



Article

Determination of Lichens' Bioaccumulation Capacity for Radioactive Isotopes Under Laboratory Conditions as a Basis for Their Application as Biomonitors

Michał Saniewski ^{1,*}, Michał Hubert Węgrzyn ², Patrycja Fałowska ^{2,3}, Patrycja Dziurawicz ^{2,3}, Karolina Waszkiewicz ^{2,3} and Tamara Zalewska ¹

¹ Institute of Meteorology and Water Management—National Research Institute, Waszyngtona 42, 81-342 Gdynia, Poland; tamara.zalewska@imgw.pl

² Institute of Botany, Jagiellonian University, Gronostajowa 3, 30-387 Kraków, Poland; michal.wegrzyn@uj.edu.pl (M.H.W.); patrycja.falowska@uj.edu.pl (P.F.); patrycja.dziurawicz@uj.edu.pl (P.D.); karolina.waszkiewicz@uj.edu.pl (K.W.)

³ Doctoral School of Exact and Natural Sciences, Jagiellonian University, Prof. S. Łojasiewicza 11, 30-348 Kraków, Poland

* Correspondence: michal.saniewski@imgw.pl

Abstract: This study investigates the bioaccumulation capacity of the lichen *Cladonia uncialis* for radioactive isotopes, aiming to establish its potential as a biomonitor. Conducted under controlled laboratory conditions, the experiment utilised gamma-emitting isotopes to simulate fallout. The lichens were exposed to varying concentrations of these isotopes in three aquariums. The results demonstrated that the bioaccumulation of ⁵⁴Mn, ⁵⁷Co, ⁶⁰Co, ⁶⁵Zn, ¹³⁷Cs, and ²⁴¹Am by *C. uncialis* was proportional to their initial deposition levels, and this occurred in the first days of the experiment. Analysis of isotope activity in washed and unwashed lichens showed that retention primarily occurred intracellularly, indicating effective bioaccumulation. The study derived the Aggregated Transfer Coefficient (Tag) for each isotope, which ranged from 0.34 to 0.64, and the absorption capacity of the elements increased in the following order: ⁵⁴Mn < ⁵⁷Co < ⁶⁰Co ≤ ⁶⁵Zn < ²⁴¹Am < ¹³⁷Cs. Absorption efficiency, amounting to approximately 50% for ¹³⁷Cs and ²⁴¹Am, highlights the potential for lichens to serve as reliable biomonitors for environmental monitoring and estimation of deposition when knowing only the activity of the isotopes in lichen.

Keywords: radioisotopes bioaccumulation; lichens; Aggregated Transfer Coefficient (Tag); *Cladonia uncialis*; environmental monitoring



Citation: Saniewski, M.; Węgrzyn, M.H.; Fałowska, P.; Dziurawicz, P.; Waszkiewicz, K.; Zalewska, T. Determination of Lichens' Bioaccumulation Capacity for Radioactive Isotopes Under Laboratory Conditions as a Basis for Their Application as Biomonitors. *Appl. Sci.* **2024**, *14*, 11455. <https://doi.org/10.3390/app142311455>

Academic Editors: Roger Narayan and Francesco Caridi

Received: 28 October 2024
Revised: 20 November 2024
Accepted: 7 December 2024
Published: 9 December 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Precipitation plays a key role in the environment's transport and distribution of radionuclides [1]. Along with dry and wet precipitation, contaminants, including radioactive isotopes, are transported to the earth's surface, which then undergo deposition and bioaccumulation in various biotic and abiotic elements of the environment [2,3]. Knowledge of the dependence of the level of bioaccumulated substances in selected organisms on the amount of deposition is a key element for the possibility of using such organisms as biomonitors, which is particularly applicable in accidental situations but also in areas where deposition studies are difficult or impossible, as is the case in polar regions, where there are logistical and financial problems of providing continuous monitoring. Therefore, optimal in such cases is the use of descriptive quantitative relationships, through the determination of appropriate coefficients. Over the past 30 years, many studies have highlighted the possibility of using lichens as biomonitors of air quality due to their sensitivity to various environmental factors [4–6]. Lichens are symbiotic organisms of fungi and algae or cyanobacteria without a root system or a waxy cuticle. Their growth and survival depend

on water and nutrients delivered mainly from the atmosphere. Lichens bioaccumulate several elements through a combination of mechanisms, including the physical uptake of particulate matter in the intercellular spaces of the core, extracellular accumulation, and intracellular accumulation [7,8]. The entire surface of the thallus participates in absorption, allowing elements from both the atmosphere and the substrate to penetrate into the lichen bodies [8,9]. In this way, lichens can accumulate exceedingly above their physiological requirements [10]. This unique trait makes them excellent collectors of substances deposited from the atmosphere [10–12]. Lichens are diverse organisms found in extreme conditions around the world. They form the dominant biomass in the Arctic and Antarctic regions. Lichens occupy about 8% of the Earth's land surface, giving them a globally important role in plant ecology [13,14]. The large surface area of lichen thalli, relative to their mass, is one of the main reasons for their relatively high capacity to accumulate radionuclides or other elements, such as heavy metals [15]. Because they can passively absorb and accumulate contaminants and radionuclides from wet and dry atmospheric deposition through the surface of the thallus, they are considered useful biomonitors of air pollution [2,4–6,16].

Coefficients are used to describe the bioaccumulation capacity of selected organisms. One of these is the aggregated transfer coefficient (Tag), an empirical coefficient used in predictive models for natural and semi-natural ecosystems. It is defined as the ratio of isotope activity in a sample (organism) to the amount of isotope deposited per unit surface area [17]. While the determination of Tag values may be relatively simple to calculate, their application may require extensive knowledge of the ecosystem [18]. It should be noted that if effective radionuclide decay is considered, the Tag value is not constant, and in some circumstances, Tag may even increase with time. This was confirmed by the observed significant increase in ^{137}Cs activity in lichens at a very low atmospheric deposition of this isotope in the air after the Fukushima–Daiichi nuclear power plant accident [18].

The aim of the study was to determine the bioaccumulation efficiency of radioactive isotopes in the tissues of the lichen species *Cladonia uncialis* through the derivation of the Tag, which can serve as a useful tool for assessing contaminant deposition based solely on the activity measured in the lichen. The study was conducted under controlled conditions to find relationship between environmental deposition levels and accumulation within *C. uncialis*.

