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ACTIVITY CONCENTRATIONS OF ^{137}CS AND ^{40}K IN MOSSES FROM SPAS IN EASTERN SERBIA

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Abstract - With the aim of detecting the presence of radionuclides in moss samples in eastern Serbia, 129 samples of mosses were collected from 2000 to 2010 in the region of the spas: Sokobanja, Banja Jošanica and Gamzigradska banja. The data obtained show that ^{137}Cs was present in all of the 129 samples from 3 sampling areas and 15 sampling localities and in 20 moss taxa. This indicates that pollution with this radionuclide resulting from the Chernobyl accident still exists. Changes in the average activity concentrations of ^{40}K are within measurement error limits, while for ^{137}Cs changes in the average activity concentrations are more noticeable, i.e. they have decreased with time.

Key words: Mosses, radionuclides, ^{137}Cs, ^{40}K, Eastern Serbia, radioactivity

INTRODUCTION

Evolutionarily, mosses were among the first plants to colonize land (Richardson, 1981) and face the challenges of terrestrial living. Thus, they have developed many adaptations for survival in harsh environments, some of which even nowadays are fascinating (e.g. anabiosis and poikilohidry, metal ion accumulations in the thallus with no cuticles etc.). The structure of mosses makes them suitable for monitoring the aerial metal burden. Mosses have a slow growth rate and a relatively large surface area-to-biomass ratio; the lack of waxy cuticles and associated stomata means that many contaminants can be absorbed over the whole external surface; they do not have a rooting system; they obtain nutrients from dry and wet precipitation by absorption through their leaves which lack cuticle (Richardson, 1981). The use of mosses in biomonitoring has significantly increased since 1968, when Rühling and Tyler first used them as biomonitors of atmospheric quality (Rühling and Tyler, 1968). Mosses may be considered the most commonly applied organisms for biomonitoring studies of radioactive contamination (Chakrabortty and Paratkar, 2006; Batias and Siontorou, 2007; Berg and Steinnes, 1997; Basile et al., 2008; Čučulović and Veselinović, 2008; Čučulović and Veselinović, 2009; Čučulović et al., 2010).

Mosses are among the pioneering plant species on burnt land, secondary bare surfaces and in tundra. They prevent land erosion and represent mini-ecosystems due to the number of organisms that depend on them. Moss utilization is high in industry, medicine and pharmacology (Longton, 1992; Delfanti at al., 1999).

Air pollutants are deposited on mosses in three forms: as an aqueous solution, in gaseous form or as attached particles. The accumulation of pollutants in mosses occurs through a number of different mechanisms.

Potassium is a lithophilic element and is monovalent under natural conditions. Of the naturally occurring primordial radionuclides, ^{40}K ($T_{1/2}=1.28x10^9$ y) is very abundant in soil as the molar fraction of ^{40}K is 0.0117 (Veselinović et al., 2004), and the average content in the Earth's core is 25.9 kg/t (Mason, 1996). In a living organism, potassium is evenly distributed. The greatest part of internal radiation coming from natural sources of radioactivity originates from potassium (United Nations Committee, 2000). Cs^+ and K^+ are competing ions.

Cesium-137 (^{137}Cs) is one of the most frequent artificial radionuclides in the environment, arising from different anthropogenic discharges such as atmospheric nuclear weapon testing, accidental release from nuclear power plants, chronic emissions from nuclear power plants and chronic emissions from nuclear reactors and fuel-reprocessing plants (Eisler, 2000.; Davis, 1963; Whicker, 1997). Cesium-137 is an emitter of gamma and beta radiation, with a long radioactive half-life (30.17 years).

