UNIWERSYTET OPOLSKI

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PRACA DOKTORSKA

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ZASTOSOWANIE MCHÓW W BIOMONITORINGU AKTYWNYM NA TERENACH ZURBANIZOWANYCH

APPLICATION OF MOSSES IN ACTIVE BIOMONITORING IN URBAN AREAS

Praca napisana pod kierunkiem
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PODZIĘKOWANIA

Według niemieckiego pedagoga Friedricha Wilhelma Fröbela „*Wychowanie to przykład i miłość - nic więcej*”. Miałem to szczęście, że mogłem tego doświadczyć dlatego w pierwszej kolejności dziękuję moim **Kochanym Rodzicom**. Za wiarę, za miłość, za przykład, za wsparcie, za cierpliwość. Opatrzność w tym życiu powierzyła nam wspólny los, który razem dzieląc dzielnie przechodzimy. Mógłbym pisać dużo i długo, tak jak tę rozprawę doktorską, ale to i tak nie odda chociaż cienia miłości i wdzięczności dla Was, Drodzy Rodzice, za to wszystko co sprawiło, że dzięki Wam dzisiaj to piszę… Kocham Was i dziękuję!

Podziękowania chciałbym również złożyć na ręce **Pana Profesora Arkadiusza Nowaka**, Promotora tej pracy, ponieważ bez jego udziału ta praca nie byłaby biologiczna. Botanik, niezłomny badacz i wytrwały człowiek, który podjął się współpracy na rzecz połączenia zagadnień biologicznych z analitycznymi, które dają skutek w postaci tej pracy. Chciałbym Panu Profesorowi bardzo serdecznie podziękować za każdy komentarz, uwagę, konstruktywną krytykę, wsparcie, pomoc, zaangażowanie, motywację do dalszego działania. Pańska historia i przykład pokazują, że można osiągnąć wszystko i ze wszystkimi będąc odpowiednio zdeterminowanym – dziękuję za ten botaniczny wzór do naśladowania na kolejne (mam nadzieję) etapy mojego rozwoju naukowego.

Chciałbym też podziękować osobie, która dekadę temu stanęła na mojej drodze i ukształtowała mnie na kolejne lata w nauce czego owocem jest ta kolejna praca, już czwarta, pod Jej przewodnictwem. Chciałbym serdecznie podziękować **Pani Profesor Małgorzacie Rajfur** za wieloletnią współpracę, która rozpoczynając się na studiach pierwszego stopnia trwa po dziś dzień z pełnym powodzeniem. Dziękuję Pani Profesor za zaangażowanie, walkę, wytrwałość, motywację, za dzielenie się doświadczeniem i włączaniem mnie w kolejne inicjatywy i działania naukowe. Któż jak nie my lepiej zrozumie sens łacińskiej sentencji: „*Ad augusta per angusta*”… Za wszystko Pani Profesor dziękuję - było warto!

AUTOREFERAT

1. Wskazane osiągnięcie wynikające z art. 187 ust. 3 Ustawy z dn. 20 lipca 2018 (Dz. U. z 2018, poz. 1668, z późn. zm.)
2. Wykaz publikacji przedstawionych do oceny w przewodzie doktorskim
3. Oświadczenia współautorów odnośnie ich udziału w powstawaniu publikacji przedstawionych do oceny w przewodzie doktorskim
4. Wykaz dorobku naukowego

1. WSKAZANE OSIĄGNIĘCIE WYNIKAJĄCE Z ART. 187 UST. 3 USTAWY Z DN. 20 LIPCA 2018 (Dz. U. z 2018, poz. 1668, z późn. zm.) PRAWO O SZKOLNICTWIE WYŻSZYM I NAUCE

Podstawą do ubiegania się o uzyskanie stopnia doktora w dyscyplinie nauki biologiczne jest cykl 9 jednotematycznych publikacji (artykuły oznaczone w wykazie dorobku naukowego – ON.1. – ON.9.) zatytułowany:

Zastosowanie mchów w biomonitoringu aktywnym na terenach zurbanizowanych

Poniżej zamieszczam wybór publikacji do oceny w przewodzie doktorskim stanowiących jednotematyczny cykl (Pełne teksty publikacji zostały zamieszczone w *Wykazie publikacji przedstawionych do oceny w przewodzie doktorskim*):

[ON.1.] **Świsłowski P**., Nowak A., Wacławek S., Daniele Silvestri, Rajfur M.: *Bioaccumulation of Trace Elements from Aqueous Solutions by Selected Terrestrial Moss Species*. Biology. 2022; 11:1692. DOI: 10.3390/biology11121692. IF = 5,168; 100 pkt MEiN.

[ON.2.] **Świsłowski P**., Kosior G., Rajfur M.: *The influence of preparation methodology on the concentration of heavy metals in Pleurozium schreberi moss samples prior to use in active biomonitoring studies.* Environ. Sci. Pollut. Res 2021;28(8):10068-10076. DOI: 10.1007/s11356-020-11484-7. IF = 3,056; 100 pkt MEiN.

[ON.3.] **Świsłowski P**., Nowak A., Rajfur M.*: Comparison of exposure techniques and vitality assessment in active biomonitoring for suitability in assessing atmospheric aerosol heavy metal pollution*. Environ. Toxicol. Chem 2022;41(6):1429-1438**.** DOI: 10.1002/etc.5321**.** IF = 3,742; 100 pkt MEiN.

[ON.4.] **Świsłowski P**., Nowak A., Rajfur M.*: Is Your Moss Alive during Active Biomonitoring Study?* Plants 2021;10(11):2389. DOI: 10.3390/plants10112389. IF = 3,935; 70 pkt MEiN.

[ON.5.] **Świsłowski P**., Nowak A., Rajfur M.*: The influence of environmental conditions on the lifespan of mosses under long-term active biomonitoring*. Atmos. Pollut. Res

2021;12(10):101203. DOI: 10.1016/j.apr.2021.101203. IF = 4,352; 70 pkt MEiN.

[ON.6.] **Świsłowski P**., Śmiechowicz B., Rajfur M.: *Effects of tobacco smoke on indoor air quality: the use of mosses in biomonitoring*. J. Environ. Health Sci 2022. DOI: 10.1007/s40201-022-00794-2. IF = 2,130; 100 pkt MEiN.

[ON.7.] **Świsłowski P**., Vergel K., Zinicovscaia I., Rajfur M., Wacławek M.: *Mosses as a biomonitor to identify elements released into the air as a result of car workshop activities*. Ecol. Indic 2022;138:108849. DOI: 10.1016/j.ecolind.2022.108849. IF = 4,958; 140 pkt MEiN.

[ON.8.] **Świsłowski P**., Ziembik Z., Rajfur M.: *Air Quality during New Year's Eve: A Biomonitoring Study with Moss*. Atmosphere 2021;12(8):975. DOI: 10.3390/atmos12080975. IF = 2,686; 70 pkt MEiN.

[ON.9.] **Świsłowski P**., Nowak A., Wacławek S., Ziembik Z., Rajfur M.: *Is active moss biomonitoring comparable to air filter standard sampling?* Int. J. Environ. Res. Public Health 2022;19(8):4706. DOI: 10.3390/ijerph19084706. IF = 3,390; 140 pkt MEiN.

Sumaryczny Impact Factor publikacji wchodzących w skład rozprawy doktorskiej wynosi **33,417** a liczba punktów MEiN to **890**.

Mój wkład w powstanie wyżej wymienionych publikacji jest dominujący. Deklaracje współautorów odnośnie ich udziału w powstawaniu publikacji zamieściłem w wykazie *Oświadczeń współautorów odnośnie ich udziału w powstawaniu publikacji przedstawionych do oceny w przewodzie doktorskim*.

Zakres tematyczny zaprezentowany w wymienionych powyżej pracach dotyczy badania kinetyki oraz wpływu czynników biotycznych i abiotycznych na procesy sorpcji metali ciężkich na trzech gatunkach mchów: *Pleurozium schreberi* (Willd. ex Brid.) Mitt., *Sphagnum fallax* (Klinggr.) Klinggr. i *Dicranum polysetum* Sw., w celu oceny możliwości ich wykorzystania w aktywnym biomonitoringu zanieczyszczenia powietrza na terenach zurbanizowanych. W przedstawionych pracach wskazuję na sposoby i metody optymalizacji techniki *moss bag* w ramach unifikacji zastosowania tej techniki w monitoringu jakości aerozolu atmosferycznego a także prezentuję możliwości praktycznego zastosowania

aktywnej metody biomonitoringu. W swoich pracach podkreślam wpływ czynników środowiskowych na żywotność mchów, ich aktywność fotosyntetyczną a tym samym ich przydatność jako bioindykatorów w kontroli jakości powietrza.

Szczegółowy zakres badań zawarty w indywidualnym Planie badawczym i zrealizowany dotyczył:

1) Wpływu metali ciężkich obecnych w układzie mchy – roztwór wodny (imitujący aerozol atmosferyczny) [roztwory jedno pierwiastkowe oraz mieszanina równomolowa wszystkich analitów] na kondycję trzech gatunków mchów – eksperyment laboratoryjny. [ON.1]

   Badania laboratoryjne kinetyki i równowag sorpcji w układzie mchy – roztwór wodny (pomiary na absorpcyjnym spektrometrze atomowym (AAS) [doświadczenie prowadzone było na mchach: *Pleurozium schreberi, Sphagnum fallax* i *Dicranum polysetum*; przeprowadzenie badań właściwości sorpcyjnych mchów dotyczących kinetyki procesu sorpcji wybranych kationów metali ciężkich: Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} i Pb^{2+}. [ON.1]
2) Oceny jednorodności składu chemicznego mchów: *Pleurozium schreberi, Sphagnum fallax* i *Dicranum polysetum* na podstawie ich analizy pod względem zanieczyszczenia metalami ciężkimi. [ON. 2]
3) Przetestowania wybranych sposobów ekspozycji próbek mchów: *Pleurozium schreberi, Sphagnum fallax i Dicranum polysetum* w terenie (metoda *moss bag*: wariant 1: mchy w woreczkach, wariant 2: mchy w woreczkach osłoniętych od działania opadu atmosferycznego, wariant 3: mchy w woreczkach osłoniętych od działania wiatru) oraz transplantacja mchów w plastikowych pojemnikach wraz z podłożem). Badania pozwoliły na wybór optymalnego sposobu ekspozycji materiału biologicznego w terenie. [ON. 3]
4) Wpływu metali ciężkich na kondycję witalną trzech gatunków mchów (pomiar zawartości chlorofilu a i b w gametofitach mchów oraz aktywności fotosyntetycznej) – badania w środowisku naturalnym. [ON. 4]
5) Oceny wpływu czynników środowiskowych tj. kierunku wiatru i odległości od źródła emisji na stężenia metali ciężkich zakumulowanych w mchach (wykorzystany został gatunek mchu najbardziej odporny na zanieczyszczenia środowiska) eksponowanych wybraną metodą. Oddziaływanie tych czynników było kluczowe, aby badania mogły być porównywalne z metodą klasyczną jaką jest ocena zanieczyszczenia powietrza

poprzez analizę metali ciężkich w pyle zawieszonym. Miejsce ekspozycji próbek oraz czas ekspozycji są podstawowymi czynnikami, które wpływają na miarodajność wyników biomonitoringowych. [ON. 5]

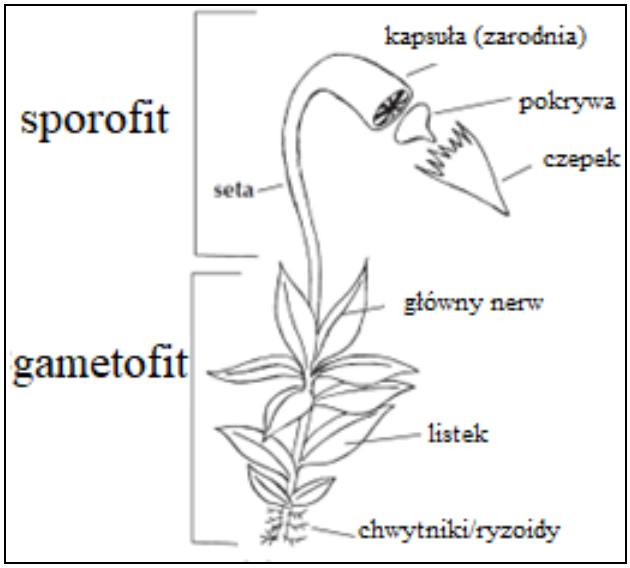
6) Aplikacyjnego zastosowania badań biomonitoringu do oceny zanieczyszczenia powietrza w pomieszczeniach zamkniętych [ON. 6], identyfikacja punktowych źródeł zanieczyszczeń [ON. 7] oraz krótkoterminowy biomonitoring aktywny na terenie zurbanizowanym [ON. 8]
7) Porównania wyników badań biomonitoringowych (wykorzystany został gatunek mchu najbardziej odporny na zanieczyszczenia środowiska) z danymi uzyskanymi w ramach monitoringu klasycznego (pobornik próbek całkowitego pyłu zawieszonego TSP - *Total Suspended Particulates*). [ON. 9]

Omówienie celu naukowego oraz postawionej hipotezy badawczej w pracach wchodzących w skład jednotematycznego cyklu publikacji

Mszaki to niewielka grupa składająca się z trzech klas nienaczyniowych roślin lądowych (mchy, wątrobowce i glewiki) i obejmuje około 20 000 gatunków (Magill, 2014). Pomimo tego, że mszaki to jedna z najstarszych grup roślin lądowych liczących ponad 330 milionów lat (wczesny karbon) to ich długa historia nie jest udokumentowana odpowiednio liczną bazą skamieniałości (Shelton et al., 2015). Briologia (gr. *bryon* – mech, *logos* – nauka) jest nauką o mszakach stanowiącą jeden z działów botaniki. Pierwsze opisy mchów sporządzano już w XVII wieku, niemniej jednak systematyczny przyrost wiedzy i początek profesjonalnych badań briologicznych rozpoczyna się od prac Johanna Hedwiga (XVIII-XIX w.) uważanego za ojca briologii (J. Shaw & Renzaglia, 2004).

Mchy (*Bryophyta*) są eukariotycznymi roślinami telomowymi niewielkich rozmiarów. Są to rośliny nienaczyniowe i zazwyczaj nie mają wewnętrznego systemu transportu. Nie posiadają kwiatów ani „prawdziwego" systemu korzeniowego lecz jedynie chwytniki (Rys. 1). Można je odnaleźć nie tylko w lasach, ale jako pionierskie organizmy zajmują tereny zurbanizowane, jak również miejsca, gdzie rośliny wyższe nie mogą przetrwać z powodu: wysokiej temperatury (pustynia), wysokości nad poziomem morza czy ograniczonego dostępu światła na obszarach takich jak tundra (Haynes et al., 2019; Schaaf et al., 2018). Obecnie różnorodność mchów to około 12-13 tysięcy gatunków (Y. Liu et al., 2019), nadal jednak w różnych częściach świata wciąż odkrywa się nowe gatunki mchów dla obszarów słabo zbadanych pod względem briologicznym (Stebel et al., 2018).

Mchy generalnie występują w dwóch formach. Pierwsza z nich - pionowa, zazwyczaj nierozgałęziona forma generuje sporofit z czubka łodygi (akrokarpy) i zazwyczaj rosną na ziemi. Z kolei te, które rozprzestrzeniają się poziomo, są rozgałęzione i produkują sporofity z boku łodygi, określane są mianem pleurokarpów. Ten rodzaj mchu zazwyczaj rośnie na drzewach, innych roślinach i ściółce. Wyjątek stanowi mech torfowy (*Sphagnum* sp.), który nie ma sety. Sporangium wznosi się na łodydze bezlistnej, gametofitycznej tkance macierzystej- sporangium pęka, wybucha i wyrzuca do powietrza zarodniki (Hallingbäck & Hodgetts, 2015; United States Government Publishing Office, 2008).



Rys. 1. Budowa morfologiczna mchu (United States Government Publishing Office, 2008)

Jeżeli chodzi o funkcje użytkowe mchów, to mogą stanowić odpowiedni materiał do badań metabolicznych w szczególności do rekombinacji genów (J. Liu et al., 2016). Mają także zastosowanie medyczne, np. służą do tamowania krwawienia (Drobnik & Stebel, 2018).

Ze względu na zmiany wywoływane przez człowieka w środowisku (głównie lata 50-te i 60-te XX w. - rozwój przemysłu i motoryzacji) wzrosło zanieczyszczenie powietrza toksycznymi pierwiastkami, określanymi mianem metali ciężkich (Cd, Pb czy Hg). Pociągnęło to za sobą negatywne skutki głównie dla środowiska biotycznego. W związku z tym zaczęto poszukiwać biologicznych metod do oceny poziomu zanieczyszczenia środowiska przyrodniczego (Zechmeister et al., 2003).

Pod koniec lat 60-tych XX wieku, dwóch szwedzkich naukowców Å. Rühling i G. Tyler odkryło, że mchy są dobrymi bioindykatorami zanieczyszczenia metalami ciężkimi w atmosferze, dając podwaliny pod pierwsze badania biomonitoringowe. Z kolei pierwsze

europejskie badania na szeroką skalę to dopiero lata 90-te ubiegłego wieku, później powtarzane okresowo co 5 lat (Paliulis & Blagnytė, 2010).

W biomonitoringu zanieczyszczenia powietrza metalami ciężkimi najczęściej stosowanymi gatunkami mchów są: *Pleurozium schreberi*, *Hypnum cupressiforme* czy *Hylocomium splendens* (Shetekauri et al., 2018).

Główne zalety wykorzystania mchów jako biomonitorów skażenia środowiska to:
- brak skórki, czyli tkanki mchów są łatwo przepuszczalne dla mikro- i makroelementów ,
- wysoka zdolność wymiany kationowej,
- łatwa dostępność mchów jako roślin kosmopolitycznych - powszechnie występują w różnych siedliskach,
- niski koszt pozyskania materiału badawczego,
- w szybki sposób można uzyskać informację o jakości środowiska,
- relatywnie szybkie tempo wzrostu i zasiedlania nowych terenów (Cenci, 2008; Macedo-Miranda et al., 2016).

Pomimo wielu zalet dotyczących wykorzystania mchów w biomonitoringu, nie brakuje też kilku ograniczeń związanych z zastosowaniem tych organizmów. Do nich zaliczamy m.in.: ograniczoną wiedzę na temat wewnętrznego rozmieszczenia metali ciężkich w tkankach mchów (Šoltés & Gregušková, 2013). Największe jednak problemy występują z ujednoliceniem protokołu wykorzystania mchów w monitorowaniu terenów miejskich (Zinicovscaia et al., 2018), czy też wciąż niezakończone dyskusje na temat naukowych kryteriów wyżej wymienionych protokołów (Miteva et al., 2017). W badaniach wykorzystujących metodę *moss bag* (siatka/worek z mchami) aktywnego biomonitoringu kładzie się nacisk na standaryzację protokołów badawczych oraz walidację procedury przygotowawczej próbek przed ekspozycją (Arndt & Planer-Friedrich, 2018; Iodice et al., 2016). Niejednokrotnie, wnioski jakie można wyciągnąć z doświadczeń są bardzo ograniczone, nie ma możliwości porównania ich do wyników badań z monitoringu klasycznego, przez co biomonitoring jest mało wiarygodnym narzędziem, nie uwzględnianym przy opracowywaniu i wdrażaniu działań na rzecz ochrony środowiska czy też jego monitoringu (Fernández et al., 2015).

Za celowe uznałem więc przeprowadzenie analiz dotyczących opracowania i przetestowania metody prowadzenia badań monitoringowych jakości powietrza pod względem zanieczyszczenia metalami ciężkimi (Mn, Fe, Ni, Cu, Zn, Cd, Hg i Pb) z wykorzystaniem trzech gatunków mchów: *Pleurozium schreberi, Sphagnum fallax* i *Dicranum polysetum*, w ramach biomonitoringu aktywnego. Zastosowanie jej pozwoliłoby na

uzyskanie miarodajnych wyników, skorelowanych z danymi otrzymywanymi metodami klasycznymi. W literaturze brak jest prac, których autorzy podejmowaliby próbę porównania wyników biomonitoringu z wykorzystaniem mchów z wynikami badań uzyskanymi w ramach np. Państwowego Monitoringu Środowiska (ocena zanieczyszczenia powietrza poprzez analizę metali ciężkich w pyle zawieszonym metodami instrumentalnymi). Istotą eksperymentu jest zwalidowanie metody biomonitoringu aktywnego i wykazanie, że biomonitoring może skutecznie konkurować z klasycznymi metodami monitorowania jakości aerozolu atmosferycznego. Kluczowa jest również kontrola parametrów życiowych mchów, aby móc mówić o biomonitoringu środowiska z wykorzystaniem organizmu żywego. Dlatego też za celowe uznałem przeprowadzenie badań podstawowych dotyczących oceny właściwości sorpcyjnych mchów oraz dokonania oceny wpływu metali ciężkich obecnych w roztworze na ich kondycję. Dopiero szczegółowa analiza wyników badań przeprowadzonych na trzech gatunkach mchów pozwoliła na opracowanie metody oceny zanieczyszczenia aerozolu atmosferycznego metalami ciężkimi na podstawie analizy składu chemicznego mchów.

Szczegółowe cele przeprowadzonych przeze mnie badań:

1. Poznanie wpływu metali ciężkich obecnych w układzie mchy – roztwór wodny na kondycję mchów (m.in. pomiar zawartości chlorofilu w gametofitach mchów).
2. Dokonanie oceny jednorodności składu chemicznego organizmów mchów na podstawie ich analizy pod względem zanieczyszczenia metalami ciężkimi.
3. Porównanie wybranych sposobów ekspozycji próbek mchów w terenie pod względem różnic w akumulacji pierwiastków przez mchy.
4. Dokonanie oceny wpływu czynników środowiskowych na stężenia metali ciężkich zakumulowanych w mchach eksponowanych wybraną metodą.
5. Przeprowadzenie oceny zanieczyszczenia powietrza metalami ciężkimi z zastosowaniem mchów, w ramach biomonitoringu aktywnego oraz metodą klasyczną [ocena zanieczyszczenia powietrza poprzez analizę metali ciężkich w pyle zawieszonym].

Hipoteza badawcza:

Główne założenia prezentowanej rozprawy doktorskiej oparłem na następujących hipotezach badawczych:

1. Gametofity mchów eksponowane w terenie w ramach biomonitoringu aktywnego odznaczają się w okresie ekspozycji witalnością życiową i zdolnością do przeprowadzania procesu fotosyntezy.
2. Stężenia metali ciężkich zakumulowanych w mchach są proporcjonalne do ich stężenia w aerozolu atmosferycznym.
3. Wyniki badań biomonitoringowych z wykorzystaniem mchów korelują z wynikami stężeń metali ciężkich (zawartych w całkowitym pyle zawieszonym) otrzymywanymi metodami klasycznymi.

Syntetyczna prezentacja osiągniętych wyników zamieszczonych w pracach wchodzących w skład jednotematycznego cyklu publikacji [ON.1. – ON.9.]

Materiały, metody oraz techniki analityczne zastosowane w pracach [ON.1. – ON.9.]

Badania w ramach rozprawy doktorskiej prowadziłem z wykorzystaniem trzech gatunków mchów naziemnych: *P. schreberi* (Pl), *S. fallax* (Sp) i *D. polysetum* (Dp). Zbierałem je w lasach południowo-wschodniej Polski w województwie świętokrzyskim. Mchy pochodziły z mezoregionu Puszczy Świętokrzyskiej, Nadleśnictwa Stąporków w okolicach wsi Stary Janów, Błotnica i Wąsosz. Do badań mchy zbierałem również w województwie opolskim (Nadleśnictwo Prószków, Bory Niemodlińskie). Mchy pobierałem zgodnie z obowiązującymi przepisami prawa oraz wytycznymi zawartymi w rozporządzeniu Ministerstwa Środowiska z 2014 r (Environment, 2014).

Przed ekspozycją próbki mchów poddawałem obróbce wstępnej, oczyszczałem z liści, igieł, ziemi. Mchy zbierałem w odległości co najmniej 5 m od koron drzew, aby nie były bezpośrednio narażone na opady atmosferyczne (pobierałem tylko zielone części mchów) zgodnie z wytycznymi programu ICP Vegetation (ICP Vegetation, 2020). Przed ekspozycją mchy kondycjonowałem w wodzie demineralizowanej zgodnie z opracowaną metodyką zawartą w osiągnięciu [ON.2.]. Następnie odpowiednią gramaturę mchów pakowałem do torebek siatkowych (technika *moss bag*) i eksponowałem w danym terenie, przez określony czas na wysokości około 1,5-2 metrów od poziomu gruntu.

Po danym czasie ekspozycji, w zależności od eksperymentu, mchy zawoziłem do laboratorium i suszyłem w temperaturze pokojowej do uzyskania suchej masy (s.m.) przed ich

mineralizacją w pojemnikach teflonowych. Tak przygotowaną każdą próbkę mchu o masie 0,400/0,500/1,000 ± 0,001 g s.m. poddałem mineralizacji w mieszaninie kwasu azotowego (V) i nadtlenku wodoru (HNO_3 65%:H_2O_2 37% w proporcji 5:3) przy użyciu mineralizatora mikrofalowego Speedwave Four Berghof, DE. Proces mineralizacji prowadziłem w temperaturze 180°C, ok. 45-50 minut. Stężenia metali oznaczałem w roztworze po mineralizacji i przesączeniu do kolb miarowych.

Stężenia metali ciężkich (Mn, Fe, Ni, Cu, Zn, Cd i Pb) oznaczałem przy użyciu techniki absorpcyjnej spektroskopii atomowej, atomizacji w płomieniu (F-AAS), na aparacie typu iCE 3500 (seria 3000) firmy Thermo Scientific, USA. Stężenie rtęci w próbkach mchów o gramaturze 0,040 g ± 0,001 g s.m. oznaczałem za pomocą analizatora rtęci AMA 254 firmy Altec Ltd., CZ. Do tych analiz mchy nie były mineralizowane - pomiar rtęci następował w próbkach bezpośrednio po ich zebraniu. Do pomiaru stężeń pozostałych pierwiastków analizowanych w [ON.7] próbki poddano neutronowej analizie aktywacyjnej w reaktorze IBR-2 (Dubna, Rosja).

W przypadku zawartości chlorofilu pomiary w terenie przeprowadziłem przy użyciu przenośnego miernika zawartości chlorofilu CCM-300 firmy Opti-Sciences, Inc (Hudson, NH, USA). Do analiz laboratoryjnych do pomiaru stężenia chlorofilu w mchach wykorzystałem spektrofotometr Cary 3500 UV-Vis Compact Peltier firmy Agilent Technologies (Santa Clara, CA, USA).

Fluorescencja chlorofilu PS II była monitorowana przy użyciu modulowanego przenośnego fluorometru (Opti-Sciences, Hudson, NH, USA). Rzeczywistą wydajność fotochemiczną mierzyłem w warunkach oświetlenia otoczenia. Pomiarów dokonywałem w terenie (warunki naturalne).

Szczegółowa metodyka badań została omówiona w każdym z artykułów [ON.1. – ON.9.].

Omówienie wyników zamieszczonych w pracach [ON.1. – ON.9.]

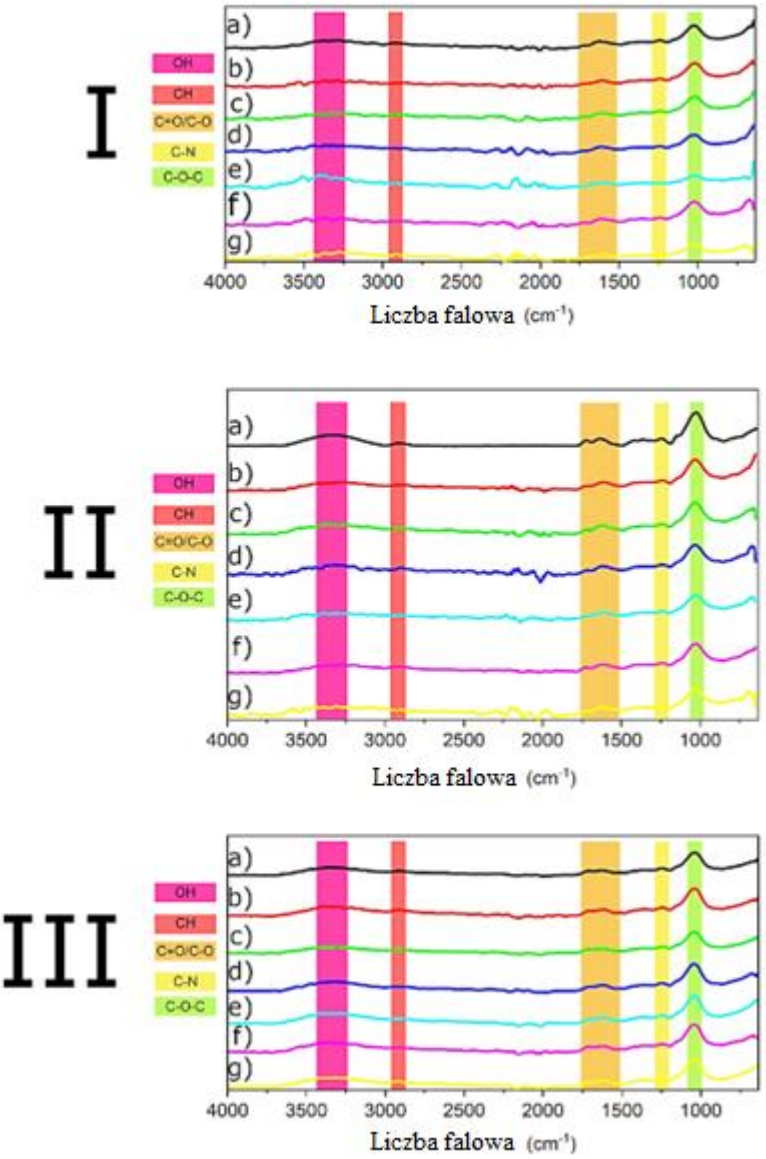
[ON.1.] Zastosowanie różnych technik analitycznych i laboratoryjnych umożliwia ocenę biokoncentracji związków i pierwiastków śladowych oraz ich wpływu na żywotność biomonitorów (Basile et al., 2008; Byun et al., 2021; Li et al., 2014). Pomimo wielu zalet stosowania klonów i dewitalizowanych biomonitorów (Di Palma et al., 2019; Fortuna et al., 2021) podtrzymuję stanowisko pomiaru i kontrolowania parametrów życiowych mchów podczas badań biomonitoringowych (Capozzi et al., 2020; Chen et al., 2015) w celu poznania rzeczywistej reakcji żywego organizmu na zanieczyszczenie środowiska, a nie tylko martwej

tkanki jako absorbentu chemicznego (Boquete et al., 2017; Świsłowski et al., 2021). W środowiskach o dużym poziomie zanieczyszczenia aerozolu atmosferycznego metalami ciężkimi fotosynteza *Pseudoscleropodium purum* jest hamowana, a ekspresja białek w tym mchu jest podatna na modyfikacje związane z warunkami środowiskowymi (Boquete et al., 2014). Zatem zrozumienie wpływu analitów na cechy morfofizjologiczne mszaków może mieć fundamentalne znaczenie dla optymalizacji ich wykorzystania w biomonitoringu (Bellini et al., 2021). Dlatego, oprócz badania sorpcji powierzchniowej pierwiastków śladowych przez mchy, konieczne jest skupienie się na biokoncentracji tych pierwiastków w ich wnętrzu i poszukiwanie związków między cząstkami przyczepionymi do powierzchni a tymi bioakumulowanymi formami w ich tkankach (Bustamante et al., 2015). W związku z tym, celem pierwszej pracy [ON.1] było poznanie zmienności biokoncentracji wybranych metali w mchach trzech gatunków: *P. schreberi, S. fallax* i *D. polysetum*, z wodnych roztworów tych pierwiastków. Przeanalizowałem sorpcję pięciu metali antropogenicznych, które często znajdują się w aerozolu atmosferycznym. Zweryfikowałem hipotezę badawczą, że mchy aktywnie gromadzą anality w swoich tkankach podczas ekspozycji na zanieczyszczenia poprzez analizę kinetyki sorpcji jonów metali ciężkich w ich gametofitach oraz poprzez identyfikację głównych grup funkcyjnych obecnych w tkankach mchów, które są odpowiedzialne za akumulację metali.

