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INVESTIGATION OF THE POSSIBILITY OF APLICATION OF BIOINDICATORS IN REDUCTION LEVEL CONTCENTRATIONS OF RADIOLOGICAL CONTAMINANTS

NATAŠA PAJIĆ
Military Tehnical Institute, Belgrade, natasa.pajic969@gmail.com

ŽELJKO SENIĆ*
Military Tehnical Institute, Belgrade, Belgrade, zsenic1@gmail.com

SONJA BAUK
Military Tehnical Institute, Belgrade, Belgrade, bauk.sonja@gmail.com

Abstract: *Bioindication and biomonitoring imply the use of biological parametars to monitor the state and quality of environment. The main task of biomonitoring program is to define appropriate indicators, whose presence and specific behavior will indicate effects of environmental and ecosystem distubances. Biological indicators are unique indicators of state of environment,which give a signal about the ecological status of ecosystem. Using bioindicatore as an early warning sign of radiological accident can help in conservation environment.The presented results indicate that lichens are good bioindicators of environmental pollution with radioactive isotope (^{137}Cs). Higher level of (^{137}Cs) on our theritory was indecated on Chernobyl accident, what is shown by messurment of activities of samples in this study.*

Key words: *biomonitors, indicators , cesium, ecosystem*

1.INTRODUCTION

The appearance of radioactivity dates back to the beginning of the world. The world we live in has been radioactive since its inception. Radioactive elements can be found in soil, air, water, food, and therefore in all living beings. According to the method of origin, they are divided into those that are present on Earth, and those that arise as a result of cosmic radiation, as well as those that arise as a result of human influence. Natural radioactivity is the result of decay of naturally unstable nuclides. All these radionuclides have a long half-life. An exception is radon gas, which has a half-life of 3.8 days. They are divided into three series: uranium ^{238}U, neptunium ^{237}Np and thorium ^{232}Th. Primary radioactive elements in nature are uranium, thorium and potassium. Potassium-40 is a beta-gamma emitter, with a physical half-life of $T_{1/2}$= 1.28x10^{9} years [1].

Radioactive radiation changes the structure and properties of the material it passes through. The most significant effect is ionization. The earliest and most common use of ionizing radiation is to diagnose injuries and diseases. However, ionizing radiation has enough energy to ionize some atoms in the body. Such ions disrupt biochemical processes in cells, which can lead to various disorders in the functioning of cells in their division, which causes serious diseases. For the safety of the human population, it is necessary to monitor the level of radionuclides in the environment.

Systematic measurement of natural radionuclides in soil, food, animal feed, air and water can determine the radionuclides that enter the human body through food. Some bioindicator species can also be used for monitoring; such as mosses, lichens, ferns and mushrooms.

Mosses accumulate a high dose of radioactivity. They are one of the most important collectors of radionuclides from the air and as such represent a bioindicator of radioactive fallout. They have the ability to retain a large part of radioactive fallout. Given the prevalence and simplicity of sampling, mosses provide a complete insight into the state of contamination of a certain area.

2. MOSSES AND LICHES AS BIOMONITORS

Biomonitoring of the environment represents a series of methods that monitor the impact of contamination on biological processes in organisms. Given the frequent distribution of organisms in a certain area, this allows biomonitoring to have great advantages over other methods.Some species are very sensitive to pollution, while others are very tolerant of air pollution and are used as a kind of indicator. Mosses grow in groups, forming a cluster, small "cushions" or carpet like forms. [2]

Corresponding author: N. Pajić;
E-mail: natasa.pajic969@gmail.com
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Figure 1. Moss colony

Mosses are perennial plants of slow growth, they are extremely sensitive biological organisms from the point of view of radioactivity monitoring. They can be used to collect reliable information about air and environmental pollution with radioactive substances, especially in the conditions of radiological accidents. The main content of radionuclides is in the upper green parts of the plant and it is estimated that the accumulation of radionuclides is concentrated in the upper parts of the plant. Direct deposition of radionuclides on that type of plants is the most important way of their contamination. From the point of biomonitoring, it is necessary to pay attention to the following properties of bioindicators

