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Accumulation of <sup>137</sup>Cs by Thallus of Epiphytic Lichen *Hypogymnia physodes* (L.) Nyl on Different Trunk Height in Pine Stands Oleksandr Orlov<sup>1\*</sup>, Oleh Zhukovskyi<sup>2</sup>, Ihor Ivaniuk<sup>3</sup>, Volodymyr Ustimenko<sup>4</sup>, Vasyl Martynenko<sup>5</sup> <sup>1</sup>State Institution "Institute of Environment Geochemistry of the NAS of Ukraine" 03142, 34a Academician Palladin Ave., Kyiv, Ukraine <sup>2</sup>Poliskyi Branch of the Ukrainian Research Institute of Forestry and Forest Melioration named after G. M. Vysotsky 10004, 2 Neskorenykh Str., v. Dovzhyk, Zhytomyr region, Ukraine <sup>3</sup>Malyn Applied College 11643, 1 M. Maklai Str., v. Hamarnia, Zhytomyr region, Ukraine <sup>4</sup>Poliskyi National University 10002, 7 Staryi Ave., Zhytomyr, Ukraine <sup>5</sup>Nature Reserve "Drevlianskiy" 11401, 188 Zamkova Str., v. Narodychy, Zhytomyr region, Ukraine

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Orlov, O., Zhukovskyi, O., Ivaniuk, I., Ustimenko, V., & Martynenko, V. (2022). Accumulation of <sup>137</sup>Cs by thallus of epiphytic lichen *Hypogymnia physodes* (L.) Nyl on different trunk height in pine stands. *Scientific Horizons*, 25(5), 48-59. Abstract. Lichens are considered by researchers as bioindicators of environmental pollution by artificial radionuclides, including <sup>137</sup>Cs. One of these bioindicator species is epiphytic lichen Hypogymnia physodes (L.) Nyl, the use of which for radioecological monitoring can be modified by several factors: tree species, placement on the tree (branches - trunk), height of sampling, etc. The purpose of this study was to investigate the specific activity of <sup>137</sup>Cs in thalli of hypogymnia in pine stands: on trunks of Pinus sylvestris L. and Betula pendula Roth at different heights of sampling in 3 height ranges: 0-65, 65-130, and 130-195 cm, and to analyse the intensity of radionuclide accumulation in the "lichen thallus - tree bark" link for both tree species in all height ranges. The study was conducted in August 2021 in the Korosten district of the Zhytomyr Oblast, Zhytomyr Polissia, on 4 test plots, in the Drevlianskyi nature reserve, mainly in middle-aged pine stands with an admixture of birch, where 130 samples of soil, tree bark, and lichen were taken. Specific activity of <sup>137</sup>Cs in the samples was measured using a SEG-001 "AKP-s"-150 gamma-ray spectrometer with a BDEG-20R2 scintillation detector (NaI(Tl). It was demonstrated that the content of <sup>137</sup>Cs in the thalli of Hypogymnia physodes at different altitude ranges did not differ statistically significantly at the 95% confidence level in all test plots on pine. A preliminary conclusion was made on the possibility of sampling Hypogymnia physodes thalli on pine trunks in the height range of 0-195 cm; however, for the convenience of sampling, it is recommended to perform sampling within 130-195 cm. It has been proven that both the specific activity of <sup>137</sup>Cs in *Hypogymnia physodes* thalli and the intensity of  $^{137}$ Cs accumulation in the chain "Hypogymnia physodes – tree bark" are approximately 2 times higher on pine compared to birch. It was found that on a birch, the content of <sup>137</sup>Cs in lichen between the height ranges of 0-65 and 65-130 cm, and 0-65 and 130-195 cm differs significantly at the 95% confidence level; therefore, it is recommended to sample Hypogymnia physodes on a birch in the range of 65-130 cm

**Keywords**: *Pinus sylvestris* L., *Betula pendula* Roth, deciduous lichen, tree bark, accumulation coefficient, specific activity, radionuclide



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#### INTRODUCTION

Lichens, as symbiotic organisms with an atmospheric feeding strategy, have long been considered by researchers as biological indicators of environmental pollution by a wide range of pollutants found in the air (Ferry *et al.*, 1973; Bargagli *et al.*, 1991; Koroleva & Revunkov, 2017), and as convenient test objects for their monitoring (Richardson, 1994; Shukla *et al.*, 2014), including radioecological ones (Seaward, 1995; Biazrov, 2005; Kondratiuk, 2008).

From the late 1950s to the present, lichens have also been used to monitor radioactive contamination of various isotopic compositions and sources of entry into the biosphere. Radioecologists have demonstrated the high efficiency of using lichens to monitor global radionuclide precipitation as a result of nuclear weapons testing in the open earth environment (Eberhardt, 1964; Nevstrueva et al., 1967), as well as migrations of technogenic radionuclides, primarily <sup>137</sup>Cs, food chains, e.g., "lichen - reindeer - human" (Tuominen & Jaakkola, 1973; Mattsson, 1975); in the monitoring zone around nuclear power plants during their regular operation in the Nordic countries (Ingemansson et al., 1981) and the former Soviet Union (Nifontova & Kulikov, 1981; Nifontova & Kulikov, 1984; Bossew et al., 2000), as well as after nuclear accidents, the largest of which is Chernobyl. Notably, lichens are characterised by high resistance to a wide range of pollutants, including radionuclides (Richardson, 1994; Biazrov, 2005; Suno et al., 2021).

