetic stirrer. All aqueous extracts from the third experiment were left at 4°C for three days and crystalline products were obtained. Masses and specific radioactivities of lichen samples and crystals were determined. Radioactivity was measured on a gamma spectrometer (CANBERRA-ORTEC, 8192 channels). The resolution was 6.8%; the energy used was 661.6 keV and the efficiency was 8.7% for ^{137}Cs.

RESULTS

The results of the first experiment demonstrated that increasing the concentration of a certain chemical in solution may or may not improve the efficiency of ^{137}Cs isolation. Thus, increasing concentrations of ammonium chloride practically had no effect (see Table I). Hydrochloric and sulfuric acids exhibited rather low effects as expressed by specific radioactivity, since there was a significant loss of lichen mass due to the hydrolytic activity of these strong acids (as much as 50% with 5% solutions). Ammonium oxalate and phosphoric acid extracted more radiocesium as their concentrations in solution increased and 5% solutions exhibited similar potentials. The wetting behavior of the three lichens was similar and the volume of the extracts obtained varied by up to 10%, but no regularity was noticed concerning lichen species.

TABLE I The efficiency of ^{137}Cs extraction from the lichen *Cetraria islandica* using different solutions

Solution (concentration)	*% Extracted ^{137}Cs*		
	1%	*2%*	*5%*
Ammon. chloride	48.9	49.0	53.1
Ammon. oxalate	47.0	60.2	75.5
Hydrochloric acid	20.4	26.8	37.0
Sulfuric acid	23.5	32.1	39.1
Phosphoric acid	49.5	50.3	74.0

In the second experiment the combination of ammonium oxalate and phosphoric acid in the same solution was tested on different lichen species. The mixture that contained 5% of both chemicals was a better extraction medium than solutions of lower concentrations (see Table II), but the investigated species behaved quite differently. With the (5 + 5)% solution, 75.7% of ^{137}Cs was isolated from *C. islandica*, 34.7% from *C. fimbriata* and 44.6% from *U. barbata*. The overall effect of (5 + 5)% solution on *C. islandica* was not better than that achieved by 5% ammonium oxalate or 5% phosphoric acid solution alone. The specificity of (5 + 5)% extracts was precipitation of crystals upon storage, even at room temperature.

TABLE II The effect of the concentration of combined ammonium oxalate/phosphoric acid solutions (1%, 2% and 5%) on ^{137}Cs extraction from the lichens: *Cetraria islandica, Cladonia fimbriata and Usnea barbata*

Lichen species (sol. concentration)	*%*	*Extracted*	*^{137}Cs*
	1%	*2%*	*5%*
C. islandica	50.2	62.0	75.7
C. fimbriata	9.7	27.6	34.7
U. barbata	25.0	30.2	44.6

TABLE III The effect of stirring on ^{137}Cs extraction with solutions containing 5% ammonium oxalate and 5% phosphoric acid (three steps)

Lichen species (extraction step)	*%*	*Extracted*	*^{137}Cs*
	I	*II*	*III*
C. islandica	50.1	71.6	77.5
C. fimbriata	28.5	46.8	47.6
U. barbata	33.3	45.0	46.4

TABLE IV Crystalline products that precipitated from 5% ammonium oxalate/5% phosphoric acid extracts (three steps)

Lichen species (extraction step)	*Crystals (g), (Bq/g)*					
	I		*II*		*III*	
C. islandica	2.6	2.1	3.0	0.8	3.6	0.2
C. fimbriata	1.7	2.3	2.8	1.1 1	3.4	<0.1
U. barbata	2.5	1.5	2.9	0.4	3.2	<0.1

The effect of including stirring during isolation on the efficiency of radiocesium extraction with (5 + 5)% solution was analysed. Each extraction step was characterized separately. It can be seen in Table III, that total extraction was improved slightly by mixing, the improvement being more significant for *C. fimbriata* than for the other two lichens. Moreover, two extraction steps were almost sufficient to solubilize all the ^{137}Cs that could be isolated from the lichens in the described manner. The amounts and specific radioactivities of the crystalline products that were obtained are presented in Table IV. In the first extraction step the yield of crystals was the least but their specific radioactivity was the greatest. However, the total quantity of crystals and their specific radioactivity differed between the lichen species. Thus, crystals obtained from the first extracts of *C. islandica* and *C. fimbriata* had similar radioactivities, i.e. 2.1 and 2.3 Bq/g respectively, while the radioactivity of *U. barbata* crystals was much lower, i.e. 1.5 Bq/g. On the other hand, nearly the same amount of crystals was obtained in the first step from *C. islandica* and *U. barbata*, i.e. 2.6 and 2.5 g respectively, while the yield from *C. fimbriata* was smaller, i.e. 1.7 g.

