MOSSES AS BIOMONITORS FOR RADIOACTIVITY FOLLOWING THE CHERNOBYL ACCIDENT

ANA ČUČULOVIĆ¹, R. ČUČULOVIĆ², TIJANA CVETIĆ ANTIĆ³ and D. VESELINOVIĆ⁴

INEP- Institute for the Application of Nuclear Energy, 11080 Zemun, Serbia ²The Faculty for the Applied Ecology Futura, Singidunum University, 11000 Belgrade, Serbia ³Faculty of Biology, University of Belgrade, 11000 Belgrade, Serbia ⁴Faculty of Physical Chemistry, University of Belgrade, 11001 Belgrade, Serbia

Abstract - In this work ¹³⁷Cs and ⁴⁰K radionuclide concentrations in moss collected at NP Djerdap in the period from 1996 to 2009 are presented. Values of the substrate-moss transfer factor for ¹³⁷Cs and ⁴⁰K were calculated. The effective and biological half-life of ¹³⁷Cs in *Homalothecium sericeum* moss collected in the period from 1996 to 2008 on the archeological locality of Lepenski Vir was also calculated.

Key words: NP Djerdap, mosses, 137Cs, 40K

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INTRODUCTION

Biomonitoring is the use of properties of an organism - a bioindicator - or part of it to obtain information about certain characteristics of the biosphere. The term bioindicator generally refers to all organisms that provide information on the environment or the quality of environmental changes, and biomonitors are organisms that provide quantitative information on the quality of the environment. Biomonitoring is a passive method and provides a measure of integrated exposure over a period of time (for review see Walterbeek, 2002; Szczepaniak and Biziuk, 2003).

To be suitable for application as a monitor for air particulate matter, specific requirements have to be met by a biological tissue or monitor (Market and Weckert, 1989; Chakrabortty and Paratkar, 2006). Biomonitoring species for radionuclides, trace elements and other air pollution are selected on the basis of specificity so that accumulation is consid-

ered to occur from the atmosphere only (Rühling, 1994). There are many other selection criteria, such as general occurrence, a well-defined representation of sampling site (Wolterbeek and Bode, 1995), and the accumulation ratio (Sloof, 1993). Generally, a biomonitor should concentrate the elements of interest, and it should quantitatively reflect its ambient elemental conditions, without any significant impact on the monitor behavior (Wolterbeek, 2002).

The mosses essentially meet most of the requirements for an organism to be used in biomonitoring. One-cell thick leaves, thick cell walls and the absence of cuticles provide effective adsorption of trace elements from air and deposited material by ion exchange (Brown and Brown, 1990; Wolterbeek, 2002). The large surface to weight ratio in mosses (Mishev et al., 1996, Zechmeister et al., 2006) improves trace element adsorption. The absence of roots ensures absorption mainly from air deposition, and it has been shown that mosses concentrate

particulates and dissolve chemical species from dry and wet deposition (Stainnes, 1995). The deposition and removal of radioactive elements in mosses is also related to rainfall (Taylor and Witherspoon, 1972; Krmar et al., 2009). It seems that adsorbed elements are transported into the protoplasts (Tyler, 1990; Wells and Brown, 1990), and from older to younger parts of moss (Brown and Brown, 1990). This process probably prevents further rainfall-related loss. According to Dragović et al. (2004), most of the ¹³⁷Cs seems to be distributed in membrane and cell wall fractions.

However, care should be taken regarding the species used in investigations, since it has been shown that for heavy metal deposition pleurocarpous mosses reflect short-term, and acrocarpous mosses long-term deposition (Sabovljević et al., 2005).

Together with lichens, mosses have been the most commonly applied biomonitors for atmospheric pollution (Tyler, 1990; Wolterbeek, 2002; Szczepaniak and Biziuk, 2003). Both mosses and lichens are thought to absorb trace elements passively. The advantage of mosses is that their age can be easily determined, in contrast to certain lichen species (Sawidis et al., 2009). Also, moss biomonitoring has been more popular because it causes fewer technical and analytical problems than lichens (Szczepaniak and Biziuk, 2003).