To monitor the radioactive fallout of <sup>137</sup>Cs after the Chernobyl disaster, the researchers used as test objects different types of lichens of different morphological structure – leafy, bushy, powdery, crusty, etc., as well as of different ecological and topical groups - epiphytic, epigeal, epilithic and others (Biazrov, 2005). The choice of indicator species in these studies was largely determined by the natural conditions of the territory and the ability to select the required number of lichen thalli of a particular species for the analysis of <sup>137</sup>Cs content in samples and getting a representative sample. The researchers recognised *Hypogymnia Physodes* (L.) Nyl, which belongs to the Parmeliaceae family (hereinafter – hypogymnia), as one of such indicator species. It is an epiphytic leafy lichen characterised by a wide geographical range (forests of the boreal and temperate zones of the North hemisphere), with a considerable ecological amplitude, grows on the trunks and branches of many types of trees, including Scots pine (Pinus sylvestris L.) (hereinafter – pine) and silver birch (Betula pendula Roth) (hereinafter - birch), which are common in Ukrainian Polissia and Forest Steppe.

The advantages of this species of lichen when used as a test object are as follows: ease of identification in the field; the formation of multi-year macroscopic thalli available for observation and sampling throughout the year; growth in large numbers on tree trunks up to 2 m high; relatively insignificant dissection of the thalli, which allows measuring their area quite accurately; the convenience of sampling thallium samples, which are easily separated from the substrate – tree bark, branches, dead wood, etc.; the availability of a methodology for estimating the age of a particular thallus and its annual growth; direct absorption of <sup>137</sup>Cs from dry (dust) and wet (rain, snow) aerial precipitation, as well as crown and trunk run-off, which can be parameterised; availability of special scales for quantifying biomonitoring results obtained with lichens (Cecconi *et al.*, 2019).

The purpose of this study was to identify the influence of the sampling height of the indicator species – the leafy epiphytic lichen hypogymnia on the content of <sup>137</sup>Cs in its thalli – to determine the optimal sampling height in the long-term monitoring of radioactive contamination of forest ecosystem components.

The task was to study the specific activity of <sup>137</sup>Cs in the hypogymnia thalli in pine stands: on the trunks of Scots pine and hanging birch at different sampling heights in 3 height ranges: 0-65 cm, 65-130 cm, and 130-195 cm; to analyse the intensity of radionuclide accumulation in the link "lichen thalli – tree bark" for both tree species in all altitude ranges.

The scientific originality of this study is that for the first time, statistically reliable data were obtained on the effect of the height of the hypogymnia sample selection on the content of <sup>137</sup>Cs in them, and the optimal height of the sampling was determined.

### LITERATURE REVIEW

Before the Chernobyl disaster, the <sup>137</sup>Cs content in hypogymnia thalli was mainly determined by global radionuclide precipitation. On the territory of the Central Urals (1981), specific activity of <sup>137</sup>Cs in hypogymnia thalli ranged within 480-750 Bq·kg<sup>-1</sup> (Nifontova, 1998); on the territory of Lower Austria at an altitude of 720 m above sea level (1981) – 115-200 Bq·kg<sup>-1</sup> (Eckl *et al.,* 1984), in northern Greece (1985) – 130 Bq·kg<sup>-1</sup> (Sawidis *et al.,* 1997).

The Chernobyl disaster radically changed the levels of anthropogenic radionuclides in the biosphere, which found a clear imprint in specific activity of <sup>137</sup>Cs in lichen thalli, including hypogymnia. According to L.G. Biazrov (2005), the specific activity of <sup>137</sup>Cs in lichen thalli in the exclusion zone of the Chernobyl Nuclear Power Plant in 1986 increased by 10<sup>2</sup>-10<sup>4</sup> times compared to pre-accident levels. However, even at a considerable distance from the Chernobyl NPP, namely along the western trail of emergency radioactive fallout in 1986, the specific activity of <sup>137</sup>Cs in hypogymnia thalli increased considerably: in Poland – by 10-100 times (up to 36.6 kBq·kg<sup>-1</sup>) (Seaward et al., 1988), in Romania (Carpathians) – about 100 times (up to 88-211 kBq·kg<sup>-1</sup>) (Bartok & Mocsy, 1990). In 1986, after the Chernobyl accident in Germany (Bavaria), the specific activity of the mentioned radionuclide in hypogymnia thalli reached 9.6 kBq·kg<sup>-1</sup> (Heinrich,1987), in Austria at different altitudes of the Alps – from 11-13. 2 kBq·kg<sup>-1</sup> (Hofmann *et al.*, 1993) to 79.6 kBq·kg<sup>-1</sup> (Heinrich, 1987), in central Norway – 8.5-38.8 kBq·kg<sup>-1</sup> (Steinnes & Njastad, 1993), in the Urals, near the location of the Beloyarsk NPP – 15-25 kBq·kg<sup>-1</sup> (Nifontova & Kulikov, 1990). Sharp increase in the specific activity of <sup>137</sup>Cs was also observed in hypogymnia thalli after the Fukushima NPP accident (Dohi *et al.*, 2021).