DISCUSSION

The experimental results obtained demonstrated that the analysed lichen species were specific in respect to chemical binding of ^{137}Cs. The samples examined were chosen to have similar quantities of ^{137}Cs, so that the efficiency of radiocesium isolation could be directly compared. The difference in behaviour of radiocesium upon extraction should therefore be a consequence of the type of chemical interaction between radiocesium and specific binding sites in each lichen. Lichen, as an integrated association of fungal and algal components, may behave differently from each organism when they are separated. As the proportion of the two components may differ within the same species taken from dissimilar habitats, under different environmental conditions or with age, it is difficult and sometimes inaccurate to make general conclusions. Lange and Ziegler,[11] for example, noted that upper toleration limits for iron and copper in fungi and algae were far below the amounts found in lichens they studied. Investigations of cation metabolism, therefore, must take into account the nature of the cation involved, biochemical structures and the physiological state of the lichen at the time. Of the greatest importance are radii of the hydrated ions (Cs^+ is small in spite of hydration)[12] and the three-dimensional structure of the binding sites.

A dilemma whether passive or active ion uptake and accumulation predominates still remains. Sloof found that transplants of the lichen *Parmelia sulcata*

Taylor and impregnated cloth exhibited similar responses to the bulk deposition of cobalt, scandium and zinc and concluded that mainly passive processes are involved in uptake and/or release mechanisms.[13] The similarity in behaviour of lichen and cloth was explained by the existence of the same functional groups at the surface, i.e. free carboxylic groups from pectic compounds. If uronic acids are present in lichens they are most likely to be associated with the algal component.[12] As algae usually comprise less than 10% of the total thallus weight, the contribution of uronic acids to ion uptake can be expected to be small. Fungal cell walls have no such cation-binding agents, as deduced from their chemical composition. In the living lichen, therefore, cesium ions are probably incorporated, at least to a certain extent, by an active process and transfered into the cell cytoplasm by the carrier normally used by the chemical homologue, potassium.[12] Relative affinities of a particular ion for specific carriers in the cell membrane system determine the proportion entering the cell and, as shown in this work, also the proportion leaving the cell upon extraction.

Lichens are sources of many specific substances, some of which are found in many lichens while others are confined to only few species.[14,15] Biochemical constituents differ in the species studied in this work.[16–19] Molecules that donate hydroxyl, carboxylic and aldehyde groups (particularly depsides and depsidones) are able to form complexes with cations and may play an important role in trace element uptake and accumulation. Ions may be bound to different sites within the lichen, which would explain their gradual release upon isolation. Tuominen and Jaakkola showed that there are acidic groups in lichen which have different pK values.[12] A group at pK 3.3 resembles pectic carboxyls, but identification of these sites still remains uncertain. Organo-phosphorus compounds, side-chain carboxylic groups of proteins as well as nitrogen donors might be responsible for binding sites with pK values 5.0 – 7.0.[20] According to Sterling, binding sites consist of a pair of acidic groups.[21] In that case metal complex formation and possibly chelation could occur. The results of the described experiment clearly showed that extraction potentials of the applied solutions were different for each species studied, which could be explained only by the existence of different bonds between ^{137}Cs and the corresponding binding sites. Efficiency of ^{137}Cs extraction could be further improved by grinding[10] and heating, although heating may cause evaporation of the isolated ^{137}Cs compounds.[22] Passive diffusion is definitely not the predominant route of radiocesium transportation in *C. fimbriata* and *U. barbata*.

Solubilized ^{137}Cs was incorporated in crystalline structures that were formed from ammonium, oxalate and phosphate ions, probably together with some other ions isolated from lichens. No regularity in respect to the amount and specific radioactivity of these crystals was observed which could again be explained by

the presence of specific ions in each lichen that either participated in crystal formation or inhibited the process. ^{137}Cs is most probably incorporated as Cs^+ instead of NH_4^+. The crystals that were formed could not be identified by crystallographic analysis using published data,[23] but ammonium phosphates (including known hydrates) were excluded. Ammonium dodecamolybdo-phosphate (AMP) is the most frequently used inorganic exchanger for determination of cesium in water[24] and it is not impossible that some kind of heteropolyacid was formed in this experiment as well. Both AMP and crystals formed from ammonium oxalate and phosphoric acid have ammonium and phosphate ions in common. The potentials of AMP are extraordinary; the separation factor for the Cs^+/Na^+ ion pair is more than 6000, while that for the Cs^+/K^+ ion pair is approximately 1800.

Finally, this *in situ* incorporation of extracted ^{137}Cs into a crystalline lattice may be a method for quantitative isolation and "concentration" of radiocesium from lichens and, perhaps, a route to correlate mass and radioactivity of ^{137}Cs.

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