2. Materials and Methods

2.1. Experiment

The experiment conducted in 2023 involved preparing cultures of a selected species of the lichen *C. uncialis*, which inhabits nutrient-poor, sandy, or rocky soils, and which is commonly found in boreal forests, heathlands, sand dunes, coastal areas, rocky outcrops, and inhospitable areas where conventional monitoring methods can be challenging to implement [19–21]. To achieve this, *C. uncialis* was collected from the Tuchola Forest region near the Przymuszewo Forest District in Poland, along with the soil on which the lichens were growing. In the laboratory, the lichens and soil were cleaned of dead plant remnants and other vegetation. The soil substrate was classified as medium to fine sand. The largest proportion of soil particles was in the 0.5–0.25 mm size class, comprising 84.2% of the total sample mass. The 1–0.5 mm fraction represented 8.8%, while finer fractions, such as 0.25–0.125 mm, 0.125–0.063 mm, and below 0.063 mm, accounted for 14.5%, 1.1%, and 1.5%, respectively. Coarser fractions were less abundant, with particles larger than 2 mm comprising only 0.4%, and fractions between 2 and 1.6 mm and 1.6 and 1 mm representing 0.2% and 0.5%, respectively. The organic matter content in the soil (LOI) was 1.6%. The experiment was conducted using three aquariums (20 cm × 30 cm × 20 cm), each with a 5 cm layer of sand at the bottom, onto which the lichens were anchored. Approximately 100 g of lichens were placed in each aquarium. For one week, the lichen cultures were maintained under controlled conditions, with regulated lighting and hydration every three days, using an aerosol generated by a humidifier placed in each aquarium. After a one-week acclimatisation period, a gamma mixed standard solution (Standard solution

of gamma-emitting isotopes, code BW/Z-63/19/20) was added to each humidifier on 17 March 2023, to initiate the contamination process. The gamma radionuclides included in the mix were ^{241}Am ($T_{1/2}$ 432.2 years), ^{57}Co ($T_{1/2}$ 0.744 years), ^{137}Cs ($T_{1/2}$ 30.05 years), ^{54}Mn ($T_{1/2}$ 0.855 years), ^{65}Zn ($T_{1/2}$ 0.668 years), and ^{60}Co ($T_{1/2}$ 5.271 years). The experiment was carried out in three parallel replicates, with 1 g of the gamma mix solution added to aquarium A, 2 g to aquarium B, and 3 g to aquarium C. The added gamma mix solution was sprayed along with the water used for hydration, and the deposition of isotopes in each aquarium is presented in Figure 1. Increasing concentrations of isotopes were intended to test experimentally whether there is a dependence of isotope bioaccumulation efficiency on their initial concentration in fallout.

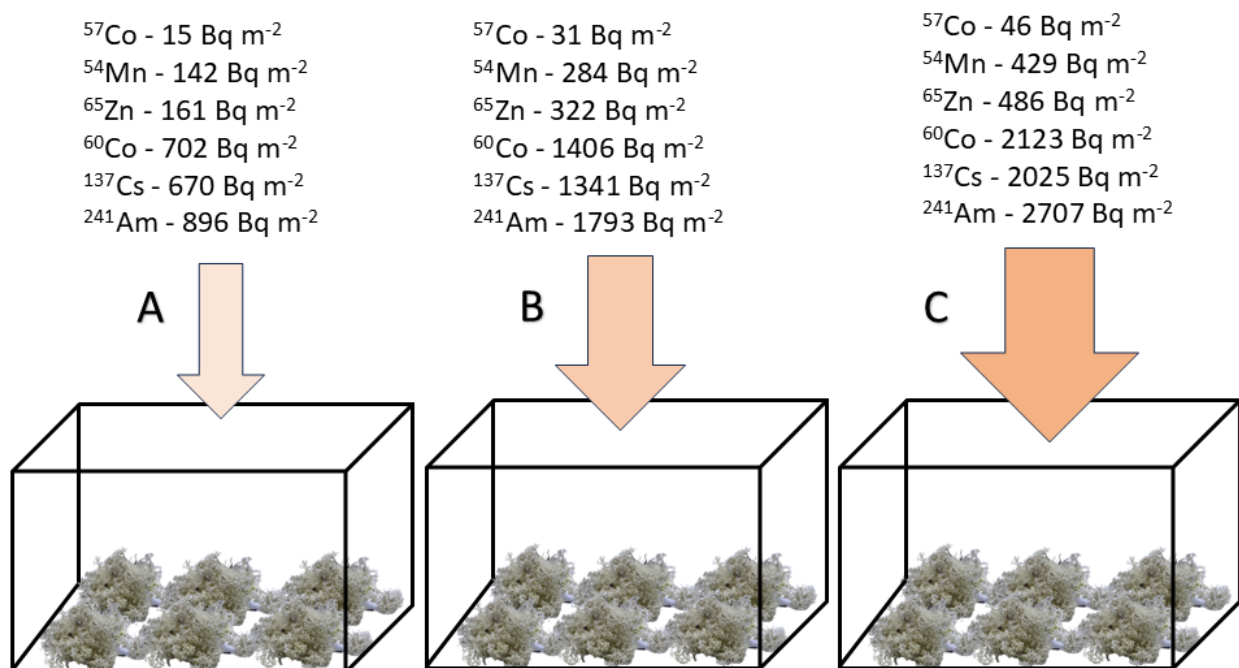


Figure 1. Diagram of the experiment showing three aquariums with the lichen *Cladonia uncialis*, where isotopes were applied in different concentrations ((A)—1 g gamma mix solution, (B)—2 g gamma mix solution, (C)—3 g gamma mix solution).

Throughout the entire experiment, the lichens were irrigated using a humidifier twice a week, with excess water draining from the aquarium through a specially prepared drain at the bottom. Prior to starting the experiment, the initial activities of the studied isotopes in the lichens and soil were measured to establish reference values, which were used to adjust the observed values of the studied elements during the experiment (Table 1).

Table 1. Initial activity of the studied isotopes in the soil and *C. uncialis* used in the experiment.

	^{54}Mn	^{57}Co	^{60}Co	^{65}Zn	^{137}Cs	^{241}Am
	Bq kg^{-1} d.w.					
Soil	<0.36	<0.18	<0.44	<1.03	2.83 ± 0.25	<0.42
<i>Cladonia uncialis</i>	<0.52	<0.41	<0.46	<1.43	7.38 ± 0.60	<0.58

Samples of contaminated lichens were collected one week after contamination, on 24 March 2023, and then at approximately monthly intervals on the following dates: 20 April 2023; 19 May 2023; 21 June 2023; and 24 August 2023. The activity of the analysed isotopes at all time points was recalculated to the reference date of 17 March 2023, considering the different half-lives ($T_{1/2}$) of each isotope. This approach enabled the direct comparison of long-term trends. Each sample from each aquarium was divided into

two subsamples. One of the subsamples was rinsed in distilled water for 6 h to remove loosely deposited contaminants from the surface of the lichens, while the other subsample was analysed without any treatment. A simple wash in distilled water does not remove isotopes trapped intracellularly or bound to cell walls and outer membranes; it only removes soluble particles loosely deposited on the surface. The material of both washed and unwashed samples was then dried at 105 °C and ashed at 400 °C to obtain homogeneous samples, and was then placed in vessels with geometries identical to the calibration geometry. In this form, the samples were measured using gamma spectrometry.

To study isotope concentrations in the substrate, soil samples were collected at the time of lichen collection, from the site directly below the collected lichen. The soil samples were then dried at 105 °C and ashed at 400 °C.