Intensywność akumulacji analitów w mchach zależy między innymi od ich powinowactwa do grup funkcyjnych tworzących związki budujące ścianę komórkową, a także od budowy i rozwoju powierzchni gametofitu mchu (Stanković et al., 2018). W warunkach przeprowadzonych badań akumulacja pierwiastków śladowych zależy od gatunków mchów w kolejności: *S. fallax* < *D. polysetum* < *P. schreberi.* Dane literaturowe wskazują, że proces akumulacji metali ciężkich w mchach zachodzi głównie poprzez wymianę jonową i ma charakter powierzchniowy (Printarakul & Meeinkuirt, 2022). Ten sam efekt zaobserwowano podczas procesów sorpcji metali ciężkich na porostach (Kłos et al., 2005). Należy jednak zauważyć, że równolegle lub wtórnie metale gromadzą się w wewnątrzkomórkowych strukturach mchów (Fasani et al., 2022; Stanković et al., 2018).

Analizę FTIR (*Fourier Transform Infrared Spectroscopy* - transformacja Fouriera spektroskopii w podczerwieni) przeprowadziłem aby lepiej zrozumieć mechanizmy sorpcyjne związane z usuwaniem kationów metali z roztworów (w warunkach środowiskowych z aerozolu atmosferycznego). Dlatego porównałem mchy przed adsorpcją i po procesie akumulacji przeprowadzonym w warunkach laboratoryjnych. Analizę FTIR głównych grup

funkcyjnych występujących w mchach odpowiedzialnych za akumulację metali przedstawiłem na rysunku 2.



Rys. 2. FTIR z I) *P. schreber*i a) przed adsorpcją, b) po adsorpcji Ni^{2+}, c) po adsorpcji Cu^{2+}, d) po adsorpcji Zn^{2+}, e) po adsorpcji Cd^{2+}, f) po adsorpcji Pb^{2+} i g) mieszanina kationów. II) *D. polysetum* a) przed adsorpcją, b) po adsorpcji Ni^{2+}, c) po adsorpcji Cu^{2+}, d) po adsorpcji Zn^{2+}, e) po adsorpcji Cd^{2+}, f) po adsorpcji Pb^{2+} i g) mieszanina kationów. III) *S. Fallax* a) przed adsorpcją, b) po adsorpcji Ni^{2+}, c) po adsorpcji Cu^{2+}, d) po adsorpcji Zn^{2+}, e) po adsorpcji Cd^{2+}, f) po adsorpcji Pb^{2+} i g) mieszanina kationów.

Wykazałem, że mchy składają się głównie z węglowodanów (>50%), lipidów i białek (Lewis et al., 2013; Maksimova et al., 2013), które zostały zidentyfikowane przez widma FTIR w *P. schreberi, D. polysetum* i *S. fallax*. Jak pokazałem na Rysunku 2, po adsorpcji różnych kationów metali, kilka pików zmieniło intensywność w porównaniu do widm mchów przed adsorpcją. W przypadku *P. schreberi* i *D. polysetum* pasmo O-H/N-H jest ogromnie

zmienione po procesie adsorpcji. W ten sam sposób pasma na ~1716 i ~1616 cm^{-1} ulegają zmianom. Wreszcie, dwa ostatnie pasma na ~1250 i ~1040 cm^{-1} również wykazują redukcję intensywności, ale w mniejszym stopniu niż poprzednia. Adsorpcja metali na mchach może być spowodowana oddziaływaniami elektrostatycznymi między jonami i ujemnie naładowanymi na nich grupami funkcyjnymi, jak wcześniej informowali Vinod i Sashidhar (Vinod & Sashidhar, 2011). Widoczny spadek intensywności piku O-H/N-H może wskazywać, że związane grupy funkcyjne O-H i ugrupowania amidowe mogą odgrywać istotną rolę w sorpcji jonów metali. Podobną obserwację odnotowano dla adsorpcji Ni^{2+} przez adsorbent gumy węglowodanowej (Vinod et al., 2010). Ponadto spadek intensywności piku ~1716 i ~1616 cm^{-1} po adsorpcji może wskazywać na udział grup C=O i N-H w koniugacji kationów metali.

Wyniki badań wskazują, że proces bioakumulacji metali ciężkich w mchach zachodzi głównie poprzez wymianę jonową, o czym świadczy między innymi spadek stężenia jonów metali w roztworze, z którym mają kontakt i jednoczesny wzrost przewodności w roztworze. Należy jednak zauważyć, że równolegle lub wtórnie metale gromadzą się w wewnątrzkomórkowych strukturach mchów. Analiza FTIR próbek mchów potwierdziła udział grup hydroksylowych, aminowych i karbonylowych w procesie biosorpcji kationów metali. Przedstawione wyniki badań wskazują na zależność pomiędzy stężeniem kationów w mchach i wokół nich (w roztworze/aerozolu atmosferycznym). Jednocześnie przeprowadzona analiza właściwości sorpcyjnych mchów pozwoliła na wskazanie odpowiednich gatunków/gatunku mchów do badań biomonitoringowych.

W kolejnym etapie dokonałem oceny jednorodności gametofitów mchów pod kątem ich zanieczyszczenia metalami ciężkimi oraz skupiłem się na opracowaniu metody ich przygotowania do badań biomonitoringowych.

[ON.2] Biomonitoring aktywny z wykorzystaniem mchów (*moss bag*) jest powszechnie stosowany w całej Europie do oceny zanieczyszczenia środowiska metalami ciężkimi, wielopierścieniowymi węglowodorami aromatycznymi (WWA) lub innymi zanieczyszczeniami organicznymi (Aničić, Tasić, Frontasyeva, Tomašević, Rajšić, Mijić, et al., 2009; Kosior et al., 2017; Vuković et al., 2015). Realizacja takich badań wymaga jednak usystematyzowania procedury eksperymentalnej. We wszystkich doświadczeniach wykorzystujących metodę *moss bag* podkreślane jest, że element standaryzacji protokołów badawczych i procedur przygotowania próbek mchu przed narażeniem na zanieczyszczenie w terenie jest kluczowy dla otrzymania wiarygodnych wyników (Arndt & Planer-Friedrich, 2018; Iodice et al., 2016; Salo et al., 2016). Przygotowanie materiału najczęściej obejmuje

zbieranie mchów ze stosunkowo czystych/niezanieczyszczonych obszarów i usuwanie po ich zbiorze wszystkich typów zanieczyszczeń (gleba, liście, igły itp.) (Di Palma et al., 2017; Vuković et al., 2015) a także płukanie wodą przed ekspozycją w workach (Giordano et al., 2013; Varela et al., 2016). Odbywa się to w celu lepszego oczyszczenia materiału i usunięcia wszelkich resztek roślinnych lub cząstek gleby (Ares et al., 2014), ale głównie w celu otrzymania odpowiednio niskiego początkowego poziomu pierwiastków śladowych w mchach przed ekspozycją. Ze względu na powyższe, celem badań [ON.2.] była ocena jednorodności materiału biologicznego, w oparciu o metodę przygotowania, do późniejszej ekspozycji w ramach aktywnego biomonitoringu. Uzyskane dane umożliwiły opracowanie znormalizowanych procedur przygotowania mchów do ekspozycji. Na podstawie podjętych badań należy stwierdzić, że metoda uśredniania i kondycjonowania materiału okazała się najlepszą metodą przygotowania próbek mchu przed ekspozycją. Wynika to z faktu, że mieszanie różnych próbek mchu (mieszanie podpróbek zebranych w miejscu pobierania próbek) daje bardziej uśredniony materiał. Stwierdziłem, że niejednorodność materiału spowodowana jest głównie osadzaniem się różnych rozmiarów cząstek pyłu na powierzchni mchów, co wpływa na wyniki w zakresie poziomu zanieczyszczenia metalami ciężkimi (Aboal et al., 2008). Kondycjonując próbki w wodzie zdemineralizowanej usuwamy cząsteczki pyłu z powierzchni i eliminujemy interakcję cząstek gleby na zawartość niektórych pierwiastków w mchach (Fernández et al., 2010) dzięki czemu otrzymujemy czystszą próbkę (bez jonów pierwiastków na jej powierzchni) gotową do ekspozycji np. na terenach zurbanizowanych. Ma to kluczowe znaczenie, ponieważ niewłaściwie przygotowane próbki mchu prowadzą do fałszywych wyników, błędnych interpretacji, a tym samym do niewłaściwych wniosków.

W pracy opisałem cztery sposoby przygotowania mchu przed ekspozycją. Wyniki przeprowadzonych badań laboratoryjnych wskazują, że spośród czterech zastosowanych technik, najlepszą jest opracowana metoda uśredniania materiału z jednoczesnym kondycjonowaniem mchów w wodzie zdemineralizowanej. Procedura ta powoduje zmniejszenie współczynnika zmienności *CV* (*Coefficient of Variation*) < 10 % dla większości metali oznaczonych w próbkach mchu. Analiza wyników z testem Wilcoxona potwierdziła statystycznie istotne różnice pomiędzy metodami stosowanymi dla większości metali i wykazała, że odpowiednia preparatyka mchu zwiększa jednorodność próbek. W artykule zastosowałem tę metodę jedynie na mchu *P. schreberi*, ale jest ona uniwersalna i możliwa do zastosowania dla innych gatunków co potwierdziłem przeprowadzając kolejne badania na dwóch pozostałych gatunkach (skuteczność opracowanej metodyki została potwierdzona w

kolejnych publikacjach [ON.3-ON.9]).

[ON.3.]. Stosując metodę *moss bag* musimy uwzględniać wiele czynników takich, jak: gatunek mchu, czas, miejsce lub wysokość ekspozycji, kształt worka z mchami, które muszą być stale standaryzowane, a wiele z tych elementów zostało doprowadzonych do unifikacji, a inne nadal stanowią wyzwanie (Ares et al., 2012; Sorrentino et al., 2021). W literaturze można znaleźć różne porównania dotyczące wybranych metod, podejść lub technik biomonitoringu z wykorzystaniem mchów (Capozzi et al., 2017; Salo et al., 2016). Niemniej jednak w tych pracach nie ma odpowiedzi na pytanie, która technika jest najlepsza, ponieważ wpływa na nią wiele czynników (w tym środowiskowych), a dodatkowo każda praca ma inny punkt odniesienia (Iodice et al., 2016; Kosior et al., 2018). Żywotność mchów jest prawie całkowicie ignorowana w tych pracach (Capozzi et al., 2020; Tretiach et al., 2007). Zgodnie natomiast z definicją biomonitoringu wykorzystuje on całość lub części organizmów żywych, które służą jako biomonitor jakości środowiska, a nie tylko jako adsorbent chemiczny (Boquete et al., 2017; Markert & Wünschmann, 2011). Tymczasem wiele prac wykorzystuje materiał dewitalizowany (Debén et al., 2016; Di Palma et al., 2017; Morales-Casa et al., 2019) uzasadniając to doskonałymi właściwościami sorpcyjnymi próbek (Ares, Aboal, et al., 2015), ale jak się okazuje nie dla wszystkich rodzajów zanieczyszczeń (Varela et al., 2016). Wykazano również, że istnieją różnice między mchami wysuszonymi i utrzymywanymi przy życiu (hydroponika) w sorpcji zanieczyszczeń (Szczepaniak et al., 2007). Konieczne jest więc skupienie się na wykorzystaniu żywego organizmu, aby mówić o autentycznym biomonitoringu wykorzystującym mech jako biomonitor (Fernández et al., 2009). Celem kolejnych badań, dotyczących ekspozycji mchów w ramach aktywnego biomonitoringu na terenach miejskich [ON.3] była ocena porównawcza czterech metod ekspozycji, oparta na pomiarach stężeń wybranych pierwiastków. Jednocześnie celem był pomiar parametrów witalności mchu (fluorescencja chlorofilu), co do tej pory było marginalnie wykonywane w tego typu badaniach, ale jest bardzo ważne biorąc pod uwagę wspomnianą wcześniej definicję biomonitoringu. W Tabeli 1 porównuję znaczenie zastosowanej metody ekspozycji w stosunku do gatunku i metalu. Zbierając wszystkie analizy testu t-Studenta i testu Wilcoxona należy stwierdzić, że metoda *moss bag* ma najbardziej znaczący wpływ w stosunku do innych technik ekspozycji mchów w zależności od badanego zanieczyszczenia dla poszczególnych gatunków. Spośród wszystkich możliwych porównań metoda *moss bag* (mb) była istotna statystycznie (wynik był wyższy) w 39,7 % przypadków w porównaniu z pozostałymi metodami przy porównywaniu stężenia danego pierwiastka dla danego gatunku. W przypadku innych metod wynik stężenia był wyższy (siła jego działania w stosunku do innych technik

ekspozycji) przy wariancie, gdzie mchy były w woreczkach osłoniętych od działania opadu atmosferycznego (dd), wariantu mchów w woreczkach osłoniętych od działania wiatru (wd) i metody transplantacji mchów w plastikowym pojemniku wraz z podłożem (bo) tylko w 19,1 %, 14,3 %, 11,1 % przypadków, odpowiednio.

Tabela 1. Test t Studenta porównania metody *moss bag* (mb*)* z innymi zastosowanymi metodami ekspozycji mchów

M1	M2	t	df	p
Cu mb *Pl*	- Cu dd *Pl*	-0.085	8	0.533
	- Cu wd *Pl*	4.587	8	**<0.001**
	- Cu bo *Pl*	2.358	8	**0.023**
Zn mb *Pl*	- Zn dd *Pl*	-0.612	8	0.721
	- Zn wd *Pl*	2.156	8	**0.032**
	- Zn bo *Pl*	1.253	8	0.123
Pb mb *Pl*	- Pb dd *Pl*	-0.075	8	0.529
	- Pb wd *Pl*	1.134	8	0.145
	- Pb bo *Pl*	0.427	8	0.340
Zn mb *Sp*	- Zn dd *Sp*	2.507	8	**0.018**
	- Zn wd *Sp*	-0.147	8	0.556
	- Zn bo *Sp*	3.039	8	**0.008**
Mn mb *Sp*	- Mn dd *Sp*	0.982	8	0.177
	- Mn wd *Sp*	1.620	8	0.072
	- Mn bo *Sp*	4.490	8	**0.001**
Hg mb *Sp*	- Hg dd *Sp*	6.828	8	**<0.001**
	- Hg wd *Sp*	5.405	8	**<0.001**
	- Hg bo *Sp*	1.192	8	0.134
Cu mb *Dp*	- Cu dd *Dp*	0.528	8	0.306
	- Cu wd *Dp*	2.204	8	**0.029**
	- Cu bo *Dp*	2.352	8	**0.023**
Zn mb *Dp*	- Zn dd *Dp*	3.394	8	**0.005**
	- Zn wd *Dp*	3.964	8	**0.002**
	- Zn bo *Dp*	6.456	8	**<0.001**

M1	M2	t	df	p
Hg mb *Dp*	- Hg dd *Dp*	-0.123	8	0.547
	- Hg wd *Dp*	1.578	8	0.077
	- Hg bo *Dp*	0.909	8	0.195

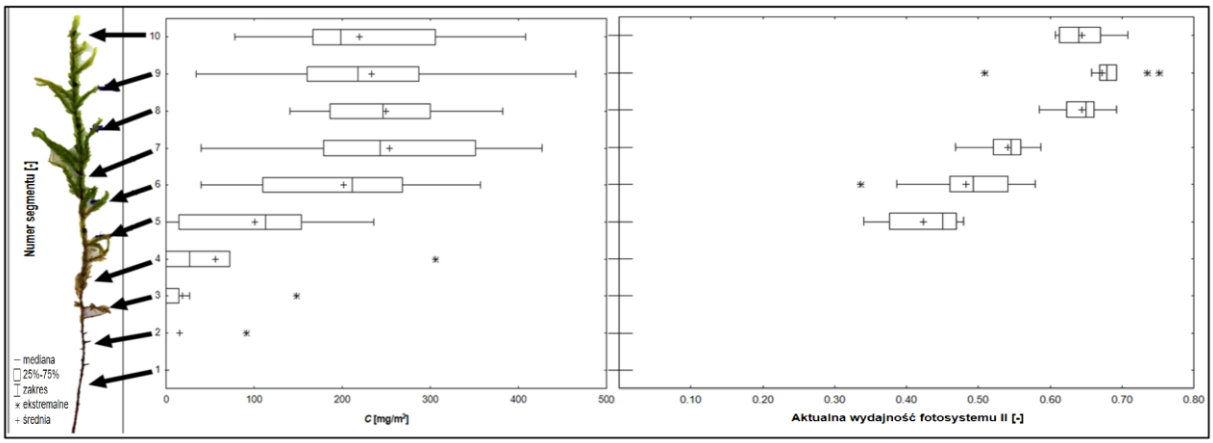
hipoteza to jeden pomiar M1 większy niż pomiar dwa M2 (M1>M2); wartości p zaznaczone **pogrubioną** czcionką są wartościami istotnymi statystycznie

Głównymi czynnikami wpływającymi na stężenie metali ciężkich podczas ekspozycji mchów były: wykorzystane gatunki mchów, charakterystyka miejsca badania oraz zastosowana metoda ekspozycji (Ares et al., 2012; Dmuchowski et al., 2011; Tabors et al., 2004). Mój eksperyment potwierdza, że zdecydowana większość wysokich stężeń metali w mchach dotyczy metody „klasycznego" worka z mchem (*moss bag*) niż wariantu suchej depozycji (wariant z woreczkami osłoniętymi od działania opadu atmosferycznego). Wskazują na to również inne badania, w których odkryte worki z mchami miały wyższe stężenia niż te zakryte, ale przez to worki odsłonięte z mchami były narażone na wymywanie przez deszcz a także osadzanie się pyłu z góry i z dołu, w porównaniu z mchami przykrytymi (Arndt et al., 2014). Porównując różne metody ekspozycji mchów, inni badacze również stwierdzili (tak jak ja), że metoda *moss bag* wykazała wyższe stężenia rozważanych pierwiastków w porównaniu z innymi technikami (Ares, Varela, et al., 2015). Skuteczność danej metody zależy jednak głównie od rodzaju biomonitorowanego zanieczyszczenia (Varela et al., 2016) oraz wariantu jego zastosowania (Aničić, Tasić, Frontasyeva, Tomašević, Rajšić, Strelkova, et al., 2009). Wyniki eksperymentu wskazały, że spośród czterech zastosowanych technik ekspozycji najskuteczniejsza okazała się metoda *moss bag* (biorąc również pod uwagę czas ekspozycji i zastosowane gatunki). Doniesienia literaturowe dla gatunku *Dicranum* sp. jak i moje wyniki badań wskazują, iż gatunek *D. polysetum* powinien być włączony do eksperymentów biomonitoringowych w zakresie monitorowania stężenia rtęci w środowisku ze względu na najlepszą akumulację tego pierwiastka na tle pozostałych analizowanych gatunków mchów. Wyniki przeprowadzonych eksperymentów i ich analiza pozytywnie zweryfikowały założone hipotezy badawcze, co pozwala na dalszy rozwój w kierunku optymalizacji tej techniki i jej zastosowania na terenach miejskich.

[ON.4.]. Należy pamiętać, że biomonitoring wykorzystuje organizmy żywe lub ich części (tkanki) w celu określenia warunków środowiska lub zmian, jakie zaszły w nim pod wpływem antropopresji (De Agostini et al., 2020; Markert, 2008). Powstaje jednak pytanie: czy można powiedzieć, że przed badaniem i po pewnym okresie ekspozycji materiał ten jest

jeszcze żywym organizmem i czy w związku z tym można mówić o BIOmonitoringu w przedmiotowej metodzie (Adamo et al., 2007)? Istotne jest także w jaki sposób witalność organizmu może wpływać na wyniki sorpcji i biomonitoringu. Pomiar zawartości chlorofilu jest ważny w analizach fizjologicznych i ekologicznych roślin, ponieważ zmiany jego zawartości są związane z różnymi kluczowymi funkcjami życiowymi, w tym wzrostem, zdolnością fotosyntezy, produkcją metabolitów i reakcjami na stres środowiskowy u roślin wyższych (Datt, 1999). Czynniki środowiskowe mogą wpływać bezpośrednio na zawartość chlorofilu w mchu torfowym (temperatura, natężenie światła) (Hyyryläinen et al., 2015; Tuba et al., 2012). Nie ma jednak badań wykazujących, że przed i po ekspozycji mchów w aktywnym biomonitoringu przez długi czas i podczas ich narażania na różnego rodzaju stresy (jak np. działanie metali ciężkich) mchy są nadal żywe i dlatego można je nadal określić jako bioindykatory. Celem moich kolejnych badań [ON.4.] było ustalenie, w jakim stopniu warunki środowiskowe przed ekspozycją wpłynęły na żywotność mchu i sorpcję metali ciężkich podczas aktywnego biomonitoringu oraz w jaki sposób trzymiesięczna ekspozycja wpływa na zawartość chlorofilu i pomiar jego fluorescencji w mchach, będąc wyznacznikiem ich kondycji w warunkach zanieczyszczenia środowiska.

Na Rysunku 3 przedstawiłem zmiany zawartości chlorofilu i jego fluorescencji w pionowym profilu gametofitu *P. schreberi.*



Rys. 3. Zmiany zawartości chlorofilu i rzeczywistej wydajności fotoukładu II w pionowym profilu gametofitu mchu

W dolnych segmentach nie ma chlorofilu lub jego stężenie jest bardzo niskie (segmenty 1–4/5). W segmentach 1–4 nie określono także rzeczywistej wydajności fotosystemu II. U *P. schreberi* średnia zawartość chlorofilu w segmentach 7–10 jest bardzo podobna (219–254 mg/m^2), przy czym najwyższą wartość odnotowano w segmencie 9 (465 mg/m^2). Wartości

rzeczywistej wydajności fotoukładu II wzrastają w wyższych partiach, podobnie do zawartości chlorofilu, gdzie w segmencie 9 odnotowano najwyższą średnią (0,666) i medianę (0,679). Budowa anatomiczna nie jest jedynym czynnikiem decydującym o stężeniu chlorofilu w pędzie mchu zależy również od stopnia rozwoju i jego wieku (Bates, 1979). W końcu ostatnie, wierzchołkowe (najwyższe) segmenty reprezentują dwu-/trzyletni okres wzrostu mchu (Harmens et al., 2012). Inne badania również potwierdzają wyniki dotyczące najwyższej zawartości chlorofilu w górnych częściach pędu, ponieważ fotosyntetycznie aktywne części to głównie 4 cm od góry, które stanowią żywe części pędu mchu w *Pleurozium schreberi* (Jägerbrand, 2015). Dlatego też do dalszych analiz pobrałem próbki mchów z wyższych segmentów (8–10), które na podstawie omawianych wyników okazały się najlepszymi miejscami do lokalnych pomiarów zawartości chlorofilu i rzeczywistej wydajności fotosystemu II.

Narażenie mchów na stres środowiskowy, taki jak zanieczyszczenie i zmieniające się warunki pogodowe (temperatura), powoduje zmniejszenie zawartości chlorofilu w czasie. Średnia zawartość chlorofilu *a* spadła o 54,3 %, podczas gdy chlorofilu *b* - o 66,4 % po 12 tygodniach ekspozycji. Obliczona wartość współczynnika korelacji liniowej Pearsona $r_{x,y}$ wynosiła 0,94 – istniała istotna korelacja między chlorofilem *a* oraz wydajnością (p = 0,02); współczynnik determinacji R^2 wyniósł 0,89. Dla korelacji między chlorofilem *b* oraz wydajnością $r_{x,y}$ wyniósł 0,89 – istniała istotna korelacja (p = 0,04) z R^2 = 0,79.

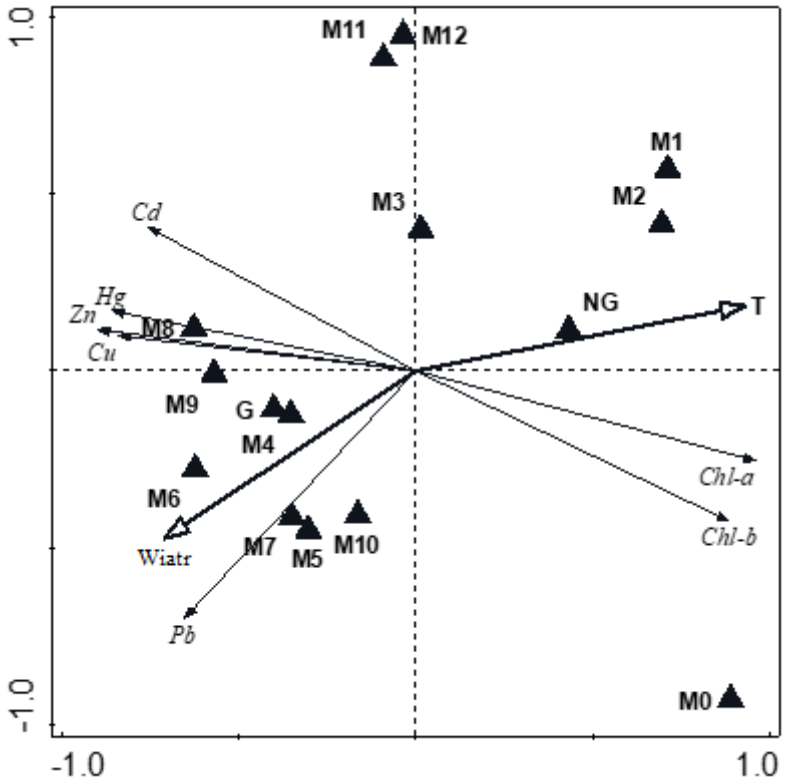
Należy uznać, że pomiary zawartości chlorofilu za pomocą fluorescencji odzwierciedlają żywotność mchów i wskazują, że podczas badania mchy wykazują cechy żywego organizmu. Aktywny biomonitoring z wykorzystaniem mchów koncentruje się głównie na oznaczaniu i analizie pierwiastków zgromadzonych przez mchy oraz wskazaniu źródeł emisji zanieczyszczeń (Adamo et al., 2007; Makholm & Mladenoff, 2005; Spagnuolo et al., 2011; Tremper et al., 2004). Pamiętajmy jednak, że nawet krótka ekspozycja powoduje uszkodzenie komórek gametofitowych (Adamo et al., 2007; Bartels et al., 2011), natomiast ekspozycja w silnie zanieczyszczonych obszarach lub przez długi czas może doprowadzić do śmierci biomonitora. Po sześciu tygodniach ekspozycji zaobserwowano postępujące zaburzenie cytoplazmatyczne przy użyciu transmisyjnego mikroskopu elektronowego, co wskazywało na śmierć mchu (Proctor et al., 2007). Spadek zawartości chlorofilu jest wynikiem postępującego zanieczyszczenia mchu i narażenia mchów na inne czynniki stresowe, które powodują ich śmierć. Fakt, że mchy utrzymują przez badany okres (trzy miesiące w okresie zmiennych warunków pogodowych), swoją witalność i zdolność do ciągłej sorpcji zanieczyszczeń z powietrza może być związany z wejściem w stan

kryptobiozy. Przez cały cykl życia są w stanie wegetować w ten sposób przez bardzo długi czas (Cesa et al., 2014; Demková et al., 2017).

W badaniach biomonitoringowych z wykorzystaniem mchów często zapomina się, że z definicji bioindykator jest żywym organizmem, a nie chemicznym adsorbentem (Boquete et al., 2017; Szczepaniak et al., 2007). Dlatego ważną rolę należy przypisać faktowi, że podczas eksperymentu lub przynajmniej przed ekspozycją mech powinien być żywą tkanką: powinien być poddawany tylko metodom przygotowawczym, które nie doprowadzą do jego śmierci. Dlatego, zgodnie z definicją biomonitoringu, konieczne jest wykluczenie dewitalizacji, która uczyniłaby mech tylko martwym adsorbentem analitów. Aktywny biomonitoring należy prowadzić z bioindykatorem, który pozostaje żywy (zgodnie z jego definicją jako żywy organizm), a ekspozycja mchu przez trzy miesiące, jak pokazują przedstawione wyniki pomimo spowodowania zmniejszenia zawartości chlorofilu w gametofitach, nie prowadzi do śmierci bioindykatora. Parametr ten był również związany z jego żywotną aktywnością w postaci rzeczywistej wydajności fotochemicznej fotosystemu II. W przyszłości należy zwrócić uwagę na kontrolę żywotności mchu podczas eksperymentów i obserwacji przejścia mchów w stan kryptobiozy.

[ON.5.]. Kolejnym aspektem, o którym już wcześniej wspomniałem jest wpływ warunków środowiskowych i czasu ekspozycji na jakość życia mchów narażonych na działania zanieczyszczenia w trakcie ekspozycji na terenach miejskich. Badania z wykorzystaniem aktywnego biomonitoringu techniką *moss bag* charakteryzują się różnymi przedziałami czasowymi (czasem ekspozycji), jak wskazuje literatura. Ekspozycja mchów w środowisku zanieczyszczonym np. metalami ciężkimi może trwać na przykład kilka dni (Cesa et al., 2014), cztery tygodnie (Demková et al., 2017; Tremper et al., 2004), dwa miesiące (Culicov et al., 2016; Milićević et al., 2017), 12 tygodni (Dmuchowski & Bytnerowicz, 2009; Giordano et al., 2009) lub nawet sześć miesięcy (Aničić Urošević & Milićević, 2020; De Agostini et al., 2020; Markert, 2008). Istnieje niewiele badań opisujących ekspozycję mchów w dłuższym okresie (Ares et al., 2012; Saitanis et al., 2013). Ze względu na brak w literaturze badań dotyczących długookresowego (6 miesięcy i więcej) aktywnego biomonitoringu metodą *moss bag* połączonego z pomiarem parametrów witalności mchu, uznano go za interesujący, ważny i uzupełniający dotychczasowe doniesienia w tym zakresie. Celem kolejnej pracy [ON.5.] była ocena stanu fizjologicznego mchu *Pleurozium schreberi* poddanego ciągłej ekspozycji przez rok, metodą *moss bag*, w zmiennych warunkach środowiskowych, w tym aerozolu atmosferycznego zanieczyszczonego wybranymi analitami: Cu, Zn, Cd, Hg i Pb.

Analizując wyniki badań, w pierwszej kolejności oceniłem wpływ wybranych czynników na żywotność *P. schreberi*, w tym czasu ekspozycji mchu w odniesieniu do zmieniających się warunków środowiskowych, takich jak wiatr i temperatura (Rys. 4).