In Ukraine, after the Chernobyl accident, the levels of <sup>137</sup>Cs content in lichen thalli, including hypogymnia, have also been studied. However, these studies were fragmented, being conducted in different years, different biotopes, using different methods, and under different radiation conditions, so the results obtained are difficult to compare. O.B. Blum (2001) cited data that in 1991, the specific activity of <sup>137</sup>Cs in the hypogymnia thallus in the 5-km zone of the Chernobyl NPP reached 470-1,061 kBq·kg<sup>-1</sup>, and 60 km from the accident unit - 1,253-1,631 Bq·kg<sup>-1</sup>. In 2020, in Zhytomyr Polissia, in a wet pine forest (B3) with a soil pollution density of 138.99  $\pm$  11.091 kBq·m<sup>-2</sup>, the specific activity of <sup>137</sup>Cs in the hypogymnia thalli on pine trunks was 14.7±0.80 kBq·kg<sup>-1</sup>, and in dry lichen barren (A1) at 17.7± 0.68 kBq·m<sup>-2</sup>-954±89.7 Bq·kq<sup>-1</sup> (Pavlenko & Orlov, 2020).

However, when lichens are used in forest biogeocoenoses as test objects of radioecological monitoring, there are several factors that can considerably modify the results of observations, e.g., the specific activity of <sup>137</sup>Cs in hypogymnia thalli, which should be considered in the process of selecting lichen samples (Orlov & Krasnov, 2007). Thus, for epiphytic leafy lichens, it was demonstrated that the content of heavy metals increases from the periphery of the thallus to its centre, i.e., it is maximal in the oldest parts (Seaward, 1980; Wang *et al.*, 1997; Nimis *et al.*, 2001). Therefore, these researchers proposed for biomonitoring of heavy metals to use thalli of foliaceous lichens in a certain size range, which generally corresponds to their certain age. Researchers F. Gailey & O. Lloyd (1986) suggested using hypogymnia thalli with a diameter of 0.8 to 2 cm. The Italian monitoring protocol for heavy metals and radionuclides includes the sampling of not the whole thalli of leafy lichens, but only their peripheral parts. According to the researchers, this part of lichens more accurately characterises the content of pollutants in the air as of the current period (Nimis et al., 2001).

Among the modifying factors of the content of atmospheric pollutants, including radionuclides, in the thalli of epiphytic lichens, the researchers attributed the location of the thalli on a certain part of the tree (Wang *et al.*, 1997). According to their data, the content of radionuclides in the same species of lichen on tree trunks was 2-3 times lower compared to a similar indicator obtained for lichen on branches in the tree crown.

According to the results of the analytical review, the authors of this paper did not find any publications on the influence of the height of the hypogymnia sample selection on the content of  $^{137}$ Cs in it.

## MATERIALS AND METHODS

The study was conducted in August 2021 in the Korostensky District of Zhytomyr Oblast. Four trial plots were laid out in the Narodytskyi branch of the Drevlianskyi Nature Reserve, their brief characteristics are presented in Table 1.

Table 1. Brief description of test areas									
Test area No. (block/ allocation)	Geographical coordinates	Stand composition/ origin	Age, years	Height, m	Diameter, cm	Density	Forest type	Quality class	Density of soil contamination <sup>137</sup> Cs, kBq·m <sup>-2</sup>
<b>TA-1</b> (11/5)	51°10'5410"N 29°06'1315"E	10 Scots pine/artificial	52	19	23	0.94	B <sub>2</sub> OP	Ι	133.4±8.92
<b>TA-2</b> (94/2)	51°13'6449"N 29°07'2946"E	10 Scots pine, ind. Silver birch/artificial	56	16	20	0.95	A <sub>2</sub> P	11	113.1±9.77
<b>TA-3</b> (98/3)	51°14'1920"N 29°13'0947"E	10 Scots pine, ind. Silver birch/natural	91	24	34	0.80	A <sub>2</sub> P	11	198.6±6.93
<b>TA-4</b> (27/4)	51°10'3220"N 29°06'2738"E	10 Scots pine, ind. Silver birch/artificial	61	18	22	0.92	A <sub>2</sub> P	II	158.2±5.16

The data in Table 1 show that the test areas were laid out in the predominant forest types of the Zhytomyr Polissia – fresh pine forest ( $A_2P$ ) and fresh oak-pine subforest ( $B_2OP$ ), were highly dense (0.80-0.95), were described by considerable productivity – I-II quality classes. In all areas, the coenosis of pine forest with green moss (*Pinetum hylocomiosum*) was represented. The density of  $^{137}\text{Cs}$  soil contamination in the experimental areas was in the range of 113.1-198.6 kBq·m-².

On each trial area, 3 pine trees were found, and, if available, birch trees close to the average. Their trunks were marked with a white cord in the height ranges: 0-65 cm, 65-130 cm, and 130-195 cm (Fig. 1). From each height range, samples of hypogymnia thalli were taken

evenly over the entire surface of the trunk, they were carefully cleaned from the bark, and samples of the upper layers of the bark of the tree were taken from the same ranges. In the projection of the crown of each of the trees under study, according to the standard method (SOU 74.14-37-425:2006, 2006), soil samples were

taken – with a cylindrical drill, 5 cm in diameter, without forest litter, to a depth of 20 cm, in 5 repetitions, after which these samples were mixed, and an aliquot was taken – a collective soil sample for radiation studies, with a volume of  $1,000 \text{ cm}^3$ .