Pollution with anthropogenic radionuclides is mainly regional in character, but it can be wider in the case of strong nuclear explosions. Radiocesium (137Cs) is one of the most important artificial radionuclides produced by nuclear fission. It has been introduced into the terrestrial environmental by nuclear weapons testing, authorized discharge of nuclear waste and accidental release from nuclear facilities. Isotopes of potassium are chemical analogues of cesium and consequently they are included in the nutrient cycle and cause long-term irradiation to biota and man (Ciuffo et al., 2002). A long half-life together with its chemical and ecophysiological similarity with potassium makes 137Cs one of the most important and harmful radionuclides released into

the environment by the nuclear industry (Sawidis et al., 2009).

The accident in the Chernobyl nuclear power plant has marked the XX century (26.04.1986, Ukraine). The most significant and dangerous radionuclides released into the atmosphere were ¹³¹I, ¹³⁴Cs and ¹³⁷Cs. Soon after the Chernobyl accident, extensive research started on radionuclide concentrations in mosses, lichens and other bioindicators (Elstner et al., 1987; Papastefanou et al. 1989; Marović et al. 2008, and many more). In moss in Finland in 1986 (Ilus et al., 1987) ¹³⁷Cs radionuclide concentrations were 28000 Bq/kg, while in Bavaria and Munich they were 12370 Bq/kg, i.e. 30000 Bq/kg (Elstner et al., 1987; Heinzl et al., 1988).

While monitoring of pollution of the environment by radionuclides in moss was expanding in the world, in ex-Yugoslavia research in this field was neglected. According to Saračević et al. (1989), ¹³⁷Cs radionuclide concentrations in moss in 1985 (before the Chernobyl accident) in hunting ground in Bosnia and Herzegovina were from 267 Bq/kg to 508 Bq/kg (mean value 429 Bq/kg), while in 1987 (after the accident) they were from 858 Bq/kg to 4604 Bq/kg (mean value 2645 Bq/kg).

¹³⁷Cs radionuclide concentrations in moss samples from areas in ex-Yugoslavia after the Chernobyl accident were different. In *Plagiothecium sp.* moss collected on the territory of the National Park Durmitor (Montenegro) in 1993 ¹³⁷Cs radionuclide concentrations were 5181 Bq/kg. (Stanković et al., 1999), while in 1996, in *Isothecium myurum* Brid. moss from the territory of NP Kopaonik they were 2183 Bq/kg, and 873 Bq/kg in *Amblystegium serpens* (Hedw) Br. Evr. moss from southern Serbia (Grdelica) (Stanković et al., 1997).

Radionuclides released in the Chernobyl accident are still present in ecosystems. It has been shown that in Belgrade and its surroundings, 24 years after the accident both soil and moss samples still contain relatively high concentrations of ¹³⁷Cs (Grdović et

al., 2010). The aim of this study was to establish radionuclide concentrations, soil-to-moss transfer, and the biological half-life for ¹³⁷Cs and ⁴⁰K, over a period of 13 years (1996-2009) in mosses collected from the Djerdap National Park.

MATERIALS AND METHODS

The samples of mosses and substrates were collected from 1996 to 2009 on the territory of the National Park Djerdap. The samples were airdried and then homogenized, and the activities were measured gamma-spectrometrically. The specific activity of radionuclides was measured using an HPGe gamma-ray spectrometer (ORTEC-AMETEK, with 8192 channels, resolution of 1.65 keV and relative efficiency of 34% at 1.33 MeV for ⁶⁰Co). Samples were measured in Marinelli vessels. Sample weight was about 0.1 kg. The counting time for each sample was 60000s. The relative error for sample preparation and measurement was 10%. Gamma Vision 32 MCA emulation software, was used to analyze gamma-ray spectra. The specific activity of the artificially produced radionuclide 137 Cs was measured via the γ -line at the energy of 661.6 keV. The specific activity of the 40K radionuclide was determined from its 1460.8 keV gammaray line. Nuclides were identified using a librarydriven search routine, and quantitative analyses were carried out using the appropriate detector calibration. Radionuclide results were reported in Bq/kg on a dry weight basis.

