

EXTRACTION OF ^{137}Cs FROM MOSS USING SOLUTIONS SIMILAR TO ACID RAIN

by

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Extraction of ^{137}Cs from: *Hypnum cupressiforme* and *Isoetes myurum* mosses was investigated using the following solutions: H_2SO_4 , HNO_3 , and a mixture of H_2SO_4 and HNO_3 at three pH values (4.61, 5.15, and 5.75) and distilled water (pH = 6.50). After five successive desorption processes lasting 24 hours the following amounts of ^{137}Cs [%] were extracted from mosses: using acid solutions 19.1-34.7 and 18.4-30.5, and using distilled water 29.1 and 26.9. Sorption of ^{137}Cs was performed at no less than two sorption points in moss tissue. In the investigated pH region no significant influence of H_2SO_4 or HNO_3 on ^{137}Cs extraction was noted in relation to water extraction, *i. e.* extraction with acid rain with pH > 4.6.

Key words: H_2SO_4 , HNO_3 , H_2SO_4 - HNO_3 mixture, distilled water, ^{137}Cs extraction, moss

INTRODUCTION

Mosses are old, primitive, non-vascular plant species, with a specific structure (they have no formed root, leaves, trunk or stoma), but also a specific ecology and physiology in comparison with other species [1, 2]. In the present research *Hypnum cupressiforme* and *Isoetes myurum* mosses were taken as the model system. *Hypnum cupressiforme* is a common and widespread species of moss. It grows in a wide variety of habitats and climatic zones and is found on all continents. It grows on tree trunks, logs, walls, rocks and other surfaces, but it prefers acidic environments and is tolerant to pollution. It was formerly used as a filling for pillows and mattresses. *Isoetes myurum*, with its modern name *Isoetes alopecuroides*, appears in woodlands, on stream banks and in sheltered places, most commonly on lower parts of tree trunks and on roots, rocks and stones, especially that are base-rich. It does not grow on acidic substrates.

Acid rain is formed of SO_2 and also of NO_2 formed from H_2SO_4 and HNO_3 after oxidation during atmospheric fallout. Acids formed in the atmosphere fall on the earth's surface with fallout or by free fall due to the effect of gravitation force. The term acid rain refers to any acid precipitation, regardless whether it is wet or dry precipitation [3-7].

If moss sorbs a certain substance (for example ^{137}Cs) it becomes polluted with it [8-26]. When moss is

then submerged in water or is periodically soaked with solutions not containing the sorbed substance, this substance will be extracted. The amount of the extracted substance will depend on the type of extraction solutions that influence spreading of pollution by this substance into the environment. In our previous work the extraction of ^{137}Cs and metals was monitored in *Cetraria islandica* lichen by using an acid solution with pH values ranging from 2.00 to 5.75, and also the extraction of ^{137}Cs from *Homalothecium sericeum* (Hedw.) Schimp. moss and *Brachythecium mildeanum* moss [27-36].

A system for monitoring the extraction of ^{137}Cs with acid solutions similar to acid rain was set up. In this study, the extraction of this isotope from two common moss species was investigated with the aim to confirm the determined possibility of using moss as pollution bioindicators and also as sources of ^{137}Cs pollution.

MATERIALS AND METHODS

In this work the following chemicals were used: H_2SO_4 p.a. (*pro analysi*), Merck, HNO_3 p.a. Alkaloid, distilled water having pH values 6.50, buffer solutions with pH 4.00 and 7.00, Carlo Erba and standard Merck filter paper.

Mass was measured on METTLER analytical scales (sensitivity 0.1 mg).

Extraction solutions were made by adding concentrated H_2SO_4 (I) or HNO_3 (II) into 200.0 mL of dis-

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tilled water until pH values of 4.61, 5.15, and 5.75 were reached (solutions H_2SO_4 : I₁, I₂, I₃, and HNO_3 : II₁, II₂, and II₃, respectively). Solution (III) was made by mixing 100.0 mL of solution (I) and 100.0 mL of solution (II), with identical pH values (solutions III₁, III₂, and III₃).

Samples of Hypnum cupressiforme (A) and *Isoetes myurum* (B) moss species were collected from the Djerdap National Park (Serbia) in 2006 and kept in paper bags. Moss samples were dried spontaneously in air, cleaned from visually noticeable impurities and then mechanically ground. Extraction samples weighing 10.00 grams were put in plastic vessels (7.50 cm in diameter and 150.0 cm³ in volume). Initial radioactivity levels of sorbed ^{137}Cs were measured in these samples.