Rys. 4. Relacje między zmiennymi i próbkami. T - temperatura, czas M0 - próbka kontrolna mchu (nie eksponowana), czas M1-M12 – czas ekspozycji mchu od pierwszego do dwunastego miesiąca, sezon G - sezon grzewczy (październik-kwiecień), sezon NG - sezon bez ogrzewania (maj-wrzesień), Chl-a i Chl-b – odpowiednio chlorofil a i chlorofil b

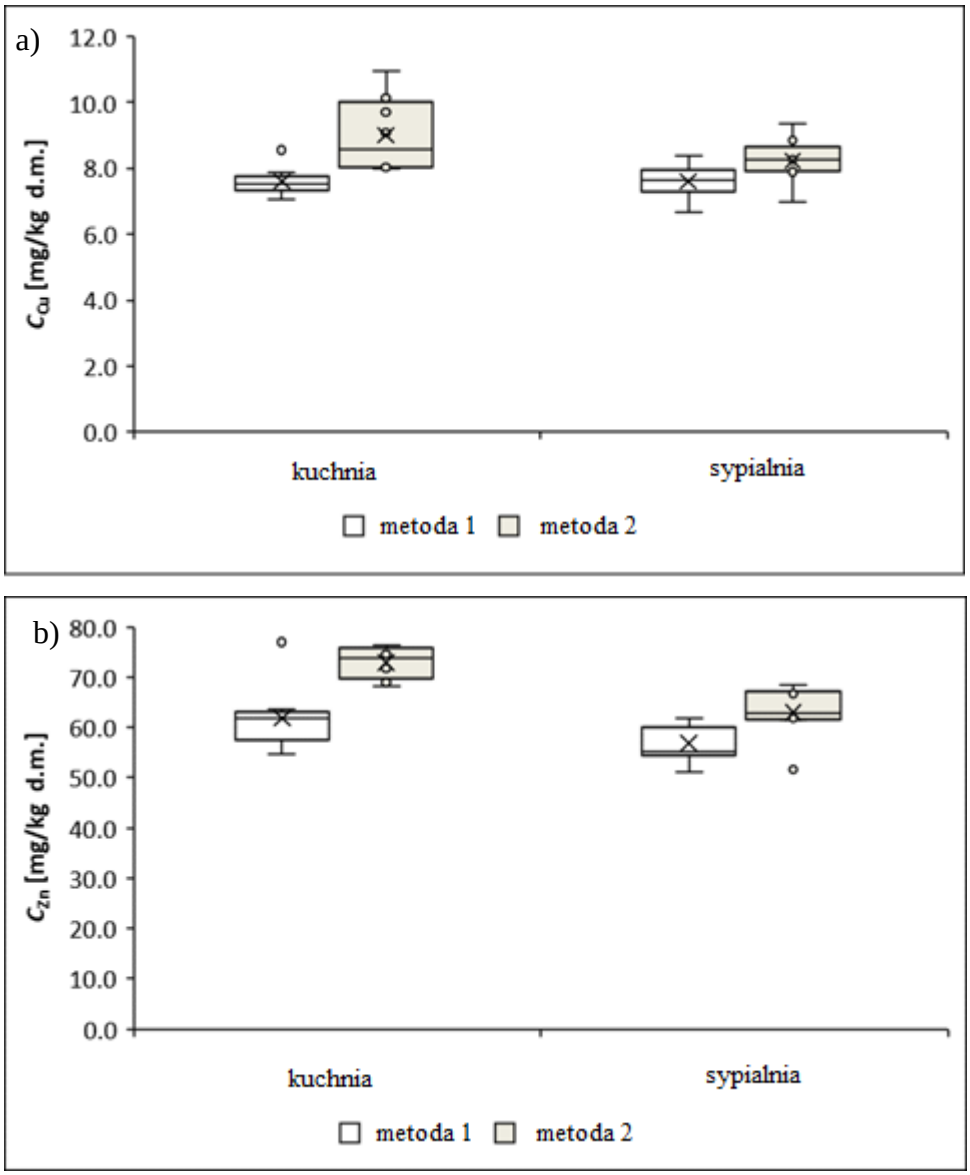
Na podstawie uzyskanych danych należy stwierdzić, że najważniejszą zmienną objaśniającą stan mchu (akumulacja metali, zawartość chlorofilu) jest temperatura (a więc pora roku), która wyjaśnia aż 60% zmienności w próbkach. Jednak drugim najważniejszym czynnikiem jest czas ekspozycji mchu. Najmłodsze i najstarsze próbki mają największą wartość wyjaśniającą, co świadczy o tym, że czas ekspozycji ma istotne znaczenie dla stanu witalności mchu. Trzecim czynnikiem jest sezon grzewczy, z oddziaływaniem 3%. Wyniki Analizy Głównych Składowych (PCA) wskazują na wpływ zmiennych środowiskowych (temperatura, sezon grzewczy, wiatr) na żywotność mchów. Całkowita zmienność wynosi 47,81 a opisana skumulowana zmienność stanowi 99,73% tej różnicy. Zawartość chlorofilu jest silnie skorelowana z porą nieogrzewania i temperaturą, natomiast wysokie stężenia metali wyraźnie korelują z sezonem grzewczym, a sam ołów (Pb) głównie z poziomem wietrzności.

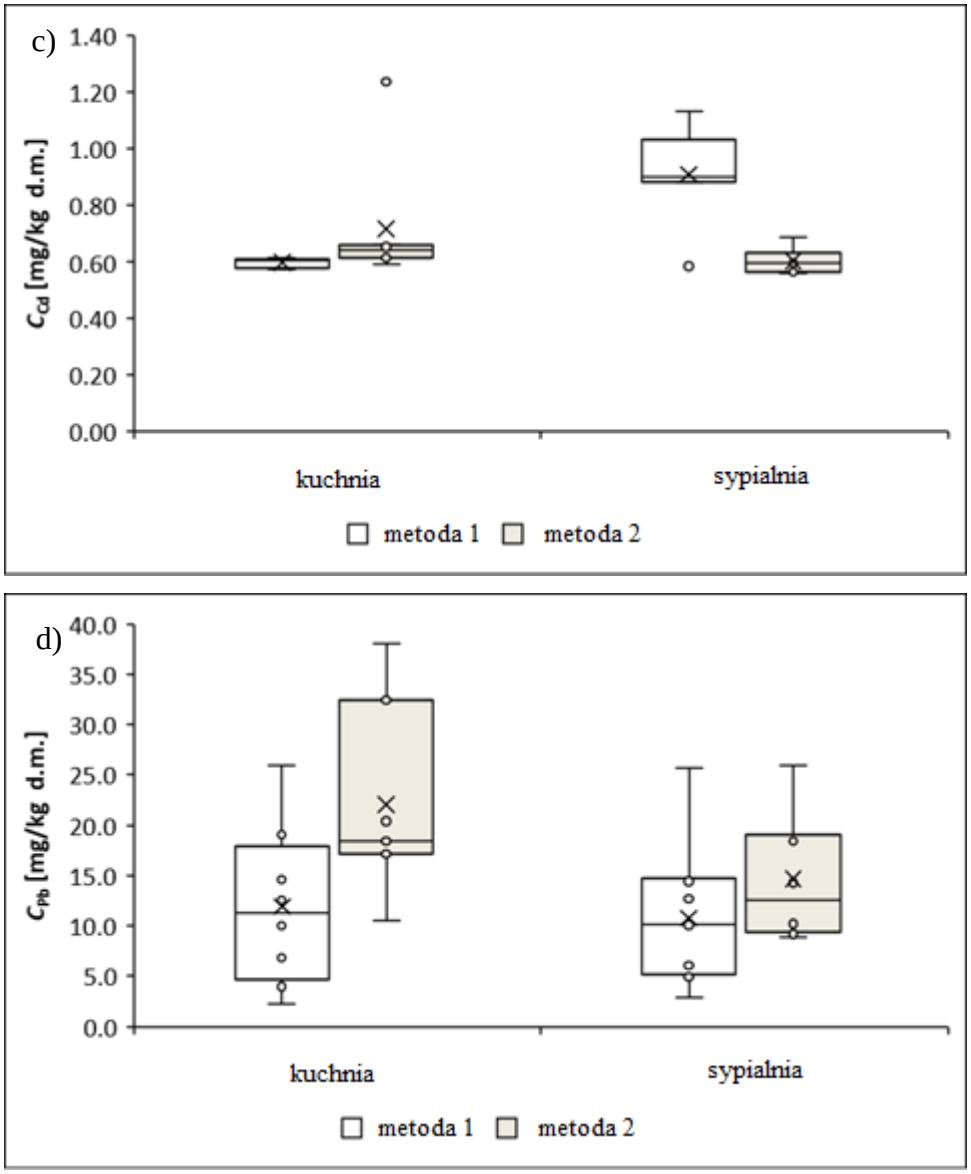
Czynniki środowiskowe mają istotny wpływ na życie roślin (w tym mchów) (Van Gaalen et al., 2007). W przypadku mchu *P. schreberi* eksperymenty ujawniły znaczące oddziaływanie temperatury na wydajność wzrostu (Jägerbrand et al., 2014). Należy również pamiętać, że zarówno odpowiednia temperatura, jak i natężenie światła wpływają na szybkość fotosyntezy w mchach (Haraguchi & Yamada, 2011; Tuba et al., 2012) a sezonowa zmienność zawartości chlorofilu może być związana z wymaganiami środowiskowymi danego gatunku (Hyyryläinen et al., 2015). W moich badaniach stwierdziłem oddziaływanie czasu ekspozycji mchu/wpływ jego starzenia się na zawartość chlorofilu, co jest ważne w aktywnym biomonitorowaniu środowiska i wniosek ten należy uwzględnić podczas przechowywania mchu przed ekspozycją (sposób przechowywania, czas) (Dołęgowska & Migaszewski, 2020).

W artykule mchy *P. schreberi* eksponowane w workach były narażone na działanie aerozolu atmosferycznego przez 12 miesięcy na terenie miasta Opola. Przed tym rocznym badaniem nie było danych na temat depozycji pierwiastków w atmosferze na tym obszarze przez tak długi czas z wykorzystaniem badań biomonitoringowych. Istotny wpływ na żywotność mchów mają czynniki środowiskowe, dlatego należy wziąć pod uwagę odpowiedni okres aktywnego biomonitoringu. Mchy *P. schreberi* skutecznie gromadzą zanieczyszczenia powietrza w okresie ekspozycji wynoszącym jeden rok i dobrze znoszą różne warunki pogodowe w Polsce - mchy narażone na stres środowiskowy wykazywały cechy żywego organizmu (zawartość chlorofilu). Wykazałem wpływ zależności temperatury i czasu ekspozycji mchów na zawartość fotosyntetycznie czynnego pigmentu.

W pracach **[ON.1-ON.5.]** przedstawiłem wyniki badań metodologicznych związanych z biomonitoringiem aktywnym. Badania rozpocząłem od porównania właściwości akumulacyjnych wybranych gatunków mchów. Następnie opracowałem metodykę optymalnego ich przygotowania do ekspozycji. Kolejno przetestowałem wybrane metody ekspozycji mchów, po przeprowadzeniu badań związanych z żywotnością mchów w trakcie badań terenowych. Kolejnym etapem jest praktyczne przetestowanie dotychczas nabytej wiedzy, opanowanych technik ekspozycji, w celu wykazania możliwość i przede wszystkim skuteczność tej techniki badania zanieczyszczenia aerozolu atmosferycznego – wyniki prezentuję w pracach [ON.6-ON.9.]. Dlatego też celem kolejnego eksperymentu było zastosowanie mchów do oceny zanieczyszczenia powietrza w przestrzeni zamkniętej (dom) spowodowanego paleniem papierosów. Badania przeprowadziłem z zastosowaniem mchów *Pleurozium schreberi* jako bioindykatorów zanieczyszczenia aerozolu atmosferycznego w pomieszczeniach mieszkalnych (kuchni i sypialni), metalami pochodzącymi z dymu tytoniowego emitowanego z różnych rodzajów papierosów: papierosów konwencjonalnych,

e-papierosów i wyrobów podgrzewających tytoń (HTP - *heated tobacco product*). Badania literaturowe dotyczące e-papierosów i podgrzewanych wyrobów tytoniowych nie pokazują praktycznie żadnych danych na temat emisji metali ciężkich z aerozolu/dymu z takich urządzeń. Omawiają przede wszystkim zawartość nikotyny lub innych związków chemicznych, ale nie ma danych na temat stężeń pierwiastków śladowych, takich jak metale (Jankowski et al., 2019; Simonavicius et al., 2019). W pierwszym etapie badań porównałem dwie metody przygotowania próbki mchu w celu uzyskania wiarygodnych wyników aktywnego biomonitoringu w pomieszczeniach zamkniętych. Na Rysunku 5 przedstawiłem wyniki stężeń wybranych metali oznaczonych w mchach, przygotowanych do analiz dwiema metodami i poddanych działaniu HTP w dwóch pomieszczeniach mieszkalnych (kuchnia, sypialnia).





Rys. 5. Stężenia wybranych metali: a) Cu, b) Zn, c) Cd, d) Pb oznaczone w mchach narażonych w dwóch pomieszczeniach wewnętrznych różniących się poziomem zanieczyszczenia bezdymnym HTP; X - średnia, —— - mediana, ° - punkt odstający

Dym HTP zawiera anality, o czym świadczy stężenie w próbkach mchu (Rys. 5). W rezultacie każdy, kto pozostaje w pobliżu palaczy HTP, jest narażony na dym tytoniowy (ETS - *environmental tobacco smoke*). Metoda przygotowania w aktywnym biomonitoringu z wykorzystaniem mchów wpływa na jakość uzyskiwanych wyników, a prawidłowe przygotowanie próbek skutkuje jednorodnością materiału (nawiązanie do pracy [ON.2.]).

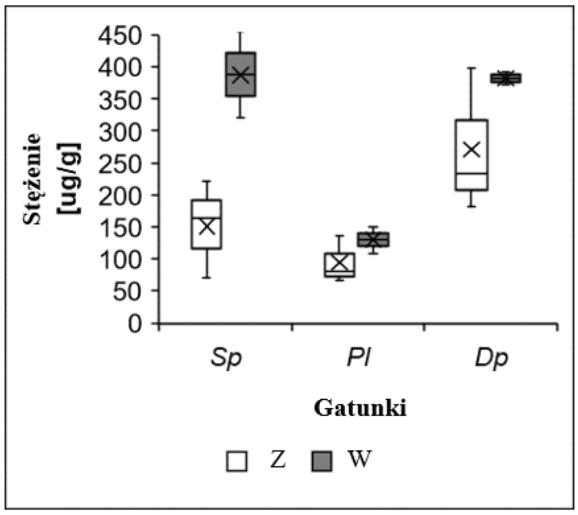
Dyskusja na temat zmniejszenia szkodliwości takich produktów jak e-papierosy i HTP wciąż trwa, ale uzyskane wyniki wskazują na ich negatywny wpływ na jakość powietrza w pomieszczeniach. Zawartość poszczególnych metali w aerozolu (z HTP) może być nawet wyższa w porównaniu z konwencjonalnym dymem papierosowym (Rajfur et al., 2018).

Stwierdziłem, że ETS pochodzący z HTP w podobny sposób zanieczyszcza aerozol atmosferyczny metalami w pomieszczeniach zamkniętych. To sprawia, że narażenie ludzi na niego może mieć negatywny wpływ na ich zdrowie. Wyniki zaprezentowane na Rysunku 5 wskazują, iż metoda przygotowawcza faktycznie ma istotne znaczenie dla uzyskiwanych wyników, co było już wcześniej udowodnione ([ON.2.]) a teraz na przykładzie praktycznym wykazałem. Aplikacyjność techniki *moss bag* w codziennym życiu wskazuje na możliwość jej wielorakiego zastosowania.

Kolejnym etapem badań było „wyjście" poza pomieszczenie zamknięte i przetestowanie opracowanej metody na innym terenie i w innych warunkach [ON.7-ON.9.]. Tym razem celem było przeanalizowanie stężeń pierwiastków zakumulowanych przez mchy narażone na ekspozycję w dwóch środowiskach – w warsztacie samochodowym - jako punktowym źródle zanieczyszczeń charakterystycznych dla działalności tego typu obiektu oraz na zewnątrz w jego otoczeniu **[ON.7.**]. Elementem nowości dotyczącym innych prac z zakresu aktywnego biomonitoringu było jednoczesne wykorzystanie trzech gatunków mchów (możliwość porównania właściwości akumulacyjnych – nowa wiedza w tym zakresie dotycząca rzadko wykorzystywanych gatunków w aktywnym biomonitoringu takich jak *Dicranum polysetum*), kontrola dwóch różnych przestrzeni (zewnętrznej - podwórko i wewnętrznej - warsztat samochodowy – wyznaczanie różnic pomiędzy badanymi przestrzeniami) oraz korelowanie wpływu stężeń zakumulowanych pierwiastków przez mchy z ich witalnością poprzez pomiar ich żywotności (fluorescencja chlorofilu). Do tej pory badania biomonitoringowe pomijały istotny element korzystania z żywego biomonitora a moje badania zajmują się tym aspektem.

Na Rysunku 6 przedstawiłem porównanie międzygatunkowe mchów, z uwzględnieniem zmienności stężeń danego pierwiastka, w podziale na próbki wystawione na zewnątrz (Z) i wewnątrz warsztatu (W).

a)


Stężenie [ug/g]
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Sp
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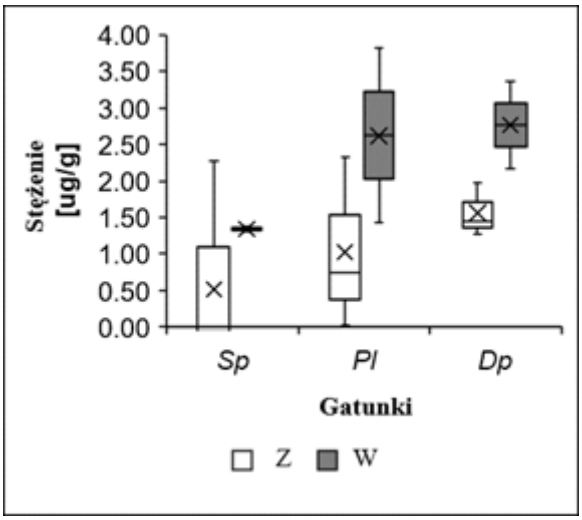
b)


Stężenie [ug/g]
4.00
3.50
3.00
2.50
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Sp
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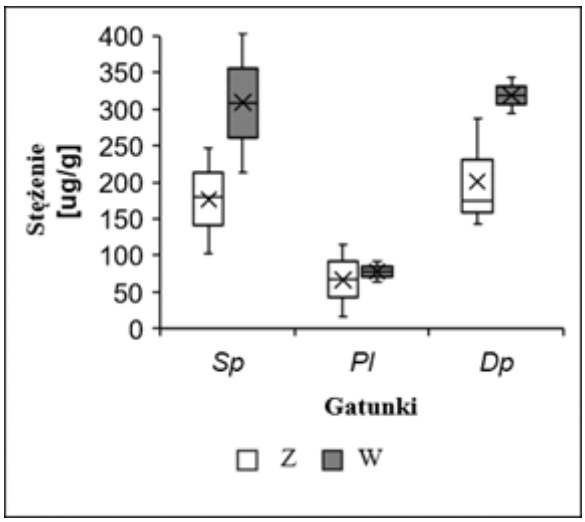
c)


Stężenie [ug/g]
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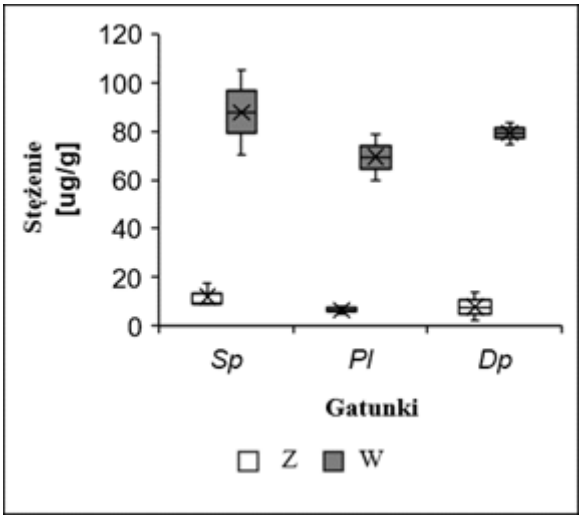
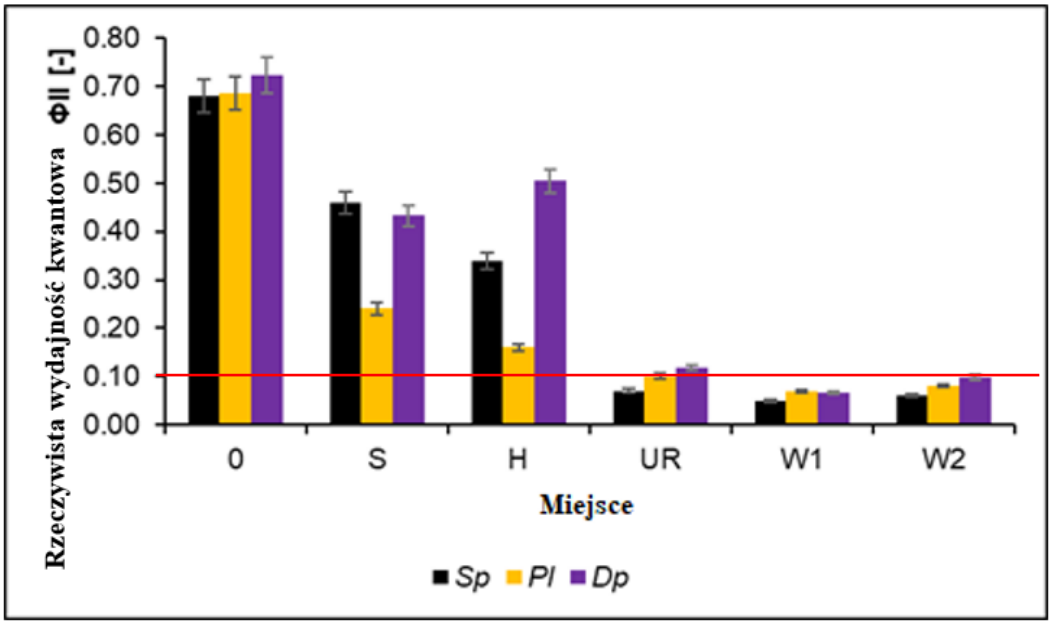
d)



Rys. 6. Zmiany stężeń: a) glinu, b) chromu, c) żelaza, d) baru w mchach po ekspozycji według gatunku i miejsca ekspozycji

Na Rysunku 6 prezentuję stężenia wybranych pierwiastków, które wewnątrz warsztatu (dwa punkty w warsztacie) były wyższe podczas ekspozycji w porównaniu z próbkami wystawionymi na zewnątrz (punkty pomiarowe zlokalizowane przy drodze, na posesji oraz pod dachem warsztatu). Wyniki wskazują, że pierwiastki, których emisja jest spowodowana aktywnością warsztatu samochodowego, gromadzą się w mchach w stężeniach wyższych niż obecne w aerozolu atmosferycznym na zewnątrz.

Kolejnym parametrem, który zależy od warunków ekspozycji i gatunków mchu, jest ich żywotność, której zmiany pokazałem na Rysunku 7.



Rys. 7. Zmiany rzeczywistej wydajności kwantowej przed ekspozycją "0" i po ekspozycji w poszczególnych punktach pomiarowych dla trzech gatunków mchu. Kolumna oznacza średnie stężenie (n = 5), wąsy reprezentują odchylenie standardowe. Punkt "0" reprezentuje pomiar w lesie przed zebraniem mchów. Czerwona linia wskazuje wartość graniczną dla witalności (Lichtenthaler et al., 2005). S - punkt zlokalizowany przy drodze obok posesji warsztatu, H - punkt na posesji; UR - punkt pod dachem warsztatu, W1, W2 – dwa punkty pomiarowe zlokalizowane w warsztacie

Rzeczywista wydajność kwantowa mchów zależała od gatunku i miejsca ich ekspozycji. Próbki wystawione bezpośrednio na zewnątrz miały znaczącą różnicę w żywotności w porównaniu z mchami wystawionymi w punkcie UR, a także w warsztacie. W stosunku do kontroli ("0") spadek żywotności mchu po ekspozycji tylko w punkcie "S" wynosił odpowiednio 32,5 %, 64,9 % i 40,2 % dla *S. fallax, P. schreberi* i *D. polysetum* odpowiednio. Mchy w punktach wewnątrz warsztatu charakteryzowały się spadkami wartości poniżej poziomu 0,1, co czyni je jedynie naturalnymi sorbentami analitów po trzymiesięcznej ekspozycji.

Mchy w aktywnym biomonitoringu służą głównie do oceny poziomu zanieczyszczenia powietrza w miastach (Rivera et al., 2011). Ważne jest jednak porównanie zanieczyszczenia środowiska zewnętrznego (otwarte przestrzenie) ze środowiskiem wewnętrznym (np. wnętrza pomieszczeń), koncentrując się na poszukiwaniu wewnętrznych źródeł zanieczyszczeń (Capozzi et al., 2019; Ndong Ba et al., 2019). Wskazują na to również wyniki moich badań porównujące stężenie pierwiastków w obu tych środowiskach. Wyższe stężenia chromu w mchach w warsztacie niż na zewnątrz są wynikiem zużycia hamulców (Suvarapu & Baek, 2017). Ruch drogowy stanowi również źródło emisji tego analitu (Vuković et al., 2016). Emisje pierwiastków takich, jak Ba i Zn są związane ze zużyciem hamulców samochodowych i wraz z Cu będą pochodzić z pyłu hamulcowego (Vuković et al., 2014). Różnica w stężeniach pierwiastków między otoczeniem zewnętrznym a warsztatem (Rys. 6) wynika głównie ze specyficznych warunków ekspozycji mchów wewnątrz pomieszczenia (Zechmeister et al., 2020). Słaby przepływ/ruch powietrza (niedostateczna wentylacja), a tym samym jego stagnacja wraz z osadzaniem pyłu, będą odpowiadać za te różnice (Vuković et al., 2014).

Istnieją istotne różnice między właściwościami akumulacyjnymi różnych pierwiastków przez suche i żywe mchy (Astel et al., 2008). Dlatego należy zwrócić uwagę na pomiar żywotności mchu podczas badań biomonitoringowych (Capozzi et al., 2020; Świsłowski et al., 2021). Na przykład w mchach akumulujących chrom, po wystawieniu ich na działanie jego soli doszło do znacznego spadku wydajności fotosyntezy i oczywistej

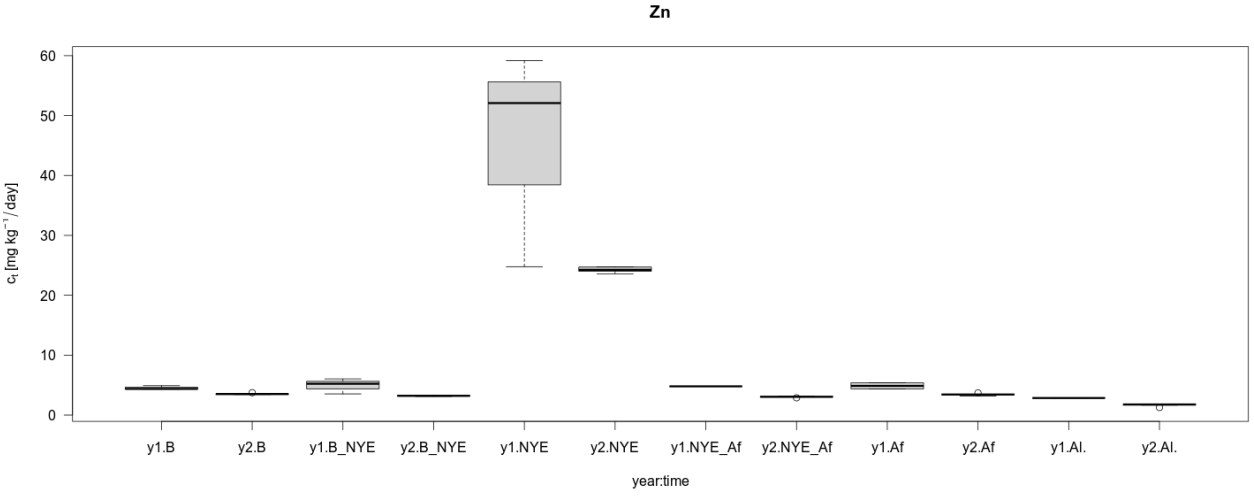
śmierci komórek (Chen et al., 2018). Narażenie na zanieczyszczenie powietrza prowadzi do utraty Cl, K i Rb, co oznacza uszkodzenie błony komórkowej mchu (Zinicovscaia et al., 2018).

Zidentyfikowano pierwiastki, których emisje wewnątrz warsztatu są bezpośrednio związane z jego aktywnością i działaniem deklarowanym przez mechanika. Podczas dwunastotygodniowej ekspozycji mchy wystawione na zewnątrz były bioindykatorami, ale ekspozycja w pomieszczeniach oznaczała, że mchy były tylko pasywnym naturalnym sorbentem, co wskazuje, że podczas monitorowania jakości powietrza w pomieszczeniach powinny one być nawadniane. *Sphagnum fallax* jako torfowiec ma potrzebę funkcjonowania w wilgotnym środowisku dlatego też powinien być wykorzystywany jedynie na terenach, gdzie będzie miał zapewnioną odpowiednią wilgotność. Ekspozycja na zewnątrz w czasie, kiedy było wilgotno zapewniła mu najlepsze warunki życia. Bates, argumentował, że mchy z rodzaju *Sphagnum* stanowią jedną z najbardziej efektywnych pułapek na cząstki unoszące się w powietrzu, ponieważ około dwie trzecie ich całkowitej suchej biomasy stanowią phyllidia (Bates, 2000). To mogło zatem przyczynić się do dobrych wyników akumulacyjnych na tle innych gatunków. W przyszłości należałoby zintensyfikować badania biomonitoringowe nad identyfikacją źródeł emisji zanieczyszczeń do powietrza i ich kontrolą wewnątrz pomieszczeń/budynków, gdyż oznaczone przeze mnie pierwiastki mogą mieć negatywny wpływ na zdrowie człowieka.

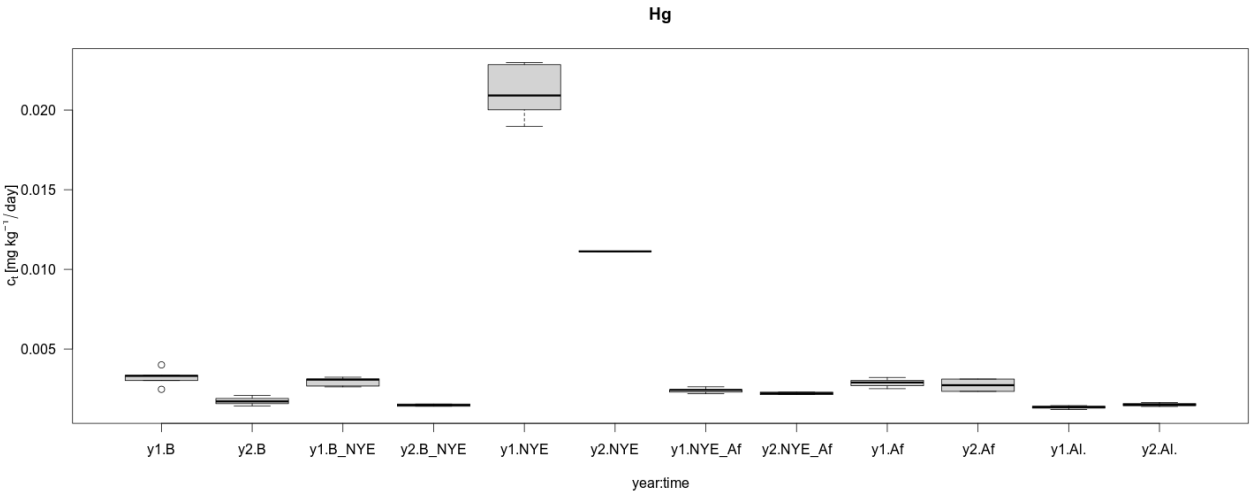
Negatywne oddziaływanie na zdrowie człowieka ma życie w zanieczyszczonym środowisku, do którego sami się przyczyniamy. Jest wiele różnych źródeł, które powodują zanieczyszczenia powietrza, i na wiele z nich nie mamy bezpośrednio wpływu a na inne już tak. Działalność antropogeniczna w dużym stopniu przyczynia się do zanieczyszczenia aerozolu atmosferycznego, jednak poza tak specyficznym źródłem jak dym papierosowy istnieje wiele innych. Wśród nich można wymienić tak niecodzienne, choć krótkotrwałe jakim jest dym pochodzący z wystrzeliwanych fajerwerków, zazwyczaj w trakcie witania nowego roku (Pongpiachan et al., 2018). Jest to jedno z najbardziej niezwykłych źródeł zanieczyszczenia powietrza, które było badane dość często i na dużą skalę w literaturze (Drewnick et al., 2006; Resmi et al., 2019; Tanda et al., 2019). Pozwala to na identyfikację konkretnych zanieczyszczeń związanych wyłącznie z wystrzeliwaniem fajerwerków (Hu et al., 2020; Wang et al., 2007). Do tej pory w literaturze spotkałem się jedynie z eksperymentem laboratoryjnym (Bowden et al., 2012), w którym mech był używany jako biomonitor analitów jakości powietrza z wypalanych fajerwerków. Uznałem to za przestrzeń do wykorzystania w badaniu środowiskowym dotyczącym wykorzystania mchu jako

bioindykatora do oceny stężeń zanieczyszczeń emitowanych przez fajerwerki, gdy są one odpalane w Sylwestra [**ON.8.**]. Celem pracy była ocena zanieczyszczenia aerozolu atmosferycznego wybranymi metalami ciężkimi (Ni, Cu, Zn, Cd, Hg i Pb) pochodzącymi z dymu fajerwerków stosowanych podczas Sylwestra w latach 2019/2020 i 2020/2021. Mech był eksponowany przez 14 dni przed 31 grudnia, 1 stycznia i 2 tygodnie po Nowym Roku. W przypadku próbek mchu czas narażenia na depozycję był zmienny. Aby oszacować intensywność osadzania, obliczyłem stosunek stężenia pierwiastków chemicznych (c_t) do czasu osadzania (t). Na Rysunku 8 przedstawiłem wykresy pudełkowe obliczonego c_t. W przypadku badanych metali obserwuje się wyraźne różnice w intensywności osadzania.