RESULTS

Table 1 shows the radionuclide concentrations (Bq kg-1 dry weight) of 137Cs and 40K in moss samples collected in 2003 in NP Djerdap (Ploče, Veliki kazan, Duboki potok, Šomrda, Alibegov potok, PRC) (Čučulović et al., 2005). Radionuclide contamination due to the Chernobyl accident was not homogenous on the territory of NP Djerdap which resulted in great differences in moss contamination. Maximal radionuclide concentrations of 137Cs were measured in samples from the Veliki kazan locality (4923 Bg/kg), while minimal levels were measured in samples collected after a fire on the Šomrda locality (76 Bq/kg). Concentrations of the natural radionuclide 40K in moss samples from the NP Djerdap territory are from 95 Bq/kg (sample 6) to 992 Bq/kg (sample 5).

Table 2 shows ¹³⁷Cs radionuclide concentrations in moss samples collected in 2006 on the territory of NP Djerdap. Analysis of the obtained results shows that in all samples in 2006 ¹³⁷Cs was still present and the ¹³⁷Cs radionuclide concentrations in the moss samples were not uniform. In moss samples from 2006, ¹³⁷Cs radionuclide concentrations were from 72 Bq/kg (*Ctenidium molluscum*, from the Čezava locality) to 3463 Bq/kg (*Brachythecium mildeanum*, from the Crni vrh locality) (Čučulović and Veselinović, 2008). The activity concentrations of analyzed radionuclides varied with moss species. The mean ¹³⁷Cs radionuclide concentrations in moss

Table 1. Radionuclide concentrations	(Bq/kg d.	w.) in moss samp	oles from the NP D	jerdap collected in 2003.
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No.	Site	¹³⁷ Cs	$^{40}{ m K}$
1	Asphalt road, turning for Ploče	4756	184
2	Veliki kazan, Ploče	511	110
3	Duboki potok	1671	226
4	Veliki kazan, viewpoint	4923	217
5	Šomrda	2721	992
6	Šomrda	192	95
7	Šomrda, relay	1239	143
8	Šomrda, fire	76	389
9	Alibeg's stream	2365	261
10	Alibeg's stream	592	733
11			145

Table 2. Radionuclide concentrations (Bq/kg d.w) in moss and substrate samples in the NP Djerdap territory collected in 2006.

No.	Site	Sample	¹³⁷ Cs	$^{40}\mathrm{K}$	TF ¹³⁷ Cs	TF 40K
1	Djerdap, 8a, Monastery wood	Isothecium myurum	83	154		
2	Djerdap, 25a, Prapazešće	Brachythecium mildeanum	1410	153		
2a	Djerdap, 25a, Prapazešće	substrate	1539	199	0.92	0.77
3	Djerdap, 41a, Brzujka	Bryum argenteum	508	191		
3a	Djerdap, 41a, Brzujka	substrate	660	231	0.77	0.83
4	Djerdap, 48b, Faca Tekija	Hypnum cupressiforme	365	172		
5	Djerdap, 67i	Hypnum cupressiforme	186	471		
6	Djerdap, 75b	Homalothecium lutescens	131	284		
7	Djerdap, 78f, Popovac	Dicranum scoparium	124	108		
8	Crni vrh, 21c	Brachythecium mildeanum	3463	481		
8a	Crni vrh, 21c	substrate	3892	521	0.89	0.92
9	Čezava, 36	Ctenidium molluscum	72	386		
10	Čezva, 37	Brachythecium mildeanum	208	442		
10a	Čezva, 37	substrate	770	546	0.27	0.81
11	Štrbac stream bed, 65a, Alibeg's stream	Hypnum cupressiforme	2737	58		
11a	Štrbac stream bed, 65a, Alibeg's stream	substrate	3220	192	0.85	0.30
12	River on the right, 37	Brachythecium mildeanum	205	133		
13	River on the right, 46	Homalothecium sp.	174	106		
14	River on the right, 47	Hypnum cupressiforme	190	174		
15	River on the left, 27	Pseudoleskeella nervosa	463	304		
15a	River on the left, 27	substrate	552	367	0.84	0.83
16	River on the left, 29	Sphagnum fuscum	166	233		
16a	River on the left, 29	substrate	422	378	0.39	0.62
17	Private wood, 18f, Tekija	Dicranum scoparium	1029	526		
17a	Private wood, 18f, Tekija	substrate	1132	551	0.91	0.95
18	Private wood, 22a, Tekija	Ctenidium molluscum	405	493		
19	Private wood, 30a, Tekija	Isothecium myurum	1040	202		
19a	Private wood, 30a, Tekija	substrate	1233	307	0.84	0.66
20	Private wood, KO Tekija, Kosovica	Hypnum cupressiforme	116	79		

collected in 2006 from the territory of NP Djerdap was 1225 Bq/kg. ⁴⁰K radionuclide concentrations in moss collected in 2006 was from 58 Bq/kg (*Hypnum cupressiforme*, Štrbac stream bed, Alibeg's stream) to 526 Bq/kg (*Dicranum scoparium*, private woods, Tekija). The mean ⁴⁰K radionuclide concentrations in moss collected in 2006 from the NP Djerdap territory was 258 Bq/kg.