After measuring the initial activity, samples were soaked in 200.0 mL of distilled water and a solution with composition similar to acid rain, (I), (II), and (III), or (D), and then left to rest for 24 hours. Five successive extractions were performed. The extractions were performed at room temperature (22 °C) with periodical mixing. After the first extraction a dark yellow solution was obtained. The second extraction produced a lighter solution, and the last obtained solution was almost colorless. After each extraction, moss samples were dried at room temperature in an open vessel until a constant mass was reached. Sample activities were measured, as well as the activities of the obtained extracted solutions. All series were repeated twice.

The activities of ^{137}Cs in the samples were determined by an ORTEC-AMETEK gamma spectrometer having an HPGe detector, 8192 channels, resolution of 1.65 keV and efficiency 34 % at 1.33 MeV ^{60}Co with a measurement error below 5.0 %. Gamma Vision-32 software was used for spectral analysis [37]. Energy calibration and efficiency calibration of the gamma spectrometer were performed prior to measurements by using a calibration source containing a mixture of radionuclides with known activity. The detector was calibrated with three different radioactive reference materials in the Marinelly geometry: Silicone resin (Czech Metrological Institute Praha (CMI Praha), Cert. No. 1035-SE-40517-17, Type MBSS 2 (^{241}Am , ^{109}Cd , ^{139}Ce , ^{57}Co , ^{60}Co , ^{137}Cs , ^{113}Sn , ^{85}Sr , ^{88}Y , ^{51}Cr), 490.0 g, density: 0.98 0.01 gcm³, vol. 500.0 5.0 cm³ ref. date 1.9.2017); silicone resin CMI Praha, Cert. No. 1035-SE-40661/14, Type MBSS 2 (^{241}Am , ^{109}Cd , ^{139}Ce , ^{57}Co , ^{60}Co , ^{137}Cs , ^{113}Sn , ^{85}Sr , ^{88}Y , ^{51}Cr), 490.0 g, density: 0.98 0.01 gcm³, vol. 500.0 5.0 cm³, ref. date 1.10.2014; and Vegetation (Inst. Radiological Protection, Belgrade: QAP 9709, 23.12.2002).

The activity of ^{137}Cs was measured in the samples before and after each extraction under the same geometrical conditions lasting one hour. The ^{137}Cs

content in each sample was expressed as the percentage of remaining cesium in the sample after each extraction in relation to its content in the initial sample.

Mass reduction of samples after each extraction was lower than the measurement error. The measurement error is the standard deviation of all individual measurements when solutions with the same pH value are used regardless of the solution type.

With successive extractions with equal volumes of desorbance solution the amount of the remaining sorbed substance, C_x , changes with the number of extractions, n_x , according to the following equation [35]

$$\ln C_x = \ln C_0 - a n_x \quad (1)$$

where is the C_0 sorbed substance content in the sample before extraction, a – the a constant, n_x – the number of successive extractions with equal volumes of extraction solution, and C_x – the amount of sorbed substance for a certain value of n_x .

RESULTS AND DISCUSSION

The content of ^{137}Cs in each sample is expressed as the percentage of the activity of the remaining ^{137}Cs in the sample after each of the five successive extractions in relation to the initial sample activity.

Table 1 shows the activity of ^{137}Cs [Bqkg⁻¹] in *Hypnum cupressiforme* (A) and *Isoetes myurum* (B) mosses before extraction and the percentage of the remaining ^{137}Cs in mosses after each of the five successive extractions using solutions (I), (II), and (III). Each value in the table is the average value of two measurement series.

The activities of ^{137}Cs in moss were as follows: 2682 and 2757 (A) and 2002 and 2040 (B) Bqkg⁻¹, that is in the interval of the measurement error, indicating the homogeneity of moss samples after preparation for measurements.

After five extractions solutions (I), (II), and (III) extracted between 19.1 % (II, pH 5.75) and 34.7 % (I, pH 4.61) of ^{137}Cs from moss (A), and 18.4 % (II, pH 5.75), and 30.5 % (I, pH 4.61) from moss (B). The first extraction, in accordance with the previous researches [34, 35], removed the highest percentage of ^{137}Cs in relation to the initial activity in the moss (12.0 %-16.9 %).

From tab. 1 it is obvious that changes in the pH value of the solution affect the extraction of radiocesium from mosses, *i. e.*, the significant role of H^+ ion in the exchange of Cs^+ ion. The obtained results indicate that in all cases with increased pH values of the extraction solution the amount of extracted ^{137}Cs is reduced. This indicates that hydrogen ions have an influence on ^{137}Cs extraction from moss.