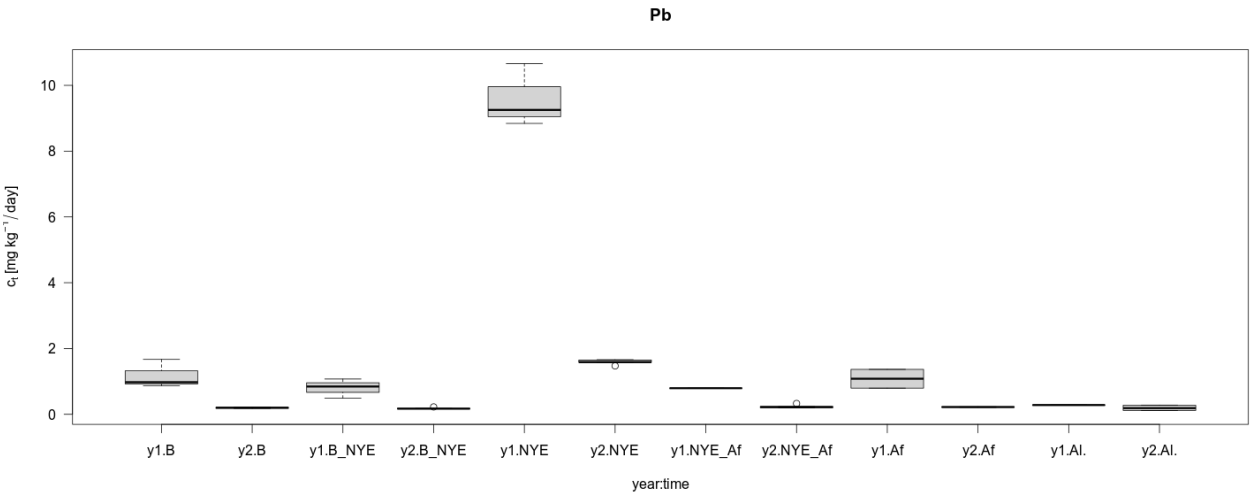
a)



b)



c)



Rys. 8. Stężenia metali ciężkich w mchach: a) Zn, b) Hg i c) Pb w zależności od czasu ekspozycji (odpowiednio y1, y2- okres 2019/2020 i 2020/2021). B- ekspozycja 14 dni przed sylwestrem; B_NYE- ekspozycja 14 dni przed sylwestrem oraz w sylwestra; NYE- ekspozycja tylko w Sylwestra (31.12/01.01); NYE_Af- ekspozycja w sylwestra oraz 14 dni po nowym roku; Af- ekspozycja tylko przez 14 dni po nowym roku; Al- próbki eksponowane przez cały okres eksperymentu

Na Rysunku 8 widzimy, że stężenia metali oznaczone w mchach różnią się znacząco między okresami badań, odnosząc je do czasu ekspozycji (liczba dni). W przypadku Zn, Hg i Pb ich stężenia oznaczone w mchach eksponowanych w czasie NYE w altach 2019/2020 znacznie przekraczają wartości stężeń oznaczone w próbkach w kolejnym roku eksperymentu.

Zmiany stężeń między dwoma okresami badania wynikają również z globalnej sytuacji blokady spowodowanej ograniczeniami pandemii koronawirusa. W literaturze można znaleźć rezultaty wskazujące, że zanieczyszczenie powietrza znacznie spadło w tym okresie w porównaniu z wartościami wcześniej raportowanymi (Giani et al., 2020; Skirienė & Stasiškienė, 2021). Wpływ pandemii na zanieczyszczenie powietrza w aspekcie monitoringu biologicznego opisał do tej pory tylko jeden znany mi autor. Wskazał on, że stężenia wybranych metali zakumulowanych przez mech *P. schreberi* w ramach pasywnego biomonitoringu zmniejszyły się w wyniku lockdownu (Yushin et al., 2020). Moje badania potwierdzają to, że stężenia metali ciężkich w okresie "NYE" i ogólnie w drugim roku badania 2020/2021 były niższe w porównaniu z tym samym okresem rok wcześniej (Rys. 8). Wpływ na to miały krajowe regulacje związane z ograniczoną możliwością gromadzenia się ludzi i udziału w imprezach masowych z okazji Nowego Roku, co oddziaływało na ograniczenie emisji z wypalanych fajerwerków. Wiadomo z literatury, że stężenia metali ciężkich, takich jak Cu, Zn i Pb, w powietrzu, są związane z ich emisją podczas spalania fajerwerków (Do et al., 2012; Fu et al., 2021; Lin & Wang, 2020; Tanda et al., 2019; Vecchi et

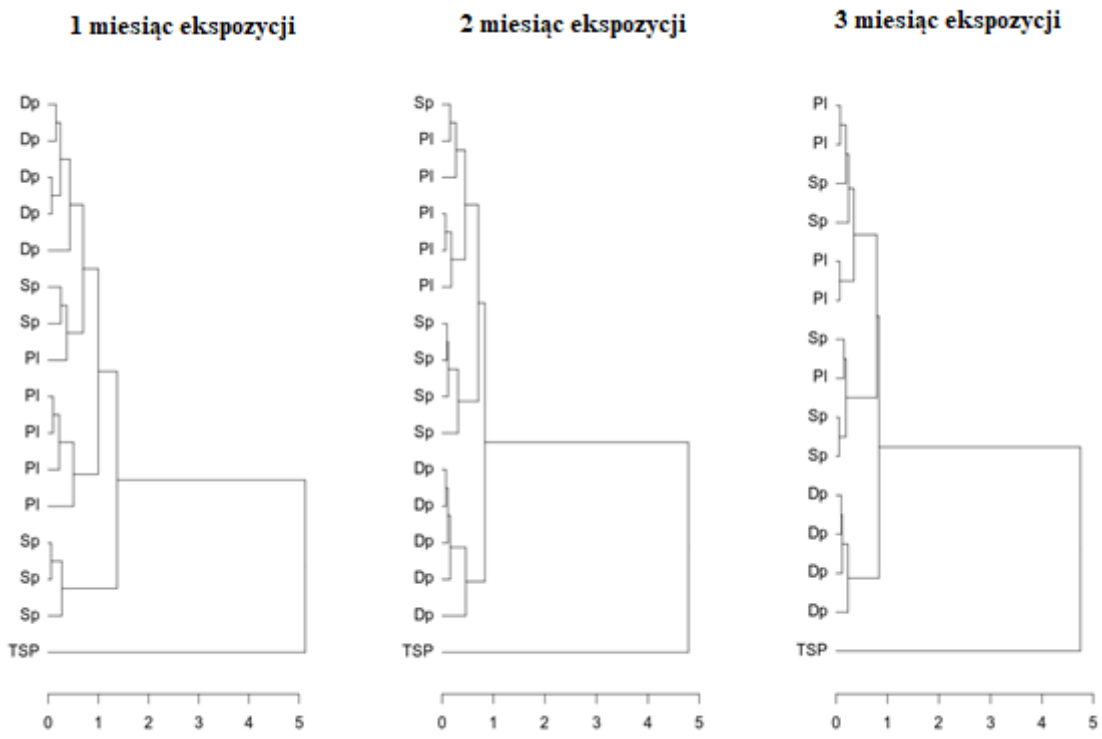
al., 2008; Wang et al., 2007). Potwierdzają to moje badania. Podczas ekspozycji mchu tylko przez 2 dni (okres NYE) stężenia tych pierwiastków charakteryzowały się wartościami porównywalnymi lub wyższymi do okresów dłuższej ekspozycji na zanieczyszczenie mchu tymi analitami (Rys. 8.).

Wyniki badań biomonitoringowych wskazują na zmienną jakość powietrza w mieście w okresie sylwestrowym w okresie 2019-2021. Zwiększone stężenia miedzi i ołowiu tylko podczas 2-dniowej ekspozycji "NYE" potwierdzają wpływ wypalonych fajerwerków na gromadzenie się tych pierwiastków w mchu *P. schreberi*. Świadczy to o dobrej zdolności akumulacji tego gatunku do stosowania w krótkich okresach narażenia na zanieczyszczenie powietrza.

W literaturze opisano już zastosowanie klasycznych metod oznaczania stężenia metali w powietrzu podczas pokazu sztucznych ogni (Moreno et al., 2007). Moje badania potwierdzają możliwość wykorzystania bioindykatorów w monitorowaniu jakości powietrza, jako metody uzupełniającej.

Rośliny są szeroko stosowane w biomonitoringu środowiskowym zanieczyszczenia pyłem PM (*Particulate Matter*) (Clough, 1975; Rai, 2016) a liście drzew zostały wykorzystane w krajowym systemie długoterminowego biomonitoringu metali ciężkich w powietrzu (Ştefănuţ et al., 2018). Rumuńskie Ministerstwo Środowiska wdrożyło ten system jako narzędzie uzupełniające Krajową Sieć Monitorowania Jakości Powietrza (Ştefănuţ et al., 2018). Ci sami autorzy włączyli również technikę *moss bag* do długoterminowego monitorowania metali ciężkich w powietrzu w ramach projektu BioMonRo (projekt: *Long-term National Monitoring System of Bioaccumulation of Airborne Heavy Metals*) (Ştefănuţ et al., 2019). Niewiele badań obejmuje bezpośrednie porównanie aktywnego biomonitoringu z wykorzystaniem mchów z wynikami uzyskanymi z poborników pyłu (Vuković et al., 2014) w celu zintegrowania tych dwóch różnych metod przy jednoczesnym pomiarze żywotności bioindykatorów (Capozzi et al., 2020). Do tej pory dokonano porównań pasywnego biomonitoringu z TSP (ang. *Total Suspended Particulates* - całkowita ilość zawieszonych cząstek stałych) (Pöykiö, 2002; Sheppard et al., 2007). W związku z tym porównałem wyniki aktywnego biomonitoringu z wykorzystaniem mchów z pomiarami instrumentalnymi. TSP został specjalnie wybrany, ponieważ na mchach osadza się pył o różnych frakcjach (Kupiainen & Tervahattu, 2004; Vanicela et al., 2021).W pracy [**ON.9.**], porównałem skład metali zgromadzonych w mchach i na filtrach. Dla podkompozycji złożonej z Mn, Fe, Cu, Zn i Pb (Aitchison, 2003) obliczyłem odległości między punktami reprezentującymi zawartość

metali w próbkach mchu i TSP. Na dendrogramie (Rys. 9.) pokazałem strukturę odległości. Aby określić strukturę klastrów, zastosowałem metodę kompletnego połączenia.



Rys. 9. Analiza skupień metali ciężkich w trzech gatunkach mchów i w TSP na filtrze

Obserwuje się dwa główne skupiska reprezentujące mech i TSP. W gromadzie mchu można rozpoznać trzy podgromady. Jeden z nich to *D. polysetum*, niezależnie od okresu ekspozycji. Pozostałe skupiska reprezentują inne gatunki mchów, nie przypisane jednoznacznie do grup.

Anatomiczne i strukturalne cechy rośliny wpływają na to, które i ile pyłów jest wychwytywanych (Weerakkody et al., 2018a, 2018b). Chociaż na mchach osadzają się głównie drobne cząstki (< 2,5 μm), wyniki z mchu *P. purum* wskazują, że cząstki uwięzione przez mchy reprezentują różne frakcje, a ilość PM była silnie związana ze stężeniem pierwiastków (Di Palma et al., 2017). Inne badania potwierdzają również, że mchy *Hypnum cupressiforme* zatrzymują występowanie potencjalnie wdychanych cząstek (≤ PM10), gdzie dominowały najmniejsze klasy cząstek (Tretiach et al., 2011). Konieczność porównania wyników biomonitoringu z innymi metodami jest również podkreślana w przypadku TSP (Kupiainen & Tervahattu, 2004; Stojanowska et al., 2021). Dlatego postanowiłem zbadać pył w całej frakcji TSP, a nie tylko wybranych PM.

W większości cytowanych prac badania odnoszą się do PM10 zarówno w pyle, jak i w mchu (Vuković et al., 2014). Szczególnie trudno mi zrozumieć, w jaki sposób w tym przypadku metale ciężkie zostały określone tylko w PM10 w odniesieniu do mchów. Autorzy stwierdzili, że nie ma statystycznie istotnej różnicy między obiema metodami (worki z mchem *S. girgensohnii* i próbki PM10); nie przedstawili jednak żadnej analizy statystycznej potwierdzającej ten wniosek (Vuković et al., 2014).

W moich badaniach przetestowałem trzy gatunki mchów: *P. schreberi, S. fallax* i *D. polysetum* w celu zweryfikowania hipotezy: stężenia metali ciężkich zgromadzonych w mchach są proporcjonalne do stężenia tych zanieczyszczeń w pyle TSP osadzonym na filtrze. Klastry obserwowane w dendrogramach składu mchu są wyraźnie oddzielone od klastra składu TSP. Ta obserwacja narzuca wniosek, że składy pierwiastkowe mchu i TSP są znacząco różne. Wpływ aktywności biologicznej mchu na jego powinowactwo do związków chemicznych implikuje różnicę między TSP a składem mchu. Powszechna tendencja zmian stężeń Pb i Zn wskazuje na źródła niskiej emisji jako główne źródło tych metali w TSP. Mech *D. polysetum* powtórnie okazał się lepszy w akumulacji rtęci na tle pozostałych gatunków, co wskazuje na możliwość jego włączenia do wykrywania rtęci w środowisku w badaniach biomonitoringowych.

W artykule wskazuję, że wyniki uzyskane dwiema metodami (aktywnego biomonitoringu i osadzania pyłu na filtrze) mają różne znaczenia. Mchy gromadzą biodostępne formy metali i są pod wpływem wielu czynników zewnętrznych podczas ekspozycji (zmieniając w ten sposób ich stopień witalności), dlatego wyniki różnią się od tych uzyskanych za pomocą urządzenia automatycznego.

Spośród trzech gatunków, mech *Pleurozium schreberi* jest najbardziej odpowiedni do monitorowania zanieczyszczenia powietrza na terenach miejskich. Jest on efektywnym biomonitorem zarówno w kilkudniowym (Sylwester), jak i kilkumiesięcznym okresie ekspozycji (do roku czasu). Ze względu na swoje zdolności adaptacyjne, jest w stanie przetrwać zmienne warunki środowiskowe przy zachowaniu witalności, co w połączeniu z jego specyfiką struktury organizmu zapewnia mu bardzo dobre właściwości akumulacyjne.

Najważniejsze osiągnięcia oraz wkład mojej pracy badawczej przedstawionej w omawianym cyklu publikacji dotyczącym wykorzystania mchów w rozwój metody biomonitoringu aktywnego na terenach zurbanizowanych, są następujące:

1. Dokonałem rzetelnego przeglądu literatury dotyczącej tematyki moich badań w ramach części wprowadzającej każdego z artykułów jak i dyskusji wyników (zacytowałem średnio 70 prac w każdym artykule) [ON.1-ON.9].
2. Opracowałem optymalną metodykę preparowania próbek mchów przed ekspozycją metodą biomonitoringu aktywnego, zapewniającą jednorodność mchów pobranych z lasów pod względem stężeń metali ciężkich. Umożliwia ona uzyskanie wiarygodnych i powtarzalnych wyników w ramach badań z wykorzystaniem mchów. Wykazałem, że ważnym elementem przygotowawczym przed ekspozycją próbek mchów jest ich wcześniejsze odpowiednie kondycjonowanie w wodzie zdemineralizowanej [ON. 2]. Skuteczność opracowanej metodyki została potwierdzona na trzech gatunkach mchów w kolejnych badaniach [ON.1., ON.3, ON.7, ON.9].
3. Porównałem ze sobą różne techniki ekspozycji mchów (stosowane przez różnych autorów) w trakcie biomonitorowania na terenie miejskim. Wskazałem najskuteczniejszą/optymalną metodę: jest nią technika *moss bag* [ON.3].
4. Wykazałem istotność pomiarów i kontroli parametrów życiowych mchów w oparciu o pomiar stężenia chlorofilu połączonego z aktywnością fotosyntetyczną. Biomonitoring z wykorzystaniem mchów jest skuteczny, kiedy eksponowany materiał to organizm żywy a nie dewitalizowany sorbent naturalny [ON.4].
5. Przeanalizowałem wpływ warunków środowiskowych i parametrów abiotycznych oddziałujących na witalność mchów w trakcie ekspozycji. Ustaliłem optymalny jej czas, dla zachowania przy tym właściwości akumulacyjnych żywych mchów [ON.5].
6. Zastosowałem wcześniej zoptymalizowaną technikę *moss bag* i wykazałem możliwość praktycznego wykorzystania mchów do monitorowania zanieczyszczenia powietrza w pomieszczeniach zamkniętych i identyfikacji metali ciężkich zawartych w dymie pochodzących z trzech typów papierosów. Dodatkowo wykazałem, że typ wyrobu tytoniowego nie ma wpływu na zanieczyszczenie powietrza wybranymi metalami ciężkimi, zatem nieprawdą jest, aby obecnie modne wyroby podgrzewające tytoń były mniej uciążliwe dla środowiska [ON.6].
7. Wykazałem oddziaływanie miejsca ekspozycji mchów, na przykładzie warsztatu i jego otoczenia zewnętrznego, na wynik badań biomonitoringowych przedstawiając istotne

zmiany w witalności mchu w zależności od miejsca ekspozycji próbek. Mchy okazały się czułem biomonitorem zanieczyszczeń emitowanych z punktowego źródła jakim była specyficzna działalność warsztatu samochodowego. Stężenia poszczególnych pierwiastków zawartych w mchach po ekspozycji odpowiadały profilowi źródeł zanieczyszczeń wynikających z działalności warsztatu [ON.7].

8. Wykazałem, że mchy mogą być stosowane w badaniach krótkoterminowych do identyfikacji źródeł zanieczyszczających. Zastosowałem aktywny biomonitoring w krótkookresowej ekspozycji mchów w Sylwestra wykazując praktyczny aspekt tego typu badań. Dowiodłem, iż wystrzeliwanie fajerwerków znacznie wzbogaca mech w różne pierwiastki, których stężenia były uzależnione od roku doświadczenia (mniejsze stężenia analitów odnotowałem w trakcie lockdown'u na przełomie 2020/2021) [ON.8].
9. Podsumowanie moich badań z wykorzystaniem mchów w biologicznym monitoringu aerozolu atmosferycznego zawarłem w artykule [ON.9]. Powiązałem aplikacyjność powyższych badań biomonitoringowych z klasyczną metodą monitoringu powietrza. Skład pierwiastkowy mchu i TSP są znacząco różne. Porównując wyniki uzyskane dwoma zastosowanymi metodami stwierdziłem niepełną zgodność metody biomonitoringu aktywnego z metodą instrumentalną. Monitoring biologiczny z wykorzystaniem mchów musi być dokładnie przemyślany w zależności od celów kontroli, wymaganego poziomu czułości oraz rodzaju zanieczyszczenia.
10. Prace wchodzące w skład cyklu 9 jednotematycznych publikacji (artykuły oznaczone w wykazie dorobku naukowego – ON.1. – ON.9.; wydane w latach 2021-2022) zatytułowanego *Zastosowanie mchów w biomonitoringu aktywnym na terenach zurbanizowanych* wzbudzają zainteresowanie wśród innych autorów. Dotychczas były one cytowane ponad 35 razy (Web of Science - 36; Scopus - 37; czy ResearchGate - 40) stan na 18.03.2023 r.

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WYKAZ PUBLIKACJI PRZEDSTAWIONYCH DO OCENY W PRZEWODZIE DOKTORSKIM

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Bioaccumulation of Trace Elements from Aqueous Solutions by Selected Terrestrial Moss Species

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Simple Summary: In this study, the kinetics of elemental sorption in moss species were investigated under laboratory conditions. Functional groups responsible for binding metal cations to the internal structures of the mosses were also identified. Based on the conducted research, it was found that regardless of the moss species, a state of equilibrium in the moss–solution system was reached after 60 min, as indicated by the stable readings of measuring instruments. Under the conditions of the experiment, in the first 10 min of the process about 70.4–95.3% of metal ions were sorbed from the solution into the moss gametophytes with respect to the concentration of this analyte accumulated in the mosses at equilibrium. The results of the study indicate that the process of bioaccumulation of heavy metals in mosses occurs mainly through ion exchange, as evidenced—among other things—by a decrease in the concentration of metal ions in the solution with which they are in contact and a concomitant increase in the conductivity in a solution. The presented results indicate the interrelationship between concentrations of cations in and around mosses (solution/atmospheric aerosols). At the same time, the presented results make it possible to identify and select appropriate moss species for biomonitoring purposes.

Abstract: The interrelationship between metal concentrations in mosses and their surroundings prompts research toward examining their accumulation properties, as it is particularly important for their usage in biomonitoring studies that use mosses. In this study, the kinetics of elemental sorption in three moss species (*Pleurozium schreberi*, *Dicranum polysetum*, and *Sphagnum fallax*) were investigated under laboratory conditions. Sorption from metal salt solutions was carried out under static conditions with decreasing elemental concentration. Functional groups responsible for binding metal cations to the internal structures of the mosses were also identified. It was shown that the equilibrium state was reached after about 60 min. Under the conditions of the experiment, in the first 10 min of the process, about 70.4–95.3% of metal ions were sorbed from the solution into the moss gametophytes by *P. schreberi* (57.1–89.0% by *D. polysetum* and 54.1–84.5% by *S. fallax*) with respect to the concentration of this analyte accumulated in the mosses at equilibrium. It can be assumed that the exposure of mosses with little contamination by heavy metals in an urbanized area under active biomonitoring will cause an increase in the concentration of these analytes in proportion to their concentration in atmospheric aerosols. In the case of *P. schreberi* and *D. polysetum*, the O-H/N-H band was enormously affected by the adsorption process. On the other hand, FTIR (Fourier transform infrared spectroscopy) analysis of *S. fallax* after adsorption showed slight changes for most of the bands analyzed. Based on this study, it can be concluded that mosses can be used as, for example, a biomonitor in monitoring of urban ecosystems, but also in the phytoremediation of surface waters.

Keywords: bioaccumulation; sorption; mosses; heavy metals; FTIR; urban areas

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1. Introduction

Over the past 50 years, studies of the application of mosses and lichens in biomonitoring have grown significantly and have either expanded or should be still expanded in sampling techniques [1,2]. With the continuous systematization and improvement in research methodologies, biomonitoring with mosses and lichens continues to find new opportunities for their application in environmental quality monitoring. Recent examples include magnetic biomonitoring with lichens in assessing the impact of dust pollution on cultural heritage [3] or the mobile air quality monitoring capabilities of the moss-bag technique [4]. In this research, it is important to determine whether we are studying bioconcentration, surface adsorption of trace elements, or both. [5].

Most biomonitoring studies emphasize the high percentage of contaminants and particle deposition on mosses' surfaces [6,7]. The different sizes of these particles and their ability to adhere to biological material is strongly related to the structure of the leaf. The fact that the more particles are deposited on the moss leaves the higher the concentration of trace elements is confirms that their uptake by mosses is mainly based on passive mechanisms (depending on the properties of the moss surface) [8,9]. Only a small percentage of their total concentration is accumulated inside tissues, either in soluble form or bound to the inner plasma membrane [10]. On one hand, there is evidence that washing samples has proven to be ineffective for the determination of the bioaccumulated fraction in terrestrial mosses [11]. On the other hand, we have to consider that washing samples is necessary for determining the polycyclic aromatic hydrocarbon (PAH) bioconcentration in mosses [12]. The use of various analytical and laboratory techniques makes it possible to assess the bioconcentration of compounds and trace elements and their impact on the viability of biomonitors [13–15]. Despite the many advantages of using clones and devitalized biomonitors [16,17], we stand by our position of measuring and controlling the vital parameters of mosses during biomonitoring studies [18,19] in order to determine the real response of a living organism to environmental pollution and not just that of dead tissue as a chemical adsorbent [20,21]. In environments with high levels of atmospheric heavy metal deposition, the photosynthesis of *Pseudoscleropodium purum* is inhibited and protein expression in this moss is susceptible to modifications related to environmental conditions [22]. Other studies have ruled out the possibility of using the moss *Ptychostomum capillare* in biomonitoring of air pollution due to a defense mechanism mediated by lipophilic substances (probably waxes) that act as a barrier to prevent metal from entering the cells, which could distort direct estimates of environmental copper content through levels detected in its tissues [23]. Thus, understanding the effects of analytes on bryophyte morphophysiological traits may be fundamental to optimizing their use in biomonitoring [24]. Therefore, in addition to just studying the surface sorption of trace elements by mosses, it is necessary to focus on the bioconcentration of these elements in their interior, and look for relationships between particles attached to the surface and those bioaccumulated in their tissues [12].

The aim of this study is to determine whether a variation in the bioconcentrations of selected metals in mosses of three species: *P. schreberi*, *S. fallax*, and *D. polysetum* accumulated from aqueous solutions of these elements. The sorption (physical and chemical process [absorption, adsorption, ion exchange] by which a metal's cations accumulated in the mosses) of five anthropogenic-derived metals that are often found in atmospheric aerosols was analyzed. We have tried to verify the research hypothesis that mosses actively accumulate analytes into their tissues during exposure to pollutants. We expect to provide evidence supporting this hypothesis by several means: (1) analysis of heavy metal ion sorption kinetics in moss gametophytes, and (2) identification of the main functional groups present in the tissues of mosses that are responsible for the accumulation of metals.

2. Materials and Methods

The species used for this study were the moss *P. schreberi* (Pl), *S. fallax* (Sp), and *D. polysetum* (Dp). They were collected in October 2021 from forests in the Swietokrzyskie Voivodship in southeastern Poland.

Moss samples were taken and treated before exposure as part of active biomonitoring in accordance with standard guidelines [25]. The mosses were prepared before exposure according to a previously developed methodology [26]. The concentrations of metals naturally accumulated in the mosses used for the experiments [mg/g dry weight] are presented in the Supplementary Materials (Table S1).

This research was conducted on whole, live/fresh (quantum yield of chlorophyll fluorescence was 0.5–0.7, see below) moss gametophytes of *P. schreberi*, *S. fallax*, and *D. polysetum*. Moss samples weighing 0.200 ± 0.001 g were placed in a perforated container with a volume of about 15 cm^3 and immersed in a salt solution of the selected metal with a volume of 200 cm^3. The solution was stirred using a magnetic stirrer (VELP Scientifica Srl, Usmate, IT; RPM: 250). Periodically, the solution was drawn directly from the vessel in which the experiment was conducted to determine (AAS) the concentration of the metal. Metal salts were dissolved in deionized water. The uncertainty of the measurements of metal concentrations using AAS did not exceed 5%. The process was carried out for about 60 min, as previous studies have shown that a state of equilibrium in the moss–solution system (stable readings of measuring instruments) is achieved within this time [27]. The concentrations of analytes were chosen so that it would be possible to follow the kinetics of the process using F-AAS and also so that the concentrations would be comparable to those found in the environment. During the process of metal accumulation in moss gametophytes the measurement of changes in the conductivity and pH of the solution were also conducted. After the process, the mosses were rinsed with deionized water and left for 60 min under laboratory conditions, and then their viability (measure of how much plant material in a lot are alive) and actual photochemical efficiency (yield) were measured. The actual quantum efficiency of photosystem II (PSII) photochemistry in the light measures the fraction of the absorbed light energy that is actually being used to drive photochemistry at PSII [28]. Estimates of the efficiency at which light absorbed by PSII are used for photochemistry; at a given light intensity, it provides an estimate of the quantum efficiency of linear electron transport through PSII [29]. The samples were dried at room temperature and were then destined for Fourier transform infrared spectroscopy (FTIR) analysis to study the moss gametophytes before and after the metal accumulation process ($c_{Mt\prime 0}$ = 2.66 mg/L—initial concentration of metal salt solutions to which the mosses were introduced). For this purpose, 0.200 ± 0.001 g of moss was dried at room temperature and then homogenized using a mortar.

2.1. Devices and Reagents

Heavy metals were determined using an iCE 3500 atomic absorption spectrometer from Thermo Electron Corporation (Grand Island, NY, USA). A CP551 pH meter (Elmetron Sp. j. Zabrze, PL) and CC551 conductivity meter (Elmetron Sp. j. Zabrze, PL) were used, whose absolute error of indication was ΔpH = 0.02 and Δκ = 0.1 μS/cm, respectively, to test the conductivity and pH of the solutions in which the mosses were immersed. Solutions of metal salts of Ni, Cu, Zn, Cd, and Pb were prepared using reagents from MERCK. The uncertainty in the readings of the laboratory balance used was ±0.001 g. For identification of the potential functional groups and possible binding sites associated with the accumulation of Ni(II), Cu(II), Zn(II), Cd(II), and Pb(II), IR analysis was performed using an FTIR spectrometer (Fourier transform infrared spectrometer, Cary 630 FTIR spectrometer, NICOLET IZ10, Thermo Scientific, Waltham, MA, USA).

The chlorophyll fluorescence of PSII was monitored using a modulated portable fluorometer (Opti-Sciences, Hudson, NH, USA). Actual photochemical efficiency (yield) was measured under ambient light [30]. Actual photochemical efficiency (yield) below 0.1 is the critical value below which the moss is only a natural sorbent and not a biomonitor because it loses its viability [31].

2.2. Quality Control

Table 1 shows the limits of detection and limits of quantification of heavy metals characterizing the iCE 3500 spectrometer [32]. Values of the highest concentrations of standards (ANALYTIKA Ltd., Prague, Czech Republic) used for calibration (2.0 mg/dm^3 for Cd, 5.0 mg/dm^3 for Cu, Zn, Ni, and Pb) were taken as the limit of the linear dependence of the signal on concentration.

Table 1. The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the iCE 3500 spectrometer [mg/L] [32].

Metal	*IDL*	*IQL*
Ni	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

Table 2 shows the concentrations of trace elements determined in the certified reference material BCR-482 *lichen*, produced by the Institute for Reference Materials and Measurements, Belgium.

Table 2. Comparison of measured and certified concentrations in BCR-482 *lichen*.

Metal	BCR-482 *lichen* (Certified)		AAS (Measured)		*Dev.***
	Concentration	±Uncertainty	Mean	±*SD* *	
		[mg/kg dry weight]			[%]
Ni	2.47	0.07	2.16	0.32	−13
Cu	7.03	0.19	6.63	0.17	−5.7
Zn	100.6	2.2	95.1	2.3	−5.5
Cd	0.56	0.02	0.53	0.03	−5.3
Pb	40.9	1.4	38.2	1.0	−6.6

Note: * standard deviation. ** relative difference between the measured (c_m) and certified (c_c) concentration 100% (c_m—c_c)/c_c.

3. Results and Discussion

The use of several moss species at once is crucial for monitoring elemental deposition in the environment due to the possibility of comparing their accumulation capacities [33]. Studies of the kinetics of the metal ion accumulation process were carried out under static conditions, with continuous stirring of the solution. In the first stage of the study, the concentrations of selected metals were evaluated in solutions of their salts into which mosses were immersed. The process was followed using the AAS technique until an equilibrium state (condition resulting from a heterophasic double exchange reaction between mobile cations bound in the cell wall of mosses and the composition of the solution with which the mosses come into contact) was reached between the mosses and the solution in which they were immersed. For this purpose, samples of mosses were immersed in salt solutions of selected metals with a volume of 200 cm^3 and a specified initial concentration of metal in solution $c_{s,0}$. Table 3 shows the initial concentrations of metals $c_{s,0}$, the concentrations of trace elements after the sorption process in solution $c_{s,1}$, and accumulated in mosses at equilibrium state $c_{M,1}$. The concentrations of sorbed metals per unit mass of mosses were determined from the relationship

$$c_{M,1} = \frac{(c_{s,0} - c_{s,1}) * V}{m} \quad (1)$$

where V = the volume of solution from which sorption was carried out and m = the mass of the moss.