2.2. Gamma Spectrometry Analysis

The activities of radionuclides were measured using a gamma spectrometry system (Extended Range Coaxial Ge Detector (XtRa) model GX4018 (Canberra, Meriden, CT, USA) with a relative efficiency of 40% and a resolution of 1.8 keV for the 1332 keV peak of ^{60}Co). The detector was coupled with an 8192-channel computer analyser and GENIE 2000 software. The Coldfinger of the detector was inserted into a Cryo-Cycle II cryostat (model 7500SL Canberra) with electrical cooling supported by liquid nitrogen (LN_2). The total time measurements for each sample were 80,000. The detector system was calibrated using the supplied gamma mixed standards (Standard solution of gamma-emitting isotopes, code BW/Z-63/19/20). The radionuclides used in the reference solution during equipment calibration were: ^{241}Am , ^{109}Cd , ^{57}Co , ^{51}Cr , ^{113}Sn , ^{85}Sr , ^{137}Cs , ^{54}Mn , ^{65}Zn , and ^{60}Co , and approximation errors were at a level of 0.8–2.1%. This reference solution was used to prepare reference samples for equipment calibration. For equipment calibration, reference samples were analysed in cylindrical plastic containers (40 mm diameter) with the same geometry as those used for environmental samples. The calibration was carried out using standard solutions with a density of approximately 1 g cm^{-3} with different heights: 1, 3, 5, 7, 10, 15, 20, and 25 mm, to match samples varying in thickness layer. The reliability and accuracy of the measurements, as well as the comparability, were verified by participation in 2023 in the intercalibrations organised by the IAEA (International Atomic Energy Agency) [22] (Table 2).

Table 2. The reliability and accuracy of the measurements obtained by the laboratory in the intercalibrations organised by IAEA (International Atomic Energy Agency).

		Reported Laboratory Value [Bq kg^{-3}]	Assigned Value [Bq kg^{-3}]	Trueness Evaluation	Precision Evaluation	Final Score	Sample Matrix
Reference sample 1	^{60}Co	14.90 ± 0.16	14.7 ± 0.7	A	A	A	Spiked Water
	^{241}Am	21.73 ± 0.35	22.3 ± 1.1	A	A	A	
Reference sample 2	^{90}Sr	12.04 ± 0.46	14.2 ± 0.7	A	A	A	Spiked Water
	^{134}Cs	41.50 ± 0.60	40.0 ± 2.0	A	A	A	
	^{137}Cs	44.21 ± 0.70	44.1 ± 2.2	A	A	A	
Reference sample 4	^{134}Cs	6.50 ± 0.37	6.93 ± 0.50	A	A	A	Japanese Soil
	^{137}Cs	260 ± 4.5	270 ± 17	A	A	A	

3. Results

3.1. Lichen

The activity of isotopes in *C. uncialis* thalli was directly proportional to their radioactive concentrations in the aerosol spray. The lowest activities of ^{57}Co , ^{54}Mn , ^{65}Zn , ^{60}Co , ^{137}Cs , and ^{241}Am in lichens was observed in Aquarium A in which the deposition was the lowest. In aquaria B and C, with the increase in deposition, a proportional increase in isotope activity was observed (Table 2). Comparing isotope activities in samples before and after rinsing, the activity of ^{54}Mn decreased by 10%, 20%, and 23% in Aquariums A, B, and

C, respectively, indicating rinsing. ^{57}Co showed greater divergence in decreases (24% in B, 19% in C), while in Aquarium A, the activity in the rinse sample was 7% higher compared to the non-rinse sample. ^{60}Co had substantial reductions across all aquaria (7% in A, 17% in B, and 18% in C). The activity of ^{65}Zn remained at a similar level in Aquarium A and decreased in B and C. ^{137}Cs activity increased in Aquariums A and C but decreased in B, indicating inconsistencies in rinsing effectiveness. ^{241}Am showed minimal decreases in A and B, with a significant increase in C. Overall, rinsing generally reduced a negligible amount of isotope activities that are within the measurement error limit (Table 3). Approximate activities in washed and unwashed samples suggests that the accumulation of all isotopes occurred intracellularly and not merely by being deposited onto the lichen's surface (Table 1, Figure 2).

Table 3. Activities of six isotopes (^{57}Co , ^{54}Mn , ^{65}Zn , ^{60}Co , ^{137}Cs , and ^{241}Am) measured in three aquaria (A, B, and C) before and after rinsing, showing the percentage changes in activity.

	A	A-Rinsed	% Change	B	B-Rinsed	% Change	C	C-Rinsed	% Change
					^{54}Mn [Bq kg ⁻¹]				
average	62	56		113	90		190	145	
(min–max)	(43–97)	(36–80)	–10	(89–139)	(71–113)	–20	(123–233)	(35–197)	–23
Median	59	47		111	85		200	166	
					^{57}Co [Bq kg ⁻¹]				
average	5.9	6.3		12.5	9.4		22	17.8	
(min–max)	(3.9–6.9)	(3.2–9.6)	7	(10.1–14.1)	(0–14.3)	–24	(17.9–25.4)	(4.5–27.1)	–19
Median	6.3	5.2		13.3	10.3		22.2	17.7	
					^{60}Co [Bq kg ⁻¹]				
average	303	282		584	481		943	773	
(min–max)	(282–371)	(210–382)	–7	(462–693)	(407–574)	–17	(722–1145)	(401–949)	–18
Median	286	243		609	447		950	826	
					^{65}Zn [Bq kg ⁻¹]				
average	71(56–82)	73(52–99)		147	135		262	202	
(min–max)			3	(112–167)	(103–157)	–8	(209–303)	(56–313)	–23
Median	71	68		127	142		264	241	
					^{137}Cs [Bq kg ⁻¹]				
average	336	363		704	636		1299	1473	
(min–max)	(291–376)	(229–480)	8	(667–732)	(466–824)	–9	(1104–1510)	(635–2063)	14
Median	356	415		713	617		1254	1563	
					^{241}Am [Bq kg ⁻¹]				
average	405	397		951	898		1721	1982	
(min–max)	(345–456)	(332–521)	–2	(832–1048)	(834–998)	–6	(1612–1863)	(1898–2043)	15
Median	421	354		947	898		1754	2001	

Initial activity levels vary significantly between the three aquariums, with Aquarium C showing the highest levels, followed by Aquarium B, and then Aquarium A. This caused the activity in the lichen to be proportional to the initial fallout deposition. Despite the different activities in all aquaria, a similar trend in bioaccumulation was observed. The activities of isotopes in all samples showed variability over time, with some isotopes, like ^{60}Co and ^{65}Zn , exhibiting more significant fluctuations. The mean activity of ^{54}Mn and ^{57}Co were with a relatively high standard deviation, indicating noticeable fluctuations over time. For ^{65}Zn , activity also varied, though less drastically than ^{54}Mn and ^{57}Co , and it stabilised toward the later sampling periods. The activities of ^{137}Cs , and ^{241}Am in lichens in each aquarium were at similar levels regardless of sampling time, suggesting that accumulation was most effective during the first days of the experiment (Figure 2). Overall, these results indicate that while there are changes over time, many of these values are within the margin of variability, suggesting that a long-term survey would provide a more stable assessment of trends (Figure 2).