The results obtained for total extraction of ^{137}Cs for all pH values show that the highest extraction occurred when solution (I) was used, followed by solution (III), and extraction was the lowest when solution

Table 1. Activity (average values of two measured samples) of ^{137}Cs in mosses *H. cupressiforme* (A) and *I. myurum* (B) before extraction, percentage of remaining ^{137}Cs in mosses after each extraction and total extracted ^{137}Cs from moss [%], using solution I, II, and III

Solution	H_2SO_4 (I)					
pH	4.61		5.15		5.75	
Mosses	A	B	A	B	A	B
	Activity levels of ^{137}Cs in mosses before extraction [Bqkg^{-1}]					
Extraction	2757	2028	2697	2002	2737	2040
	Percentage of remaining ^{137}Cs in mosses after extraction [%]					
I	84.4	86.9	83.1	84.0	86.9	87.2
II	73.8	77.3	77.8	78.3	81.9	82.4
III	68.2	74.0	75.7	76.7	79.8	79.8
IV	65.9	71.2	73.5	75.2	78.1	79.2
V	65.3	69.5	73.1	74.8	77.5	79.0
	Total extracted ^{137}Cs from mosses [%]					
	34.7	30.5	26.9	25.2	22.5	21.0
Solution	HNO_3 (II)					
pH	4.61		5.15		5.75	
Mosses	A	B	A	B	A	B
	Activity levels of ^{137}Cs in mosses before extraction [Bqkg^{-1}]					
	2749	2016	2703	2011	2741	2034
	Percentage of remaining ^{137}Cs in mosses after each extraction [%]					
I	85.5	86.4	89.9	88.0	87.9	88.1
II	80.1	80.8	83.7	83.3	84.9	84.4
III	77.4	78.2	81.5	82.2	83.8	82.8
IV	76.3	77.9	79.2	81.1	82.1	81.9
V	76.0	77.3	78.7	80.8	80.9	81.6
	Total desorbed ^{137}Cs from mosses [%]					
	24.0	22.7	21.3	19.2	19.1	18.4
Solution	$\text{H}_2\text{SO}_4\text{-HNO}_3$ (III)					
pH	4.61		5.15		5.75	
Mosses	A	B	A	B	A	B
	Activity levels of ^{137}Cs in mosses before extraction [Bqkg^{-1}]					
Extraction	2737	2021	2682	2017	2720	2025
	Percentage of remaining ^{137}Cs in mosses after each extraction [%]					
I	85.4	87.3	84.1	85.3	87.6	87.4
II	81.0	78.5	79.9	78.0	82.3	83.2
III	78.3	74.8	77.7	76.5	79.7	80.1
IV	76.1	73.1	76.9	75.1	78.5	79.2
V	75.0	72.3	76.8	74.4	78.0	78.7
	Total extracted ^{137}Cs from mosses [%]					
	25.0	27.7	23.2	25.6	22.0	22.3

(II) was used. This means that anion type affects extraction, regardless of the fact that the used acids were strong. In this case the sulfate anion has a stronger effect. Since cesium is an alkali metal, the effect of the acid anion type on extraction is the consequence of acid anions on corresponding components of the organic molecule in moss tissue to which the sorbed cesium was bonded.

In our previous analyses, the same solutions, after five successive extractions, extracted from 22.8 % (III, pH 5.75) to 33.2 % (III, pH 4.61) of ^{137}Cs from *H. sericeum* moss and from 20.5 % (III, pH 5.75) to 37.6 % (I, pH 4.61) of ^{137}Cs from *B. mildeanum* moss [34, 35]. The comparison of the current results with those obtained for *H. sericeum* moss and for *B. mildeanum*

moss, leads to the conclusion that moss type has no effect on ^{137}Cs extraction type.

The ^{137}Cs desorption mechanism using acid solutions can also be applied to other alkali metals.

Table 2 contains data collected when distilled water (D) was used as the extraction agent. Distilled water (D) extracted 29.1 % ^{137}Cs from moss (A) and 26.9 % from moss (B). The first extraction, in accordance with the previous researches, removed the highest percent of ^{137}Cs in relation to the initial activity in the moss, 16.9 % (A) and 11.7 % (B). The comparison of the current results with those obtained for *H. sericeum* moss (31.3 %) and for *B. mildeanum* moss (30.7 %), leads to the conclusion that moss type does not affect ^{137}Cs extraction type [34, 35].

