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On monitoring anthropogenic airborne uranium concentrations and $^{235}\text{U}/^{238}\text{U}$ isotopic ratio by Lichen – bio-indicator technique

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Abstract

Lichens are widely used to assess the atmospheric pollution by heavy metals and radionuclides. However, few studies are available in publications on using lichens to qualitatively assess the atmospheric pollution levels. The paper presents research results applying epiphytic lichens as bio-monitors of quantitative atmospheric contamination with uranium. The observations were conducted during 2.5 years in the natural environment. Two experimental sites were used: one in the vicinity of a uranium contamination source, the other one – at a sufficient distance away to represent the background conditions. Air and lichens were sampled at both sites monthly. Epiphytic lichens *Hypogimnia physodes* were used as bio-indicators. Lichen samples were taken from various trees at about 1.5 m from the ground. Air was sampled with filters at sampling stations. The uranium content in lichen and air samples as well as isotopic mass ratios $^{235}\text{U}/^{238}\text{U}$ were measured by mass-spectrometer technique after uranium pre-extraction. Measured content of uranium were 1.45 mg kg^{-1} in lichen at $2.09\text{E}-04 \text{ } \mu\text{g m}^{-3}$ in air and 0.106 mg kg^{-1} in lichen at $1.13\text{E}-05 \text{ } \mu\text{g m}^{-3}$ in air.

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The relationship of the uranium content in atmosphere and that in lichens was determined, $C_{\text{AIR}} = \exp(1.1 \times C_{\text{LICHEN}} - 12)$.

The possibility of separate identification of natural and man-made uranium in lichens was demonstrated in principle.

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1. Introduction

There is a rich literature on the use of lichens as bio-monitors of atmospheric contamination (Conti and Cecchetti, 2001). Lichens are also good radionuclide bio-accumulators (Notter, 1988). For example, lichens were used to assess radionuclide fall-out after the Chernobyl accident (Barci et al., 1988; Seaward et al., 1988; Mihok et al., 1989; Sloof and Wolterbeek, 1992; Hofmann et al., 1993; Triulzi et al., 1996; Sawidis et al., 1997; Conti and Cecchetti, 2001; Byazrov et al., 1993). In the suburbs of St. Petersburg Russia in the samples of *Parmelia sulcata* lichens gathered in the summer of 1991 contained ^{134}Cs levels in agreement with levels in soils in the same area (Khromov, 1991; Malysheva, 1996, 1998). In Norway, the content of ^{134}Cs and ^{137}Cs in lichens was two orders higher than in vascular plants (Bretten et al., 1992; Conti and Cecchetti, 2001). Natural radionuclide decrease (self-cleaning) occurs in lichens. The average biological self-cleaning period for ^{137}Cs in lichens *Xanthoria parietina* constituted 58.6 months; demonstrating it to be the best bio-indicator of radioactive fall-out (Topcuoglu et al., 1995; Conti and Cecchetti, 2001). Lichens are distinguished by high, when compared to vascular plants, resistance to ionizing radiation, at both chronic and acute levels (Rao et al., 1977). Radiation affects the composition and density of lichen associations (Brodo, 1964; Woodwell and Whittaker, 1968). However, the studies discussed above revealed correlations between lichens and environment but found no well predictive relationship between radionuclide concentration in lichens and the atmosphere.

The mechanism of metal penetration of lichen depends on the origin of elements and, hence, on the source of their environment entry. The lichens gathered in the Balkans have been used to demonstrate that uranium contained in the lichens did not originate from weapons with depleted uranium (Loppi et al., 2003). The mechanism of nutrient accumulation by lichens is well understood (Edwards et al., 1991; Clark et al., 2000). Lichens obtain nutrients for growth and metabolism from wet and dry precipitates (Nash and Egan, 1988; Nash, 1996; Rodrigo et al., 1999) that penetrate the lichen thallus, primarily in cation form (Kershaw, 1985; Nash, 1996). Accumulation of dust particles which settle in intercellular space, can occur during the lichen thallus growth. For example, the main mechanism of uranium accumulation with lichens is the trapping of dry fall-out (Looney et al., 1985). A multitude of dust particles accumulated in the inter-cellular space in the core of lichens; the composition of the particles corresponds to the composition of particles

found on the thallus surface (Garty et al., 1979; Richardson et al., 1985). Contaminants can deposit onto lichens directly or indirectly from fog, dew, sedimentation or gas absorption (Knops et al., 1991; Conti and Cecchetti, 2001). Therefore, when examining the problem of using lichens to assess atmospheric contamination, it is essential to understand the mechanism of metal absorption and accumulation by lichens.

This paper presents the study of the application of lichens to the quantitative assessment of atmospheric contamination with radionuclides, in particular with uranium. At low concentration of man-made uranium in the air, accumulation of man-made and natural uranium in lichens was shown to proceed by the different pathways.