(a) the absence of a root system prevents the reception of ions from the soil or some other substrate,

(b) an underdeveloped cell membrane allows the absorption of metal ions along the entire surface of the cell,

(c) the specific composition of the partition wall contributes to rapid ion exchange,

(d) the ability to accumulate and tolerate a high concentration of metals,radioactive contamination of an area after a nuclear,

Mosses have several advantages over settling tanks used for pollution detection. Mosses are very widespread, and their sampling is very simple and inexpensive. Likewise, some types of mosses are capable of accumulating heavy metals to high concentrations, significantly higher than those obtained in samples from settling collectors. Thus, various analytical techniques can be used to obtain more precise results, because the concentrations of radionuclides in mosses are generally well above the detection limit. [3]

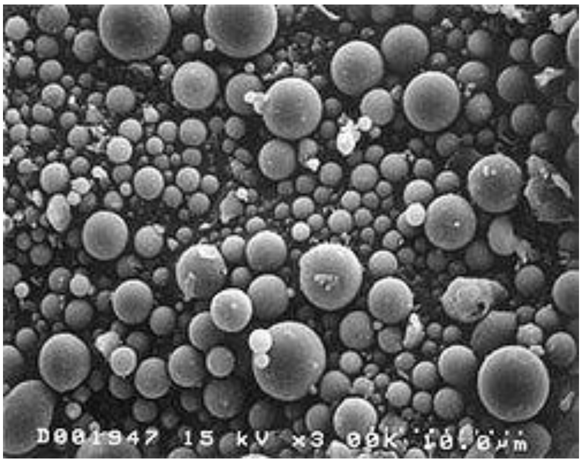

Figure 2. Scanning electron microscope (SEM) images of aerosol particles detected on the surface of mosses

In areas where mosses occur in their natural habitats, air pollution can be assessed by means of active biomonitoring, using moss bags. Samples are collected from uncontaminated areas in bags that are transported to the region that is in the process of monitoring. The measurement procedure is performed before and after exposure of the sample to radiological contamination.

Radioactive elements can accumulate in mosses to very high concentrations, so they represent an effective biological indicator of radionuclides.

2.1. Liches as biomonitors

Lichens are organisms adapted to survive in extreme conditions. They survive in areas of high humidity and drought, large light and temperature changes, high levels of radioactivity and high concentrations of polluting substances.

Information on the spatial and temporal distribution of radionuclide pollution is obtained by exposure to the emission of fission products.

Figure 3. Lichens colony

Lichens are specific organisms in the body of which lichen acid compounds are formed. the acid that is formed is usnic acid, which has found great use in medicine, the perfume industry, and the cosmetic industry. In addition to lichen acids, lichens contain and build compounds such as antioxidants, which have found application in pharmacology.

Lichens are considered accumulators of radionuclides, heavy metals and pesticides from the environment, which is most likely a consequence of the reaction of organic acids created by lichens. [4]

Due to the ability of lichens to absorb ^{137}Cs from the environment and to concentrate it in their body, lichens are an important element of radioecological studies of ecosystems, and environmental monitoring using lichens is important for assessing the radiation load of organisms living in that area.

3. MATERIAL AND METHODS

Samples of bioindicators (moss and lichen) were collected in the area of Belgrade parks and forests (Kosutnjak, Makis, Zvezdara forest, Avala, Topcider park).

The activity was determined by the gamma spectrometric method (HPGe detector with an efficiency of 56% and a resolution of 1.72 keV for ^{60}Co at 1.3 MeV).

The samples are delivered to the laboratory, cleaned of visible impurities (soil, grass), dried and measured in Marinelli bowl with a volume of 1l.

Spectrum processing was performed using the software package GammaVision 32. The relative measurement uncertainty of sample preparation and measurement is up to 10%. The activity of ^{134} Cs was determined via the γ-lines at energies of 604 KeV, 796 KeV and 802 KeV, ^{137} Cs via the γ-line at the energy of 661.6 KeV, and ^{40}K via the γ-line at energy of 1460 KeV.

The measured activity was converted into a dose assuming that all emitted particles (gamma and beta) were absorbed in the tissue that accumulated ^{137} Cs.