Table 2. Activity (average values of two measured samples) of ^{137}Cs in mosses *H. cupressiforme* (A) and *I. myurum* (B) before extraction (Bqkg^{-1}) with distilled water (pH 6.50), percentage of remaining ^{137}Cs in mosses after each extraction and total extracted ^{137}Cs from moss [%]

Mosses	A	B
	Activity levels of ^{137}Cs in mosses before extraction [Bqkg^{-1}]	
	2650	2061
Extraction	Percentage of remaining ^{137}Cs in mosses after extraction [%]	
I	83.2	87.3
II	76.7	79.3
III	73.3	75.9
IV	72.0	73.9
V	70.9	73.1
	Total extracted ^{137}Cs from mosses [%]	
	29.1	26.9

The obtained results indicate that hydrogen ions have an effect on ^{137}Cs extraction from moss.

The extraction of ^{137}Cs with distilled water, tab. 2, showed that water is also an extraction medium with significant efficiency, as the percentage of totally desorbed ^{137}Cs in all cases was higher than in the case of acid solutions, except in a single case when H_2SO_4 solution with $\text{pH} = 4.61$ was used. This implies that ^{137}Cs extraction is a complex process, influenced by water as a solvent, as well as by a series of other components present in the extraction solution, *i.e.* in acid rain that leads to extraction in natural conditions. The obtained results are in accordance with the ones obtained for the *H. sericeum* moss and *B. mildeanum* moss [34, 35].

If the results of our previous research on the extraction of ^{137}Cs from *Cetraria islandica* lichen using solutions with the same pH values and compositions are compared with the results obtained in this experiment, it can be concluded that two times more ^{137}Cs is extracted from lichen compared to mosses and this is probably the consequence of different structures of these species, the selectivity of semipermeable membranes, sorption of ^{137}Cs in the thallus, plant age and location. As *Cetraria islandica* lichen was collected in 1994, it was also probably more contaminated with ^{137}Cs .

Figure 1 shows the change in ^{137}Cs content in (A) and (B) moss species depending on the number of successive extractions, n_x . Exponential curves were obtained for all extraction solutions, (I), (II), and (III), and their form does not depend on pH value and solution type, fig. 1. Analogous curves were obtained when distilled water was used for extractions. The obtained results were analyzed by applying the same procedure as in our previous papers [34, 35].

The dependence of the logarithm of the amount of sorbed substance in the solid sorbent phase on the number of successive extractions, n_x (eq. 1) is in all cases linear, fig. 2, showing that only one sorption type

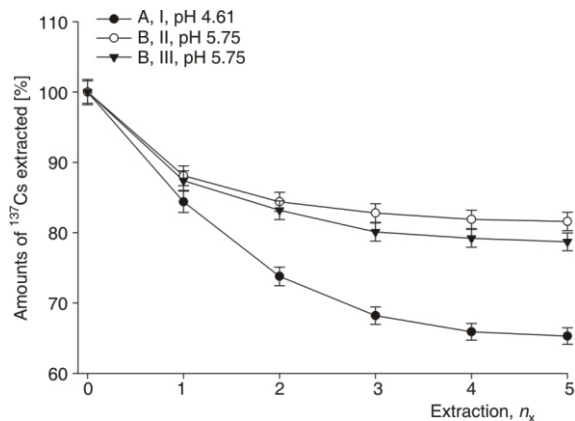


Figure 1. Content [%] of ^{137}Cs in moss A – *Hypnum cupressiforme* and B – *Isoetecium myurum* in relation to the initial content (100%), depending on the number of successive extractions, n_x , with solutions (I), (II), and (III)

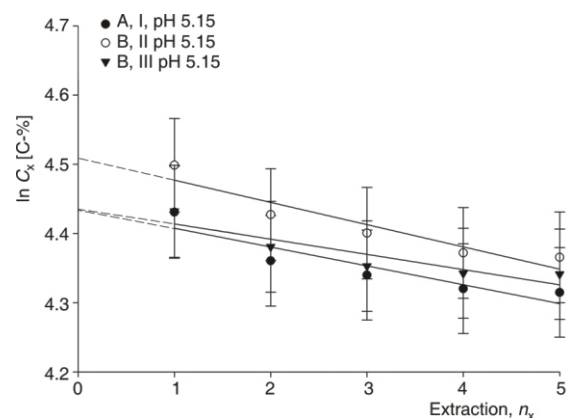


Figure 2. Logarithm of the content [%] of ^{137}Cs in moss (A) *Hypnum cupressiforme* and (B) *Isoetecium myurum*, $\ln C_x$, depending on the number of successive extractions [n_x], using solutions: (I), (II), and (III), initial content $C_0 = 100\%$

is active, or one of more sorption types prevail (the rest are negligible). The results of this research are in accordance with our previous researches [34, 35].