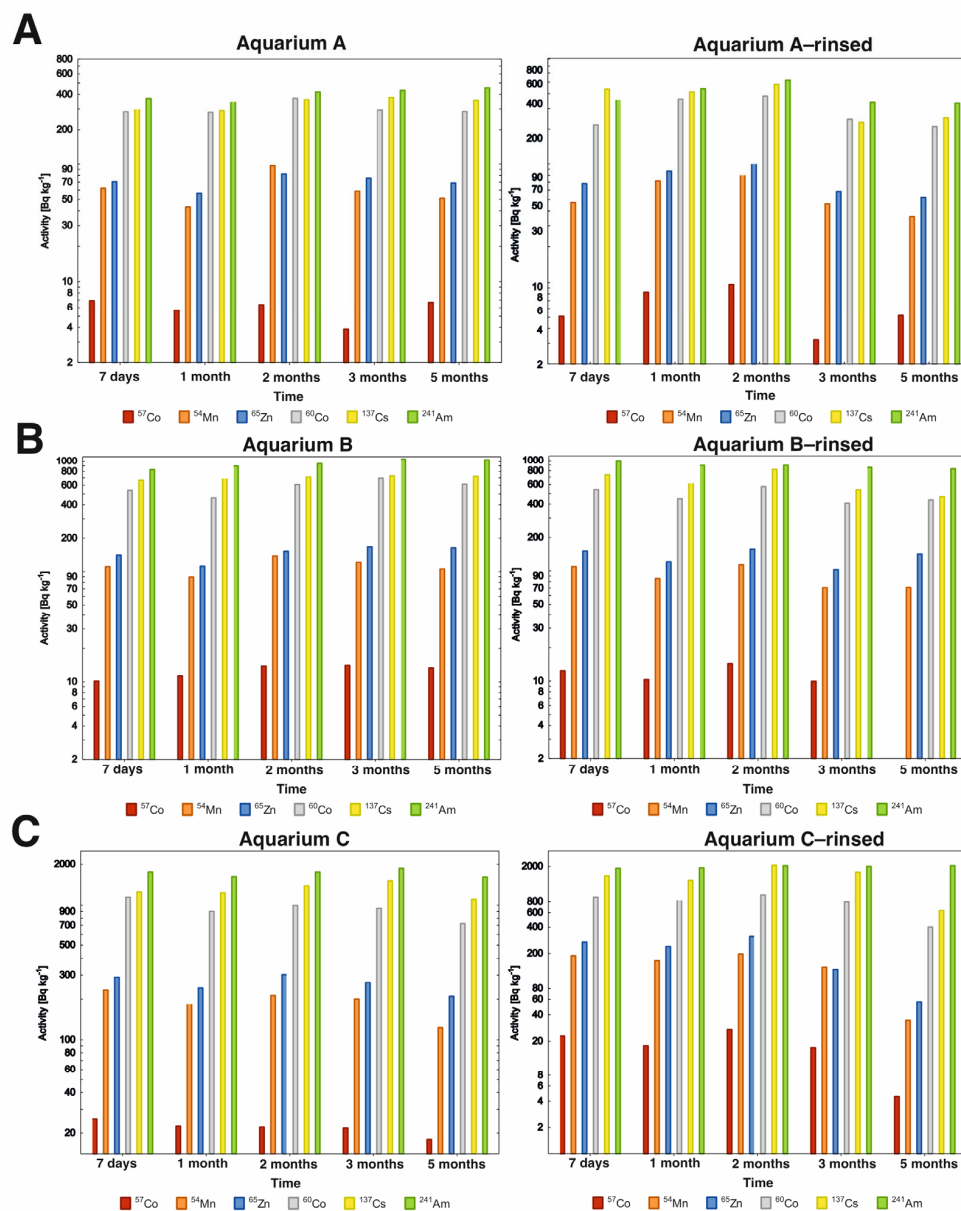


Figure 2. Temporal change (7 days, 1 month, 2 months, 3 months, 5 months) in the concentration activity of isotopes (⁵⁷Co, ⁵⁴Mn, ⁶⁵Zn, ⁶⁰Co, ¹³⁷Cs, and ²⁴¹Am) in thallus of *Cladonia uncialis* (unrinsed and rinsed) in relation to deposition (A–C).

3.2. Soil

The activity of isotopes in the soil, as in the case of lichens, depended on the initial conditions of the fallout. In the case of Aquarium A, in which contamination was the lowest, only three isotopes, ⁶⁰Co, ¹³⁷Cs, and ²⁴¹Am, were observed in the soil sample, and their average activities were 2.90 Bq kg⁻¹, 4.48 Bq kg⁻¹, and 2.20 Bq kg⁻¹ (Figure 3); the concentrations of the others were below the detection limit. In Aquarium B, where contamination was two times higher than in Aquarium A (Figure 1), the average activities of ⁶⁰Co, ¹³⁷Cs, and ²⁴¹Am were 7.42 Bq kg⁻¹, 10.9 Bq kg⁻¹, and 7.84 Bq kg⁻¹, respectively. In Aquarium C, where the contamination was three times higher than in Aquarium A, in addition to the isotopes observed in aquariums A and B, the concentrations of ⁵⁴Mn, ⁵⁷Co and ⁶⁵Zn were also measured in the soil. In all aquaria, the activity of ¹³⁷Cs and ²⁴¹Am in the soil increased with time, suggesting that a small amount of the isotopes that had been deposited on the surface were leached from the surface of the lichens into the soil during irrigation with a humidifier.