Table 3. Changes in elemental concentrations in solutions [mg/L] and in mosses [mg/g d.w.] during the sorption process (n = 3).

Pleurozium schreberi			*Dicranum polysetum*			*Sphagnum fallax*		
$c_{s,Ni(0)}$	$c_{s,Ni(1)}$	$c_{M,1}$	$c_{s,Ni(0)}$	$c_{s,Ni(1)}$	$c_{M,1}$	$c_{s,Ni(0)}$	$c_{s,Ni(1)}$	$c_{M,1}$
0.07	<0.05	>0.02	0.07	<0.05	>0.02	0.07	<0.05	>0.02
0.30	<0.05	>0.25	0.30	<0.05	>0.25	0.30	<0.05	>0.25
2.67	0.52	2.15	2.67	0.69	1.98	2.67	0.49	2.18
$c_{s,Cu(0)}$	$c_{s,Cu(1)}$	$c_{M,1}$	$c_{s,Cu(0)}$	$c_{s,Cu(1)}$	$c_{M,1}$	$c_{s,Cu(0)}$	$c_{s,Cu(1)}$	$c_{M,1}$
0.05	<0.03	>0.02	0.05	<0.03	>0.02	0.05	<0.03	>0.02
0.30	<0.03	>0.27	0.30	<0.03	>0.27	0.30	<0.03	>0.27
2.60	0.20	2.40	2.60	0.19	2.40	2.60	0.33	2.30
$c_{s,Zn(0)}$	$c_{s,Zn(1)}$	$c_{M,1}$	$c_{s,Zn(0)}$	$c_{s,Zn(1)}$	$c_{M,1}$	$c_{s,Zn(0)}$	$c_{s,Zn(1)}$	$c_{M,1}$
0.03	<0.01	>0.20	0.03	<0.01	>0.02	0.03	<0.01	>0.02
0.30	0.02	0.28	0.30	0.02	0.28	0.30	0.01	0.29
2.81	0.40	2.41	2.81	0.64	2.17	2.81	0.49	2.32
$c_{s,Cd(0)}$	$c_{s,Cd(1)}$	$c_{M,1}$	$c_{s,Cd(0)}$	$c_{s,Cd(1)}$	$c_{M,1}$	$c_{s,Cd(0)}$	$c_{s,Cd(1)}$	$c_{M,1}$
0.03	<0.01	>0.02	0.03	<0.01	>0.02	0.03	<0.01	>0.02
0.30	<0.01	>0.29	0.30	<0.01	>0.29	0.30	0.07	0.23
2.67	0.09	2.58	2.67	0.27	2.40	2.67	0.23	2.44
$c_{s,Pb(0)}$	$c_{s,Pb(1)}$	$c_{M,1}$	$c_{s,Pb(0)}$	$c_{s,Pb(1)}$	$c_{M,1}$	$c_{s,Pb(0)}$	$c_{s,Pb(1)}$	$c_{M,1}$
0.09	<0.07	>0.02	0.09	<0.07	>0.02	0.09	<0.07	>0.02
0.24	<0.07	>0.17	0.24	<0.07	>0.17	0.24	<0.07	>0.17
2.70	0.08	2.62	2.70	0.10	2.60	2.70	0.13	2.57

From these experiments, aqueous solutions of analytes with concentrations of about 2.60–2.80 mg/dm^3 were used for the second stage of the study, which aimed to follow the kinetics of the sorption process in the moss–solution system using AAS. The use of solutions with such concentrations of individual metals made it possible to run the tests until equilibrium was reached in the moss–aqueous solution system; at the same time, the concentrations of analytes were close to those found in the environment [34]. Mosses, due to their specific anatomical structure and specific mode of nutrition, sorb nutrients contained in atmospheric aerosols [35]. The process of trace element accumulation occurs mainly from aqueous solutions; as the concentration of an analyte in solution increases, its concentration in moss gametophytes increases [36], as shown in Table 3.

Figure 1 shows the changes in elemental concentration, conductivity, and pH in salt solutions of the analyzed metals after immersing selected moss species in them and conducting the sorption process.

It can be concluded from the experiments that regardless of the moss species, in the moss–solution system a state of equilibrium was reached after 60 min, as indicated by stable readings of conductivity and pH of the solution and the absence of significant changes in the concentrations of trace elements in the solutions (see Table 3 and Figure 1a). A slight increase in the copper (60 min) concentration in solution with *D. polysetum* could be due, for example, to the dissolution of salts/dust naturally accumulated on the moss. Subsequent measurements of concentrations were stable. In comparison, the state of dynamic equilibrium during the process of sorption of Cu^{2+} ions in the marine algae *Palmaria palmata* was reached after about 70 min, and in the freshwater algae *Spirogyra* sp. after about 30 min [37]. In the moss *P. schreberi*, 80.5–97.0 % of the metal ions found in the initial solution (in *D. polysetym*—74.2–96.3%; in *S. fallax*—81.6–95.2%) accumulated during the 60 min process (Table 3). In the first 10 min of the process, about 70.4–95.3% of metal ions were sorbed from the solution to the moss gametophytes by *P. schreberi* (57.1–89.0% by *D. polysetum*; 54.1–84.5% by *S. fallax*) relative to the concentration of this metal accumulated in the mosses at equilibrium (Supplementary Materials—Figures S1–S4). The intensity of analyte accumulation in mosses depends, among other things, on their affinity for the functional groups that make up the compounds that form the cell wall, as well as the structure and development of the surface of the moss gametophyte [38]. Under the conditions of the research conducted, the accumulation of trace elements depended on the species of

moss, increasing in a series: *S. fallax* < *D. polysetum* < *P. schreberi*. Evaluation of sorption properties is important when using different moss species in biomonitoring studies.

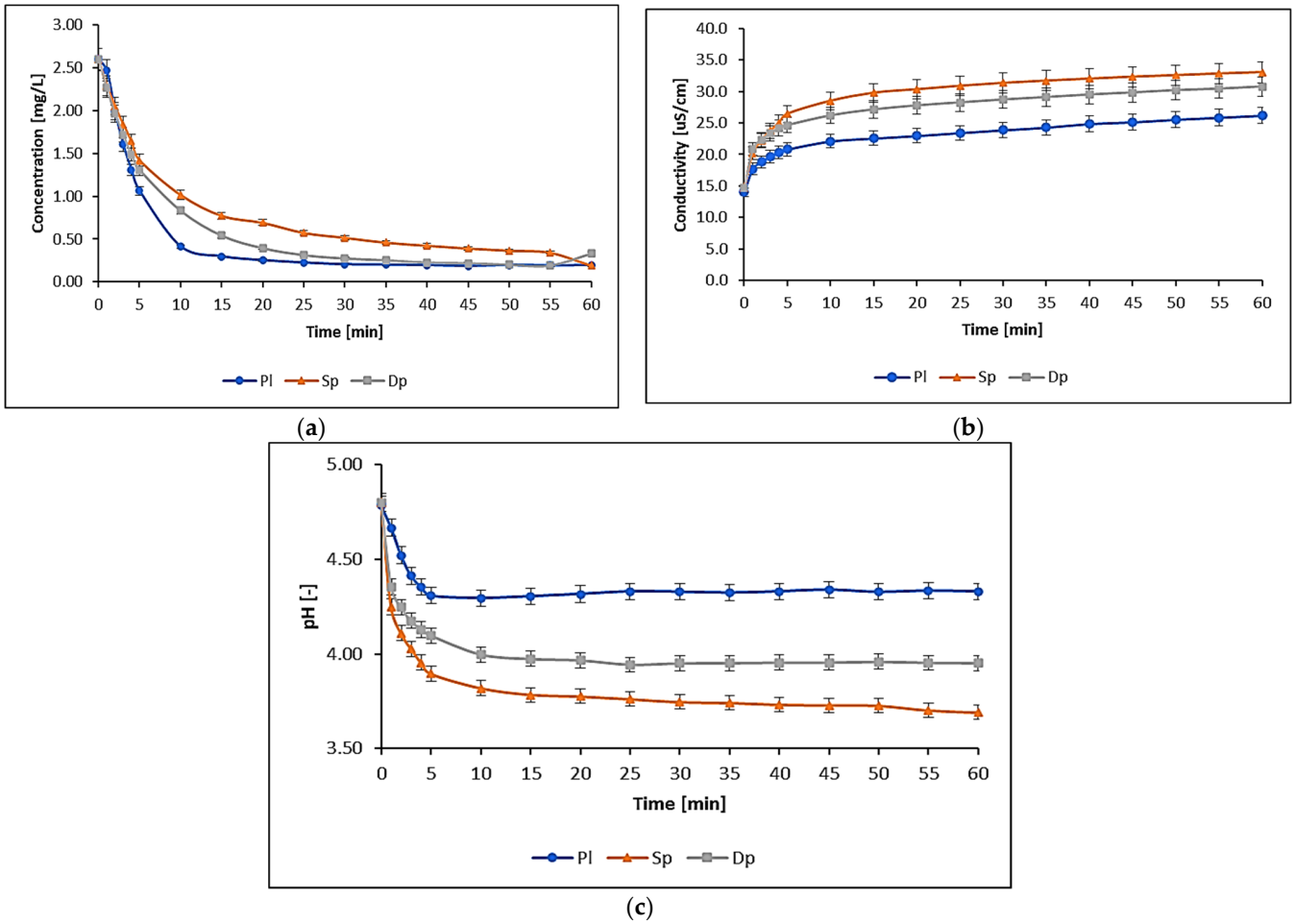

Figure 1. Changes in physicochemical parameters in Cu solution during the accumulation process of moss gametophytes: (**a**) concentration; (**b**) conductivity; (**c**) pH (Pl—*P. schreberi*, Sp—*S. fallax*, Dp—*D. polysetum*). Whiskers indicate standard deviation levels.

Figure 1c shows that regardless of species, the process of Cu sorption by mosses is accompanied by the sorption of H^+ ions. An increase in conductivity was also spotted during the process (Figure 1b). Figures of physicochemical changes for the remaining trace elements are presented in the Supplementary Materials (Figures S1–S4). The increase in the solution's conductivity after the insertion of mosses into the solution was caused, among other things, by the dissolution of salts naturally accumulated on the surface of the mosses and progressive, irreversible changes in the structure of cell membranes over time, which cause the leakage of ionic substances from moss cells to the solution [39–41]. This process can be observed using conductivity measurements to track the kinetics of metal sorption on mosses that have been stored for 6 months (Figure 2).

The changes in the structures of cell membranes, occurring over time and causing an increase in the amount of ions in solution, were observed through an increase in conductivity as well as a decrease in the pH of the solution (an increase in the concentration of hydrogen ions), which also led to a reduction in Cu sorption on moss gametophytes by about 5% (under experimental conditions, regardless of moss species). At the same time, the reduction in the vitality of mosses after a period of 6 months was confirmed by studies using the actual photochemical efficiency of PSII. Live/fresh mosses were characterized by a vitality of 0.6–0.7, while for mosses stored for a period of 6 months the vitality dropped

to 0.1 (anabiosis state) but they did not lose their accumulative properties [20]. Various devitalized matrices (mosses, lichens) are commonly used in the literature because of the ease of working with such material, as well as precisely because of their lack of loss of uptake ability [18,42].

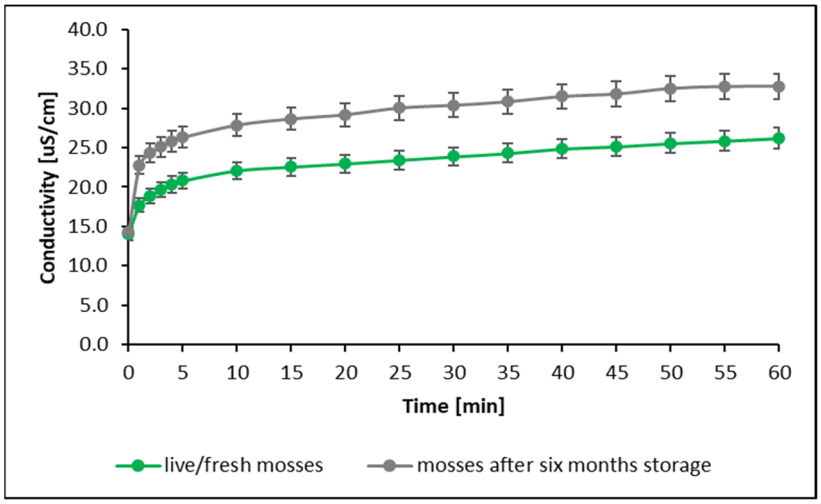

Figure 2. Conductivity changes during the process of Cu sorption on the moss *P. schreberi* (mosses stored in the laboratory for 6 months were used for the study).

Literature data indicate that the process of heavy metal accumulation in mosses occurs mainly through ion exchange and is surface-based [43]. The same effect was observed during the study of heavy metal sorption processes in lichens [44]. However, it should be noted that in parallel or secondarily, metals accumulate in the intracellular structures of mosses [38,45].

FTIR analysis of the main functional groups found in the mosses that are responsible for metal accumulation was carried out (Figure 3).

An FTIR analysis was carried out to better understand the adsorption mechanisms involved in removing metal cations. Therefore, mosses before adsorption and after the removal of metals were compared (Figure 3). Mosses have been reported to be composed mainly of carbohydrates (>50%), lipids, and proteins [46,47], which were all identified by FTIR spectra in *P. schreberi, D. polysetum*, and *S. fallax*. Overall, the mosses' spectra have various regions of interest. The first one (~3700–~3000 cm^{-1}) represents O-H and N-H bonds [48] (symmetric and asymmetric stretch) from carbohydrates and proteins. Following peaks could be observed at ~2913 cm^{-1} C-H (stretch) from polysaccharides, lipids, and carbohydrates, while at ~1716 and ~1616 cm^{-1} the peaks were ascribed respectively to C=O [49] and N-H [50]. At ~1243 cm^{-1} another peak appeared that could be attributed to the presence of amide (C-N stretch) [51] from proteins and glycoproteins. Then, at ~1040 cm^{-1}, we could identify the signal of C-O-C functionalities [52] (stretch) from oligosaccharides, glycoprotein, and carbohydrates.

As Figure 3 shows, after adsorption with various metal cations, several peaks changed in intensity compared to the spectra of mosses before adsorption. In the case of *P. schreberi* and *D. polysetum*, the O-H/N-H band is tremendously affected after the adsorption process. In the same way, the bands at ~1716 and ~1616 cm^{-1} underwent changes. Finally, the last two bands at ~1250 and ~1040 cm^{-1} also show an intensity reduction but to a lesser extent than the previous one. On the other hand, FTIR of *S. fallax* after adsorption showed slight changes for most of the bands analyzed. In Figure 3 III, the dominant changes are in the region of ~3700–~3000 cm^{-1}. The adsorption of metals in mosses could be due to electrostatic interactions between the ions and negatively charged functional groups on them, as reported previously by Vinod and Sashidhar [53]. The apparent decrease in intensity of the O-H/N-H peak could indicate that the bonded O-H functional groups

and amide moieties may play an essential part in the sorption of metal ions. A similar observation was reported for the adsorption of Ni^{2+} by a carbohydrate gum adsorbent [54]. In addition, a decrease in intensity of the ~1716 and ~1616 cm^{-1} peaks after adsorption could indicate participation of the C=O and N-H groups in conjugating the metal cations.

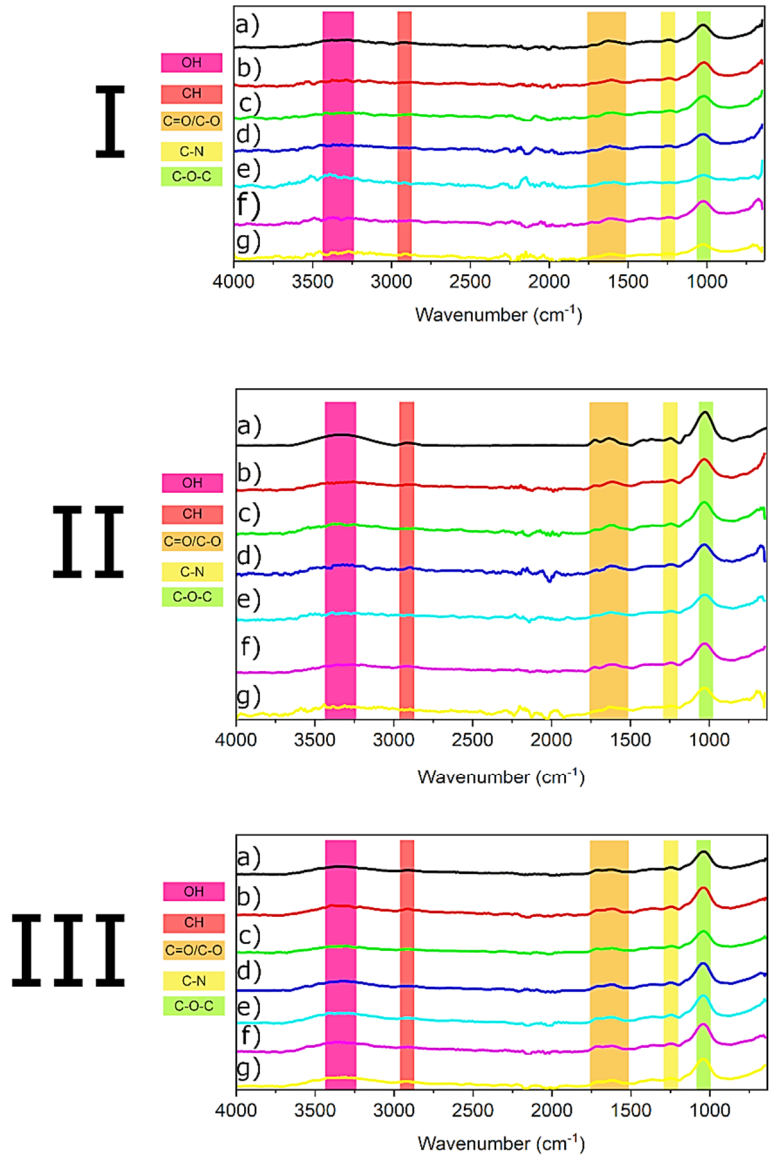

Figure 3. FTIR of (**I**) *P. schreber*i (**a**) before adsorption, (**b**) after Ni^{2+} adsorption, (**c**) after Cu^{2+} adsorption, (**d**) after Zn^{2+} adsorption, (**e**) after Cd^{2+} adsorption, (**f**) after Pb^{2+} adsorption, and (**g**) mixture of cations. (**II**) *D. polysetum* (**a**) before adsorption, (**b**) after Ni^{2+} adsorption, (**c**) after Cu^{2+} adsorption, (**d**) after Zn^{2+} adsorption, (**e**) after Cd^{2+} adsorption, (**f**) after Pb^{2+} adsorption, and g) mixture of cations. (**III**) *S. fallax* (**a**) before adsorption, (**b**) after Ni^{2+} adsorption, (**c**) after Cu^{2+} adsorption, (**d**) after Zn^{2+} adsorption, (**e**) after Cd^{2+} adsorption, (**f**) after Pb^{2+} adsorption, and (**g**) mixture of cations.

As reported by González and Pokrovsky [55], metal cations can bind to the surface layers of the cell wall via cation exchange. Several substances make up the cell walls of mosses, such as cellulose and glycoproteins, which can be involved in the adsorption of metals, as reported by Nag [56] and Maruyama [57], respectively. Therefore, based on FTIR analysis and the literature [55], it seems likely that the surface layers of the mosses

are responsible for metal adsorption. In addition, the slightly different mosses' cell wall compositions can influence metal adsorption [58].

4. Conclusions

The results of this study indicate that the process of bioaccumulation of heavy metals in mosses occurs mainly through ion exchange as evidenced, among other things, by a decrease in the concentration of metal ions in the solution with which they are in contact and a concomitant increase in the conductivity of a solution. Based on the conducted research, it was found that regardless of the moss species, in the moss–solution system, a state of equilibrium was reached after 60 min, as indicated by stable readings of conductivity and pH of the solution and the absence of significant changes in the concentrations of trace elements in the solution. It should be noted, however, that in parallel or secondarily, metals accumulate in the intracellular structures of mosses. FTIR analysis of moss samples confirmed the participation of hydroxy, amine, and carbonyl groups in the biosorption process of metal cations.

The presented results of this study indicate the interrelationship between the concentration of cations in and around mosses (solution/atmospheric aerosols). At the same time, the presented results make it possible to identify and select appropriate moss species for biomonitoring purposes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biology11121692/s1, Figure S1: Physicochemical changes in Ni solution during the accumulation process on moss gametophytes: (a) concentration, (b) conductivity, (c) pH. Figure S2: Physicochemical changes in Zn solution during the accumulation process on moss gametophytes: (a) concentration, (b) conductivity, (c) pH. Figure S3: Physicochemical changes in Cd solution during the accumulation process on moss gametophytes: (a) concentration, (b) conductivity, (c) pH. Figure S4: Physicochemical changes in Pb solution during the accumulation process on moss gametophytes: (a) concentration, (b) conductivity, (c) pH. Table S1: Concentrations of metals naturally accumulated in the mosses used for the experiments [mg/g d.w.].

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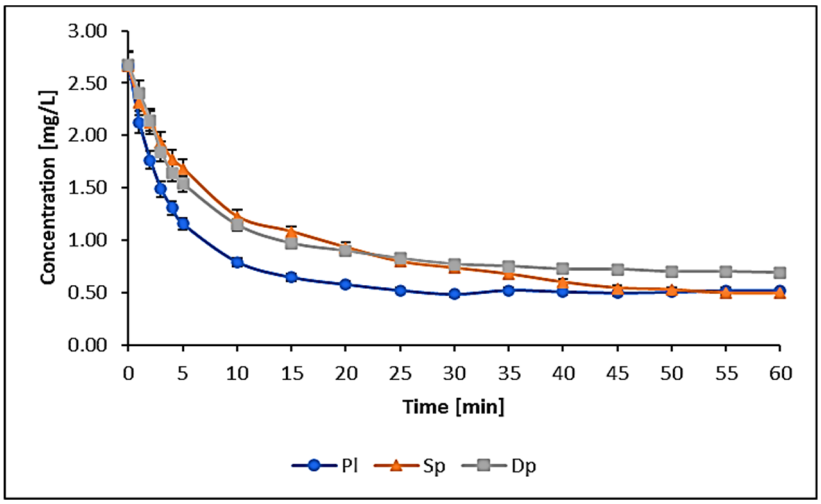
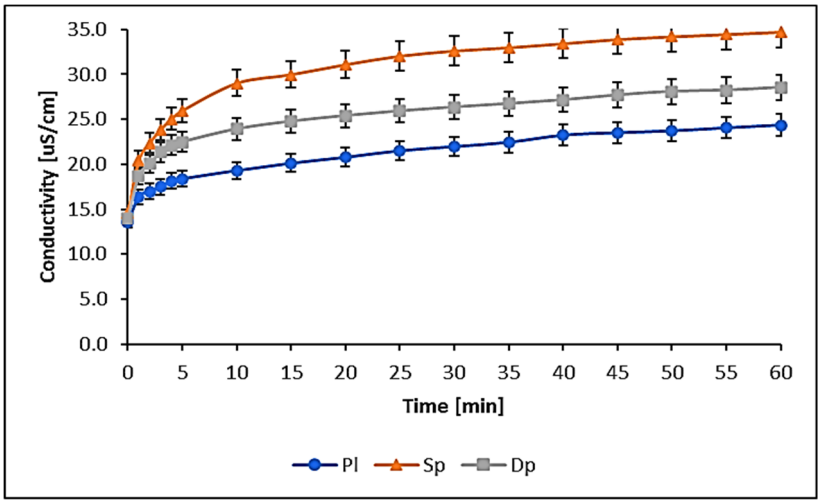
a)


Concentration [mg/L]
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0
5
10
15
20
25
30
35
40
45
50
55
60
Time [min]
Pl
Sp
Dp

b)


Conductivity [uS/cm]
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
5
10
15
20
25
30
35
40
45
50
55
60
Time [min]
Pl
Sp
Dp

c)

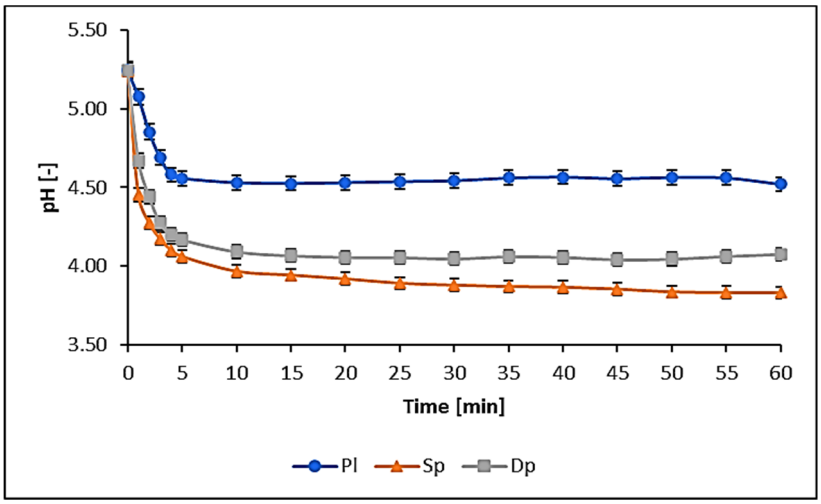
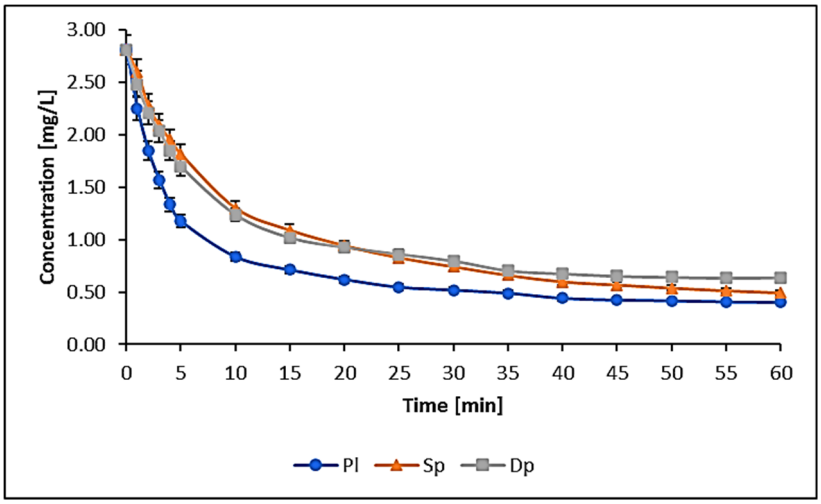
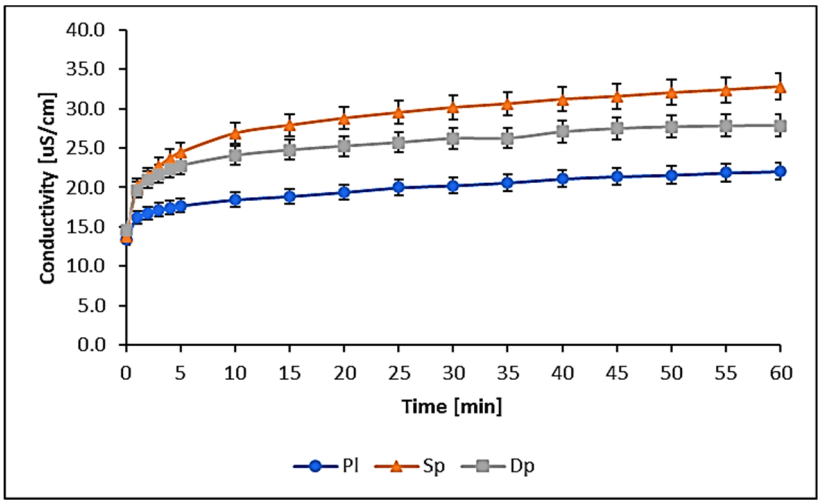


Figure S1. Changes in physicochemical parameters in Ni solution during the accumulation process on moss gametophytes: a) its concentration, b) conductivity, c) pH

a)



b)



c)

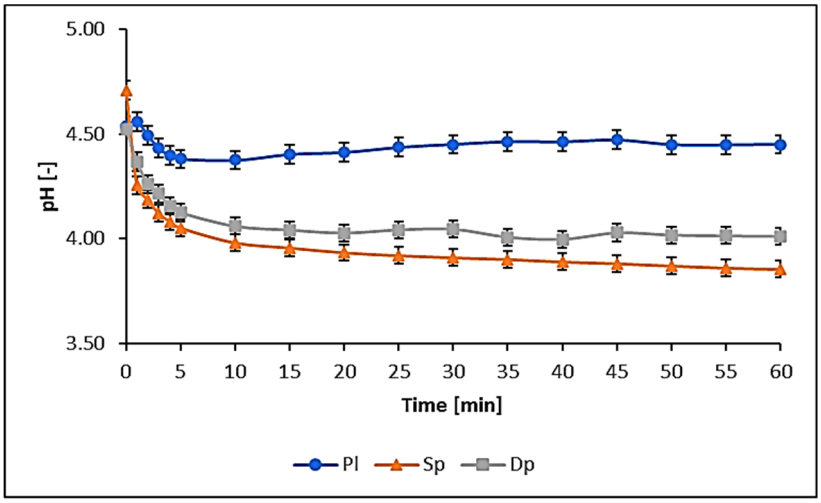
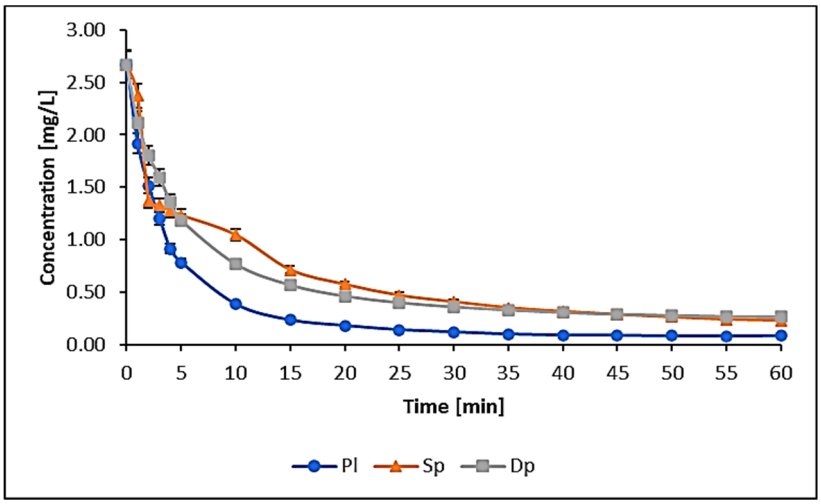
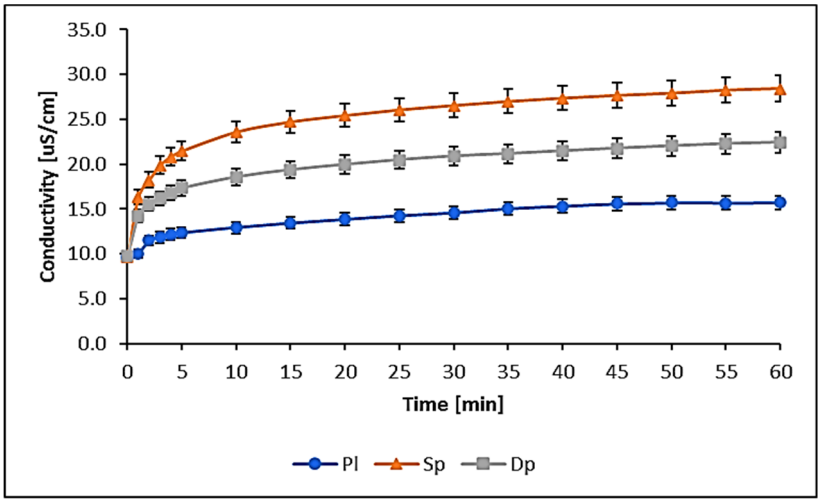


Figure S2. Changes in physicochemical parameters in Zn solution during the accumulation process on moss gametophytes: a) its concentration, b) conductivity, c) pH

a)