Figure 1. Marking of the pine trunk into height ranges for sampling

In the measurement laboratory of the Polissia National University, hypogymnia, bark and soil samples were dried at a temperature of +80°C for 96 hours, after which the hypogymnia and bark samples were ground on an LMT-2 laboratory mill, and the soil - on a PRH-1 sample preparation machine. In the laboratory of radiology of the Polissky branch of Ukrainian Research Institute of Forestry and Forest Melioration, the soil was placed in Marinelli vessels with a volume of 1,000 cm<sup>3</sup>, bark – in calibrated vessels with a volume of 135 ml ("Denta"), hypogymnia – in calibrated vessels with a volume of 80 ml (soil weighing bottle), after which they were weighed on analytical ANG-50C scales. The specific activity of <sup>137</sup>Cs in the samples was measured using a SEG-001 "AKP-S"-150 gamma spectrometer with a BDEG-20R2 scintillation detector (NaI(Tl). The relative measurement error of the mentioned indicator did not exceed 15%.

To evaluate the intensity of  $^{137}$ Cs accumulation in the system "lichen thalli – tree bark", the accumulation coefficient (AC) (=proportionality coefficient) was determined – as the ratio of the specific activity of  $^{137}$ Cs in the hypogymnia thalli (Bk·kg<sup>-1</sup>) to the specific activity of  $^{137}$ Cs in the tree bark (Bq·kg<sup>-1</sup>) (Strand *et al.*, 2009).

Statistical analysis of the data was carried out in the Excel package using generally accepted methods of variational statistics (Lakin, 1973). Average values and simple statistics of the parameters under study for each of the height ranges were found on each trial area for 3 trees of the same breed, the statistical significance of the difference in the obtained data was determined according to the method of univariate analysis of variance per Fisher's test.

Notably, the use of hypogymnia as a bioindicator for detecting radioactive contamination has recently received little attention in the scientific literature. In this regard, the authors of this study chose a source base that describes the development of the use of hypogymnia in different periods, namely after the nuclear tests of the 1960s and 1970s and before the Chernobyl disaster, in the period between the accidents at the Chernobyl NPP and Fukushima, as well as after the Fukushima accident.

## **RESULTS AND DISCUSSION**

The results of statistical processing of the specific activity values of <sup>137</sup>Cs in samples of hypogymnia and tree bark were grouped according to trial areas (Table 2). The analysis of these data shows important regularities, namely it is noticeable that in the test areas with higher levels of radioactive contamination of <sup>137</sup>Cs, the values of the specific activity of the mentioned radionuclide in the hypogymnia thalli were generally higher compared to less radioactively contaminated areas. Thus, on TA-3 with an average density of soil contamination of 198.6±6.93 kBq·m<sup>-2</sup>, the content of radionuclide in the hypogymnia thalli on pine in the height range of 0-65 cm was 12,396±1,140.6Bq·kg<sup>-1</sup>, and on TA-2 at 113.1±9.77 kBq·m<sup>-2</sup>–8,565±582.7 Bq·kg<sup>-1</sup>.

<b>Table 2</b> . Statistical indicators of the specific activity of <sup>137</sup> Cs in the samples of hypogymnia and tree bark in the trial areas					
<b>6</b> • • •		Cs in samples at different a	samples at different altitude ranges, Bq·kq <sup>-1</sup>		
Sample type	Statistical indicators	h – 0-65 cm	h – 65-130 cm	h – 130-195 cm	
	·	TA-1			
Hypogymnia on a pine tree	M±m	14,069±997.6	15,066±749.4	15,645±1,112.6	
	Std	1,727.9	1,298.0	1,927.0	
	V, %	12.29	8.62	12.32	
	P, %	7.09	4.97	7.11	
	min.	12,200	13,691	13,713	
	max.	15,608	16,270	17,567	
Pine bark	M±m	2,052±456.9	1,877±402.8	2,056±549.9	
	Std	791.3	697.6	952.4	
	V, %	38.56	37.16	46.32	
	P, %	22.26	21.46	26.74	
	min.	1,543	1,381	1,363	
	max.	2,964	2,675	3,142	
		TA-2			
Hypogymnia on a pine tree	M±m	8,565±582.7	11,049±874.6	10,615±851.1	
	Std	1,009.3	1,514.9	1,474.1	
	V, %	11.78	13.71	13.89	
	P, %	6.80	7.92	8.02	
	min.	7,402	9,397	9,000	
	max.	9,215	12,373	11,888	
Pine bark	M±m	1,887±171.9	1,724±216.4	1,534±101.4	
	Std	297.7	373.9	175.7	
	V, %	15.77	21.74	11.45	
	P, %	9.11	12.55	6.61	
	min.	1,571	1,340	1,350	
	max.	2,162	2,089	1,700	
Hypogymnia on a birch	M±m	3,934±450.4	4,022±231.2	3,919±103.3	
	Std	780.1	400.4	179.0	
	V, %	19.83	9.96	4.57	
	P, %	11.45	5.75	2.64	
	min.	3412	3761	3716	
	max.	4,831	4,483	4,054	
Birch bark	M±m	1,986±394.2	1,381±178.8	976±60.0	
	Std	682.8	309.6	104.0	
	V, %	34.39	22.43	10.65	
	P, %	19.85	12.95	6.15	
	min.	1,204	1,027	886	
	max.	2,466	1,603	1,090	
		TA-3			
Hypogymnia on a pine tree	M±m	12,396±1,140.6	14,797±1,102.9	14,099±1,853.6	
·	Std	1,975.6	1,910.3	3,210.5	
	V, %	15.94	12.91	22.77	
	P, %	9.20	7.45	13.15	
	min.	10,862	12,856	11.878	