The extrapolation of the obtained linear dependences results in values for $\ln C_{0e}$ (C_{0e} – the value of C_0 obtained by extrapolation of the straight line based on experimental data) presented in tab. 3. The values of C_0 ($C_0 = 100 - C_{0e}$) ranging from 5.6 % to 22.5 % indicate that at least two types of ^{137}Cs sorption are present in mosses, that significantly differ in bonding strength. The weakly bonded part of ^{137}Cs is extracted using the described solutions. The remaining part of ^{137}Cs is bonded much more strongly, and corresponding capacities are very full, so the amounts of extracted ^{137}Cs from these locations are much lower and they do not significantly cause changes in equilibrium concentrations in corresponding volumes of the used extraction agents. This leads to the fact that the graph of eq. 1 does not differ from a straight line.

Table 3. Values of initial activity of ¹³⁷Cs (C_{0e}), determined by extrapolation of straight lines in percentage and the difference (C₀) in relation to the measured initial activity C₀ (100 %) before extraction with solutions* (I), (II), (III), and distilled water (D), for mosses A (*H. cupressiforme*) and B (*I. myurum*)

	pH	A		B	
		C _{0e} [%]	C ₀ = 100-C _{0e} [%]	C _{0e} [%]	C ₀ = 100-C _{0e} [%]
I	4.61	85.6	14.4	82.2	17.8
	5.15	84.4	15.6	77.5	22.5
	5.75	87.8	12.2	87.8	12.2
II		C _{0e} [%]	ΔC ₀ = 100-C _{0e} [%]	C _{0e} [%]	ΔC ₀ = 100-C _{0e} [%]
	4.61	94.6	5.6	85.6	14.4
	5.15	90.9	9.1	88.7	11.3
	5.75	89.1	10.9	89.1	10.9
III		C _{0e} [%]	ΔC ₀ = 100-C _{0e} [%]	C _{0e} [%]	ΔC ₀ = 100-C _{0e} [%]
	4.61	87.4	12.6	87.8	12.2
	5.15	84.8	15.2	85.6	14.6
	5.75	86.5	13.5	88.2	11.8
D		C _{0e} [%]	ΔC ₀ = 100-C _{0e} [%]	C _{0e} [%]	ΔC ₀ = 100-C _{0e} [%]
	6.50	83.9	16.1	88.2	11.8

* Solution compositions are given in the Experimental section

CONCLUSIONS

The comparison of the obtained results with the ones obtained for *H. sericeum* moss and *B. mildeanum* moss points out to equal behavior of mosses during extraction with acid solutions. This means that acid rain will desorb ¹³⁷Cs from mosses and transfer it into the environment. This also happens when rain is not acid but very clean. As cesium is an alkali metal these conclusions can be applied to other alkali metals.

The results obtained for ¹³⁷Cs extraction from lichens show that in general there are no differences in relation to extraction from mosses, so these species can be used for the same purpose – detection of ¹³⁷Cs.

Hypnum cupressiforme and *Isothecium myurum* mosses were collected in 2006, i. e. 20 years after the Chernobyl accident, while *Homalothecium sericeum* moss was collected in 2002, 16 years after the accident, showing that mosses retain sorbed ¹³⁷Cs for a long time, though transfer of ¹³⁷Cs from a polluted environment cannot be excluded.

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AUTHORS' CONTRIBUTIONS

The experiments were done by A. A. Čučulović. Data analysis, discussion of the obtained results as well as writing of the manuscript was done by A. A. Čučulović and D. S. Veselinović.

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**ЕКСТРАКЦИЈА ^{137}Cs ИЗ МАХОВИНА
РАСТВОРИМА СЛИЧНИМ КИСЕЛОЈ КИШИ**

Испитивана је екстракција ^{137}Cs из маховина *Hypnum cupressiforme* и *Isoetes macrospora* користећи растворе: H_2SO_4 , HNO_3 и смешу $\text{H}_2\text{SO}_4\text{-HNO}_3$, на три рН вредности 4.61, 5.15 и 5.75, као и дестиловану воду, (рН = 6.50). После пет сукцесивних екстракција у трајању од по 24 часа екстраховано је ^{137}Cs [%] из маховине 19.1-34.7 и 18.4-30.5 а дестилованом водом 29.1 и 26.9. Маховине после екстракција нису претпеле уочљиве структурне промене. Сорпција ^{137}Cs врши се на најмање два сорпциона места у ткиву маховине. У испитиваној области рН, нема битног утицаја H_2SO_4 и HNO_3 на екстракцију ^{137}Cs у односу на екстракцију водом, односно екстракцију киселим кишама са вредностима рН > 4.6.

Кључне речи: H_2SO_4 , HNO_3 , смеша $\text{H}_2\text{SO}_4\text{-HNO}_3$, дестилована вода