Concentration [mg/L]
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0 5 10 15 20 25 30 35 40 45 50 55 60
Time [min]
Pl Sp Dp

b)


Conductivity [uS/cm]
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0 5 10 15 20 25 30 35 40 45 50 55 60
Time [min]
Pl Sp Dp

c)

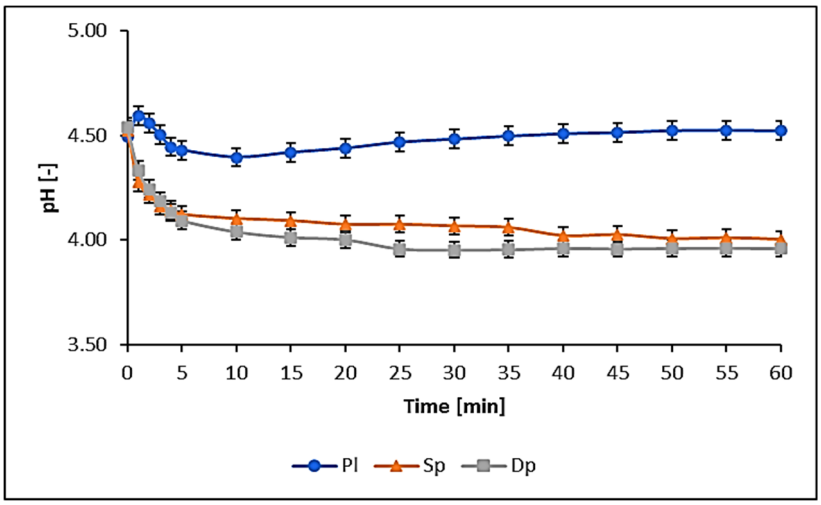
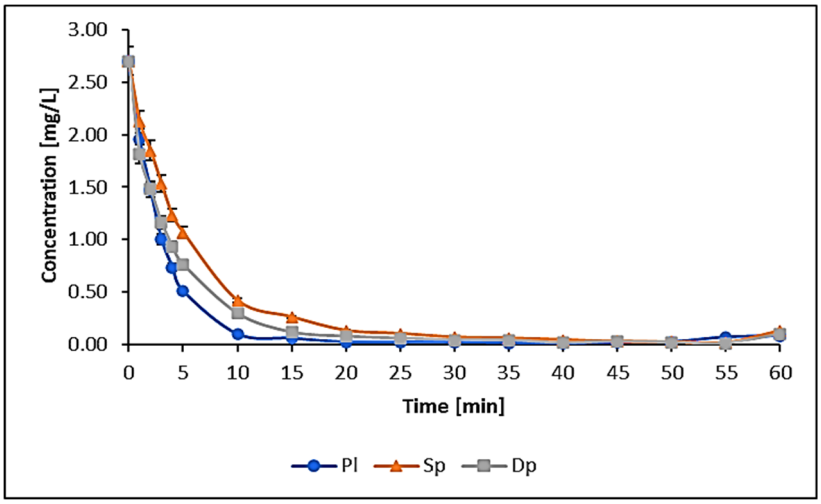
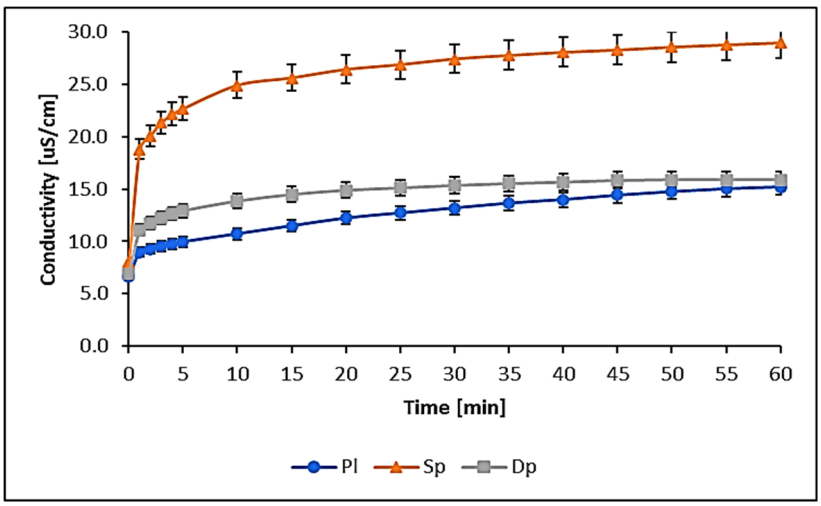

pH [-]
5.00
4.50
4.00
3.50
0 5 10 15 20 25 30 35 40 45 50 55 60
Time [min]
Pl Sp Dp

Figure S3. Changes in physicochemical parameters in Cd solution during the accumulation process on moss gametophytes: a) its concentration, b) conductivity, c) pH

a)



b)



c)

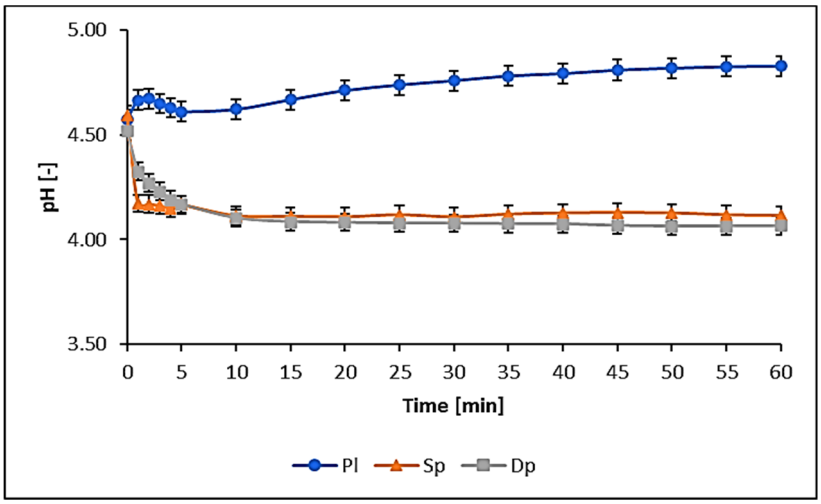


Figure S4. Changes in physicochemical parameters in Pb solution during the accumulation process on moss gametophytes: a) its concentration, b) conductivity, c) pH

Table S1. Concentrations of metals naturally accumulated in the mosses used for the experiments [mg/g d.w.]

Biomonitor	Ni	Cu	Zn	Cd	Pb
P. schreberi	< 0.001	< 0.001	0.04	< 0.0003	0.003
S. fallax	< 0.001	< 0.001	0.03	< 0.0003	< 0.002
D. polysetum	< 0.001	< 0.001	0.04	< 0.0003	< 0.002

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RESEARCH ARTICLE

The influence of preparation methodology on the concentrations of heavy metals in *Pleurozium schreberi* moss samples prior to use in active biomonitoring studies

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Abstract

Active biomonitoring is used to assess environmental pollution of elements such as heavy metals by indicator species such as mosses. They are used, among others, in urbanized areas where no indicator species are found. In such study areas, mosses collected from sites considered to be ecologically clean shall be exposed. In this context, it is very important to prepare the mosses properly before the exposure, so that the information received about the condition of the environment is reliable. In 2018, studies were conducted in the forested areas of southern Poland—in Opolskie Province. *Pleurozium schreberi* mosses were used in these studies. Atomic absorption spectrometry with flame atomiser (F-AAS) was used to determine the concentrations of Mn, Fe, Ni, Cu, Zn and Pb present. The aim was to study the influence of preparation methodology on *Pleurozium schreberi* moss samples prior to use in active biomonitoring studies. Four different methodologies were tested across four different sample locations (with varying levels of pollution). The results of the research were analysed and the coefficient of variation (*CV*) was determined. The value of the *CV* is influenced, among other things, by the location of the particular sample and the level of pollution by, for example heavy metals, in the moss. The research conducted proves that of the four methods used to prepare mosses for later exposure in active biomonitoring, the best method is averaging with simultaneous conditioning of mosses in demineralised water. This treatment causes the *CV* coefficient to fall below 10% for most of the metals determined in the moss samples. It has also been shown that maintaining moss collection methodology in accordance with ICP Vegetation standards (open/wooded area—tree canopy) also has a significant impact on the result obtained. Statistical analysis confirmed (Wilcoxon test) that the method of processing the mosses significantly influenced the results obtained. Thanks to the appropriate preparation of the mosses before exposition, they can be used in active biomonitoring of, for example, urban areas.

Keywords Mosses · Biomonitoring · Heavy metals · Coefficient of variation · Research methodology · Air pollution

Introduction

Various plant and animal species have been used in studies on monitoring air, water or soil quality (de Oliveira et al. 2016; Oishi 2018). Mosses are commonly used biomonitors to assess atmospheric aerosols (Kosior et al. 2010; Korzeniowska and Panek 2012). They are regarded as one of the main bioindicators of air pollution (Salo and Mäkinen 2014). Mosses do not have a root system and absorb nutrients and pollutants through their surfaces as a result of wet or dry deposition (Ares et al. 2012). For example, ectohydric moss *Pleurozium schreberi* has been used extensively throughout the European Union (Lequy et al. 2017; Nickel and Schröder 2017), including in Poland (Olszowski et al. 2012; Rajfur et al. 2018).

Several aspects need to be considered when analysing biomonitoring studies using mosses. One may draw different conclusions, depending on the type of study (active or passive biomonitoring) and the method of preparation.

Passive biomonitoring is a useful method for air quality assessment, including that of concentrations of heavy metals in the atmosphere. The technique enables the qualitative comparison of polluted and pollution-free areas in order to identify precisely the sources and scale of atmospheric aerosol pollution

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(Fernández et al. 2015). Passive biomonitoring studies are not fully standardized; there is still the question of the mass of a given sample and the number of subsamples. In the case of passive biomonitoring, the problem of measurement uncertainty occurs quite frequently and is related to the applied protocol/method for preparing samples for analysis (Fernández et al. 2015; Aboal et al. 2017). Examples of pollution monitoring studies of heavy metals or radionuclides indicate the heterogeneity of samples and, therefore, the inability to compare them using, for example, two moss species (Krmar et al. 2013; Boquete et al. 2014). The authors recommend the use only of the green parts of mosses in this type of biomonitoring (Dmuchowski et al. 2011; Boquete et al. 2014). A comparison of various bioindication methods showed that *P. schreberi* moss delivered distinctly different results from other mosses, and this method did not offer the highest correlation coefficient compared with other methods (Dmuchowski et al. 2011). A comparison of two biomonitoring methods (active and passive) proves that the former offers more advantages due to the standardization of the material (Fernández et al. 2000).

The second type of biomonitoring studies using mosses is active biomonitoring, the so-called moss bag method. It is very simple to carry out, reliable and financially acceptable due to the low cost of material acquisition (Debén et al. 2018). The method is used for the simultaneous monitoring of concentrations of many environmentally importance analytes. Active monitoring is commonly used throughout Europe to assess pollution associated with heavy metals, polycyclic aromatic hydrocarbons (PAHs) or other organic pollutants (Aničić et al. 2009; Vuković et al. 2015a; Kosior et al. 2017). The method is used both in urban and industrial areas (Capozzi et al. 2016). However, the implementation of such studies requires the systematization of experiment procedure. All studies which use the moss bag method emphasize the element of the standardization of the research protocols and procedures for preparing moss samples prior to exposure in the field (Iodice et al. 2016; Salo et al. 2016; Arndt and Planer-Friedrich 2018).

Preparation of the material most often includes collecting mosses from relatively clean areas and removing all impurities (soil, leaves, needles, etc.) (Vuković et al. 2015a; Di Palma et al. 2017), as well as rinsing with water prior to exposition in bags/sachets (Giordano et al. 2013; Varela et al. 2016). This is done in order better to clean the material and remove any plant remains or soil particles (Ares et al. 2014), but mainly in order to maintain the properly low initial level of trace elements in the mosses prior to exposition. The study results confirm the importance of the washing procedure prior to the use of the material in this field of study, because the analysis of the washing solutions demonstrates that the concentration of the analytes decreases considerably (Calabrese et al. 2015). The study results indicate a higher content of heavy metals in the 'wet' moss bags after exposition compared with those that were not conditioned in water. The research methodology often includes mixing samples prior to direct exposition, in order to obtain homogeneous material (Ares et al. 2015; Vuković et al. 2015b).

Based on literature sources, a study into the influence of the method of preparing biological material on the distribution of heavy metal concentration in mosses was conducted.

The objective of the research was to assess the biological material homogeneity, based on the method of preparation, for subsequent exposition within active biomonitoring of, for example, urbanized city areas. The data obtained enabled the establishment of standardized procedures for preparing mosses for exposition.

Materials and methods

Materials

Pleurozium schreberi mosses were used in the research. This is a species commonly found in Europe, including in Poland, which is used as an air quality bioindicator and also in active biomonitoring (Viskari et al. 1997; Suoranta et al. 2016; Boquete et al. 2017; Mahapatra et al. 2019). Mosses taken from the natural environment have been used in the study because, thus far, no methodology has been developed and no *Pleurozium schreberi* clones have been grown (the authors of the publication make such attempts). Mosses were collected in the Niemodlinskie Forest in Proszkow Forest Region in Poland's Opolskie Province. Samples of mosses were collected in four locations, marked A, B, C and D (Table 1; Fig. 1). The availability of unpolluted anthropogenic sites from which mosses can be collected without endangering the native population is limited. For this purpose, specimens taken from sampling points with different levels of heavy metal contamination were used for comparative testing.

The material collection was carried out along a total length of approximately 1 km—from an area adjacent to the A4 motorway to the last point, D (Table 1). The described areas is under the administration of Proszkow Forest Region—Niemodlinskie Forest (Fig. 1a) and has a majority of coniferous trees.

Moss samples were collected from an area of 1 m × 1 m at each point. Within the 1 m^2 (Fig. 1b), 12 points were marked from which mosses were collected for study (Fig. 1c). Collection of the material was carried out in spring (April) 2018. The collected moss samples were taken to a laboratory and dried at room temperature until dry mass (d.m.) was obtained. Next, the green part of the gametophyte, live and active tissues (Boquete et al. 2014), was separated from the moss to be used in the research (Jiang et al. 2018; ICP Vegetation 2020). Next, the mosses were prepared according to the methods described in Table 2.

Table 1 Measuring points

Point	GPS track	Description
A	50° 35′ 14″ N 17° 48′ 52″ E	Woodland adjacent to the A4 motorway (100 m from motorway)
B	50° 35′ 09″ N 17° 48′ 49″ E	Location near a forest road (300 m from motorway)
C	50° 34′ 53″ N 17° 48′ 31″ E	Location selected at random in deep woodland (900 m from motorway)
D	50° 34′ 53″ N 17° 48′ 22″ E	Open area selected as a reference point, in line with the recommendations of ICP Vegetation (ICP Vegetation 2020) (1 km from motorway)

Fig. 1 Locations of measuring points (**a**) with image one of the study surface (**b**) and 12 points marked in the surface area (**c**)

Table 2 Methodology of moss sample preparation

Method	Description
1	Removal of adhered materials/impurities (e.g. leaves, needles, soil)
2	As above + conditioning mosses in demineralised water (each of 12 samples with a mass of 0.6 g d.m. was conditioned and left in 1 l of demineralised water with conductivity of $\kappa = 0.5$ μS/cm for 1 h)
3	As in method 1 + averaging (manually mixing up the 12 moss samples taken from one measuring point and then separating them again)
4	As above + conditioning in 12 L of demineralised water with conductivity of $\kappa = 0.5$ μS/cm for 1 h

Methods

Mosses from measurement points A, B and C were prepared by four different methods. Table 2 presents the assumptions of the methods of moss preparation prior to determining the concentrations of heavy metals. For each method, 12 moss samples ($n = 12$) were taken. A total of 144 samples were analysed from points A, B and C. Since measurement points C and D were only 100 m apart and were less contaminated with heavy metals, the mosses taken from point D were prepared only by method 1 (12 samples). In total, 156 moss samples were prepared and analysed.

Before mineralisation, the moss samples were dried at room temperature until dry mass was obtained. Each moss sample with a mass of 0.400 ± 0.001 g d.m. was prepared in this way and mineralised in a mixture of nitric acid (V) and hydrogen peroxide (HNO_3 65%:H_2O_2 37% = 5:3) using a Speedwave Four Berghof DE microwave oven. The mineralisation process was carried out at a temperature of 180 °C.

The concentrations of heavy metals (Mn, Fe, Ni, Cu, Zn and Pb) were determined using an atomic absorption flame spectrometer (F-AAS) type iCE 3500 (series 3000) made by Thermo Scientific, USA.

Quality control

Table 3 presents the instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) of the iCE 3500 spectrometer. The results were converted for 1 kg of sample. The calibration of the spectrometer was performed with a standard solution from ANALYTIKA Ltd. (CZ). The values of the highest concentrations of the models used for calibration (7.5 mg/dm^3 for Mn, 10 mg/dm^3 for Fe, 5 mg/dm^3 for Ni, Cu, Zn, Pb) were approved as linear limits to signal dependence on concentration. The concentrations of metals were determined in solution after mineralisation and dilution and filtration into 25-cm^3 volumetric flasks.

Table 3 The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the iCE 3500 (mg/dm^3) spectrometer (Thermo Fisher Scientific Inc. 2011)

Metal	*IDL*	*IQL*
Mn	0.0016	0.020
Fe	0.0043	0.050
Ni	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Pb	0.0130	0.070

Table 4 shows the concentrations of heavy metals in certified reference materials BCR-482 *lichen*, produced at the Institute for Reference Materials and Measurements, Belgium.

Table 5 presents the method detection limit (*MDL*) and the method quantification limit (*MQL*) of the AAS analytical method, determined on the basis of the results of metal determination in mineralised moss samples, so that the concentration of metal values (C_M) would be comparable with the *IQL* values. The *IQL* value for each of the quantified metals was accepted as the expected detection limit of the analytical method. Twelve determinations were carried out, one for each metal, in line with the analytical procedure. *MDL* and *MQL* values were determined based on own research presented in this article and collected in the following table.

In order to assess the relative differentiation of the results of the concentration levels (mg/kg d.m.) of analytes in the mosses collected from the studied area, the *CV* was determined, which refers the value of standard deviation *s* (absolute differentiation of the feature distribution) to the mean value of x_{av} (Konieczka and Namieśnik 2018):

$$CV = s/x_{av} \cdot 100\ (\%) \quad (1)$$

The results were interpreted based on this coefficient, which is frequently used in analysis of biomonitoring studies ((Fernández et al. 2015; Dołęgowska 2016; Zhou et al. 2017).

The Lilliefors modifications of the Kolmogorov-Smirnov test failed to prove a hypothesis about data normality (Zar 2010). Therefore, differences between the preparation methods in terms of concentrations of elements in the mosses were evaluated by the Wilcoxon test. All calculations were carried out using Statistica 13 software (StatSoft Inc 2017).

Results and discussion

Figure 2 a–f show an analysis of the concentrations of elements in mosses, depending on the method applied, and regardless of the area type from which the mosses were collected.

Table 4 Comparison of measured and certified concentrations in BCR-482 *lichen*

Metal	BCR-482 *lichen*		AAS ($n = 5$)		*Dev.***
	Concentration (mg/kg d.m.)	Measurement uncertainty	Average	± *SD** of the concentrations	(%)
Mn	33.0	0.50	31.7	0.68	− 3.90
Fe	804	160	771	154	− 4.10
Ni	2.47	0.07	2.16	0.32	− 13.0
Cu	7.03	0.19	6.63	0.17	− 5.70
Zn	100.6	2.20	95.1	2.30	− 5.50
Pb	40.9	1.40	38.2	1.00	− 6.60

*Standard deviation

**Relative difference between the measured (c_z) and certified (c_c) concentration $100\% \cdot (c_z - c_c)/c_c$

On the basis of the results of the Wilcoxon test, it can be stated that for manganese the preparation method does not significantly influence changes in concentration distribution of this analyte. In the case of iron, considerable differences can be noticed between concentration distribution for method 1, compared with methods 2, 3 and 4. This was confirmed by statistical calculations using the Wilcoxon test (Table 6). The data presented in Table 6 confirms that appropriate preparation of the material influences the quality of the result, where statistically significant differences between methods occur. In the case of copper, it can be observed that the lowest fluctuation of results was obtained when the moss was prepared using methods 3 and 4. Zinc is a similar case where, for methods 2, 3 and 4, considerable differences can be observed between the concentration distribution of the element, compared with method 1. Similarly, as above, this is reflected in the statistical data collected in Table 6. In the case of lead, there are considerable differences between the first (simplest) method of sample preparation and the relatively small distribution of data in method 4.

The mosses collected from area C had the highest average for manganese and iron. This effect may be caused by the addition of more of these elements to mosses due to the rainfall under tree crowns and leaching from tree trunks (Krawczyk et al. 2009). Cadmium was not determined in any preparation method—it was below the quantification limits of the applied analytical method. The distribution of lead may be influenced by the polluting source as, for example, in area A—traffic (Singh et al. 2017), and its concentration in method 4, and the statistically relevant difference is the result of the appropriate preparation and homogenisation of the material, which enabled its determination (Fernández et al. 2015).

Table 5 Parameters of the heavy metal determination method in moss samples (mg/dm^3)

Metal	Mn	Fe	Ni	Cu	Zn	Pb
MDL	0.891	1.00	0.057	0.015	0.120	0.026
MQL	2.67	3.01	0.172	0.044	0.359	0.079

The coefficient of variation values was determined in the next stage of the results analysis. A comparison was carried out of the difference in heavy metals concentration values determined in the samples of moss, prepared for analysis under the four proposed methods (Table 7).

On the basis of the research undertaken, it should be stated that the material averaging and conditioning method (method 4) proved to be the best method for preparing moss samples prior to exposition in future active biomonitoring. This is because mixing different samples of moss (mixing subsamples collected in sampling site) produces more averaged material. It has been stated that the heterogeneity of the material is caused mainly by the sediment of various sized dust particles on the surface of the mosses, which influences the variability of samples as far as the heavy metal pollution level is concerned (Aboal et al. 2008). By conditioning samples in demineralised water, we remove dust particles from the surface and eliminate the influence of soil particles on the content of certain elements in mosses (Fernández et al. 2010), thanks to which we receive a purer sample (without ions of elements in ion-exchange centres) ready for exposition in, for example, urbanized areas. This is of key importance because improperly prepared moss samples lead to false results, wrong interpretations and therefore inappropriate conclusions.

Further methodological studies looked at the influence of the area from which the sample is collected on the level of heavy metals pollution. The conclusion was based on the assumptions and recommendations of the ICP Vegetation programme, which presents, among other things, the method and location from which moss samples should be collected for the purpose of research. While focusing on these recommendations, an attempt was made to analyse within our own study, how material averaging changes between mosses collected from an open area without trees in comparison with samples collected from area C—deep in the woods, under trees.

Comparing the value of the coefficient of variation for the biological material prepared for analysis in line with method 1 for the two areas, it should be stated as follows: the *CV* was lower for area D than for area C (Table 8). However, it should

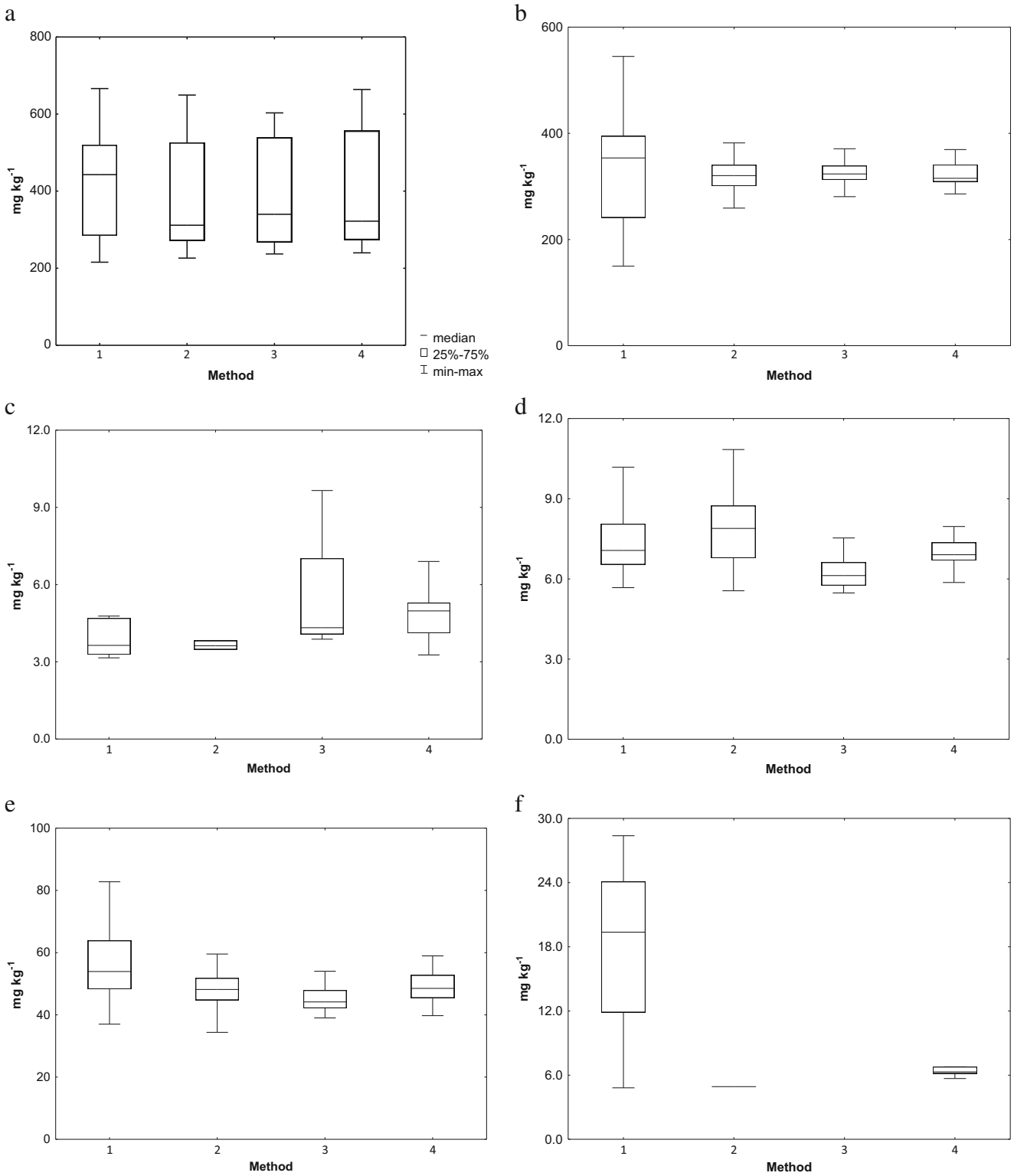

Fig. 2 Comparison of heavy metal concentrations. **a** Mn, **b** Fe, **c** Ni, **d** Cu, **e** Zn, and **f** Pb in moss samples depending on method

be emphasized that, for the samples collected from area D, it was also possible to determine the *CV* for lead (based on the determined concentration). In addition, it was only possible to determine the concentration of Ni and calculate its coefficient of variation from the samples collected from area C. These results confirm the assumptions of the ICP Vegetation programme, as regards the method of sample collection, recommending open spaces, preferably without trees.

Table 6 The results of the Wilcoxon test for heavy metals depending on method

v1	v2	*W*	*p*
Mn1	Mn2	290.000	0.509
Mn1	Mn3	277.000	0.388
Mn1	Mn4	260.000	0.258
Fe1	Fe2	659.000	***
Fe1	Fe3	618.000	***
Fe1	Fe4	633.000	***
Ni1	Ni4	89.000	0.235
Cu1	Cu2	269.000	0.323
Cu1	Cu3	560.000	***
Cu1	Cu4	494.500	*
Zn1	Zn2	521.000	**
Zn1	Zn3	608.000	***
Zn1	Zn4	465.000	*
Pb1	Pb4	44.000	**

v1, v2—value 1 and value 2; *W*—test statistical value/the sum of the signed ranks; *p* value—probability value/significant level alpha; Mn1—method 1 for Mn; Mn2—method 2 for Mn; Mn3—method 3 for Mn; Mn4—method 4 for Mn

* < 0.05; ** < 0.01; *** < 0.001

Table 7 Results of the coefficient of variation from areas A, B and C (%)

Metal	1	2	3	4
A area—method				
Mn	16.1	13.7	*5.43*	5.84
Fe	17.3	9.72	*3.99*	5.99
Ni	4.71	4.65	-*	24.1
Cu	5.35	18.0	7.41	*4.36*
Zn	7.59	7.77	6.52	*4.57*
Pb	-*	-*	-*	*8.76*
B area				
Mn	10.6	8.31	*5.38*	5.84
Fe	8.44	7.57	3.68	*2.86*
Ni	54.6	-*	-*	*14.6*
Cu	40.0	15.3	50.3	*6.34*
Zn	10.9	14.4	7.96	*7.38*
Pb	-*	-*	-*	*20.0*
C area				
Mn	9.37	9.02	*4.91*	7.70
Fe	11.5	9.75	*4.24*	5.63
Ni	44.4	-*	*6.28*	29.8
Cu	35.1	22.2	9.11	*7.77*
Zn	25.8	16.4	8.86	*7.11*
Pb	-*	-*	-*	*51.6*

Italicized values mean the lowest value

*Below the limit of quantification (mg/kg d.m)

Table 8 A comparison of *CV* for mosses collected from areas C and D and prepared for analysis in accordance with method 1 (%)

Metal	C area	D area
Mn	9.37	*6.02*
Fe	11.5	*7.59*
Ni	44.4	-*
Cu	35.1	*6.24*
Zn	25.8	*15.4*
Pb	-*	54.3

Italicized values mean the lowest value

*Below the limit of quantification (mg/kg d.m)

Therefore, it is important to select carefully the locations for sampling material for research. As illustrated in Table 8 above, it later turns out to be of key importance for the quality of the result obtained. This is also important because many publications do not include a detailed description of the sample-taking location, or samples are collected from inappropriate locations, without using any of the protocols or recommendations that have been set in moss sample-taking methodology.

Conclusions

Biomonitoring offers a cheap and simple supplement to the classical methods for assessing environmental quality and condition. However, for biomonitoring to be comparable and of equal quality to these methods, it is very important to prepare material properly prior to exposition, particularly when conducting active biomonitoring. This information is often missing from the scientific literature, or the descriptions provided in the sections on materials and methods are superficial.

This study achieved its goal by showing that the obtained result and the range of variation of biological material are influenced not only by the proper selection of the location for moss-sampling but also by the processing method used prior to exposition in, for example, urbanized areas. The study describes four methods of preparing moss for exposition. The results of the laboratory research undertaken indicate that, of the four applied methods, the best one is averaging with simultaneous conditioning of mosses in demineralised water. This procedure results in the decrease of *CV* to below 10% for most metals determined in moss samples. The analysis of the results with the Wilcoxon test confirmed statistically significant differences between the methods used for most metals and showed that appropriate moss preparations increase the homogeneity of samples.

It was also determined that applying moss sample collection methodology in line with ICP Vegetation (open area/trees, crowns) has a significant influence on the result obtained.

Authors' contributions PŚ: conceptualisation, formal analysis, investigation, methodology, resources, visualisation, writing—original draft.
GK: formal analysis, validation, writing—review and editing.
MR: methodology, project administration, resources, supervision, validation, writing—review and editing.