2. Experimental layout

This study was performed in the central region of Russia from 1999 through 2001. In the study continuous point sources of uranium emission to the atmosphere have existed for more than 40 years. Two experimental sites were selected: one in the vicinity of a uranium emission source, the other one – at the distance of about 30 km in the predominantly upwind direction. The sites were named as “contaminated” and “clean”. The sampling area of sites was 100×100 m. The prevailing type of vegetation at the sites is mixed forest, consisting of pines, birches, aspens, limes and fir-trees. At each of the sites, stationary air sampling stations were set up to sample air aerosols for uranium content. The epiphytic lichen *Hypogimnia physodes* (L.) Nyl was used as a bio-indicator.

Lichens were sampled once a month. Lichen samples were taken from tree trunks at a height of 1.5 m from the ground surface along the entire circumference of the tree. Sampling was carried out throughout each experimental site. After collection lichen samples were washed in bi-distilled water, to separate the precipitated dust and other foreign particles (Stone et al., 1995), and dried. The samples were carbonized with nitric acid with gradual heating up to the temperature of $+350$ °C. To verify the carbonized step of lichen the IAEA-336 Reference Material (RM) was used (Stone et al., 1995). Because U is not in the IAEA-336 RM the validation was made using Cr as a surrogate. The data obtained showed a good agreement between the analysis result (Cr content 1.04 ± 0.21 mg kg⁻¹) and the recommended value in IAEA-336 RM (Cr content 0.89–1.23 mg kg⁻¹). This indicates that the systematic error of the carbonized technique isn't significant. The carbonized lichen samples were used for the mass-spectrometer measurement of uranium after mineralization of samples and extraction.

Air was sampled by continuous pumping of atmospheric air through perchlorovinyl depth-type filters with ultra thin (1.5–2.5 μm) fibers. These filters are highly effective in trapping the aerosol particles we were interested. The parameters of aerosol sampler were: filter surface – 100 cm², rate of air pumping through the filter – from 70 to 75 l min⁻¹, sampling period (filter replacement frequency) 30 days, volume of air filtered – from 2800 to 3200 m³. Air sampling was made continuously;

the U content was measured monthly. The air sampling technique was developed in compliance with IAEA instructions (IAEA, 1989).

The uranium content in air filters and carbonized lichen samples was determined by mass-spectrometry method of isotopic dilution (Sisoev, 1993). The method of isotopic dilution is based on measuring the isotopic composition of an element in a sample after an exactly known amount of a standard has been introduced in it. In this work highly enriched ^{233}U (isotope content was 99.9728 atom. percent) was used as a standard. A certain amount of the standard was introduced into samples of filters and carbonized lichens. The amount of standard was chosen so that the isotope mass ratio $^{238}\text{U}/^{233}\text{U}$ would be within 0.1–10.0. Then the samples of filters and carbonized lichens were mineralized at the temperature of $+(500\text{--}600)^\circ\text{C}$. The ash was processed with a mixture of HNO_3 and HF (1:3) acids to remove Si. Uranium was extracted from the produced residue with ethyl acetate from 1 M of HNO_3 saturated with ammonium nitrate (Vinogradov, 1962). Measurements of isotopic uranium ratios were performed using the mass-spectrometer with the surface ionization source. To measure the weight of uranium in sample, this ratio is multiplied by a relationship of isotope atomic masses ($238/233 = 1.0215$) and by a mass of the introduced standard. Using of isotopic dilution method allows taking into account and minimizing the sample preparation error, because ^{233}U standard participate in sample preparation procedures too. The accuracy of the indicator dosage was not less than $\pm 2\%$. For mass-spectrometry analysis a random component of error is specified mainly by the time during which the statistics are being acquired. In this work the random component of the error was less than 1%. Thus, for air filters and for lichens the total error of U measured was 2%.

The experiments were conducted from 1999 through 2001.

3. Results and discussion

The values of U content in atmosphere and lichens for the entire period are presented in Table 1. The results show that the U content in the atmosphere in our study does not exceed the $0.075\ \mu\text{g m}^{-3}$ value considered by Canadian scientists as the standard for natural U being set based on potential toxicity to plants (Brechignac and Howard, 2001).