3.1. Equipment and calibration

Calibration of the detector for measuring plant samples was done with a reference radioactive material, a matrix of silicone resin in Marinelli geometry. As a calibration source, a 1000ml mix standard of the MBSS 2 type in Marinelli geometry of the Inspectorate for ionizing radiation Czech metrological institute (Inspectorate for ionizing radiation Czech metrological institute) was used - certificate number: Cert.No: 9031-OL-159/08 total activities 41, 48 KBq on August 31, 2023 (^{241}Am, ^{109}Cd, ^{139}Ce, ^{57}Co, ^{60}Co, ^{203}Hg, ^{88}Y, ^{113}Sn, ^{85}Sr ^{137}Cs). The time of measuring the samples is 60000 s.

3.2. Examination of radioactivity by gamma spectrometry

Gamma spectrometry is today a highly developed and applicable method for monitoring and measuring the activity of samples from the environment. Gamma spectrometry makes it possible to identify radionuclides present in the sample and determine their concentration by detecting the emission of gamma photons from a specific sample. All detector systems used in γ radiation spectrometry consist of a detector and associated electro he conversion of photon energy into the kinetic energy of electrons and positrons occurs through some of the previously mentioned interactions. The result of each individual interaction is the creation of a certain amount of charge carriers, which can be detected and measured. The generated charge carriers are collected on the detector electrodes with the help of an electric field, and the collection time itself depends on the type and geometry of the detector. In the case of gas detectors, the collection time is a few milliseconds, while with semiconductor detectors it is only a few nanoseconds.

The main characteristics of the detector come from the density, volume and atomic number of the material from which it is made. It is these quantities that are related to the probability of interaction of x radiation with the detector material and the probability that the entire energy of the gamma radiation photon will be deposited in the detector volume. [5]

Semiconductor detectors, as the most widely used detectors, can be viewed as ionization chambers in the solid state, which differ according to the density and dimension of their detector volume. Unlike the previously used gas detectors, the use of semiconductors provides an advantage, first of all, in the amount of energy used to create an electron-positron pair.

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In semiconductor detectors, this energy is about ten times less than the energy required to create an electron-ion pair in gas detectors. The advantage is also reflected in their quick response and small size.

The working principle is based on the passage of radiation through the depletion region, during which electron-hole pairs are created, which are quickly ejected from this region due to the effect of the existing electric field.
The resolution depends on the energy deposited in the detector, that is, at higher energies, the resolution is better.

The first semiconductor material used for the detection of gamma radiation was silicon (Si), and later germanium (Ge) was used, which had an exceptional energy resolution, which is why it was widely used in spectrometry of photon radiation.In order to improve the performance of germanium detectors, a new material known as high-purity germanium with an active volume about 1 cm wide was obtained. Due to germanium detectors, gamma spectrometry is a widely used measurement technique for determining the concentration of radionuclide activity in various samples.

3.3.Determination of radionuclide activity

The specific activity of radionuclides is determined based on the expression

$$A = \frac{N}{\varepsilon \cdot \gamma \cdot ts \cdot V(m)}$$

Equation means:

$N = Ns - \frac{ts}{tb} \cdot Nb$– corrected total area under a given photopeak of the corresponding gamma energy in the spectrum

Ns – the total area of the photo peak in the spectrum
Nb - the corresponding total area of the photo peak in the basic radiation spectrum
tb(s) – total time of basic radiation spectrum recording
ts(s) - total time of recording the spectrum of the sample
ε-efficiency on the gamma energy of the photo tip

3.4. Sample preparation and gamma emitter spectrometry methods

The preparation of plant culture samples consists of drying at room temperature, concentration, i.e. mineralization at a temperature of 450°C and closing with parafilm in order to establish a radioactive balance.

Mineralization is carried out for the purpose of concentrating plant cultures and water in order to reduce the overall measurement uncertainty of measurement and minimal detection of specific activity, but also to increase the efficiency of detection.

The sample preparation procedure was based on the recommendations of IAEA 25. [6]

The measurement geometry was determined depending on the type of samples and for that purpose a plastic Marinelli geometry of 1000 ml was used for non-mineralized plant, soil and sediment samples.