The first nuclear weapons were detonated in New Mexico and Japan in 1945. The partial meltdown of the reactor at Chernobyl in April 1986 released high amounts of radionuclides into the environment, especially ^{137}Cs. Following the Chernobyl reactor accident, numerous studies involving the monitoring of the long-lived fission product ^{137}Cs were undertaken using mosses as biological indicators to update the inventory of this long-lived radionuclide (e.g. Boileau at al., 1982; Giovani at al., 1994; Papastefanou at al., 1992; Butkus and Konstatinova, 2005; Sawidis at al., 2009; Elstner et al., 1987; Papastefanou et al., 1989; Marović et al., 2008; Ilus et al., 1987; Elstner et al., 1987; Čučulović et al., 2002; Čučulović et al., 2008). As mosses lack a well-developed root system, the uptake of radionuclides occurs mainly through an ion exchange process directly from wet and dry deposition on the moss body (Delfanti et al., 1999; Mishev et al., 1996).

The expansion of knowledge about contamination paths by following bioindicators is important for radiological research and comparison with available data.

MATERIALS AND METHODS

Samples of mosses were collected from 2000 to 2010 in the territories of the following spas: Sokobanja, Banja Jošanica and Gamzigradska banja.

Sokobanja is located in the central part of eastern Serbia and is part of the Zaječar region. Most of the municipality is part of the extensive Carpathian-Balkan mountain range. A smaller western part is in the zone of the old Rhodopes mass. It is located 43° 38' 29" latitude north and 21° 52' 37" longitude east at 400 m above sea level. The spa is located in a west-east direction and slopes westward. The climate is mild continental. Samples from the territory of the Sokobanja municipality were taken at the following locations: in the city of Sokobanja (hotel "Sunce") (locality SB1); Lepterija (1.5 km east from the center of Sokobanja on the left bank of the Moravica river) (SB2); in Soko grad (400 m from Lepterija, upstream the Moravica) (SB3); Ozren (about 4.5 km from the center of Sokobanja in the "Ozren" Special Hospital Complex) (SB4); Ophthalmology hospital (on the Ozren road, 5 km from Sokobanja Southeast, close to the Ozren Special hospital for treating eye problems) (SB5). In the period 2000-2010, samples were taken as follows: from locality SB1 5 samples, and one sample from each of the localities SB2 23, SB3 1, SB4 57 and SB5, giving a total of 87 samples.

Banja Jošanica is located in the northwestern part of the Sokobanja valley, between the western part of Mt.Rtanj and the eastern slopes of Mt. Bukovik, close to Sokobanja. In the territory of Banja Jošanica, samples were taken in the following locations: in the park (BJ1); close to the mineral water springs (BJ2); and on the Jošanica River banks

(BJ3). In the period 2009-2010, from each location (BJ1, BJ2 3 and BJ3) 3 samples were taken, giving a total of 9 samples.

Gamzigradska banja is located close to Zaječar, in the meandering valley of the Crni Timok River, at the outermost southwestern part of the Romanian basin close to the Carpathian Mountains of eastern Serbia. This spa is one of the oldest balneological centers in Serbia. In the territory of Gamzigradska banja samples were taken on the left (GB1) and right bank of the Crni Timok river (GB2), near the Gamzigrad Rehabilitation Center (GB3), near Hotel Kastrum (GB4), from the hotel annexes (GB5), hydroelectric power station (HE) Gamzigrad (GB6) and in the archeological locality of Felix Romuliana (GB7). In the period 2006-2010, from locality GB1 7 samples were taken, from GB2 1, GB3 5, GB4 3, GB5 3, GB6 12 and GB7 2 samples were taken, in total 33 samples.

Selection of localities was made based on the presence of large enough amounts of moss and the absence of immediate sources of pollution.