Table 2 also shows ¹³⁷Cs radionuclide concentrations in moss substrate collected in 2006 on the NP Djerdap territory. ¹³⁷Cs radionuclide concentrations in the substrate samples are not uniform (from 422 Bq/kg, Čezava, to 3892 Bq/kg, Crni vrh). The different radiocesium activity concentrations in the sub-

strates are the consequence of non-uniform contamination of locations after the Chernobyl accident. The mean ¹³⁷Cs activity concentrations in the substrates were 1491 Bq/kg. ⁴⁰K activity concentrations in the substrates were from 152 Bq/kg (Štrbac stream bed, Alibeg's stream) to 551 Bq/kg (private woods, 18f). The mean ⁴⁰K activity concentrations in substrates from the NP Djerdap territory was 366 Bq/kg.

Knowing the ¹³⁷Cs and ⁴⁰K radionuclide concentrations in mosses and their substrates enabled calculation of transfer factors given in Table 2. Transfer factors (TF) were calculated as the ratio of the radionuclide concentration in plants (Bq/kg plant) to its concentration in soil (substrate) (Bq/kg soil):

No.	Site	Sample	¹³⁷ Cs	⁴⁰ K
		2008		
1	Djurakovo	Hypnum cupressiforme	11.8	260
2	Golubac	Hypnum cupressiforme	120	242
3	Golubac	Homalothecium lutescens	76	228
4	Brnjica	Brachythecium rutabulum	21	364
5	Brnjica	Homalothecium sericeum	7.14	168
6	Lepenski vir	Brachythecium salebrosum	14.0	192
7	Lepenski vir	Isothecium myosuroides	146	484
8	Lepenski vir	Leucodon sciuroides	417	322
		2009		
1	Golubac city	Hypnum cupressiforme	7.95	184
2	Golubac city	Homalothecium lutescens	5.43	142
3	Golubac city	Homalothecium lutescens	14.5	189
3a	Golubac city	substrate	16.4	148
4	Golubac city	Hypnum cupressiforme	19.2	192
5	Golubac city	Hypnum cupressiforme	15.7	190
6	Brnjica	Brachythecium rutabulum	27	227
6a	Brnjica	substrate	38	243
7	Lepenski vir	Brachythecium salebrosum	12.7	140
8	Lepenski vir	Isothecium myosuroides	111	346
8a	Lepenski vir	substrate	105	356
9	Lepenski vir	Isothecium myosuroides	104	471

Table 3. Radionuclide activity concentrations (Bq/kg d.w.) in moss and substrate samples from the NP Djerdap territory collected in 2008 and 2009.

TF=Bq/kg plant (dry weight)/ Bq/kg soil (dry weight) [1]

The soil-to-moss transfer factor for the 137 Cs studied was from 0.27 to 0.92 (mean value for 137 Cs was 0.74). Potassium remains in homeostatic equilibrium in the plants and is readily assimilated by the plants and generally shows a TF smaller than 1. Potassium-40 TF values ranged from 0.30 to 0.95 (mean value for 40 K was 0.74).

The ¹³⁷Cs and ⁴⁰K activity concentrations in the mosses did not exhibit any competitive relationship, probably because mosses act as atmospheric filters and accumulate both radioisotopes in a directly related manner).

Analysis of the ¹³⁷Cs and ⁴⁰K activity concentrations is mosses and their substrata (Table 2) leads to the conclusion that there is a linear dependence

between ¹³⁷Cs (⁴⁰K) activity concentrations is mosses and ¹³⁷Cs (⁴⁰K) activity levels in the substrate.