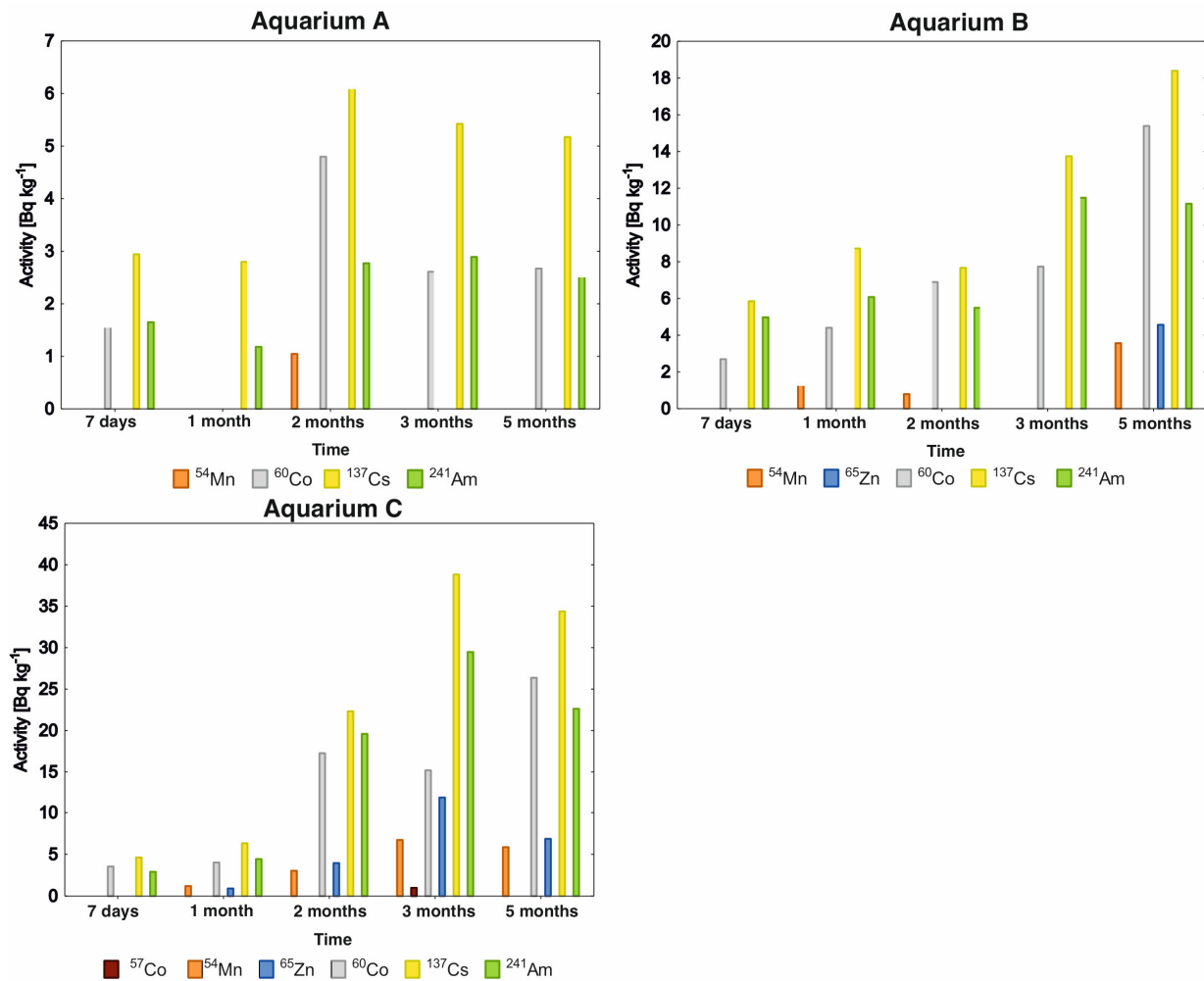


Figure 3. Temporal change (7 days, 1 month, 2 months, 3 months, 5 months) in the activity concentrations of isotopes (^{57}Co , ^{54}Mn , ^{65}Zn , ^{60}Co , ^{137}Cs , and ^{241}Am) in soil in relation to deposition (Aquarium A, Aquarium B, Aquarium C).

3.3. Aggregated Transfer Coefficient (T_{ag})

Based on the obtained results of isotope concentrations in lichen tissue and deposition rates, the Aggregated Transfer Coefficient (T_{ag}) was determined, which is an empirical coefficient that determines bioaccumulation capacity and which can be used in predictive models for both natural and semi-natural ecosystems. It is defined by a specific expression [18]:

$$T_{ag} = \frac{\text{Activity concentration of the sample (A)}}{\text{Activity of the deposition per unit area (D)}} (\text{m}^2 / \text{kg}) \quad (1)$$

The average T_{ag} value determined from the data for all sampling periods was not statistically significantly different between Aquaria A, B, and C for the three isotopes and was for ^{54}Mn (0.44, 0.40, 0.44), ^{57}Co (0.38, 0.41, 0.47), ^{60}Co (0.43, 0.42, 0.44), ^{65}Zn (0.44, 0.42, 0.54), and ^{137}Cs (0.50, 0.53, 0.64), respectively (Kruskal–Wallis test; $p > 0.05$). Only for ^{241}Am (0.45, 0.53, 0.64) was T_{ag} significantly higher in the aquarium with the highest deposition (Aquarium C) compared to Aquariums A (Kruskal–Wallis test; $p < 0.05$) (Figure 4). Since one of the components needed to calculate the T_{ag} is the activity in lichens, the coefficient's variation followed the same trend as activity in lichens.

The T_{ag} value is not fixed and may even rise over time under certain conditions. Even during a short experiment of 5 months, significant differences were observed in the calculated T_{ag} depending on the contamination and sampling period (Figure 4, Table 4).