In this work, 20 moss taxa were analyzed (129 samples), that grew in the sampling localities: *Brachythecium midleanum* (Schimp.) Schimp. (2 samples)*; Dicranum scoparium Hedw.* (3 samples); *Bryum argenteum Hedw.* (3 samples); *Ctenidium molluscum (Hedw.)* Mitt. (1 sample); *Tortella tortuosa* (Hedw.) Limpr. (2 samples); *Homalothecium philippeanum* (Spruce) Schimp. (7 samples); *Brachythecium rivulare* Schimp. (6 samples); *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr. (1 sample); *Plagiomnium cuspidatum* (Hedw.) T.J. Kop. (10 samples); *Homalothecium sericeum* (Hedw.) Schim. (26 samples); *Leucodon sciuroides* (Hedw.) Schwaegr (13 samples); *Homalothecium lutescens* (Hedw.) H. Rob (1 sample); *Hylocomium splendens* (Hedw.) Schimp (8 samples); *Hypnum cupressiforme* Hedw. (12 samples); *Syntrichia calcicola* J. J. Amann (1 sample); *Anomodon attenuatus* (Hedw.) Hübner. (3 samples); *Brachythecium rutabulum* (Hedw.) Schimp. (2 samples); *Homalothecium sp.* (12 samples); *Grimmia pulvinata* Hedw. Sm. (7 samples) and *Pylaisia polyantha* (Hedw.) Schimp. (9 samples). The nomenclature follows Sabovljevic et al. (2008). The vouchers are deposited in the bryophyte collection of the BEOU.

The moss samples were dried in air, homogenized, and the radionuclide activities measured gamma-spectrometrically. The specific activity of radionuclides was measured using an HPGe gamma-ray spectrometer (ORTEC-AMETEK, with 8192 channels, resolution 1.65 keV, relative efficiency 34% at 1.33 MeV for ^{60}Co). Samples were measured in Marinelli vessels. The sample weight was about 0.1 kg. The counting time for each sample was 60000 s. The relative error for sample preparation and measurement was 10%. Gamma Vision 32, MCA emulation software, was used to analyze the measured gamma-ray spectra. The specific activity of the artificially produced radionuclide ^{137}Cs was measured via the γ-line at energy of 661.6 keV. The specific activity of the ^{40}K radionuclide was determined from its 1460.8 keV gamma-ray line. Nuclides were identified using a library driven 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

Activity values of ^{137}Cs and ^{40}K (Bq/kg) in mosses collected on the territory of Sokobanja, Banja Jošanica and Gamzigradska banja in the period from 2000 to 2010 are given in Table 1.

Maximal and minimal values of ^{137}Cs and ^{40}K were noted on the territory of the Sokobanja municipality in the locality SB4. The minimal activity value of ^{137}Cs was 1.12 Bq/kg (locality SB4, 2010, sample 7) and ^{40}K 25 Bq/kg (SB4, 2009, sample 7). The maximal activity concentration of ^{137}Cs was 192 Bq/kg (SB4, 2006, sample 14) and ^{40}K 427 Bq/kg (SB4, 2006, sample 12).

On the territory of Banja Jošanica, the minimal activity concentration of ^{137}Cs was 72 Bq/kg (locality

Table 1. Locality (L), sampling year (Y), moss species (S), sample number (N) and activity (A) (Bq/kg) (lowest-highest (A_1-A_2), individual (A) and average (Ā)) ^{137}Cs and ^{40}K in spas in Eastern Serbia.

L	Y	S	N	^{137}Cs (Bq/kg)		^{40}K (Bq/kg)	
				A_1-A_2; A	Ā	A_1-A_2; A	Ā
SB1	2001	**14**	2	25-42	34	119-319	219
	2002	**14**	1	25		319	
	2006	**5**	1	41		250	
	2008	**9**	1	33		65	
SB2	2000	**13**	1	177		173	
	2001	**13**	2	117-177	147	173-229	201
	2002	**13**	3	93-176	129	171-229	201
	2006	**4**	1	107		325	
		5	1	98		176	
	2007	**13**	1	37		202	
	2008	**9**	2	48*	48	243-352	298
		10	1	4.40		169	
		14	1	20		167	
		16	1	45		178	
	2009	**9**	1	49		200	
		10	2	3.50-24	13.8	210	226
		16	1	28		115	
	2010	**9**	2	25-39	32	142-186	164
		16	1	54		187	
		17	2	4.72-8.45	6.59	251-323	287
SB3	2008	**10**	1	20		148	
SB4	2001	**14**	1	105		277	
		15	1	61		414	
	2002	**14**	1	100		187	
	2006	**1**	2	5.41-21	13.2	123-208	166
		2	2	2.70-124	63	83-143	113
		3	1	76		389	
		18	12	18.3		188	
		12	1	59		427	
		14	4	8.21-192	74	95-209	150
		18	1	10.5		103	
	2007	**2**	1	71		81	
		13	1	62		304	
		14	2	10-101	56	265-286	276
	2008	**6**	2	13.4-52	33	159-203	181
		7	2	2.90-3.60	3.25	184-239	212
		8	1	14.5		267	
		9	1	33		65	
		10	4	8.41-39	18.2	164-326	244
	2009	**6**	1	15.9		166	
		7	2	2.50-2.91	2.71	25	25
		9	1	8.90		156	
		10	5	5.50-25	11.4	145-255	221
	2010	**6**	4	7.21-28	18.2	159-211	186
		7	2	1.12-1.65	1.39	101-150	126
		9	2	6.84-34	20	141-178	160
		10	11	8.26-27	17.1	179-415	250