Data availability All data generated and analysed during this study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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Environmental Toxicology

Comparison of Exposure Techniques and Vitality Assessment of Mosses in Active Biomonitoring for Their Suitability in Assessing Heavy Metal Pollution in Atmospheric Aerosol

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Abstract: The most widespread and used technique is the moss-bag method in active biomonitoring of air pollution using mosses. In the literature, we can find various studies on the standardization of this method, including attempts to standardize treatments and preparation procedures for their universal application. Few works comprehensively focus on other methods or compare other techniques used in active biomonitoring with mosses, especially including measurements of their vital parameters. Our experiment aimed to assess air pollution by selected heavy metals (Cu, Zn, Cd, Pb, Mn, Fe, and Hg) using three moss species (*Pleurozium schreberi*, *Sphagnum fallax*, and *Dicranum polysetum*) during a 12-week exposure in an urban area. Mosses were exposed simultaneously using four techniques: moss bag in three variants (exposed to air for total deposition of heavy metals, exposed to air for only dry deposition, and sheltered from the wind) and transplants in boxes. Increases in heavy metal concentrations in mosses were determined using the relative accumulation factor (RAF). The actual quantum yield of photosystem II photochemical was also analyzed as the main vitality parameter. The results indicate that all moss species during the changing environmental conditions survived and retained their vitality, although it decreased by >50% during the exposure. The best biomonitor was the moss *P. schreberi*, whose RAF increments were the highest throughout the study period for the majority of elements. The moss-bag technique had a statistically significant effect (almost 40%) on the concentration value of a given metal for a certain species, and thus it is the most recommended technique that can be applied in air quality monitoring in urban areas. *Environ Toxicol Chem* 2022;41:1429–1438. © 2022 SETAC

Keywords: Air pollution; Biomonitoring; Heavy metals; Living biomonitor; Moss-bag technique

INTRODUCTION

Goodman and Roberts carried out the first study of air pollution with mosses using the moss-bag active biomonitoring technique, which was slightly modified later by Little and has been used and revised to standardize any treatments associated with it (Ares et al., 2012). This method is competitive with instrumental measurements because of its low cost, simplicity, and uncomplicated sample preparation, possibility to control exposure time and potential for a highly resolved spatial resolution of biomonitoring networks (Morales-Casa et al., 2019; Turgut et al., 2019). After 50 years of use, this method is in continuous optimization (De Nicola et al., 2013; Rogova et al., 2018), which prevents its use as a regular environmental monitoring tool by official organizations (Aničić Urošević & Milićević, 2020). It is mainly applied in urban areas, where mosses acting as biomonitors do not occur naturally and, compared to the passive method, in general, active biomonitoring methods are characterized by better repeatability of determinations—a low coefficient of variation in relation to samples collected at a given site or better standardization of material (Fernández & Carballeira, 2000; Varela et al., 2010). Nevertheless, the moss-bag method has to take into account many components (such as moss species; time, place, or height of exposure; design of moss-bag shape) that need to be continuously standardized; and many of these components have been brought to unification, and others are still challenging (Ares et al., 2012; Sorrentino et al., 2021). There are also other methods of moss exposure used in active biomonitoring (Govindapyari et al., 2010).

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One variation of the classic moss-bag technique is the canopy variant. It involves covering with plastic pots to prevent moss bags from being exposed to wet deposition (Sun et al., 2009). It is used to distinguish and prioritize accumulation pathways of a given metal (Tan et al., 2000) and to determine the degree and type of contamination, mainly in industrial areas (De Agostini et al., 2020; Morales-Casa et al., 2019). Nowadays, it is rarely applied, knowing that the pollutants accumulated by mosses are the sum of both dry and wet deposition (Schröder & Nickel, 2019; Xiao & Liu, 2011).

Another way is the transplantation of mosses together with their substrate in plastic boxes (Jóźwiak & Rybiński, 2013). The concept of transplantation (or plant translocation) also refers to the transfer of bryophytes to colonize new areas, taking into account, for example, climatic factors (Merinero et al., 2020); but in biomonitoring, it is about moving mosses with their natural substrate from an unpolluted site to an exposed area where they can become biomonitors for air pollution (Suoranta et al., 2016).

Various comparisons can be found in the literature regarding the different methods, approaches, or techniques of biomonitoring using mosses (Capozzi et al., 2017; Salo et al., 2016). Nevertheless, in these works, no answer is given as to which technique is the best because this is influenced by many factors (including environmental), and each work has a different reference point (Iodice et al., 2016; Kosior et al., 2018). The vitality of mosses is almost completely ignored in these works (Capozzi et al., 2020; Tretiach et al., 2007). According to the definition of biomonitoring, it uses whole or parts of living organisms that serve as a biomonitor of environmental quality and not just as a chemical adsorbent (Boquete et al., 2017; Markert & Wünschmann, 2011). Meanwhile, many works use devitalized material (Debén et al., 2016; Di Palma et al., 2017; Morales-Casa et al., 2019), justifying this by the excellent sorption properties of the samples (Ares, Aboal, et al., 2015), but as it turns out not for all types of pollutants (Varela et al., 2016). It has also been shown that there are differences between dried and kept alive (hydroponic) mosses in sorption of pollutants (Szczepaniak et al., 2007). So it is necessary to focus on the use of a living organism to talk about authentic biomonitoring studies using moss as a biomonitor (Fernández et al., 2009). The first studies conducted with *Sphagnum palustre* confirm the validity of using live mosses because of their better indication properties compared to dry moss (Astel et al., 2008). Measurements of analyte concentrations in the air provide information on the emission of pollutants into the atmosphere but do not indicate the effects of these substances on living organisms (Long et al., 2008). Hence, the need to monitor the biomonitor's vital functions during the study has a crucial meaning (Świsłowski, Nowak, & Rajfur, 2021a).

The aim of the present study was a comparative evaluation of four methods of moss exposure in active biomonitoring in an urban area, based on measurements of concentrations of selected elements. At the same time, our goal was to test moss vitality parameters (chlorophyll fluorescence), which so far was marginally performed in this type of research but is very important given the previously mentioned definition of biomonitoring and the biomonitor (moss) of environmental quality (Świsłowski, Nowak, & Rajfur, 2021b).

Two research hypotheses were verified: (1) of the four tested methods for moss exposure, the moss-bag technique is the optimal method in the assessment of atmospheric aerosol heavy metal contamination within the framework of active moss biomonitoring, and (2) gametophytes of the moss *Pleurozium schreberi* exposed in urban areas by the moss-bag technique have the highest actual photochemical yield of photosystem II (PSII), which indicates their best vitality in relation to the other moss species analyzed.

MATERIALS AND METHODS

Materials

The moss species used for the present study were *P. schreberi*, *Sphagnum fallax*, and *Dicranum polysetum*. They were collected in October 2020 from forests in Świętokrzyskie Province, Poland. The mosses came from the Puszcza Świętokrzyska mesoregion, Stąporków Forest District, near Stary Janów and Błotnica villages.

Methods

Before exposure, moss samples were pretreated, following the guidelines of ICP Vegetation (2020). Before exposure, the mosses were conditioned in demineralized water (Świsłowski, Kosior, & Rajfur, 2021). Subsequently, 3 g of mosses (24 samples per month, method, and species) were packed into mesh bags and exposed at a height of approximately 1.5–2 m from ground level. The first method of exposure was the most commonly used moss-bag method; the second was a variant with a cover, protecting against wet deposition; and in the third, the bags with mosses were shielded all around, protecting them from the wind. As a fourth way we established two transplant boxes of each species for a period of 3 months (16 October 2020–16 January 2021). Mosses were exposed in the downtown of Opole next to the buildings of the University of Opole (50.6709 N, 17.9220 E, Opole Province, Poland). Each month, samples were collected and heavy metal concentrations and chlorophyll fluorescence measured. A detailed description of the exposure methods along with their figures are presented in Supporting Information, Table S1 and Figure S1.

To determine the heavy metal content, the moss samples, 1 g dry mass each, were mineralized in a mixture of nitric acid (V) 68% (POCH) pure for analysis (p.a.), and hydrogen peroxide 32% (p.a.; POCH) using a Speedwave Four Berghof, DE microwave oven. The mineralization process was carried out at a temperature of 180 °C. Heavy metals were determined using an atomic absorption flame spectrometer type iCE 3500 (series 3000; Thermo Scientific). Concentrations of metals were determined in solution after mineralization and dilution, and solutions were filtered into volumetric flasks of 25 cm^3. The results were converted into 1 kg of sample. Calibration of the spectrometer was performed with a standard solution from ANALYTIKA. The values of the highest concentrations of the

models used for calibration (2 mg/dm^3 for cadmium; 5 mg/dm^3 for copper, zinc, and lead; 7.5 mg/dm^3 for manganese; and 10 mg/dm^3 for iron) were approved as linear limits to signal dependence on concentration. The concentration of mercury in the samples (0.04 g ± 0.001 g dry mass) was determined with AMA 254 mercury analyzers from Altec. The relative accumulation factor (RAF) was used to determine increases of concentrations of the analytes in the exposed mosses samples (Zinicovscaia et al., 2018).

Chlorophyll fluorescence of PSII was monitored using a modulated portable fluorometer (Opti-Sciences). Actual photochemical efficiency (yield) was measured under ambient light (Šraj Kržič & Gaberščik, 2005). Mosses were collected in the field (natural conditions), at noontime, after each month of exposure separately and transported to the laboratory. Measurements were made with 10 replicates—10 moss samples were taken (biological replicates) for each species and exposure method. During the measurements, relative humidity ranged from 24% to 36% and temperature from 20 °C to 25 °C.

Statistica (Ver 13.3), JASP 0.10.2, and Microsoft Excel 2016 software were used to process and present the data. Shapiro-Wilk's test was used to check data normality. Therefore, differences between the exposure methods in terms of element concentrations in the mosses were evaluated by the Student *t* test and Wilcoxon test. Analysis of variance (ANOVA) and multivariate ANOVA (MANOVA) were used to assess the influence of factors on contaminant concentrations in mosses (Tessier & Boisvert, 1999).

RESULTS

In the first stage of the experiment, the actual quantum yield of PSII was analyzed in relation to the time and method of exposure of mosses in the biomonitoring experiments carried out (Figure 1). Other chlorophyll fluorescence parameters are shown in Supporting Information, Table S2.

TABLE 1: Three-way analysis of variance of the effect of method, exposure time, and species on the statistical significance of *Y*(II) values

Effect for Y(II)	SS	*df*	MS	*F*	*p*
Intercept	11.6	1	11.6	1009	**<0.001**
M	2.96	3	0.987	85.6	**<0.001**
S	0.009	2	0.005	0.396	0.673
T	0.054	2	0.027	2.33	0.099
M × *S*	0.308	6	0.051	4.44	**<0.001**
M × *T*	1.41	6	0.236	20.4	**<0.001**
S × *T*	0.079	4	0.020	1.71	0.148
M × *S* × *T*	0.268	12	0.022	1.94	**0.030**
Error	3.53	306	0.012		

M = method; *S* = species; *T* = exposure time; SS, sum of squares of effects; MS = mean sum of squares of effects.
p values in bold are statistically significant.

As shown in Figure 1, the method of transplanting mosses with substrate was characterized by an actual quantum yield of PSII at an average level of 0.64–0.70 depending on the species. Subsequently, these values decreased to mean values of 0.13–0.25 after 3 months. For the other methods, the samples before exposure had half the actual quantum yield of PSII of the control samples from the box method, and the actual photochemical efficiency (yield) reached values of 0.14–0.20 in the third month of exposure. In the box method, for the species *P. schreberi*, *S. fallax*, and *D. polysetum*, the actual quantum yield of PSII values decreased by 73.8%, 59.4%, and 81.4%, respectively, in relation to the mean value for the control sample. On the other hand, for the other methods, the decreases were 55.6%, 49.5%, 65.8% for *P. schreberi*, *S. fallax*, and *D. polysetum*, respectively.

Table 1 presents a statistical analysis of the effect of selected factors on moss vitality.

The data in Table 1 confirm the statistical significance of the effect of the exposure method on the value of the actual quantum yield of PSII. Thus, the manner and form of exposure of the mosses affected the vitality of the mosses

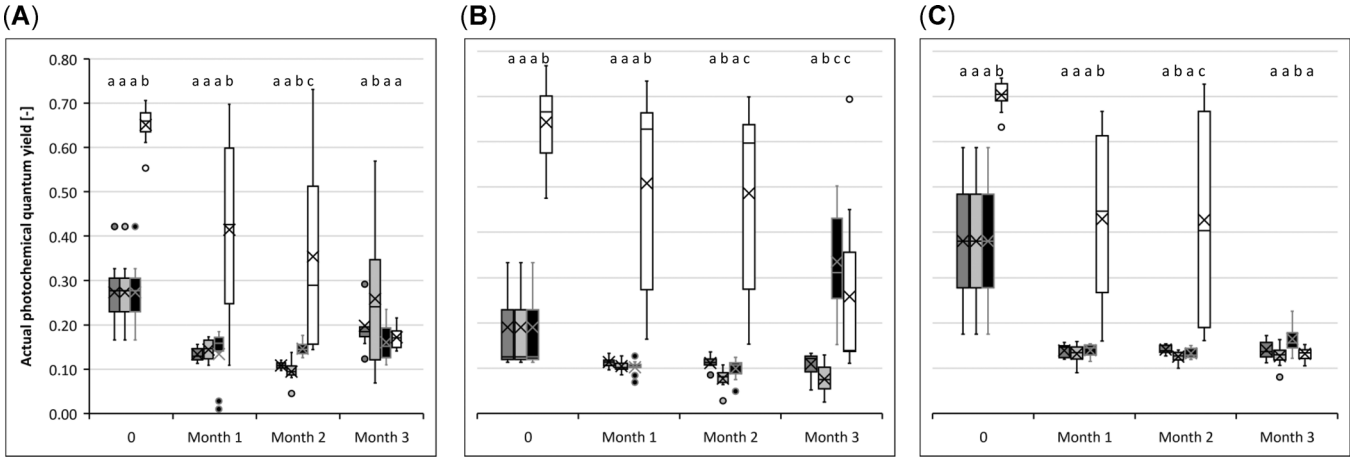

FIGURE 1: The actual photochemical efficiency (yield) of mosses: (**A**) *Pleurozium schreberi*, (**B**) *Sphagnum fallax*, and (**C**) *Dicranum polysetum*. Different letters mean statistical differences among methods in a given month of exposure ($p < 0.05$). Dark gray color is the moss-bag method, light gray is the method of covering to protect against wet deposition, black is the method of shielding to protect from wind, and white is the box method. The actual quantum efficiency of photosystem II (PSII) photochemistry in the light measures the fraction of the absorbed light energy that is actually being used to drive photochemistry at PSII (Henriques, 2009).

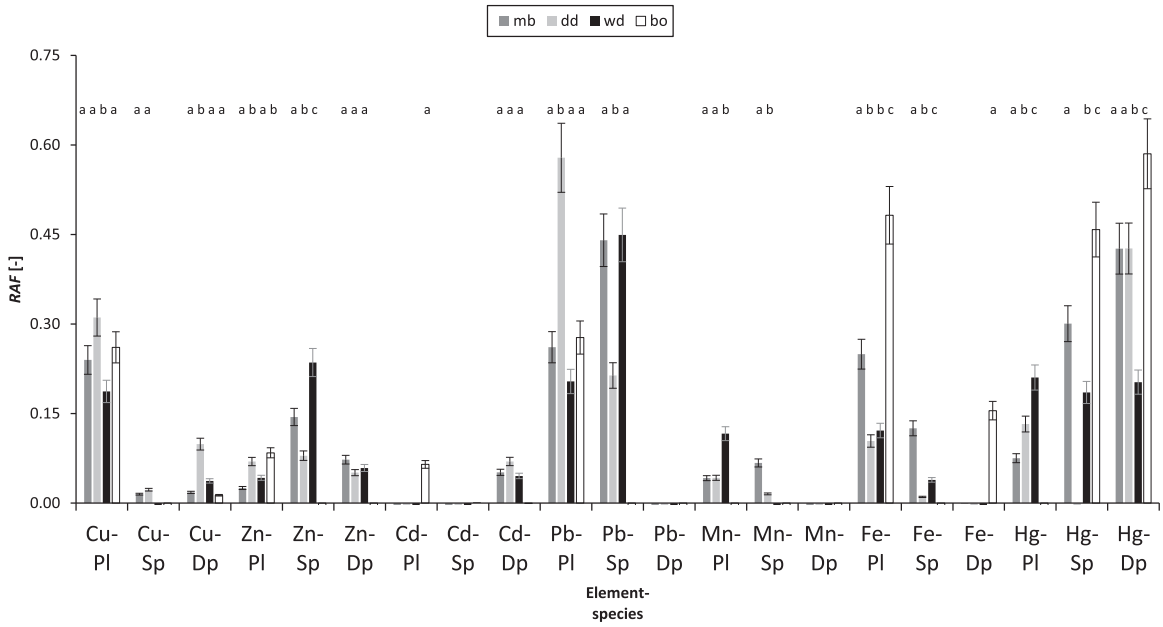

FIGURE 2: Relative accumulation factor values (±standard deviation) for mosses for the first month of exposure. Different letters mean statistical differences between methods for a given element in the following months of exposure ($p < 0.05$). RAF = relative accumulation factor; mb = moss bag; dd = covering to protect against wet deposition; wd = shielding to protect from wind; bo = box; Pl = *Pleurozium schreberi*; Sp = *Sphagnum fallax*; Dp = *Dicranum polysetum*.

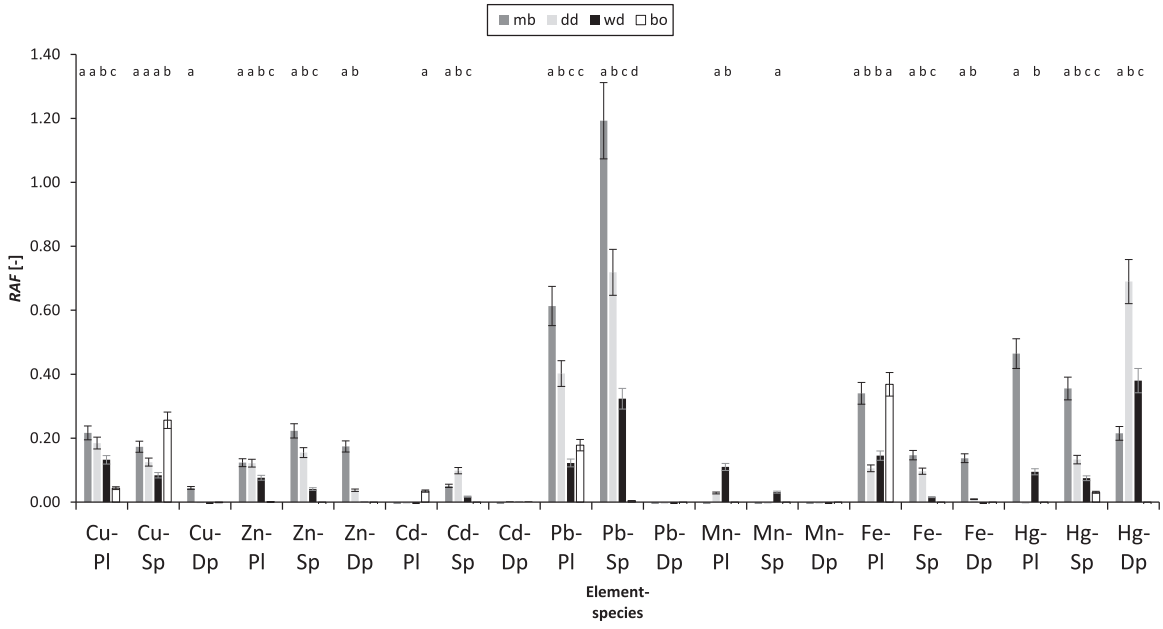

FIGURE 3: Relative accumulation factor values (±standard deviation) for mosses for the second month of exposure. Different letters mean statistical differences between methods for a given element in the following months of exposure ($p < 0.05$). RAF = relative accumulation factor; mb = moss bag; dd = covering to protect against wet deposition; wd = shielding to protect from wind; bo = box; Pl = *Pleurozium schreberi*; Sp = *Sphagnum fallax*; Dp = *Dicranum polysetum*.

during the present study. Considering species *S* and time *T* separately, it can be seen that they were not statistically significant. However, the interaction of these parameters (simultaneous effect of environmental factors) means that comparing simultaneously the method and time of exposure and a given species affects the value of photosynthetic yield of PSII.

Figures 2–4 show the RAF values calculated for the heavy metal concentrations determined in the mosses during the 12-week exposure.

The results shown in Figure 2 indicate increases in RAF in *P. schreberi* mosses throughout the experiment. Each period was characterized by an increment in at least one method, and increments for all methods for a given element over time were

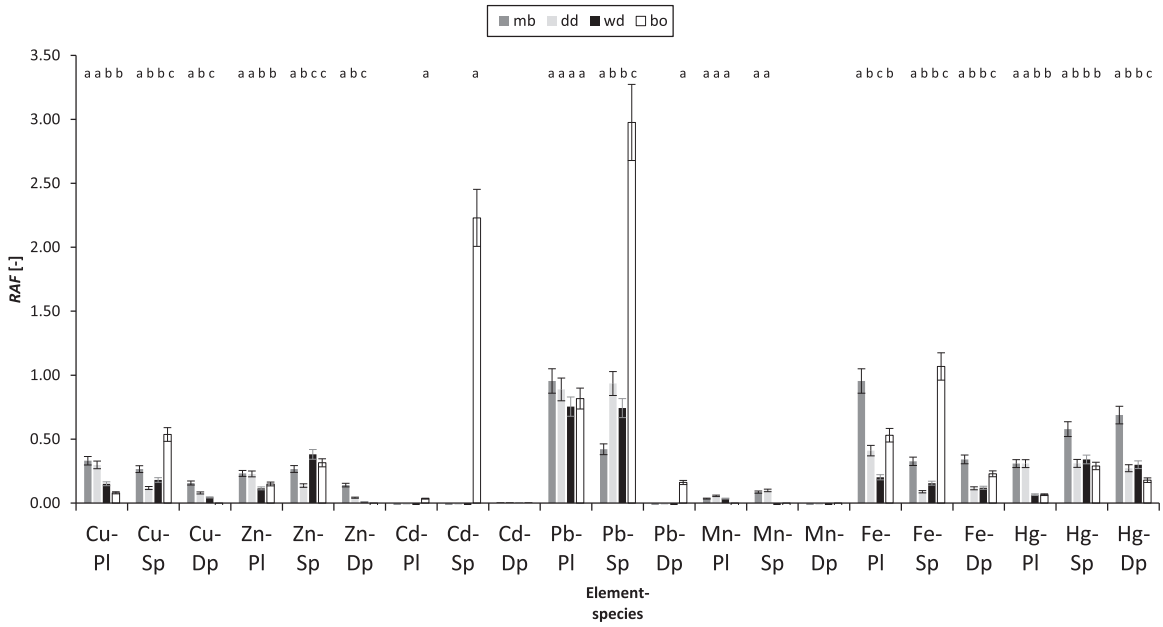

FIGURE 4: Relative accumulation factor values (±standard deviation) for mosses for the third month of exposure. Different letters mean statistical differences between methods for a given element in the following months of exposure ($p < 0.05$). RAF = relative accumulation factor; mb = moss bag; dd = covering to protect against wet deposition; wd = shielding to protect from wind; bo = box; Pl = *Pleurozium schreberi*; Sp = *Sphagnum fallax*; Dp = *Dicranum polysetum*.

recorded for copper, lead, and iron. The highest RAF values for most metals were recorded after 8 weeks and in the last month of exposure for the moss-bag method (copper, zinc, lead, iron, mercury). For *S. fallax*, after 2 months, the most significant contribution (in terms of RAF values) was recorded for the moss-bag and dry deposition methods for most of the elements analyzed. In the last month of exposure, high increases in cadmium and lead were recorded for the box method. In relation to the *P. schreberi* species, RAF values for *S. fallax* are lower. In Figure 4 the presented results show very low or no RAF increments in *D. polysetum* mosses. The exception is mercury, where RAF values are high throughout the study period from the beginning of the exposure. For the other elements (depending on the method) increments were recorded in the first and last months of exposure. Considering this, mosses by species (without differentiating between methods and exposure times) were ranked in order of decreasing RAF values: *P. schreberi* > *S. fallax* > *D. polysetum*.

Statistical evaluation by MANOVA revealed strong, statistically significant, simultaneous effects ($p < 0.001$) of method (*M*) and exposure time (*T*) and species (*S*) on the concentrations of all heavy metals. In turn, the results from the ANOVA indicated that, apart from lead ($p = 0.255$), the simultaneous consideration of the interaction of the three factors on each analyte separately has a statistically significant effect on the result obtained. The complete statistical analysis of each parameter (method, species, exposure time) per element is presented in Supporting Information, Tables S3 and S4.

Because of the statistical significance of the method of moss exposure to actual quantum yield of PSII and heavy metal concentrations, Table 2 compares the significance of the moss-bag method against the others for the species and metal.

As shown in Table 2, the statistical significance of analyte concentrations depends on both metal type and moss species (as presented earlier) when comparing given moss exposure methods. Collecting all the analyses of the Student *t* test and the Wilcoxon signed-rank test concerning this issue (see also Supporting Information, Tables S5–S11), it should be concluded that the moss-bag method has the most substantial effect relative to the other moss exposure techniques depending on the pollutant tested for particular species. Of all possible comparisons, the moss-bag method was statistically significant (the result was higher) in 39.7% of the cases compared to the other methods when comparing the concentration of a given element for a given species (see also Figures 2–4). For the other methods, the concentration result was higher (strength of its effect relative to the other exposure techniques) for the dry deposition and wind moss-bag variants and the box method in only 19.1%, 14.3%, and 11.1% of the cases, respectively.

DISCUSSION

The PSII actual photochemical quantum yield reflects the actual energy capture efficiency and the proportion of energy used during the photochemical reaction to the energy absorbed by the plant (He et al., 2020; Hückstädt et al., 2013). A number of stresses and factors influence its amount, such as an increase in metal concentration causing a decrease in vitality (Bellini et al., 2020), soil salinity during nitrogen fertilization (Song et al., 2019), or water availability and light intensity when co-occurring with other species (Nayaka & Saxena, 2014). In the investigated moss species, a decrease in actual quantum yield of PSII can be observed during their exposure, which was due

TABLE 2: Paired samples Student *t* test comparison of the moss-bag method with the other methods used[a]

M1		M2	*t*	*df*	*p*
Cu mb Pl	–	Cu dd Pl	−0.085	8	0.533
	–	Cu wd Pl	4.587	8	**<0.001**
	–	Cu bo Pl	2.358	8	**0.023**
Zn mb Pl	–	Zn dd Pl	−0.612	8	0.721
	–	Zn wd Pl	2.156	8	**0.032**
	–	Zn bo Pl	1.253	8	0.123
Pb mb Pl	–	Pb dd Pl	−0.075	8	0.529
	–	Pb wd Pl	1.134	8	0.145
	–	Pb bo Pl	0.427	8	0.340
Zn mb Sp	–	Zn dd Sp	2.507	8	**0.018**
	–	Zn wd Sp	−0.147	8	0.556
	–	Zn bo Sp	3.039	8	**0.008**
Mn mb Sp	–	Mn dd Sp	0.982	8	0.177
	–	Mn wd Sp	1.620	8	0.072
	–	Mn bo Sp	4.490	8	**0.001**
Hg mb Sp	–	Hg dd Sp	6.828	8	**<0.001**
	–	Hg wd Sp	5.405	8	**<0.001**
	–	Hg bo Sp	1.192	8	0.134
Cu mb Dp	–	Cu dd Dp	0.528	8	0.306
	–	Cu wd Dp	2.204	8	**0.029**
	–	Cu bo Dp	2.352	8	**0.023**
Zn mb Dp	–	Zn dd Dp	3.394	8	**0.005**
	–	Zn wd Dp	3.964	8	**0.002**
	–	Zn bo Dp	6.456	8	**<0.001**
Hg mb Dp	–	Hg dd Dp	−0.123	8	0.547
	–	Hg wd Dp	1.578	8	0.077
	–	Hg bo Dp	0.909	8	0.195

mb = moss bag; Pl = *Pleurozium schreberi*; Sp = *Sphagnum fallax*; Dp = *Dicranum polysetum*; dd = covered to protect against wet deposition; wd = shielded to protect from wind; bo = box.
[a]In all tests, the hypothesis is that Measurement 1 (M1) is greater than Measurement 2 (M2; M1 > M2); *p* values in bold are statistically significant.

to variable environmental conditions (including meteorological) and other factors (such as moss aging, drying, or freezing). However, in the case of mosses exposed in bags, actual photochemical quantum yield increment can be observed in the last observation period. This may have been positively influenced by hydration processes (measured during sample thawing and snowmelt) on fluorescence output (Yamakawa et al., 2018). For *Sphagnum* mosses, maximum PSII output measurements using chlorophyll fluorescence remained high until maximum water content was reduced and then decreased with further water content reduction (Van Gaalen et al., 2007). A similar variation of maximum chlorophyll fluorescence yield with water content was also reported for *Pleurozium* moss (Williams & Flanagan, 1998). Our obtained values of actual quantum yield of PSII photochemistry were comparable with the results obtained by the authors (Kangas et al., 2014) for *P. schreberi* and different species of mosses of the genus *Sphagnum*. However, the experiment was performed at a different time of the year, which may indicate the adaptation capabilities of these plants. In the present study, we measured the fluorescence of chlorophyll. The portable fluorometer can measure different parameters. These values were completed in Supporting Information, Table S2. We showed that *P. schreberi* mosses, compared to the other analyzed species, are characterized by the smallest decrease in the value of the actual photochemical yield PSII during a 3-month exposure in an urban area. This demonstrates their high resistance to environmental conditions relative to other species. At the same time, *P. schreberi* was the best biomonitor of atmospheric aerosol heavy metal pollution (Figures 2–4).

In biomonitoring of trace elements using mosses, comparisons usually concern different species and accumulation of metals by them within one transplantation method (Castello, 2007; Culicov & Yurukova, 2006). At the same time, attention is paid to the influence of environmental factors (Motyka et al., 2015), different orientations of moss bags (Dharmasiri & Deeyamulla, 2013), or the location of samples (Rogova et al., 2018) on the final result of elemental concentration in mosses. In passive biomonitoring, *D. polysetum* had the highest accumulation capacity (compared, e.g., to *P. schreberi*), which has ample rhizoid felt and dense structure of bunches that probably increases the retention of polluting components (Ryzhakova et al., 2014). The impossibility of comparing the results obtained by two different methods of monitoring atmospheric aerosol contamination was shown by Kosior et al. (2015). Nevertheless, species of the genus *Dicranum* are valuable biomonitors to evaluate total mercury levels (Davis et al., 2007); *D. polysetum* is sensitive to SO_2 concentrations (Dueck et al., 1992) and prefers undisturbed landscapes, or at least its cover decreases with increasing pollution and metal concentrations (Folkeson & Andersson-Bringmark, 1988). For the moss taxa *Dicranum* sp., using the moss-bag technique, the highest RAF values were recorded, with significant accumulation of heavy metals in this species influenced not only by its previously described structure but also by the exposure site (Demková et al., 2019). Values of RAF >1.00 indicate significant elemental enrichment (Vuković et al., 2017), so such values will be recorded in places particularly exposed to pollution, such as local industrial emissions or roads with heavy traffic (Aničić, Tomašević, et al., 2009; Vuković et al., 2015)—our location is not a heavily polluted urbanized area. Hence, the RAF values obtained are correspondingly lower. Other studies, on the other hand, confirm that *Pleurozium* moss, exposed by the moss-bag technique, in comparison with the other species analyzed, was characterized by the highest RAF increments, which indicates not only its metal accumulation capacity but also the much higher air pollution in the underground parking in relation to our location (Demková et al., 2018). The main factors influencing the concentration of heavy metals during moss exposure will be the species used, the test site characteristic, and the exposure method (Ares et al., 2012; Dmuchowski et al., 2011; Tabors et al., 2004).