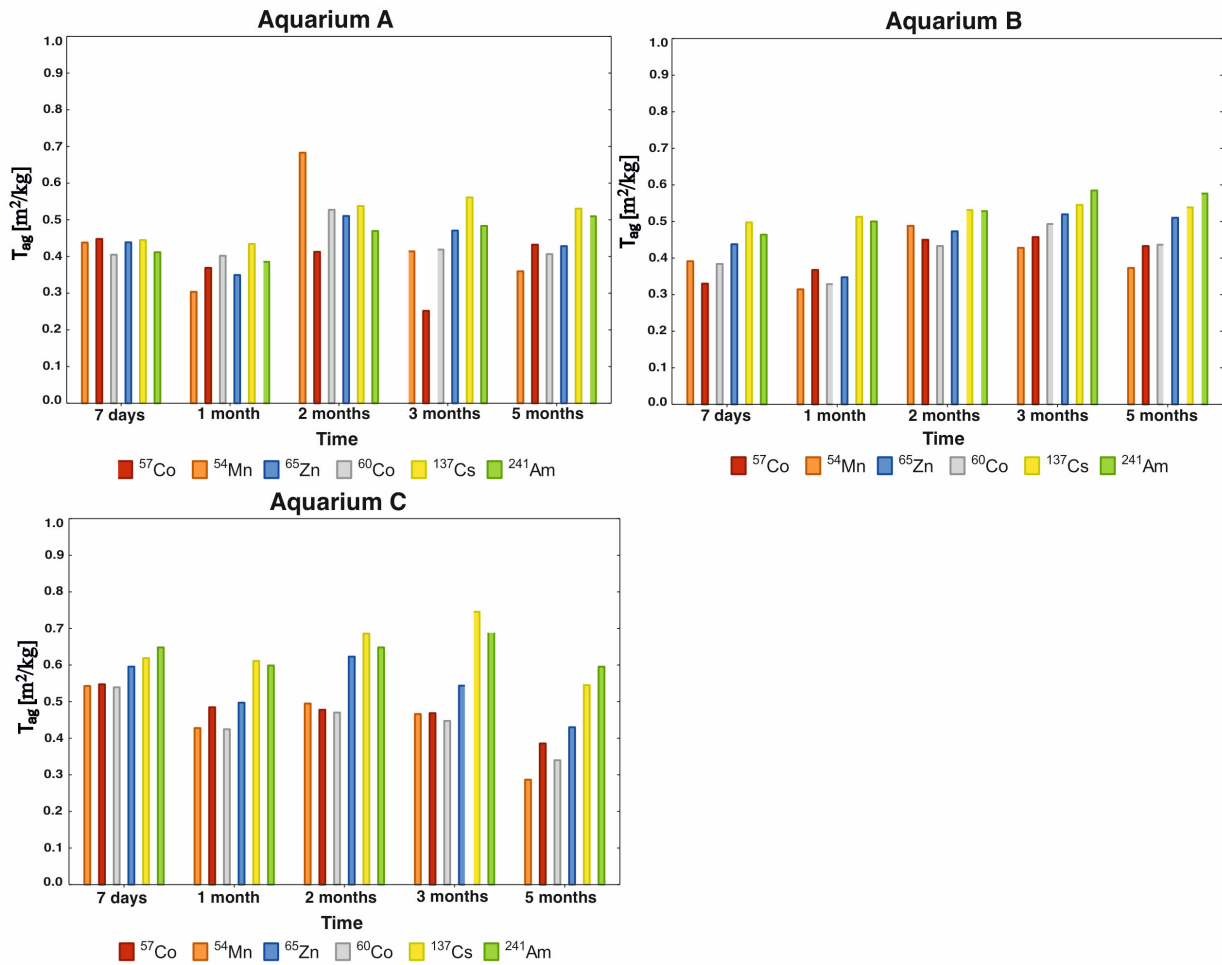


Figure 4. Temporal change (7 days, 1 month, 2 months, 3 months, 5 months) of aggregated transfer coefficient (Tag) of isotopes (^{57}Co , ^{54}Mn , ^{65}Zn , ^{60}Co , ^{137}Cs , and ^{241}Am) in *Cladonia uncialis*, in relation to deposition (Aquarium A, Aquarium B, Aquarium C).

Table 4. Average, minimum, and maximum values of the Tag in *C. uncialis* depending on the deposition.

	Deposition [Bq m ⁻²]	Tag [m ² kg ⁻¹] Average (Min–Max)
^{54}Mn	142	0.44 (0.30–0.68)
	284	0.40 (0.31–0.49)
	429	0.44 (0.29–0.54)
^{57}Co	15	0.38 (0.25–0.45)
	31	0.41 (0.33–0.46)
	46	0.47 (0.39–0.55)
^{60}Co	702	0.43 (0.40–0.53)
	1406	0.42 (0.33–0.49)
	2123	0.44 (0.34–0.54)
^{65}Zn	161	0.44 (0.35–0.51)
	322	0.42 (0.33–0.49)
	486	0.54 (0.43–0.62)
^{137}Cs	670	0.50 (0.43–0.56)
	1341	0.53 (0.50–0.55)
	2025	0.64 (0.55–0.75)
^{241}Am	896	0.45 (0.39–0.51)
	1793	0.53 (0.46–0.58)
	2707	0.64 (0.60–0.69)

4. Discussion

Experimental studies showed that the accumulation of ^{57}Co , ^{54}Mn , ^{65}Zn , ^{60}Co , ^{137}Cs , and ^{241}Am by *C. uncialis* proceeded immediately after deposition reaching equilibrium in the first days of the experiment as corroborated by our results (Figure 2). Monnet et al. 2006 reported that accumulation of metals by lichens occurs rapidly, in most cases within a few hours. In the case of Cu, maximum accumulation was observed after 3–6 h [23]. Short-term metal accumulation by lichens can be described as an “ion exchange” process involving carboxyl or hydrocarboxyl sites on outer cell walls or structural macromolecules [24]. Metals can be divided into two main categories: those essential for lichen metabolic processes and those that are trace elements with toxic properties. Essential elements for biological functions can easily cross the plasma membrane and are components of various enzymes, serving as enzyme activators [25,26]. Moreover, a well-known detoxification mechanism includes the binding of metals to organic acids, oxalate crystals, lichen secondary metabolites, polysaccharides, and melanin pigments [27,28]. Metals that are immobilised within cell wall components or situated extracellularly tend to be less detrimental to lichens. The absorption capacity of the elements increased in the order: $^{54}\text{Mn} < ^{57}\text{Co} < ^{60}\text{Co} \leq ^{65}\text{Zn} < ^{241}\text{Am} < ^{137}\text{Cs}$ (Table 4). Absorption rates were highest for ^{241}Am and ^{137}Cs , for which maximum values of Tag reached $0.64 \text{ m}^2/\text{kg}$ (Table 3). ^{137}Cs can be especially easily incorporated into the biological structure of plants due to its similarity to potassium ions [6]. ^{241}Am is an artificial element of recent origin, and thus does not have a biological role, however bioaccumulation by bacteria and fungi has been reported. The absorption of ^{241}Am by *R. arrhizus* is related to contact time. The adsorption rate increased rapidly with time and rose up to 99% in 1 h. Later, the adsorption rate grew slowly and tended to the equilibrium [29]. While the computation of Tag values is relatively straightforward, their application necessitates a comprehensive understanding of the ecosystem. Elements like the time of year or the type of soil can introduce variability in the observed Tag values. The aggregated transfer coefficient for ^{137}Cs for lichen found in the literature calculated after the Chernobyl accident ranges from $0.8 \text{ m}^2 \text{ kg}^{-1}$ to $2.3 \text{ m}^2 \text{ kg}^{-1}$ [30]. These values were much higher than those calculated in this publication. The accumulation of metals and radioactive isotopes in plants are influenced by numerous factors, including element availability, species, age, as well as environmental conditions such as temperature, humidity, substrate characteristics, and pH [31–33]. Consequently, metal transfer from the soil to lichens can also occur in nature, potentially leading to variations in results. In the case of our experiment, the activity levels in the soil were low enough that accumulation from the substrate would not have significantly impacted the results in the lichens. However, since the Tag values varied by up to twofold during the experiment, a balance of the isotopes accumulated in the lichens relative to the introduced isotopes was conducted (Figure 4, Table 4). The accumulation coefficient for each aquarium was calculated using Formula (2).

$$\text{Accumulation Coefficient} = \frac{\text{Activity of Lichen} \left[\frac{\text{Bq}}{\text{g}} \right] \times \text{Total Mass of Culture} [\text{g}]}{\text{Isotope Contamination} [\text{Bq}]} [\text{unit}] \quad (2)$$

For the four isotopes— ^{54}Mn , ^{57}Co , ^{60}Co and ^{65}Zn , the absorption efficiency at low concentrations (Aquarium A) was about 36–40% fallout. As the deposition increased (Aquarium B, C), the absorption efficiency decreased to 24–29% (Figure 5). In the case of ^{137}Cs and ^{241}Am , the absorption efficiency was significantly higher, oscillating around 50%. In addition, in the case of ^{137}Cs , a comparable absorption efficiency was observed regardless of the concentration in the fallout, suggesting that this isotope accumulated at a constant rate regardless of its initial concentration.