Table 1. *Continued*

L	Y	S	N	^{137}Cs (Bq/kg)		^{40}K (Bq/kg)	
				A_1-A_2; A	Ā	A_1-A_2; A	Ā
BJ1	2009	**18**	1	132		190	
	2010	**18**	2	123-148	136	141-201	171
BJ2	2009	**18**	1	197		251	
	2010	**18**	2	103-107	105	137-172	155
BJ3	2009	**18**	1	158		169	
	2010	**18**	2	72-79	76	208-222	215
GB1	2006	**19**	2	57-121	89	134-243	189
		20	1	112		130	
	2008	**19**	3	44-84	57	226-361	273
		20	1	77		123	
GB2	2006	**19**	1	58		95	
GB3	2006	**20**	2	59-60	60	116-128	122
	2008	**20**	3	60-63	62	144-276	230
GB4	2006	**19**	1	115		193	
		20	1	193		306	
	2008	**20**	1	83		280	
GB5	2006	**11**	2	78-178	128	178-226	233
	2008	**11**	1	53		158	
GB6	2006	**3**	1	83		112	
		11	2	145-269	207	226-239	233
	2008	**3**	1	41		101	
		11	5	170-213	189	196-351	249
	2009	**11**	3	120-199	161	209-228	216
GB7	2006	**10**	2	94-115	105	138-158	148

* same values

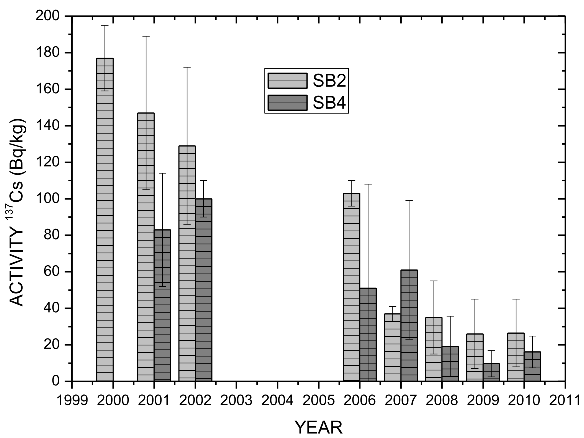

Fig. 1. Average activity of ^{137}Cs (Bq/kg) and standard deviation in moss samples collected on localities SB2 and SB4 in the period 2000-2010.

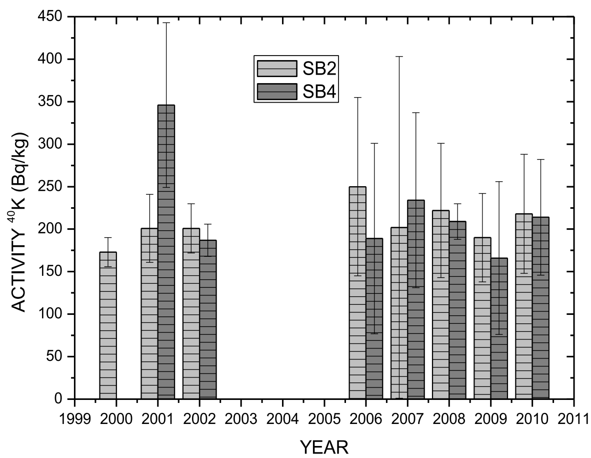

Fig. 2. Average activity of ^{40}K (Bq/kg) and standard deviation in moss samples collected on localities SB2 and SB4 in the period 2000-2010.