3.5. Sample preparation and gamma emitter spectrometry methods

Since the distribution of radionuclides in the soil is not uniform, the radium equivalent index $Raeq$ was determined. This index compares the specific activity of materials containing different amounts of ^{226}Ra, ^{232}Th and ^{40}K. Raeq was calculated using the following formula and expressed in Bqkg-1

$$Raeq = CRa + 1{,}43CTh + 0{,}07CK$$

The definition of Raeq is based on the assumption that a specific activity of 370 Bqkg-1 originating from ^{226}Ra, or a specific activity of 259 Bqkg-1 originating from ^{232}Th, or a specific activity of 4 810 Bqkg-1 originating from 40K, produces the same gamma dose rate.

External absorbed dose

Based on the results of measuring the activity of radionuclides in the soil, the strength of the external absorbed dose of gamma radiation 1 m above the ground, which originates from the detected radionuclides ^{226}Ra, ^{232}Th and ^{40}K in Bqkg-1, was determined using the following formula:

$$\dot{D}(nGyh{-}1) = 0.462CRa + 0.604CTh + 0.0417CK,$$

Equation means:
CRa, CTh, CK represent specific activities of ^{226}Ra, ^{232}Th and 40^{K} in soil

Calculation of the annual effective dose (DE)

The annual effective dose is determined using the formula:

$$DE\ (mSv) = 0.7(SvGy{-}1)\cdot\ 365\ (days)\ \cdot\ 24\ (h)\ \cdot\ \dot{D}(nGyh{-}1)\cdot\ 0.2$$

The formula for estimating the annual effective dose includes a coefficient of 0.7 $SvGy{-}1$, which represents the conversion of the absorbed dose in the air into an effective dose, as well as the external factor K (0.2), i.e. the time a person spends in the external environment, i.e. 20% [7]

3.6. Soil-plant transfer factor (TF)

The external hazard index Hex was calculated using the formula:

$$Hex = CU\ 370 + CTh\ 259 + CK\ 4810 \leq 1,$$

Equation means:

CU, CTh, CK represent the specific activity of ^{226}U, ^{232}Th and ^{40}K in Bqkg-1

The maximum value of Hex equal to unity corresponds to the upper recommended limit $Raeq$ of 370 Bqkg-1 [8]
The value of this index must be less than unity in order for the impact of radionuclides on the environment to be considered negligible.

3.6. Soil-plant transfer factor (TF)

Table 1. Average values of soil-plant transfer factors of individual elements in the soil of the wider area of Belgrade area:

Element	**Plant community**	**Parts of plant**	**Mean and standard deviation**
Be	Meadow	Halm	4,2±1,3
Cs	Grass	Halm	4,2±1,3
	Meadow	Halm	6,3±2,4
	Moss	Halm	7,5±1,8
K	Meadow	Halm	3,1±1.8

Pb	Grass	Halm	9,2±4,8
	Meadow	Halm	1,3±4
Ra	Grass	Halm	3,1±1.11
	Meadow	Halm	7,1±2,6
	Moss	Halm	6,9±4,5
Th	Lichen	Halm	4,2±3,1
	Meadow	Halm	9.9±5,5
U	Moss	Halm	1,7±9,4
	Grass	Halm	4,6±5,3
	Meadow	Halm	3,6±5,9

The International Atomic Energy Agency (1994) defined the transfer factor as the ratio of the specific activity of radionuclides in dry plant mass to the specific activity of radionuclides in soil. The soil-plant transfer factor was calculated for ^{226}Ra, ^{232}Th, ^{40}K, ^{238}U, ^{235}U, ^{137}Cs and ^{210}Pb using the formula:

$$TF=\frac{radionuclide\,activity\,per\,unit\,of\,dry\,mass\,(Bqkg-1)}{radionuclide\,activity\,per\,unit\,of\,dry\,soil\,(Bqkg-1)}$$

The quantification of radionuclide uptake by plants was monitored through the "soil-plant" transfer factor for all radionuclides, and the results are presented in Table 1. The transfer factor research results for ^{40}K are higher than for ^{226}Ra and ^{232}Th, which is in accordance with literature values. The maximum value of TF-^{40}K was found in the samples from the control area "Makis", TF-^{226}Ra in the samples of Zvezdara forest. In the case of ^{210}Pb, higher values of the transfer factor were obtained, which is in accordance with the fact that the plant, in addition to absorbing this radionuclide from the soil, can also absorb it through the leaves.