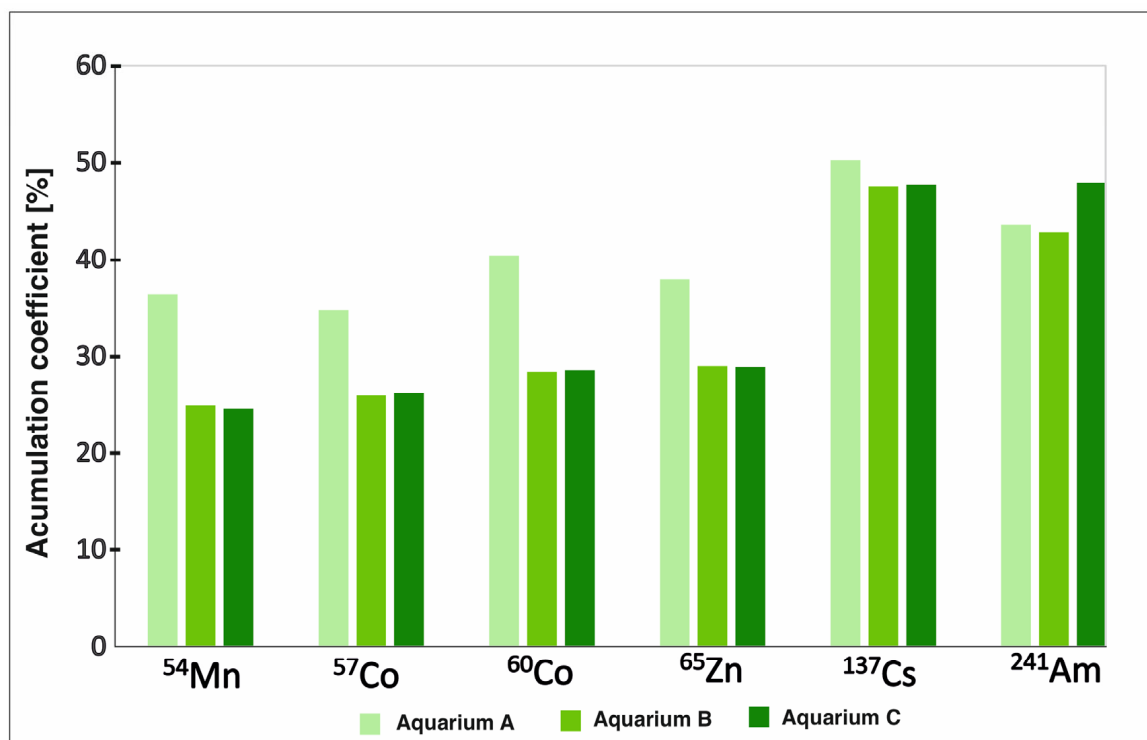


Figure 5. Accumulation efficiency at the different initial fallout concentrations.

Based on the experimental data from this study, it is possible that some Tag coefficients reported in the literature might be overestimated, and relying on them to calculate actual deposition could lead to overestimations [19]. Studies have shown that lichens respond to changes in atmospheric heavy metals within a few days, while the residence time of many elements in lichen thalli remains constant for at least 0.5 years.

5. Conclusions

Cladonia uncialis demonstrated a remarkable ability to bioaccumulate radioactive isotopes, including ⁵⁷Co, ⁵⁴Mn, ⁶⁵Zn, ⁶⁰Co, ¹³⁷Cs, and ²⁴¹Am, from the environment, with isotopic activity within lichen tissues being directly proportional to the baseline isotopic activity in the fallout. Rinsing the lichen samples with distilled water resulted in a minimal reduction of isotope activity, indicating that most isotopes bound to cell walls and outer membranes were trapped intracellularly rather than merely adhering to the surface. This finding underscores the species' capacity to absorb and retain radioactive materials effectively. The Aggregated Transfer Coefficient (Tag), which provides a quantitative measure of the lichen's bioaccumulation ability, revealed higher values for ¹³⁷Cs and ²⁴¹Am, with maximum coefficients reaching 0.63 and 0.64 m² kg⁻¹, respectively. Moreover, the activity of ¹³⁷Cs and ²⁴¹Am in lichen tissues reached equilibrium within the first few days after fallout, and the Tag values remained stable throughout the experiment. These results highlight the potential of *C. uncialis* as a reliable biomonitor for rapid and effective assessment of radioactive contamination in the environment.