0.007	
Table 2,	Continued

Council a forma	Charlie Lindiana	Specific activity of $^{\rm 137}\rm Cs$ in samples at different altitude ranges, Bq·kg $^{\rm 1}$				
Sample type	Statistical indicators	h – 0-65 cm	h – 65-130 cm	h – 130-195 cm		
		TA-1				
	max.	14,625	16,675	17,780		
Pine bark	M±m	2,395±251.3	2,889±368.1	3,262±475.8		
	Std	435.3	637.6	824.1		
	V, %	18.18	22.07	25.26		
	P, %	10.50	12.74	14.59		
	min.	1,893	2,155	2,603		
	max.	2,673	3,306	4,186		
Hypogymnia on a birch	M±m	5,957±195.8	4,898±210.3	4,032±224.5		
	Std	339.1	364.3	388.9		
	V, %	5.71	7.44	9.64		
	P, %	3.30	4.29	5.57		
	min.	5,692	4,577	3,690		
	max.	6,324	5,294	4,455		
Birch bark	M±m	1,959±538.5	1,401±356.6	991±98.2		
	Std	932.7	617.6	170.1		
	V, %	47.62	44.07	17.16		
	P, %	27.49	25.45	9.91		
	min.	1.241	791	808		
	max.	3.013	2.026	1.144		
		TA-4				
Hypogymnia on a pine tree	M±m	9.881±842.2	12.265±1.031.4	11.747±1.299.1		
	Std	1 458 8	1,786.5	2 250 2		
	V. %	14.76	14.57	19.16		
	P %	8.52	8 41	11.06		
	min.	8.256	10.219	9.545		
	max	11 077	13,516	14 042		
Pine bark	M±m	2.188±184.0	1 844±237.5	1 623±125 3		
	Std	318.8	411.4	2171		
	V %	14 57	22.31	13 38		
	P %	8 41	12.51	772		
	min	1873	1411	1 373		
	max	2409	2230	1 771		
Hypogympia on a hirch	M+m	3 645+1777	3 925+1470	3 977+39 7		
	Std	220 3	254.6	68.7		
		6.04	6.49	1 73		
	V, 70	3 /0	 z 75	1.75		
	F, 70	3,412	2 729	2.00		
	 	2,950	۵,750 ۸ ۵۱۲	J,722		
Rirch bark	M+m	3,03U	17/0+120 2	1 010+70 7		
DIICII DAFK	ا۲۱⊐[۱] ۲+م	1,0/0±302.1	L040±123.2	1,010=37./		
	Sta	525.5	225.8	68.8		
	V, %	27.86	10.60	6./6		
	<u>Р, %</u>	10.09	9.58	3.90		
	min.	1,288	1093	953		
	max.	2,287	1,512	1,090		

**Note:** M±m is the average arithmetic value with an error; Std is the standard square deviation; V is the variation coefficient; P is the relative error of the average arithmetic value (accuracy of the experiment); min., max. are the minimum and maximum value

Data in Table 2 suggests that in all trial areas on the pine and on the birch, the specific activity of <sup>137</sup>Cs in the hypogymnia thalli varied quite slightly (V<20%) within each height range (Lakin, 1973). Thus, on a pine tree in the height range of 0-65 cm, the variation coefficient of the specific activity of <sup>137</sup>Cs in the hypogymnia thalli ranged from 11.78 to 15.94%, and the accuracy of the experiment was high, within 6.80-9.20%. A similar pattern was also observed in the remaining height ranges on pine. On the birch, in the height range of 0-65 cm, the variation coefficient of the specific activity of <sup>137</sup>Cs in the hypogymnia thalli was also low and was within 5.71-19.83% with 3.30-11.45% accuracy of the experiment. Like pine, birch had a similar distribution in the rest of the altitude ranges.

The significant values of the specific activity of <sup>137</sup>Cs in the thalli of hypogymnia, which grew on a pine tree, attract attention. At TA-1, TA-3 and TA-4, at almost all height ranges, the content of the mentioned radio-nuclide in the hypogymnia thalli exceeded 10 kBq·kg<sup>-1</sup> – the level of contamination of solid radioactive waste from <sup>137</sup>Cs (Order of the Ministry of Healthcare of Ukraine No. 54, 2005) with relatively low values of radioactive contamination of the territory 133.4-198.6 kBq·m<sup>-2</sup> (3.44-5.09 Ci·km<sup>-2</sup>). This suggests that hypogymnia is an intensive accumulator of <sup>137</sup>Cs from aerial fallout, which correlates well with published data from other researchers (Richardson, 1994; Kondratiuk, 2008; Anderson *et al.*, 2022).

Notably, in all trial areas where pine and birch

grew (TA-2, TA-3 and TA-4), the specific activity of <sup>137</sup>Cs in the hypogymnia thalli on birch at all height ranges was significantly lower compared to the pine thalli. On TA-2 in the height range of 0-65 cm, the mentioned indicator on the birch was equal to 3,934±450.4 Bq·kg<sup>-1</sup>, and on pine –  $8,565\pm582.7$  Bq·kq<sup>-1</sup>; in the height range of 65-130 cm, the values were equal to 4,022±231.2 and 11,049±874.6 Bg·kg<sup>-1</sup>; in the height range of 130-195 cm - 3,919±103.3 and 10,615±851.1 Bq·kg<sup>-1</sup>, respectively. The results of the univariate analysis of variance showed that in all sample areas, the difference in the average values of <sup>137</sup>Cs content in the hypogymnia thalli on the pine and on the birch differed substantially at the 95% confidence level: on TA-2 – F<sub>fact.</sub>=39.53–61.09> > $F_{0.95}$ =7.71, p=0.001-0.003; on TA-3 -  $F_{fact.}^{Iatt.}$ =29.07-77.73> > $F_{0.95}$ =7.71, p=0.001-0.006; on TA-4 -  $F_{fact.}$ =35.74-64.08> >F<sub>0.95</sub>=7.71, p=0.001-0.004.