The transfer factor for lead in the moss samples from Avala of 1.75 is similar to the values obtained from other localities, while in the twigs it is 1.38. A lower value of TF-^{137} Cs was found in Cetraria islandica in this test compared to Pseudevenia furfuracea, which determined that the mean value of Tf in the leaves is 0.019, and in the soil 0.036. Lichens from the area of Kosutnjak show different transfer factor values, and also in a large number of samples due to very low specific activity, Tf could not be determined. Potassium, as one of the most widespread elements in nature, is part of the method of monitoring radionuclides and other indicators of the state of the environment in order to optimally manage areas covered by forest vegetation.

4. RESULTS AND DISCUSSION

4.1 Determination of activity correlation

Table 2 presents the results of the activity level of natural and artificial radionuclides in moss samples collected in the area of Belgrade parks and forests (Kosutnjak, Makiš, Zvezdara forest, Avala, Topcider park).Correlations of activity levels of radiocesium-137 and potassium-40, as well as some natural radionuclides, are graphically presented. Analyzing the data shown in table 2, it is obvious that most moss samples are most contaminated with radiocesium-137, compared to natural radionuclides, whose values are uniform. Of the natural radionuclides, ^{40}K is the most abundant in the moss samples from all the examined localities

Table 2. Radionuclide activity levels in moss samples (Bq/kg)

Sample	**Location**	**Radionuclide**					
		^{137}Cs	**^{134}Cs**	**^{40}K**	**^{226}Ra**	**^{238}U**	**^{232}Th**
H. splendens	Kosutnjak	117	< 0,5	229	15,6	8,2	14,6
H. cupressiforme	Kosutnjak	42,2	0,2	119	7,1	4,6	7,3
H. cupressiforme	Z.forest	105	0,5	277	26,6	8,6	22,4
S. calcicola	Kosutnjak	61,1	0,2	414	31,5	28,2	34,0
H. cupressiforme	Avala	24,6	< 0,8	319	17,7	<15,4	14,5
H. splendens	T. park	177	< 1,0	173	17,0	<19,0	15
S. purum	Z.forest	43,6	< 0,7	50,2	<13,4	<12,2	2,4
N. crispa	Makis	190	0,2	68,2	9,4	6,0	2,7
H. cupressiforme	Makis	326	1,9	120	10,3	12,8	2,1
H. splendens	Z.forest	109	< 0,7	78,2	<11,5	< 9,1	2,9

As cesium and potassium are metabolically similar elements, it should be expected that higher levels of ^{40}K activity imply lower levels of ^{137} Cs activity. The ratio of activity levels of these radionuclides in the tested samples is not such that any correlation can be established, especially in moss samples that are more contaminated with radiocesium. The same radionuclides were examined in soil samples and no dependence was found between the activity concentration of ^{137}Cs and ^{40}K [9].

Cesium came into the soil after the huge amount of precipitation and became more concentration, so its varied from place to place.The obtained results indicate the great capacity of mosses and lichens to absorb radiocesium. In addition, the examined ecosystems represent almost closed biological communities, in which the level of radioactive contamination is kept relatively stable for a long period of time.