Author Contributions: Conceptualisation, M.S., M.H.W. and T.Z.; methodology, M.S., M.H.W. and T.Z.; validation, M.S., M.H.W. and T.Z.; formal analysis, M.S.; investigation, M.S., M.H.W., T.Z., P.F., P.D. and K.W.; resources, M.S.; data curation, M.S.; writing—original draft preparation, M.S., M.H.W., T.Z., P.F., P.D. and K.W.; writing—review and editing, M.S., M.H.W., T.Z., P.F., P.D. and K.W.; visualisation M.S. and T.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This study has been performed within the framework of IMGW-PIB FBW-15/2023.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. International Atomic Energy Agency. *Worldwide Marine Radioactivity Studies (WOMARS): Radionuclide Levels in Oceans and Seas*; IAEA: Vienna, Austria, 2005; Volume IAEA-TECDOC-1429.
2. Saniewski, M.; Wietrzyk-Pełka, P.; Zalewska, T.; Węgrzyn, M.H. Current radioactive fallout contamination along a trans-European gradient assessed using terricolous lichens. *Chemosphere* **2022**, *304*, 135281. [[CrossRef](#)]
3. Saniewski, M.; Wietrzyk-Pełka, P.; Węgrzyn, M.H.; Saniewska, D.; Bałazy, P.; Zalewska, T. Distribution of ⁹⁰Sr and ¹³⁷Cs in biotic and abiotic elements of the coastal zone of the King George Island (South Shetland Archipelago, Antarctic Peninsula). *Chemosphere* **2023**, *322*, 138218. [[CrossRef](#)]
4. Anderson, J.; Lévesque, N.; Caron, F.; Beckett, P.; Spiers, G.A. A review on the use of lichens as a biomonitoring tool for environmental radioactivity. *J. Environ. Radioact.* **2022**, *243*, 106797. [[CrossRef](#)]
5. Conti, M.E.; Cecchetti, G. Biological monitoring: Lichens as bioindicators of air pollution assessment—A review. *Environ. Pollut.* **2001**, *114*, 471–492. [[CrossRef](#)]
6. Nimis, P.L.; Scheidegger, C.; Wolseley, P.A. Monitoring with lichens—Monitoring lichens. In *Monitoring with Lichens—Monitoring Lichens*; NATO Science Series; Springer: The Hague, The Netherlands, 2002. [[CrossRef](#)]
7. Purvis, O.W.; Pawlik-Skowrońska, B. Lichens and metals. *Br. Mycol. Soc. Symp. Ser.* **2008**, *27*, 175–200.
8. Nash, T.H. Nutrients, elemental accumulation and mineral cycling. In *Lichen Biology*; Nash, T.H., Ed.; Cambridge University Press: Cambridge, UK, 2008; pp. 234–251. [[CrossRef](#)]
9. Basile, A.; Sorbo, S.; Aprile, G.; Conte, B.; Castaldo Cobiانchi, R. Comparison of the heavy metal bioaccumulation capacity of an epiphytic moss and an epiphytic lichen. *Environ. Pollut.* **2008**, *151*, 401–407. [[CrossRef](#)]
10. Boonpeng, C.; Sangiamdee, D.; Noikrad, S.; Watthana, S.; Boonpragob, K. Metal accumulation in lichens as a tool for assessing atmospheric contamination in a natural park. *Environ. Nat. Resour. J.* **2020**, *18*, 166–176. [[CrossRef](#)]
11. Hale, M.E. *How to Know the Lichens*; Wm. C. Brown Company Publishers: Dubuque, IA, USA, 1969.
12. Garty, J.; Garty-Spitz, R.L. Lichens and particulate matter: Interrelations and biomonitoring with lichens. In *Recent Advances in Lichenology: Modern Methods and Approaches in Biomonitoring and Bioprospection*; Upreti, D.K., Divakar, P.K., Shukla, V., Bajpai, R., Eds.; Springer: New Delhi, India, 2015; pp. 47–85. [[CrossRef](#)]
13. Holt, E.A.; Nelson, P.R. Climatic, vegetative, and disturbance predictors of lichen species' height in Arctic Alaska, USA. *Polar. Biol.* **2021**, *44*, 133–145. [[CrossRef](#)]
14. Knops, J.M.H.; Nash, T.H.; Boucher, V.L.; Schlesinger, W.H. Mineral cycling and epiphytic lichens: Implications at the ecosystem level. *Lichenologist* **1991**, *23*, 309–321. [[CrossRef](#)]
15. Iurian, A.R.; Hofmann, W.; Lettner, H.; Türk, R.; Cosma, C. Long term study of Cs-137 concentrations in lichens and mosses. *Rom. J. Phys.* **2011**, *56*, 983–992.
16. Saniewski, M.; Wietrzyk-Pełka, P.; Zalewska, T.; Olech, O.; Węgrzyn, M.H. Bryophytes and lichens as fallout originated radionuclide indicators in the Svalbard Archipelago (High Arctic). *Polar Sci.* **2020**, *25*, 100536. [[CrossRef](#)]
17. Arctic Monitoring and Assessment Programme. *AMAP Assessment 2002: Radioactivity in the Arctic*; Arctic Monitoring and Assessment Programme: Oslo, Norway, 2004; ISBN 82-7971-017-5.
18. Koivurova, M.; Leppänen, A.-P.; Kallio, A. Transfer factors and effective half-lives of ¹³⁴Cs and ¹³⁷Cs in different environmental sample types obtained from Northern Finland: Case Fukushima accident. *J. Environ. Radioact.* **2015**, *146*, 73–79. [[CrossRef](#)] [[PubMed](#)]
19. Smith, C.W.; Aptroot, A.; Coppins, B.J.; Fletcher, A.; Gilbert, O.L.; James, P.W.; Wolseley, P.A. *Lichens of Great Britain and Ireland*; BLS: New Delhi, India, 2009; p. 1046.
20. Wirth, V.; Hauck, M.; Schultz, M.; De Bruyn, U.; Bültmann, H.; John, V.; Otte, V. *Die Flechten Deutschlands*; Eugen Ulmer: Stuttgart, Germany, 2013; Volume 2, 1244p.
21. Stenroos, S.; Velmala, S.; Pykälä, J.; Ahti, T. *Lichens of Finland. Norrlinia*; Botanical Museum, Finnish Museum of Natural History: Helsinki, Finland, 2016; Volume 30, 866p.
22. International Atomic Energy Agency (IAEA). *IAEA-TERC-2023-01: Technical Report*; IAEA: Vienna, Austria, 2023.
23. Monnet, F.; Bordas, F.; Deluchat, V.; Baudu, M. Toxicity of copper excess on the lichen *Dermatocarpon luridum*: Antioxidant enzyme activities. *Chemosphere* **2006**, *65*, 1806–1813. [[CrossRef](#)]
24. Haas, J.R.; Bailey, E.H.; Purvis, W. Bioaccumulation of metals by lichens; uptake of aqueous uranium by *Peltigera membranacea* as a function of time and pH. *Am. Mineral.* **1998**, *83*, 1494–1502. [[CrossRef](#)]
25. Bačkor, M.; Loppi, S. Interactions of lichens with heavy metals. *Biol. Plant.* **2009**, *53*, 214–222. [[CrossRef](#)]
26. Van Assche, F.; Clijsters, H. Effects of metals on enzyme activity in plants. *Plant Cell Environ.* **1990**, *13*, 195–206. [[CrossRef](#)]

27. Sarret, G.; Manceau, A.; Cuny, D.; Van Haluwyn, C.; Déruelle, S.; Hazemann, J.L.; Soldo, Y.; Eybert-Bérard, L.; Menthonnex, J.J. Mechanisms of lichen resistance to metallic pollution. *Environ. Sci. Technol.* **1998**, *32*, 3325–3330. [[CrossRef](#)]
28. Pawlik-Skowrońska, B.; Purvis, W.O.; Pirszel, J.; Skowroński, T. Cellular mechanisms of Cu-tolerance in the epilithic lichen *Lecanora polytropa* growing at a copper mine. *Lichenologist* **2006**, *38*, 267–275. [[CrossRef](#)]
29. Liu, N.; Yang, Y.; Luo, S.; Zhang, T.; Jin, J.; Liao, J.; Hua, X. Biosorption of ²⁴¹Am by *Rhizopus arrhizus*: Preliminary investigation and evaluation. *Appl. Radiat. Isot.* **2002**, *57*, 139–143. [[CrossRef](#)]
30. IAEA. *Modelling of Radionuclide Interception and Loss Processes in Vegetation and of Transfer in Semi-Natural Ecosystems*; IAEA-Tecdoc-857; IAEA: Vienna, Austria, 1996; pp. 49–84.
31. Golmakani, S.; Vahabi Moghaddam, M.; Hosseini, T. Factors affecting the transfer of radionuclides from the environment to plants. *Radiat. Prot. Dosim.* **2008**, *130*, 368–375. [[CrossRef](#)]
32. Wan, Y.; Liu, J.; Zhuang, Z.; Wang, Q.; Li, H. Heavy metals in agricultural soils: Sources, influencing factors, and remediation strategies. *Toxics* **2024**, *12*, 63. [[CrossRef](#)] [[PubMed](#)]
33. Saniewski, M.; Wietrzyk-Pelka, P.; Zalewska, T.; Osyczka, P.; Węgrzyn, M.H. Impact of distance from the glacier on the content of ¹³⁷Cs and ⁹⁰Sr in the lichen *Cetrariella delisei*. *Chemosphere* **2020**, *259*, 127433. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.