Table 2. Average activities of ^{137}Cs and ^{40}K (Bq/kg), standard deviation in moss samples collected on localities SB2 and SB4 in the period 2000-2010.

YEAR	SB2		SB4	
	^{137}Cs (Bq/kg)	^{40}K (Bq/kg)	^{137}Cs (Bq/kg)	^{40}K (Bq/kg)
2000	147 ± 42	201 ± 40	---	---
2001	129 ± 43	201 ± 29	83 ± 31	346 ± 97
2002	---	---	100 ± 10	187 ± 19
2003	---	---	---	---
2004	---	---	---	---
2005	---	---	---	---
2006	103 ± 7	250 ± 105	51 ± 57	189 ± 112
2007	37 ± 4	202 ± 20	61 ± 38	234 ± 103
2008	35 ± 20	222 ± 79	19.2 ± 16.5	209 ± 21
2009	26 ± 19	190 ± 52	9.66 ± 7.26	166 ± 90
2010	27 ± 19	218 ± 70	16.1 ± 8.72	214 ± 68

BJ3, 2010, sample 18) and ^{40}K 137 Bq/kg (BJ2, 2010, sample 18). The maximal activity concentration of ^{137}Cs was 197 Bq/kg (BJ2, 2009, sample 18) and ^{40}K 251 Bq/kg (BJ2, 2009, sample 18).

On the territory of Gamzigradska banja, the minimal activity concentration of ^{137}Cs was 41 Bq/kg (locality GB6, 2008, sample 3) and ^{40}K 95 Bq/kg (GB2, 2006, sample 19). The maximal activity concentration of ^{137}Cs was 269 Bq/kg (GB6, 2006, sample 11) and ^{40}K 361 Bq/kg (GB1, 2008, sample 19).

The average activity concentrations of ^{137}Cs and their standard deviations in moss samples collected on localities SB2 and SB4 in the period 2000-2010 are shown in Fig. 1. The average activity concentrations of ^{137}Cs in mosses collected on locality SB2 in most cases are higher than the average activity concentrations of ^{137}Cs in moss samples collected in locality SB4.

The average activity concentrations of ^{40}K and their standard deviations in moss samples collected on localities SB2 and SB4 are shown in Fig. 2.

DISCUSSION

The presence of the artificially produced radionuclide ^{137}Cs and the natural radionuclide ^{40}K was noted in all the investigated moss samples collected from spas in eastern Serbia in the period from 2000 to 2010.

Previous research has shown that mosses *C. molluscum* (4), *L. sciuroides* (11), H. splendens (13) and *H. cupressiforme* (14), are good bioindicators and biomonitors of radioactive pollution (Delfanti et al., 1999; Hanssen et al., 1980; Sawidis et al., 2009; Giovani et al., 1994). These mosses grow on the territory of Sokobanja, Banja Jošanica and Gamzigradska banja and they were randomly selected and used in this investigation. The results of this research confirm that besides these, other moss species can be used as bioindicators of ^{137}Cs and ^{40}K radionuclide pollution (Table 1).