Table 3 shows 137Cs activity levels in moss and substrate samples collected in 2008 and 2009 on the NP Djerdap territory. 137Cs activity concentrations (Bq/kg) in mosses collected in 2008 on the NP Djerdap territory range from 7.14 (Homalothecium sericeum, Brnjica) to 417 Bq/kg (Leucodon sciuroides, Lepenski vir). The mean activity concentration of ¹³⁷Cs in moss collected in 2008 on the NP Djerdap territory was 102 Bq/kg. The results of 137Cs activity concentrations in mosses show that there were no new contaminations of the NP Djerdap territory with this radionuclide. 40K activity concentrations (Bq/kg) in mosses collected in 2008 were from 168 (Homalothecium sericeum, Brnjica) to 484 Bq/kg (Isothecium myosuroides, Lepenski vir). The mean activity concentration of 40K in mosses collected in 2006 on the NP Djerdap territory was 283 Bq/kg.

Sample year	¹³⁷ Cs (Bq/kg)	⁴⁰ K (Bq/kg)
1996. moss	724	287
substrate	1413	
1997. moss	1417	437
1999. moss	1055	
substrate	1491	
2000. moss	1352	
2001. moss	855	405
2003. moss	745	397
2004. moss	377	253
2008. moss	234	332

Table 4. ¹³⁷Cs (Bq/kg) and ⁴⁰K (Bq/kg) activity concentrations in *Homalothecium sericeum* moss and its substrate collected on a rock close to the Lepenski vir archeological site in the period from 1996 to 2008.

These results indicate that there was no new ⁴⁰K pollution of the NP Djerdap territory.

¹³⁷Cs activity concentrations (Bq/kg) in mosses collected in 2009 on the NP Djerdap territory were from 5.43 (*Homalothecium lutescens*, Golubac city) to 111 Bq/kg (*Isothecium myosuroides*, Lepenski vir). The mean activity concentration of ¹³⁷Cs in mosses collected in 2009 NP Djerdap territory was 35 Bq/kg. ⁴⁰K activity concentrations (Bq/kg) in mosses collected in 2009 were from 140 (*Brachythecium salebrosum*, Lepenski vir) to 471 Bq/kg (*Isothecium myosuroides*, Lepenski vir). The mean activity concentration of ⁴⁰K in moss collected in 2008 on the NP Djerdap territory was 231 Bq/kg.

Table 3 shows ¹³⁷Cs activity concentrations in moss substrate samples collected in 2009 on the NP Djerdap territory (Čučulović and Veselinović, 2009). ¹³⁷Cs activity concentrations in the substrate samples were from 16.4 Bq/kg (substrate 3a, Golubac city) to 105 Bq/kg (substrate 8a, Lepenski vir). ⁴⁰K activity concentrations in the substrates were from 148 Bq/kg (substrate 3a, Golubac city) to 356 Bq/kg (substrate 8a, Lepenski vir). The different radionuclide concentrations in the substrate are the consequence of non-uniform contamination of locations by the Chernobyl accident.

Confirmation that there were no new contaminations with radiocesium on the NP Djerdap terri-

tory is found in the results presented in Table 4. It gives the results of activity concentrations (Bq/kg) of ¹³⁷Cs and ⁴⁰K in *Homalothecium sericeum* moss growing on a rock near the entrance to the Lepenski vir archeological site. *H. sericeum* moss was collected from the same spot in the summer period from 1996 to 2008. It is obvious that the radiocesium activity concentration decreases with time which shows that there were no new contaminations with this radionuclide on this area and wider.

The results presented in Table 4 indicate that in a certain time period the activity concentrations of ¹³⁷Cs decrease faster in moss than the physical half-life period (30.2 years). This reduction in radionuclide concentrations in the moss gametophyte is explained as the consequence of an increase in biomass while the radionuclide amount remains unchanged.

Using the data on ¹³⁷Cs activity concentration in moss from Lepenski vir in the period from 1997 to 2008 given in Table 2 we can calculate the biological and effective half-life period of ¹³⁷Cs in *Homalothecium sericeum* moss. The biological and effective half-life period depends on the chemical form of the radionuclide, surrounding pH, temperature, moss type and some external parameters.