It is also important to compare the average values of the <sup>137</sup>Cs content in hypogymnia thalli at each trial area on pine in all height ranges (Fig. 2). The analysis of these data shows that the amount of <sup>137</sup>Cs content in the hypogymnia thalli varied in different height ranges on pine in different trial areas. On TA-1, a monotonous increase in the mentioned indicator was observed from 0-65 cm to 65-130 cm and furtherx– to 130-195 cm. In the rest of the test plots, an increase in the specific activity of <sup>137</sup>Cs was observed in the hypogymnia thalamus from 0-65 cm to 65-130 cm, followed by a decrease in the height range of 130-195 cm.



Figure 2. The average values of the specific activity of <sup>137</sup>Cs in the hypogymnia thalli on the pine in the trial areas

However, in most of the trial areas, the errors of the arithmetic mean of the indicator under study overlapped, and therefore it is important to assess the statistical significance of the difference in the content of <sup>137</sup>Cs in the hypogymnia thalli at different height ranges in each of the trial areas. Calculations showed that in all test areas on pine, the content of <sup>137</sup>Cs in hypogymnia thalli at different height ranges did not differ statistically significantly at the 95% confidence level: on TA-1 –  $F_{fact}$  = =0.08-2.60< $F_{0.95}$ =7.71; on TA-2 –  $F_{fact}$ =3.95-5.59< $F_{0.95}$ =7.71; on TA-3 –  $F_{fact.}$ =0.61-2.29< $F_{0.95}$ =7.71; on TA-4– $F_{fact.}$ =0.10-3.20< $F_{0.95}$ =7.71. Thus, it is legitimate to draw a preliminary conclusion about the possibility of selecting hypogymnia thalli on pine trunks in the height range of 0-195 cm; however, considering the greater convenience of sampling, the authors of this paper recommend performing the sampling in the height range of 130-195 cm.

Of considerable scientific interest is the assessment of the intensity of  $^{137}\mathrm{Cs}$  accumulation in the thalli

of hypogymnia on pine at different trial areas (Fig. 3). These data clearly indicate certain trends in changes in the average values of AC at different altitude ranges of pine. On TA-1, TA-2, TA-4, the average values of AC increase with height, and on TA-3 they increase within the

height range of 0-65 cm and 65-130 cm and decrease above. However, considering the errors of the arithmetic mean, which in most cases overlap, the assessment of the statistical significance of the parameter under study is of considerable interest.



Figure 3. Average AC values of <sup>137</sup>Cs in the link "hypogymnia thalli – pine bark" in different trial areas

It was found that on TA-1, the average values of AC at different height ranges of pine did not differ statistically significantly at the 95% confidence level - $F_{fact} = 0.01 - 0.35 < F_{0.95} = 7.71$ , and on TA-3 –  $F_{fact} = 0.03$ - $0.80 < F_{0.95} = 7.71$ . However, on TA-2, the difference in the average values of AC was substantial at the 95% confidence level between the height ranges of 0-65 and 65-130 cm –  $F_{fact}$ =30.56> $F_{0.95}$ =7.71; p=0.005; in the ranges of 0-65 cm and 130-195 cm - F<sub>fact</sub>=34.59>F<sub>0.95</sub>=7.71; p= 0.004; at the same time, there was no statistically significant difference between the ranges of 65-130 cm and 130-195 cm -  $F_{fact}$  = 0.80 <  $F_{0.95}$  = 7.71. On TA-4, a significant difference in the average values of AC is shown for all pairs of height ranges: 0-65 cm and 65-130 cm - $F_{fact.}$ =34.61> $F_{0.95}$ =7.71; p=0.004; in the ranges of 0-65 cm and 130-195 cm –  $F_{fact.}$ =10.64> $F_{0.95}$ =7.71; p=0.03 and ranges of 65-130 cm and 130-195 cm –  $F_{fact.}$ =57.08> $F_{0.95}$ =7.71; p=0.002.

According to a similar scheme, hypogymnia was studied on the birch as well (Fig. 4). These data suggest that the specific activity of  $^{137}$ Cs in the thalli of

hypogymnia at different height ranges of birch in the trial areas changed differently. At TA-2, the values of the mentioned indicator at all height ranges were close -3,919-4,022 Bg·kg<sup>-1</sup>. On TA-4, a weak increase in the mentioned indicator was observed in the height ranges from 0-65 cm to 65-130 cm - 3,645-3,925 Bq·kg<sup>-1</sup> and further in the height range of 130-195 cm - up to 3,977 Bq·kg<sup>-1</sup>. Considering the overlap of the errors of the average values of the indicator under study on TA-2 and TA-4, between the height ranges on the birch, the specific activity of <sup>137</sup>Cs in the hypogymnia thalli did not differ statistically at the 5% level of significance: on TA-2 –  $F_{fact}$  =0.00-0.08< $F_{0.95}$ =7.71; on TA-4 –  $F_{fact}$  =0.12-6.22< $F_{0.95}$ =7.71. However, on TA-3, the difference of the mean values under study in all dispersion pairs was significant at the 95% confidence level: between the height ranges of 0-65 cm and 65-130 cm –  $F_{fact.}$ =13.07> $F_{0.95}$ =7.71; p = 0.02; between the height ranges 0-65 cm and 130-195 cm –  $F_{fact}$ =40.90> $F_{0.95}$ =7.71; p=0.003; between the height ranges of 65-130 cm and 130-195 cm –  $F_{fact} = 7.92 > F_{0.95} = 7.71$ ; p=0.05.