The data obtained (Table 1) shows that ^{137}Cs was present in all of the 129 samples from the 3 sampling areas and 15 sampling localities, and in 20 moss taxa. This definitely indicates that pollution by this radionuclide as a consequence of the Chernobyl accident still exists. The different activity concentrations of ^{137}Cs in mosses from spas in eastern Serbia are the consequence of different contents of ^{137}Cs in the fallout after the Chernobyl accident, moss species, substrate on which they grow, altitude, climatic and other factors. A comparison of the average activity concentrations of ^{137}Cs in the mosses collected on the territory of the Sokobanja, Banja Jošanica and Gamzigradska banja spas allows us to conclude that the lowest activity concentrations were present in the samples from the Sokobanja municipality. The highest average activity concentrations of ^{137}Cs in moss collected on the territory of the Sokobanja municipality were in the moss collected on the Lepterija locality (SB2), and they were slightly lower in the moss collected from the Ozren locality (SB4). The average activity concentrations of ^{137}Cs in moss collected on the territory of the Sokobanja municipality in the observed time period was in agreement with the findings of other authors (Sawidis et al., 2009).

The lowest activity concentrations of ^{137}Cs were measured in *B. rivulare* (7) from Ozren (1.12-3.60 Bq/kg). As this moss grows besides the Ripaljka Falls, on the Gradašnica River, and that the river water periodically floods this moss, one can assume that the river water has desorbed ^{137}Cs from the moss and this is the reason why the activity concentrations of ^{137}Cs are low in this moss. Low activity concentrations of ^{137}Cs were also noted in the substrate on which *B. rivulare* moss grows. One can assume that the water also desorbs ^{137}Cs from the substrate. Investigations of ^{137}Cs desorption from *Cetraria islandica* (L.) Ach. lichen by distilled water and solutions simulating the composition of acid rain (Čučulović et al., 2006) favor this assumption. Activity concentrations in mosses growing close to this species, for example *H. philippeanum* (6), not flooded by the river, are higher (up to 28 Bq/kg, 2010).

In *L. sciuroides* (11), collected on the territory of Gamzigradska banja, high levels of ^{137}Cs were noted in 2009 (from 120 to 199 Bq/kg). Due to construction work on the Timok riverbanks since 2009, this moss species is an endangered species and no longer grows on these localities in Gamzigradska banja. Investigations have confirmed that this moss species is a good bioindicator of radioactive pollution in the environment (Sawidis et al., 2009) and it should be used in research.

In Fig. 1 and 2, one can clearly note that changes in the average activity concentrations of ^{40}K are within measurement error limits, while for ^{137}Cs, changes in the average activity concentrations are more noticeable, i.e. they decrease with time.

As ^{40}K is a natural isotope, this similarity in activity indicates a significant even distribution of ^{40}K on the Earth's surface and in fallouts that is reflected by its content in moss. However, for ^{137}Cs, the content reduction with time can be the consequence of the radioactive decay of ^{137}Cs, and washout with water not enriched with ^{137}Cs.

The ten-year reduction in the average activity concentration of ^{137}Cs at the SB2 locality is 79.1%, while it is 80.6% at the SB4 locality, as shown in Table 2 for the period 2001-2010. Reduction in activity concentrations of ^{137}Cs due to radioactive decay was calculated using the radioactive decay equation:

$$A=A_0e^{-\lambda t} \qquad (1)$$

where A_t is the activity in the sample after t = 10 years, A_0 is the sample activity for t=0, λ is the radioactive decay constant $=\ln2/T_{1/2}=0.023$ god^{-1}, and t is the time of 10 years.

It is 20% for sample SB2, and for sample SB4 21%, which is significantly lower than previously given values. This shows that the reduction in ^{137}Cs originates from the washout of moss by atmospheric water and its transfer to the environment, i.e. expansion of pollution, while for ^{40}K this process does not occur as it is naturally distributed over all parts of our planet.

There was no correlation in accumulation between ^{137}Cs and ^{40}K in the mosses collected in Sokobanja and Gamzigradska banja. The absence of any correlation between the activity concentrations of these radionuclides in mosses has also been demonstrated in other radioecological studies (Korobova et al., 2007), but it is contrary to the findings of Cevik and Celik (2009).

Activity concentrations of 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 the 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 concentrations is due to different morphology and anatomy (Sabovljević et al., 2005; Cevik and Celik, 2009), leading to different accumulations of radioactivity from dry and wet deposition.

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

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