The non-normal nature of data inhibits application of the traditional *t*-criterion (test of differences). Therefore, when analyzing the significance of difference in U content in lichens and atmosphere for “clean” and “contaminated” sites we used non-parametric tests of difference between independent groups (Wald–Wolfowitz, Mann–Whitney tests and two-sample Kolmogorov–Smirnov test) (StatSoft Inc., 2000). All the tests demonstrated statistically significant differences in U content in the air and lichens sampled from “clean” and “contaminated” sites (Table 2). Plots of U content in lichens as the function of the U content in atmosphere obtained during the entire observation period 1999–2001, along with the confidence interval for regression line are presented in Fig. 1. The confident interval was determined

Table 1

Averaged U content in air and lichens from “clean” and “contaminated” sites

Parameter	U content			
	“Contaminated” site		“Clean” site	
	Air ($\mu\text{g m}^{-3}$)	Lichen (mg kg^{-1})	Air ($\mu\text{g m}^{-3}$)	Lichen (mg kg^{-1})
Average	2.09E-04	1.45	1.13E-05	1.06E-01
Minimum	3.63E-06	6.59E-01	1.35E-06	5.2E-02
Maximum	3.56E-03	2.41	3.33E-05	6.37E-01
Median	5.55E-05	1.46	1.09E-05	7.7E-02
Standard deviation (σ)	6.41E-04	4.02E-01	6.42E-06	1.12E-01
Amount of observations	34	30	27	29
Variation (%)	307	28	57	106

at $P = 0.95$ for the regression line for entire observation period (1999–2001). The relationship for the year 1999–2001 can be described as:

$$C_{\text{AIR}} \approx \exp(1.1 \times C_{\text{LICHEN}} - 12),$$

where C_{AIR} – is the U content in the air ($\mu\text{g m}^{-3}$),

C_{LICHEN} – is the U content in lichens (mg kg^{-1}).

The lichen-indication method was examined to monitor the atmospheric contamination with U. To this end, the regression dependences for years 1999–2000 were constructed (Fig. 1). To test the method we used the data obtained in 2001. Using the U content values measured in lichens during this period we predicted the regression dependence of the U content in the atmosphere. The obtained values were compared with the values measured by the air sampling stations. The comparison of values is presented in Table 3 and Fig. 2.

The results obtained by two methods basically differ by no more than 2.4 times for “clean” site and 6 times for the “contaminated” site. The highest difference was recorded for the point highlighted with the marker in Figs. 1 and 2 and Table 3. At this point, the lichen-indication method records the value of U content in the air by a factor of ~ 132 lower than the sampling station. At the “contaminated” site the scattering of the U concentration in aerosol samples is higher than that in the lichen samples. Probably, it is associated with biochemical features of lichens, which cannot absorb admixtures immediately or in great amounts from the atmosphere and with physical–chemical properties of uranium particles that can be in the form difficult to

Table 2

Results of significance analysis of the difference between the U content in the atmosphere and lichens sampled from the “clean” and “contaminated” sites

Subject of investigation	p -Level		
	Wald–Wolfowitz runs test	Mann–Whitney U test	Kolmogorov–Smirnov test
Atmosphere	2.5E-06	2.0E-09	<0.001
Lichens	2.0E-13	4.3E-11	<0.001

Note: Difference is significance when “ p -level” <0.05.

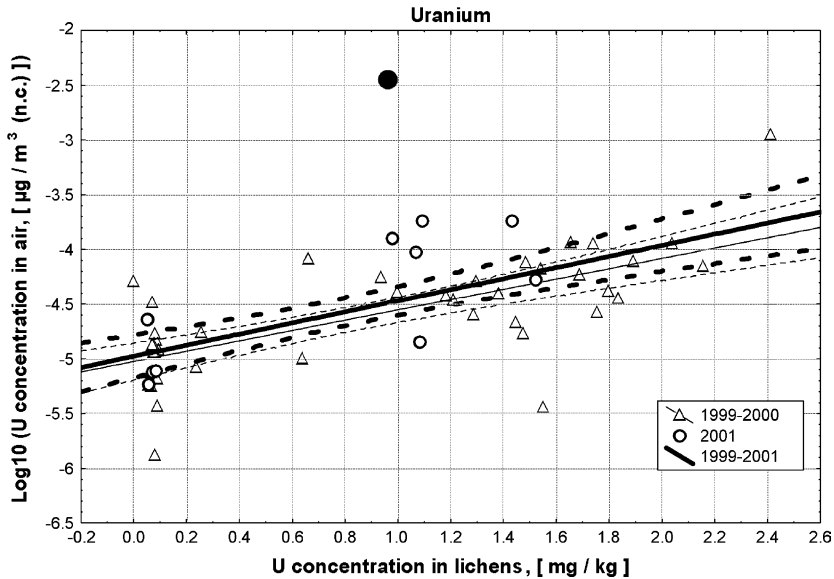


Fig. 1. Regression dependences of the U content in lichens and in the air.

assimilating lichens. In addition the concentration of U in lichen is averaged over longer period of time as compared to the air aerosol samples (one month). Perhaps, lichen samples also contain “older” contamination. U absorption with lichens can be somewhat affected by meteorological conditions such as the wind direction and

Table 3

Comparison of U assessing results by using the lichen-indication method and sampling stations