Figure 4. Average values of the specific activity of <sup>137</sup>Cs in the hypogymnia thalli on the birch in the test areas

Average values of the accumulation coefficient of <sup>137</sup>Cs in the link "hypogymnia thalli – birch bark" at all height ranges were calculated for the test areas where birch was present (Fig. 5). The data in this figure clearly show that a general trend was observed in all test areas on the birch - a monotonous increase in the average values of AC with height. For example, on TA-2, the average values of AC were as follows: in the height range of 0-65 cm  $- 2.1\pm0.43$ ; in the height range of 65-130 cm - 3.0±0.34; in the height range of 130-195 cm - 4.0±0.16. It is of great interest that on several trial areas the difference in the average values of AC at different height ranges was statistically significant at the 95% confidence level: on TA-2 between the height ranges of 0-65 cm and 130-195 cm - $F_{fact.}$ =17.10> $F_{0.95}$ =7.71; p=0.01; between the height ranges

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of 65-130 and 130-195 cm – F<sub>fact.</sub>=7.74>F<sub>0.95</sub>=7.71; p=0.05; however, there was no statistically significant difference between the height ranges of 0-65 and 65-130 cm - $F_{fact} = 2.43 < F_{0.95} = 7.71$ . Furthermore, a substantial difference in mean values was demonstrated at the 5% level of significance for all dispersion pairs of AC on TA-4 – F<sub>fact</sub>=10.64-57.08>F<sub>0.05</sub>=7.71; p=0.001-0.03. On the other hand, on TA-3, there was no statistically significant difference in the average values of AC between all height ranges on the birch –  $F_{fact}$ =0.20-0.68< $F_{0.95}$ =7.71. Furthermore, the average values of AC were calculated for the entire array of data on birch for all height ranges (Fig. 4). Calculations showed that in the height range of 0-65 cm, the average value of AC in the link "hypogymnia thalli – birch bark" was equal to 2.5±0.35; in the height range of 65-130 cm – 3.6±0.33; in the height range of 130-195 cm – 4.0±0.16.



Figure 5. Average AC values of <sup>137</sup>Cs in the link "hypogymnia thalli – birch bark" in different trial areas

It was demonstrated that between the height ranges of 0-65 cm and 65-130 cm, there was a substantial difference at the 95% confidence level –  $F_{fact.}=4.96>F_{0.95}=4.49$ ; p=0.04; between the height ranges of 0-65 cm and 130-195 cm –  $F_{fact.}=14.97>F_{0.95}=4.49$ ; p=0.001; however, there was no statistically significant difference between the height ranges of 65-130 and 130-195 cm –  $F_{fact.}=1.26<F_{0.95}=4.49$ . Therefore, the authors of this paper recommend sampling the Hypogymnia physodes on the birch in the range of 65-130 m.

A comparison of the data in Figures 2 and 4 suggests that the intensity of <sup>137</sup>Cs accumulation in the link "hypogymnia thalli – tree bark" is considerarbly higher in pine than in birch. The difference in the average values of AC in the trial areas at different altitude ranges was as follows: TA-2 – 1.8-2.1 times; TA-3 – 1.1-1.5 times; TA-4 – 1.8-2.3 times. The likely reason for this is the lower acidity of pine bark (pH=3.0-3.1) compared to birch bark (pH=3.5-3.6) (Fojcik *et al.*, 2017). Pine bark acidifies the flow of trunk and crown run-off, while <sup>137</sup>Cs is leached from the microparticles of dust it carries. In birch, the acidification of run-off is less pronounced. The leached <sup>137</sup>Cs in a water-soluble form is absorbed by the hypogymnia thalli, which, thus, returns <sup>137</sup>Cs to

the small (biological) cycle and prevents it from entering the soil – to the large (geological) cycle. Furthermore, the variation in the content of <sup>137</sup>Cs in the bark and hypogymnia thalli at different altitude ranges can be explained by the fact that the pH of the bark of both pine and birch at different altitudes can vary significantly (Grodzinska, 1976), causing the variation in the content of <sup>137</sup>Cs in components under study – both in tree bark and hypogymnia thalli.

The materials obtained in the trial areas, which represent the pine forests prevailing in Zhytomyr Polissia, expand the ideas about the ways of redistribution of <sup>137</sup>Cs in forest ecosystems, namely about the levels of accumulation of the mentioned radionuclide in the thalli of the known indicator species of epiphytic lichens – hypogymnia. This particular species was previously studied upon analysing the content of <sup>137</sup>Cs in the components of forest (Boyko & Orlov, 2012) and forest-swamp ecosystems (Orlov, 2021). Therewith, lichen sampling was always carried out on Scots pine trunks in a narrow height range – 120-150 cm. The results obtained in this paper proved that among all the components of the biota of the ecosystems under study, hypogymnia in terms of specific activity levels of <sup>137</sup>Cs

was inferior only to a part of the macromycete species, which provided the possibility of measuring this indicator in small samples of lichen with acceptable accuracy.