Knowing that

$$A_t = A_0 \exp(-(\lambda + \lambda_b)t)$$
 [2]

where: A_t is the activity per unit moss dry weight at any time

 A_0 is the activity per unit moss dry weight at t=0

 λ is the constant = $ln2/T_f = 0.023$ year⁻¹, T_f is the physical half-life of ¹³⁷Cs of 30.2 years

 $\lambda_b = \ln 2/T_b$ (year-1), T_b is the biological half-life, and

t is the time period of 9 years if we use data from 1999 to 2008.

Exchanging values for A_t , A_0 , λ and t, values for λ_b of 0. 1408 year 1 and T_b (biological half-life of 137 Cs) of 4.9 years are obtained.

Knowing that

$$T_{\text{eff}} = T_f T_b / T_f + T_b$$
 [3]

where: T_{eff} is the effective half-life of ¹³⁷Cs in moss

T_f is the physical half-life of ¹³⁷Cs

T_b is the biological half-life of ¹³⁷Cs in moss,

then the effective half-life of 137 Cs can be then calculated in *Homalothecium sericeum* moss (Sloof, 1993). In our case the effective half-life was 4.2 years. The calculated values for T_b and T_{eff} are lower than the values obtained for *Evernia prunastri* lichen collected in southern Serbia in the period from 1989 to 1993 where T_b was from 8.3 to 10.1 years and T_{eff} was from 6.5 to 7.5 years (Stanković, 1994).

DISCUSSION

In this work the presence of ¹³⁷Cs was noted in all investigated samples. Activity concentrations of the analyzed radionuclides varied with moss species, as well as among samples of the same species collected from different localities. Different activity concentrations of radiocesium in the same moss species from different locations are mostly due to non-uniform contamination of locations after the

Chernobyl accident. While intraspecific variation in radioactivity is due to different deposition and washout rates, interspecific variation of activity concentration is due to different morphology and anatomy (Sabovljević et al., 2005; Sawidis et al., 2009; Cevik and Celik, 2009), leading to different accumulation of radioactivity from dry and wet deposition.

A decreasing trend of activity concentrations has been observed in all samples. Since activity already accumulated in mosses will make some decreasing trend of activity deposition less prominent (Krmar et al., 2009), this decreasing trend is even more certain, and confirms what is usually stated: that after the Chernobyl accident there was no significant ¹³⁷Cs emission. The temporal variations in ¹³⁷Cs activity shown in *Hypnum cupressiforme* (Krmar et al., 2009) are not significant, since in our study the accumulated activity is much higher than this variation level.

The biological half-life of ¹³⁷Cs for Homalothecium sericeum moss was 4.9 years, while the effective half-life of ¹³⁷Cs for moss was 4.2 years. The calculated values for T_b and T_{eff} are lower than the values obtained for Evernia prunastri lichen collected in southern Serbia in the period from 1989 to 1993 where T_b was from 8.3 to 10.1 years and Teff was from 6.5 to 7.5 years (Stanković, 1994). This value is comparable to the mean ecological half-life for ¹³⁷Cs in mosses, which was calculated to be 4.4 years by Cevik and Celik (2009). However, for the same species in their study, the ecological half-life was much longer (10.4 years). Since ecological half-life depends on many factors (including different morphoanatomic and microclimatic differences), this introduces some uncertainty to the estimation.

There was no correlation in accumulation between ¹³⁷Cs and ⁴⁰K. The absence of any correlation between activity concentrations of these radionuclides in mosses was also demonstrated in other radioecological studies (Korobova et al. 2007), but contrary to the findings of Cevik and Celik (2009). A lin-

ear dependence of ¹³⁷Cs (⁴⁰K) activity concentrations in moss on the ¹³⁷Cs (⁴⁰K) activity concentrations in the substrate was observed. This indicates that differences between localities are mainly the consequence of different rates of deposition after the Chernobyl accident, and not different washout rates. Substratemoss transfer factors were calculated for ¹³⁷Cs and ⁴⁰K, and for both radionuclides the values were less than 1. This means that no significant transfer of radionuclides from substrates occurred, and that the radioactivity in the moss is mainly from atmospheric deposition.

In this work, mosses have again been shown to be good biomonitors for atmospheric radionuclide deposition. Although substantially decreased, ¹³⁷Cs is still present in moss tissue. This radioactivity is thought to originate mainly from the Chernobyl accident. Monitoring of ¹³⁷Cs activity in mosses thus provides a very sensitive method for detection of this radionuclide and should be performed as a means of continuous evaluation of environment pollution.

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