Experimental site	U content in the air (µg m ⁻³)		Differences between the observed and predicted U concentrations	
	Observed concentration	Concentration predicted by model	In absolute units (µg m ⁻³)	In relative units (> or < by a factor of <i>n</i> [*])
Clean site	5.94E-06	1.00E-05	-4.09E-06	<1.68
	7.63E-06	1.02E-05	-2.54E-06	<1.34
	7.77E-06	1.03E-05	-2.57E-06	<1.33
	2.32E-05	9.99E-06	1.32E-05	>2.32
Contaminated site	5.41E-05	4.92E-05	4.84E-06	>1.10
	1.44E-05	3.05E-05	-1.61E-05	<2.12
	9.55E-05	3.02E-05	6.53E-05	>3.16
	1.27E-04	2.73E-05	1.00E-04	>4.65
	1.82E-04	4.46E-05	1.37E-04	>4.08
	1.84E-04	3.09E-05	1.53E-04	>5.95
	3.56E-03	2.70E-05	3.53E-03	>131.85

Note: “> (<) *n*” means that the value by *n* times higher (lower) was measured by using the sampling station than by lichen-indication method.

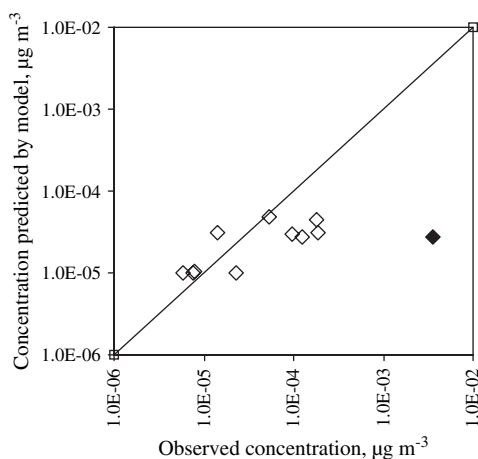


Fig. 2. Comparison of observed U concentration and concentration predicted by model.

velocity. Humidity also determines the time of particle's presence in air. All these factors can be regarded as limitations of the lichen-indication method and argue that lichens are good bio-indicators for investigating a long-term air pollution effect in a given region.

We also attempted to determine the uranium of natural and man-made origin in the lichens. Natural uranium is known to enter epiphytic lichens primarily with dust particles (Looney et al., 1985). At our experimental site the dust particles were mainly fine sand silicon compounds. Within dust micro-particles, metals are considered to have restricted solubility and accessibility for metabolic processes in lichens (Brown and Beckett, 1985; Puckett, 1985). Hence, dust particles being in inter-particle spaces of lichens do not actually change their chemical composition during the long-term lifetime of lichens. In case of low atmospheric contamination, when man-made uranium is present in environment solutions in the form of cations, it enters lichens in the form accessible for cation-exchange and metabolic processes. (Kershaw, 1985; Nash, 1996). This was the case at the “clean” site. Such U after lichen incineration is present in compositions soluble by 1 M of HNO₃. When processing the lichen ash with 1 M of HNO₃ solution, the man-made uranium will transfer into the solution and natural uranium will remain basically in the insoluble residue of dust micro-particles consisting of silicon compounds. We estimated the content of uranium and its isotopic ratio in the solution and insoluble ash residue of lichens taken from the “clean” site. The results are presented in Table 4. For comparison purposes, isotopic ratios are presented for atmospheric samples taken simultaneously from the “clean” and “contaminated” sites and for natural U.

4. Conclusion

We have studied possible monitoring of man-made atmosphere uranium by lichen-indication technique illustrated with uranium in natural environment. When

Table 4

U content in lichens, the $^{235}\text{U}/^{238}\text{U}$ isotope mass ratio in lichen samples and air filters

Sample		U content in sample	$^{235}\text{U}/^{238}\text{U}$
Lichens (mg kg^{-1} of dry weight)	Extract of 1 M of HNO_3 from ash	0.062	0.0050
	Insoluble residue of ash	0.029	0.0072
Atmosphere ($\mu\text{g m}^{-3}$)	“Clean” site	(1.22–0.86) E-05	0.0068–0.0078
	“Contaminated” site	(2.50–11.7) E-05	0.0050–0.0055
Natural U (Loppi et al., 2003)		–	0.0072

determining the U content in the atmosphere, the lichen-indication method yields good results and can be used to diagnose a long-term atmospheric contamination with U and to identify the contamination source. Factors, such as the wind direction and velocity and humidity can limit the usefulness of the lichen-indication method.

The possibility of separate assessment of man-made and natural uranium in lichens was illustrated in principle. However, these results represent an initial stage of study. The technique is to be further developed in order to isolate elements, Uranium in the soluble form in the lichen thallus, and incorporated in dust micro-particles, from lichens. The further investigation of uranium in environmental (soil, water, airborne) is necessary.

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