229	117
119	42.2
277	105
414	61.1
319	24.6
173	177
50.2	43.6
68.2	190
120	1116
132	1257

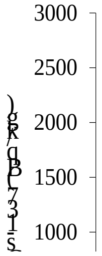

Figure 4. Dependence of ^{137}Cs activity level on ^{40}K

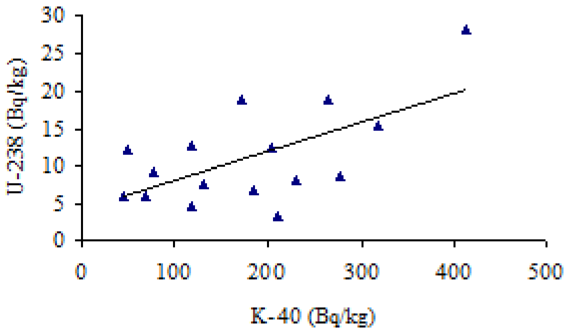

Figure 5 Dependence of ^{238}U activity level on ^{40}K

Considering the data for natural radionuclides ^{238}U, ^{226}Ra and ^{232}Th and their correlations with the activity levels of ^{40}K, a straight-line dependence of the activity levels of these radionuclides with ^{40}K is evident.

The linear dependence of ^{238}U and ^{232}Th activity levels on ^{40}K has already been investigated and confirmed in soil samples [10], and our research points to the conclusion that these regularities are also shown in bioindicator samples, such as mosses.

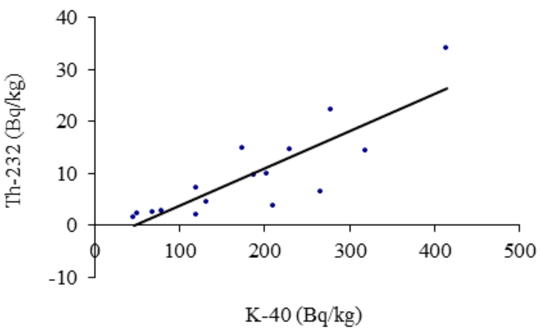

Figure 6. Dependence of ^{232}Th activity level on 40K

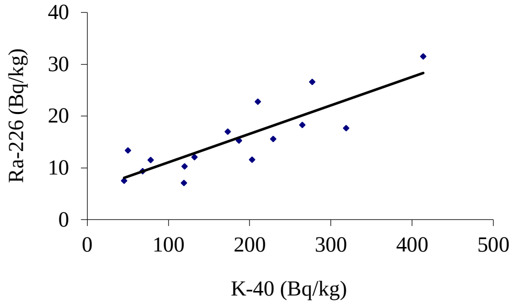

Figure 7 . Dependence of ^{226}Ra activity level on ^{40}K

By comparing the values of the strength of the absorbed doses in the bioindicators on all five ecosystems, a higher radiation load of mosses compared to lichens is observed. The difference in the degree of ^{137} Cs accumulation in mosses and lichens is caused by the difference in the morphological structure and physiological characteristics of these plant species. Mosses are characterized by a large surface-to-mass ratio (almost 10 times greater than this ratio for grass), which explains the high capacity of these species to absorb radionuclides.

Although the examined bioindicator species accumulate high activities of ^{137} Cs, the radiation load (up to 14.13 mSv/year) is below the level at which changes occur in the reproductive cycle of plants. This indicates the suitability of using mosses and lichens as bioindicator species.

When assessing the state of the ecosystem, a number of factors should be taken into account: sampling method, spatial and temporal variations for all samples and age indices of organisms. Apart from altitude, other influences are also present here. It is especially important to take into account the processes of vertical migration of radiocesium, as well as the influence of specific characteristics of the studied ecosystems associated with these processes.

Given that mosses and lichens are characterized by a wide geographical and ecological distribution of species, availability throughout the year, long life and a high degree of tolerance to pollutants, data on the radiation load caused by internal irradiation of these species can serve as a basis for evaluating different ecosystems from the aspect of radioactive contamination

All the results presented in this paper indicate that lichens are good bioindicators of environmental pollution with radiocaesium and that the activity levels of this radioactive isotope have significantly decreased since the accident in Chernobyl .Also, the results indicate that there was no new contamination with this isotope.

5. CONCLUSION

All the results presented in this paper indicate that lichens and mosses are good bioindicators of environmental pollution with radiocesium.

The activity levels of $^{137+134}Cs$ in lichens and mosses collected in the territory of Belgrade are at the level of recommended values for the territory of Serbia.

Investigations of the basic indicators of the state of the environment in the examined locations on the territory of the city of Belgrade indicate the quality of the environment, the possible degree of changes that can potentially affect humans and the entire living world.

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