The authors of this paper consider the results of this study to be pilot, since they were obtained during one growing season. Statistically reliable substantiation of the optimal sampling height of hypogymnia on tree trunks for bioindication of radioactive contamination of forest ecosystems is not only of scientific, but also of essential practical importance, since the sampling height of this lichen and the tree organ from which its samples are taken can considerably affect the <sup>137</sup>Cs content in it, and, accordingly, on the results of long-term monitoring.

Prospects for further research are to confirm the obtained results on a larger statistical sample, in diverse types of forest vegetation conditions, in plantations of different species composition (pine, oak, birch, mixed) and of different ages.

#### CONCLUSIONS

1. The specific activity of <sup>137</sup>Cs in the hypogymnia thalli varied rather weakly in all test plots on the pine and on the birch – the coefficient of variation was less than 20%.

2. In all test areas, the values of the specific activity of  $^{137}\mathrm{Cs}$  in the hypogymnia thalli on the birch at all altitude ranges were considerably lower compared to the lichen thalli on the pine and differed substantially at the 95% confidence level.

3. In all test areas on pine, the content of <sup>137</sup>Cs in hypogymnia thalli at different altitude ranges did not statistically significantly differ at the 95% confidence level. This allowed drawing a preliminary conclusion about the possibility of sampling hypogymnia thalli on pine trunks in the height range of 0-195 cm; however, considering greater convenience, the authors of this paper recommend performing sampling on pine trees in the height range of 130-195 cm.

4. The average AC values of  $^{137}$ Cs in the link "hypogymnia thalli – tree bark" throughout the data array were as follows: on pine, in the height range of 0-65 cm – 7.4±1.35; 65-130 cm – 8.7±1.76; 130-195 cm – 8.7±2.29; on the birch, in the height range of 0-65 cm – 2.5±0.35; 65-130 cm – 3.6±0.33; 130-195 cm – 4.0±0.16.

5. On TA-2 and TA-4 between the height ranges on the birch, the specific activity of  $^{137}$ Cs in the thalli of hypogymnia did not differ statistically significantly at the 5% level of significance, on TA-3 the difference was significant between all height ranges.

6. A significant difference at the 95% confidence level was observed in the average AC values on the birch between the height ranges of 0-65 cm and 65-130 cm, and 0-65 cm and 130-195 cm, but it was absent between the ranges of 65-130 cm and 130-195 cm. Therefore, the authors of this paper preliminarily recommend sampling the Hypogymnia physodes on the birch in the height range of 65-130 cm.

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## Акумуляція <sup>137</sup>Cs таломами епіфітного лишайника *Hypogymnia physodes* (L.) Nyl на різних висотах стовбура у соснових деревостанах

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Анотація. Лишайники розглядаються дослідниками як біоіндикатори забруднення навколишнього середовища штучними радіонуклідами, в т.ч. <sup>137</sup>Cs. Одним з таких видів-біоіндикаторів є епіфітний лишайник *Нуродутпіа* physodes (L.) Nyl, використання якого для радіоекологічного моніторингу може модифікуватися рядом факторів: деревною породою, розміщенням на дереві (гілки – стовбур), висотою відбору зразків та ін. Метою досліджень було вивчити питому активність <sup>137</sup>Cs у таломах гіпогімнії у соснових деревостанах: на стовбурах Pinus sylvestris L. та Betula pendula Roth на різній висоті відбору зразків у 3-х діапазонах висот: 0–65,65–130,130–195 см та проаналізувати інтенсивність акумуляції радіонукліду у ланці «таломи лишайника – кора дерева» для обох деревних порід у всіх висотних діапазонах. Дослідження проведене у серпні 2021 р. у Коростенському районі Житомирської області, Житомирському Поліссі, на 4 пробних площах, у природному заповіднику «Древлянський», переважно у середньовікових соснових деревостанах з домішкою берези, де відібрано 130 зразків ґрунту, кори дерев та лишайника. Питому активність <sup>137</sup>Cs у зразках вимірювали з використанням гамма-спектрометра СЕГ-001 «АКП-С»-150 зі сцинтиляційним детектором БДЕГ-20Р2 (Nal(Tl). Продемонстровано, що на всіх пробних площах на сосні вміст <sup>137</sup>Cs у таломах Hypogymnia physodes на різних висотних діапазонах статистично достовірно не відрізнявся на 95 % довірчому рівні. Зроблено попередній висновок про можливість відбору таломів Hypogymnia physodes на стовбурах сосни у діапазоні висот 0-195 см, однак, для зручності відбору зразків попередньо рекомендуємо проводити його у діапазоні 130-195 см. Доведено, що, як питома активність <sup>137</sup>Cs у таломах Hypogymnia physodes, так і інтенсивність акумуляції <sup>137</sup>Cs у ланці «Hypogymnia physodes – кора дерев» є приблизно у 2 рази вищою на сосні порівняно з березою. Виявлено, що на березі вміст <sup>137</sup>Ся у лишайнику між висотними діапазонами 0-65 – 65-130 см та 0-65 – 130-195 см суттєво відрізняється на 95 % довірчому рівні, тому попередньо рекомендуємо відбір зразків *Нуродутпіа physodes* на березі проводити у діапазоні 65–130 см

Ключові слова: *Pinus sylvestris* L., *Betula pendula* Roth, листуватий лишайник, кора дерева, коефіцієнт накопичення, питома активність, радіонуклід