In the studies presented earlier, only the accumulation potential of different moss species under one method of exposure was mentioned. However, comparisons of bulk and dry deposition are often found in the literature. The results of the authors of these studies indicate that it is not possible to correlate the two different methods (Boquete et al., 2020; Couto et al., 2004), and the concentration of pollutants in bulk and dust deposition depends on environmental conditions (Kosior et al., 2018). When comparing moss exposures under dry and wet deposition, dry deposition appears to be the more important pathway for accumulating metals from the air into mosses (Sun et al., 2009),

especially for mercury (Lodenius, 1998). Our experiment using these moss-bag and dry deposition methods demonstrates that the vast majority of high metal concentrations in mosses were for the moss-bag method rather than for the dry deposition moss-bag variant. This is also indicated by other studies, where uncovered moss bags had higher concentrations than covered ones; but also moss-bags were exposed to leaching by rain, as well as dust deposition from above and below, compared to covered mosses (Arndt et al., 2014). Moreover, it was practically irrelevant for mercury, although, as discussed earlier, *D. polysetum* had higher concentration increments than the other species, and its mercury accumulation potential would need to be tested more extensively in the future. In recent years, however, the moss-bag method has been standardized in active biomonitoring (Capozzi et al., 2016; Di Palma et al., 2016). The efficacy of a moss-bag technique (with *Sphagnum russowii* mosses) at detecting element deposition trends was determined, comparing these results with those of the National Acid Deposition Program/National Trends Network (Makholm & Mladenoff, 2005). When comparing different methods of moss exposure, other researchers also found (as we did) that the moss-bag method showed higher concentrations of the elements considered compared to other techniques (Ares, Varela, et al., 2015). However, the effectiveness of a given method is mainly dependent on the type of pollutant that is biomonitored (Varela et al., 2016) and the variant of its application (Aničić, Tasić, et al., 2009). The decrease of analytes in the moss bags after exposure indicated that the moss as living material in the bag might absorb and contain elements deposited from the air with the ability to exchange with elements in the atmospheric aerosol (Cao et al., 2009). The results of our study indicate that among the three versions of the moss-bag technique and the box method, the moss-bag method performs the best, which is the most frequently used method in the literature and was supported by the higher concentrations of heavy metals in mosses compared to the other methods (RAF values) and statistical analyses. Analyzing the data on the actual photochemical performance of PSII of three moss species exposed by four methods, we can conclude that mosses exposed by the moss-bag technique have the best parameters, indicating their viability. The statistical significance of the effect of exposure method on actual quantum yield of PSII is confirmed by the data in Table 1.

In perspective, it would be advisable to focus on integrating this method of active biomonitoring with instrumental measurements on the principle of searching for correlations of concentrations of analytes accumulated in mosses and on filters from dust collectors. Such research will help to integrate biomonitoring studies into state and local air quality monitoring procedures and best practices.

CONCLUSIONS

The vitality of mosses during air quality monitoring is influenced not only by environmental conditions and air pollutants but also by the way the plants are exposed. It is necessary to remember and constantly monitor their vitality parameters to fulfill the definition of biomonitoring and seek to standardize methods of air pollution assessment using living organisms. The presented results indicate that the contamination of atmospheric aerosol at the study site is low (in relation to literature reports), which makes the selected species sensitive monitors of air quality even in the case of low concentrations of analytes. *Pleurozium schreberi* proved to be the best among them (RAF values). The results also indicated that, among the four exposure techniques used, the moss-bag method proved to be the most effective (also taking into account the exposure time and the species used). The results positively verified the assumed research hypotheses, which allows further research development toward optimization of this technique and its application in urban areas.

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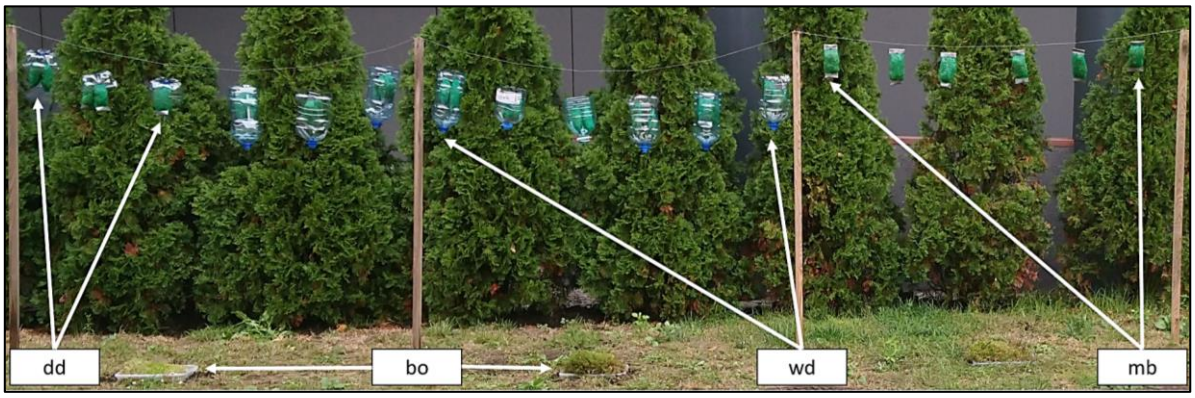
1 Table 1 SM. Methodology of moss exposure methods

Method	Number of samples	Exposure time	Sampling regime	Form of the exposition
Moss-bag technique (mb)	18	three months (16 October 2020 - 16 January 2021)	After each month of exposure, 6 bags were collected (2 bags per species) from which 3 samples for each species were prepared for chemical analyses in triplicate.	3 g of mosses (in one moss-bag) were exposed at a height of about 1.5-2 meters from ground level (without any cover).
Moss-bag technique with a cover (dd)	18			3 g of mosses (in one moss-bag) were exposed at a height of about 1.5-2 meters from ground level. Mosses had plastic cover over the top of the bag.
Moss-bag technique with shield protecting from the wind (wd)	18			3 g of mosses (in one moss-bag) were exposed at a height of about 1.5-2 meters from ground level Mosses were in a plastic tube containing a bag, uncovered at the top and bottom.
Mosses in boxes (bo)	2 boxes per species		After each month of exposure, for chemical analyses, 3 samples for each species were prepared in	Mosses were collected from the forest along with the surface soil layer and placed in boxes

		triplicate.	of dimensions: 33.5 x 22.0 x 15.4 cm. Mosses were placed at the exposure site in holes in the ground matching the dimensions of the box

a)



b)

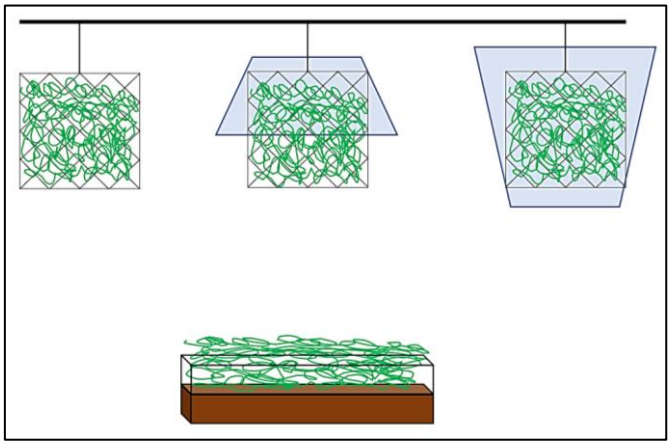


Figure 1. SM. Visualization of moss exposure methods: a) real photo b) from left: diagram of methods mb, dd, wd and bo respectively

Table 2 SM. Other measured chlorophyll fluorescence parameters (mean values ± standard deviation) in relation to the time and method of exposure of mosses.

Species	Month	Method	Fs	Fms	Ft	alpha	RH [%]	PAR [uE]
Pl	0	mb						
		dd	113 ± 27.7	158 ± 49.1	107 ± 26.1	0.59 ± 0.05	28 ± 1.14	7 ± 0.32
		wd						
		bo	176 ± 34.5	515 ± 136	178 ± 33.1	0.56 ± 0.16	72 ± 11.9	12 ± 9.63
Sp	0	mb						
		dd	246 ± 26.4	306 ± 16.4	242 ± 27.8	0.71 ± 0.01	33 ± 1.00	4 ± 0.58
		wd						
		bo	239 ± 38.8	703 ± 196	249 ± 41.1	0.75 ± 0.20	74 ± 4.89	14 ± 9.31
Dp	0	mb						
		dd	163 ± 24.0	307 ± 183	150 ± 11.3	0.70 ± 0.12	32 ± 0.71	5 ± 0.00
		wd						
		bo	152 ± 23.9	519 ± 99.8	154 ± 28.1	0.63 ± 0.15	76 ± 4.76	14 ± 8.30
Pl	1	mb	113 ± 22.8	130 ± 26.0	114 ± 22.7	0.69 ± 0.15	35 ± 0.92	2 ± 1.05
		dd	110 ± 11.9	128 ± 16.2	110 ± 10.0	0.55 ± 0.14	24 ± 0.52	2 ± 0.88
		wd	111 ± 22.6	129 ± 31.0	111 ± 21.4	0.59 ± 0.10	28 ± 1.48	6 ± 0.32
		bo	182 ± 46.5	362 ± 168	171 ± 48.7	0.56 ± 0.12	34 ± 4.45	5 ± 0.70
Sp	1	mb	172 ± 34.0	194 ± 38.3	168 ± 32.8	0.58 ± 0.08	30 ± 5.17	4 ± 1.33
		dd	164 ± 47.3	183 ± 51.9	162 ± 47.9	0.58 ± 0.10	29 ± 0.88	6 ± 0.84
		wd	166 ± 42.2	185 ± 48.3	165 ± 42.5	0.59 ± 0.11	28 ± 0.97	6 ± 0.63
		bo	168 ± 45.6	422 ± 217	147 ± 35.6	0.56 ± 0.16	28 ± 2.60	6 ± 1.77
Dp	1	mb	102 ± 10.9	119 ± 13.0	100 ± 13.5	0.65 ± 0.05	25 ± 0.95	3 ± 2.02
		dd	119 ± 33.3	136 ± 36.7	118 ± 31.7	0.65 ± 0.18	27 ± 0.79	5 ± 0.95
		wd	105 ± 14.4	123 ± 17.1	104 ± 14.9	0.69 ± 0.12	27 ± 1.93	6 ± 0.42
		bo	143 ± 48.3	271 ± 95.9	131 ± 43.8	0.54 ± 0.12	24 ± 0.57	6 ± 0.32
Pl	2	mb	98 ± 6.36	110 ± 9.19	95 ± 5.66	0.63 ± 0.04	30 ± 1.41	8 ± 0.00
		dd	100 ± 6.01	111 ± 7.92	98 ± 6.75	0.53 ± 0.11	26 ± 0.74	7 ± 0.64
		wd	110 ± 11.0	129 ± 14.1	109 ± 10.7	0.66 ± 0.06	29 ± 0.88	4 ± 2.36

		bo	119 ± 34.1	225 ± 139	109 ± 22.6	0.62 ± 0.12	28 ± 2.37	6 ± 0.84
Sp	2	mb	150 ± 29.4	169 ± 33.5	149 ± 29.1	0.64 ± 0.07	28 ± 0.82	7 ± 0.57
		dd	185 ± 23.8	200 ± 26.5	182 ± 25.0	0.64 ± 0.06	28 ± 1.71	6 ± 0.48
		wd	157 ± 36.8	175 ± 42.2	155 ± 36.0	0.62 ± 0.07	27 ± 0.42	5 ± 0.32
		bo	186 ± 67.8	444 ± 240	166 ± 54.4	0.70 ± 0.08	29 ± 2.37	5 ± 0.70
Dp	2	mb	102 ± 10.0	118 ± 11.4	103 ± 11.2	0.71 ± 0.05	28 ± 0.64	7 ± 0.35
		dd	108 ± 11.4	124 ± 12.9	107 ± 13.3	0.64 ± 0.09	28 ± 0.74	6 ± 0.53
		wd	105 ± 7.82	122 ± 9.66	103 ± 8.99	0.65 ± 0.09	27 ± 0.71	6 ± 0.53
		bo	112 ± 20.8	253 ± 145	102 ± 15.5	0.71 ± 0.12	27 ± 0.52	6 ± 0.46
Pl	3	mb	113 ± 15.0	142 ± 26.5	111 ± 12.5	0.48 ± 0.07	27 ± 1.00	2 ± 2.55
		dd	113 ± 13.0	163 ± 59.0	109 ± 12.8	0.57 ± 0.11	25 ± 0.32	1 ± 1.90
		wd	157 ± 27.6	187 ± 29.9	155 ± 29.2	0.39 ± 0.16	23 ± 0.42	1 ± 1.45
		bo	134 ± 23.3	162 ± 26.4	130 ± 24.0	0.48 ± 0.16	33 ± 3.56	2 ± 2.22
Sp	3	mb	172 ± 31.2	193 ± 38.8	169 ± 31.2	0.61 ± 0.04	25 ± 0.42	0.30 ± 0.95
		dd	163 ± 54.0	176 ± 56.9	162 ± 54.9	0.60 ± 0.08	25 ± 0.82	1 ± 1.90
		wd	166 ± 32.3	257 ± 67.2	160 ± 31.4	0.63 ± 0.13	25 ± 1.71	1 ± 0.48
		bo	157 ± 51.5	230 ± 108	150 ± 49.4	0.53 ± 0.15	29 ± 1.48	0.00
Dp	3	mb	126 ± 26.1	147 ± 30.4	124 ± 24.8	0.56 ± 0.15	26 ± 0.71	0.00
		dd	112 ± 18.2	129 ± 21.7	111 ± 19.3	0.67 ± 0.12	24 ± 0.57	0.30 ± 0.67
		wd	137 ± 28.9	164 ± 35.4	134 ± 29.1	0.53 ± 0.13	27 ± 1.25	3 ± 2.57
		bo	121 ± 23.6	139 ± 26.3	117 ± 22.4	0.63 ± 0.12	28 ± 1.06	3 ± 3.40

Fs: Fluorescence under steady state; Fms: Maximum fluorescence at steady state; Ft: Instantaneous fluorescence; alpha: leaf absorptance; RH: Relative Humidity; PAR: Photosynthetically Active Radiation

Table 3 SM. Multivariate significance test for the effect of method (M) and exposure time (T) and species (S) on the concentration of all elements

Effect	Test	Value	F	Effect df	Error df	p
Intercept	Wilksa	0.037	247	7	66.0	**<0.001**
	Pillai'a	0.963	247	7	66.0	**<0.001**
	Hotelln.	26.2	247	7	66.0	**<0.001**
	Roy'a	26.2	247	7	66.0	**<0.001**
M	Wilksa	0.050	16.6	21	190	**<0.001**
	Pillai'a	1.48	9.42	21	204	**<0.001**
	Hotelln.	9.53	29.4	21	194	**<0.001**
	Roy'a	8.61	83.6	7	68	**<0.001**
S	Wilksa	0.019	58.5	14	132	**<0.001**
	Pillai'a	1.48	27.0	14	134	**<0.001**
	Hotelln.	25.2	117	14	130	**<0.001**
	Roy'a	24.1	231	7	67	**<0.001**
T	Wilksa	0.219	10.7	14	132	**<0.001**
	Pillai'a	0.929	8.31	14	134	**<0.001**
	Hotelln.	2.90	13.5	14	130	**<0.001**
	Roy'a	2.64	25.3	7	67.0	**<0.001**
M*S	Wilksa	0.028	8.53	42	313	**<0.001**
	Pillai'a	2.00	5.06	42	426	**<0.001**
	Hotelln.	9.78	15.0	42	386	**<0.001**
	Roy'a	7.99	81.0	7	71.0	**<0.001**
M*T	Wilksa	0.105	4.59	42	313	**<0.001**
	Pillai'a	1.64	3.82	42	426	**<0.001**
	Hotelln.	3.36	5.14	42	386	**<0.001**
	Roy'a	1.92	19.5	7	71.0	**<0.001**
S*T	Wilksa	0.163	6.00	28	239	**<0.001**

	Pillai'a	1.34	4.96	28	276	**<0.001**
	Hotelln.	2.60	5.94	28	258	**<0.001**
	Roy'a	1.24	12.2	7	69.0	**<0.001**
M*S*T	Wilksa	0.043	3.30	84	412	**<0.001**
	Pillai'a	2.23	2.81	84	504	**<0.001**
	Hotelln.	4.76	3.64	84	450	**<0.001**
	Roy'a	1.67	10.0	12	72.0	**<0.001**

values in **bold** p are statistically significant values

Table 4 SM. Three-way significance test for the effect of method (M) and exposure time (T) and species (S) on elemental concentration

Efect for	SS	df	MS	F	p
Cu-Intercept	56.7	1	56.7	453	**<0.001**
M	4.85	3	1.62	12.9	**<0.001**
S	25.6	2	12.8	102	**<0.001**
T	3.48	2	1.74	13.9	**<0.001**
M*S	6.11	6	1.02	8.14	**<0.001**
M*T	3.11	6	0.52	4.15	**0.001**
S*T	7.16	4	1.79	14.3	**<0.001**
M*S*T	7.30	12	0.61	4.87	**<0.001**
Error	9.00	72	0.13		
Zn-Intercept	963	1	963	207	**<0.001**
M	506	3	169	36.3	**<0.001**
S	316	2	158	34.0	**<0.001**
T	326	2	163	35.1	**<0.001**
M*S	190	6	31.7	6.82	**<0.001**
M*T	158	6	26.2	5.65	**<0.001**
S*T	158	4	39.5	8.51	**<0.001**
M*S*T	249	12	20.8	4.48	**<0.001**
Error	334	72	4.64		
Cd-Intercept	1.41	1	1.41	121	**<0.001**
M	0.862	3	0.287	24.7	**<0.001**
S	2.31	2	1.15	99.3	**<0.001**
T	0.006	2	0.003	0.240	0.787
M*S	0.752	6	0.125	10.8	**<0.001**

M*T	0.168	6	0.0281	2.41	**0.035**
S*T	0.030	4	0.008	0.651	0.628
M*S*T	0.317	12	0.026	2.27	**0.016**
Error	0.837	72	0.011		
Pb-Intercept	173	1	173	13.0	**<0.001**
M	41.8	3	13.9	1.04	0.379
S	433	2	217	16.2	**<0.001**
T	188	2	94.0	7.05	**0.002**
M*S	47.9	6	7.98	0.598	0.731
M*T	209	6	34.8	2.61	**0.024**
S*T	45.3	4	11.3	0.849	0.499
M*S*T	204	12	17.0	1.27	0.255
Error	961	72	13.3		
Mn-Intercept	17191	1	17191	168	**<0.001**
M	24816	3	8272	80.7	**<0.001**
S	4937	2	2469	24.1	**<0.001**
T	136	2	68.1	0.665	0.518
M*S	5426	6	904	8.82	**<0.001**
M*T	5802	6	967	9.43	**<0.001**
S*T	1459	4	365	3.56	**0.010**
M*S*T	5435	12	453	4.42	**<0.001**
Error	7380	72	103		
Fe-Intercept	147749	1	147749	93.1	**<0.001**
M	100823	3	33608	21.2	**<0.001**
S	60374	2	30187	19.0	**<0.001**
T	112185	2	56093	35.4	**<0.001**

M*S	18498	6	3083	1.94	0.085
M*T	31086	6	5181	3.27	**0.007**
S*T	6850	4	1712	1.08	0.373
M*S*T	73568	12	6131	3.86	**<0.001**
Error	114224	72	1586		
Hg-Intercept	0.015	1	0.015	144	**<0.001**
M	0.002	3	0.001	7.59	**<0.001**
S	0.002	2	0.001	11.7	**<0.001**
T	0.002	2	0.001	7.65	**<0.001**
M*S	0.005	6	0.001	7.46	**<0.001**
M*T	0.004	6	0.001	6.10	**<0.001**
S*T	0.001	4	0.0002	1.51	0.208
M*S*T	0.007	12	0.001	6.04	**<0.001**
Error	0.007	72	0.0001		

SS - sum of squares of effects; MS - mean sum of squares of effects; values in **bold** p are statistically significant values

93 Table 5 SM. Wilcoxon signed-rank test comparing the moss-bag method (mb) with the others

M1		M2	W	p
	-	Cd dd *Pl*	10.0	0.886
Cd mb *Pl*	-	Cd wd *Pl*	42.0	**0.012**
	-	Cd bo *Pl*	0.00	0.997
	-	Mn dd *Pl*	6.00	0.980
Mn mb *Pl*	-	Mn wd *Pl*	4.00	0.990
	-	Mn bo *Pl*	44.0	**0.004**
	-	Fe dd *Pl*	44.0	**0.004**
Fe mb *Pl*	-	Fe wd *Pl*	45.0	**0.002**
	-	Fe bo *Pl*	9.00	0.951
	-	Hg dd *Pl*	35.0	0.077
Hg mb *Pl*	-	Hg wd *Pl*	20.0	0.417
	-	Hg bo *Pl*	45.0	**0.004**
	-	Cu dd *Sp*	38.0	**0.037**
Cu mb *Sp*	-	Cu wd *Sp*	44.0	**0.004**
	-	Cu bo *Sp*	12.0	0.898
	-	Cd dd *Sp*	17.5	0.306
Cd mb *Sp*	-	Cd wd *Sp*	30.5	0.185
	-	Cd bo *Sp*	6.00	0.438
	-	Pb dd *Sp*	29.0	0.248
Pb mb *Sp*	-	Pb wd *Sp*	29.0	0.248
	-	Pb bo *Sp*	28.0	0.285
	-	Fe dd *Sp*	44.0	**0.004**
Fe mb *Sp*	-	Fe wd *Sp*	45.0	**0.002**
	-	Fe bo *Sp*	21.0	0.590
	-	Cd dd *Dp*	2.00	0.750
Cd mb *Dp*	-	Cd wd *Dp*	3.00	0.607

M1		M2	W	p
	-	Cd bo *Dp*	6.00	0.125
	-	Pb dd *Dp*	28.0	0.285
Pb mb *Dp*	-	Pb wd *Dp*	7.00	0.973
	-	Pb bo *Dp*	8.00	0.963
	-	Mn dd *Dp*	25.0	0.410
Mn mb *Dp*	-	Mn wd *Dp*	18.0	0.715
	-	Mn bo *Dp*	43.0	**0.006**
	-	Fe dd *Dp*	45.0	**0.002**
Fe mb *Dp*	-	Fe wd *Dp*	42.0	**0.010**
	-	Fe bo *Dp*	28.0	0.285

all tests, hypothesis is measurement one: M1 greater than measurement two: M2 (M1>M2); values in **bold** p are statistically significant values

113 Table 6 SM. Paired Samples Student t-test comparing the dry deposition method (dd) with the others

M1	M2	t	df	p
	- Cu mb *Pl*	0.085	8	0.467
Cu dd *Pl*	- Cu wd *Pl*	4.205	8	**0.001**
	- Cu bo *Pl*	3.073	8	**0.008**
	- Zn mb *Pl*	0.612	8	0.279
Zn dd *Pl*	- Zn wd *Pl*	2.156	8	**0.032**
	- Zn bo *Pl*	2.708	8	**0.013**
	- Pb mb *Pl*	0.075	8	0.471
Pb dd *Pl*	- Pb wd *Pl*	1.070	8	0.158
	- Pb bo *Pl*	0.508	8	0.313
	- Zn mb *Sp*	-2.507	8	0.982
Zn dd *Sp*	- Zn wd *Sp*	-1.744	8	0.940
	- Zn bo *Sp*	1.785	8	0.056
	- Mn mb *Sp*	-0.982	8	0.823
Mn dd *Sp*	- Mn wd *Sp*	1.190	8	0.134
	- Mn bo *Sp*	4.288	8	**0.001**
	- Hg mb *Sp*	-6.828	8	1.000
Hg dd *Sp*	- Hg wd *Sp*	-0.920	8	0.808
	- Hg bo *Sp*	-1.177	8	0.863
	- Cu mb *Dp*	-0.528	8	0.694
Cu dd *Dp*	- Cu wd *Dp*	1.561	8	0.079
	- Cu bo *Dp*	3.056	8	**0.008**
	- Zn mb *Dp*	-3.394	8	0.995
Zn dd *Dp*	- Zn wd *Dp*	1.039	8	0.165
	- Zn bo *Dp*	7.198	8	**<0.001**
	- Hg mb *Dp*	0.123	8	0.453
Hg dd *Dp*				
	- Hg wd *Dp*	1.141	8	0.143

M1	M2	t	df	p
	- Hg bo *Dp*	0.711	8	0.249

all tests, hypothesis is measurement one: M1 greater than measurement two: M2 (M1>M2); values in **bold** p are statistically significant values

142 Table 7 SM. Wilcoxon signed-rank test comparing the dry deposition method (dd) with the others

M1		M2	W	p
	-	Cd mb *Pl*	26.0	0.144
Cd dd *Pl*	-	Cd wd *Pl*	28.0	**0.011**
	-	Cd bo *Pl*	0.00	0.997
	-	Mn mb *Pl*	39.0	**0.027**
Mn dd *Pl*	-	Mn wd *Pl*	6.00	0.978
	-	Mn bo *Pl*	45.0	**0.002**
	-	Fe mb *Pl*	1.00	0.998
Fe dd *Pl*	-	Fe wd *Pl*	31.0	0.180
	-	Fe bo *Pl*	0.00	1.000
	-	Hg mb *Pl*	10.0	0.939
Hg dd *Pl*	-	Hg wd *Pl*	12.0	0.819
	-	Hg bo *Pl*	28.0	**0.011**
	-	Cu mb *Sp*	7.00	0.973
Cu dd *Sp*	-	Cu wd *Sp*	24.5	0.429
	-	Cu bo *Sp*	9.00	0.951
	-	Cd mb *Sp*	10.5	0.751
Cd dd *Sp*	-	Cd wd *Sp*	22.0	0.547
	-	Cd bo *Sp*	6.00	0.438
	-	Pb mb *Sp*	16.0	0.787
Pb dd *Sp*	-	Pb wd *Sp*	24.0	0.455
	-	Pb bo *Sp*	30.0	0.213
	-	Fe mb *Sp*	1.00	0.998
Fe dd *Sp*	-	Fe wd *Sp*	20.0	0.633
	-	Fe bo *Sp*	21.0	0.590
	-	Cd mb *Dp*	4.00	0.375
Cd dd *Dp*	-	Cd wd *Dp*	5.00	0.250

M1		M2	W	p
	-	Cd bo *Dp*	5.00	0.250
	-	Pb mb *Dp*	17.0	0.752
Pb dd *Dp*	-	Pb wd *Dp*	12.0	0.898
	-	Pb bo *Dp*	9.00	0.951
	-	Mn mb *Dp*	20.0	0.633
Mn dd *Dp*	-	Mn wd *Dp*	9.00	0.951
	-	Mn bo *Dp*	42.0	**0.010**
	-	Fe mb *Dp*	0.00	1.000
Fe dd *Dp*	-	Fe wd *Dp*	29.0	0.248
	-	Fe bo *Dp*	13.0	0.875

all tests, hypothesis is measurement one: M1 greater than measurement two: M2 (M1>M2);); values in **bold** p are statistically significant values

162 Table 8 SM. Paired Samples Student t-test comparison of wind method (wd) with the others

M1		M2	t	df	p
	-	Cu mb *Pl*	-4.587	8	0.999
Cu wd *Pl*	-	Cu dd *Pl*	-4.205	8	0.999
	-	Cu bo *Pl*	0.327	8	0.376
	-	Zn mb *Pl*	-2.156	8	0.968
Zn wd *Pl*	-	Zn dd *Pl*	-2.156	8	0.968
	-	Zn bo *Pl*	0.034	8	0.487
	-	Pb mb *Pl*	-1.134	8	0.855
Pb wd *Pl*	-	Pb dd *Pl*	-1.070	8	0.842
	-	Pb bo *Pl*	-1.324	8	0.889
	-	Zn mb *Sp*	0.147	8	0.444
Zn wd *Sp*	-	Zn dd *Sp*	1.744	8	0.060
	-	Zn bo *Sp*	3.788	8	**0.003**
	-	Mn mb *Sp*	-1.620	8	0.928
Mn wd *Sp*	-	Mn dd *Sp*	-1.190	8	0.866
	-	Mn bo *Sp*	2.910	8	**0.010**
	-	Hg mb *Sp*	-5.405	8	1.000
Hg wd *Sp*	-	Hg dd *Sp*	0.920	8	0.192
	-	Hg bo *Sp*	-1.060	8	0.840
	-	Cu mb *Dp*	-2.204	8	0.971
Cu wd *Dp*	-	Cu dd *Dp*	-1.561	8	0.921
	-	Cu bo *Dp*	1.011	8	0.171
	-	Zn mb *Dp*	-3.964	8	0.998
Zn wd *Dp*	-	Zn dd *Dp*	-1.039	8	0.835
	-	Zn bo *Dp*	6.500	8	<**0.001**
	-	Hg mb *Dp*	-1.578	8	0.923
Hg wd *Dp*					
	-	Hg dd *Dp*	-1.141	8	0.857

M1	M2	t	df	p
-	Hg bo *Dp*	-0.217	8	0.583

all tests, hypothesis is measurement one: M1 greater than measurement two: M2 (M1>M2); values in **bold** p are statistically significant values

191 Table 9 SM. Wilcoxon signed-rank test comparing the wind method (wd) with the others

M1		M2	W	p
	-	Cd mb *Pl*	3.00	0.991
Cd wd *Pl*	-	Cd dd *Pl*	0.00	0.993
	-	Cd bo *Pl*	0.00	0.997
	-	Mn mb *Pl*	41.0	**0.014**
Mn wd *Pl*	-	Mn dd *Pl*	39.0	**0.029**
	-	Mn bo *Pl*	45.0	**0.002**
	-	Fe mb *Pl*	0.00	1.000
Fe wd *Pl*	-	Fe dd *Pl*	14.0	0.850
	-	Fe bo *Pl*	0.00	1.000
	-	Hg mb *Pl*	16.0	0.637
Hg wd *Pl*	-	Hg dd *Pl*	24.0	0.220
	-	Hg bo *Pl*	42.0	**0.010**
	-	Cu mb *Sp*	1.00	0.998
Cu wd *Sp*	-	Cu dd *Sp*	20.5	0.617
	-	Cu bo *Sp*	3.00	0.985
	-	Cd mb *Sp*	14.5	0.845
Cd wd *Sp*	-	Cd dd *Sp*	23.0	0.500
	-	Cd bo *Sp*	6.00	0.438
	-	Pb mb *Sp*	16.0	0.787
Pb wd *Sp*	-	Pb dd *Sp*	21.0	0.590
	-	Pb bo *Sp*	27.0	0.326
	-	Fe mb *Sp*	0.00	1.000
Fe wd *Sp*	-	Fe dd *Sp*	25.0	0.410
	-	Fe bo *Sp*	20.0	0.633
	-	Cd mb *Dp*	3.00	0.607
Cd wd *Dp*	-	Cd dd *Dp*	1.00	0.875

M1		M2	W	p
	-	Cd bo *Dp*	3.00	0.186
	-	Pb mb *Dp*	38.0	**0.037**
Pb wd *Dp*	-	Pb dd *Dp*	33.0	0.125
	-	Pb bo *Dp*	16.0	0.787
	-	Mn mb *Dp*	27.0	0.326
Mn wd *Dp*	-	Mn dd *Dp*	36.0	0.064
	-	Mn bo *Dp*	45.0	**0.002**
	-	Fe mb *Dp*	3.00	0.994
Fe wd *Dp*	-	Fe dd *Dp*	16.0	0.787
	-	Fe bo *Dp*	9.00	0.951

all tests, hypothesis is measurement one: M1 greater than measurement two: M2 (M1>M2); values in **bold** p are statistically significant values

211 Table 10 SM. Paired Samples Student t-test comparison of box method (bo) with the others

M1		M2	t	df	p
	-	Cu mb *Pl*	-2.358	8	0.977
Cu bo *Pl*	-	Cu dd *Pl*	-3.073	8	0.992
	-	Cu wd *Pl*	-0.327	8	0.624
	-	Zn mb *Pl*	-1.253	8	0.877
Zn bo *Pl*	-	Zn dd *Pl*	-2.708	8	0.987
	-	Zn wd *Pl*	-0.034	8	0.513
	-	Pb mb *Pl*	-0.427	8	0.660
Pb bo *Pl*	-	Pb dd *Pl*	-0.508	8	0.687
	-	Pb wd *Pl*	1.324	8	0.111
	-	Zn mb *Sp*	-3.039	8	0.992
Zn bo *Sp*	-	Zn dd *Sp*	-1.785	8	0.944
	-	Zn wd *Sp*	-3.788	8	0.997
	-	Mn mb *Sp*	-4.490	8	0.999
Mn bo *Sp*	-	Mn dd *Sp*	-4.288	8	0.999
	-	Mn wd *Sp*	-2.910	8	0.990
	-	Hg mb *Sp*	-1.192	8	0.866
Hg bo *Sp*	-	Hg dd *Sp*	1.177	8	0.137
	-	Hg wd *Sp*	1.060	8	0.160
	-	Cu mb *Dp*	-2.352	8	0.977
Cu bo *Dp*	-	Cu dd *Dp*	-3.056	8	0.992
	-	Cu wd *Dp*	-1.011	8	0.829
	-	Zn mb *Dp*	-6.456	8	1.000
Zn bo *Dp*	-	Zn dd *Dp*	-7.198	8	1.000
	-	Zn wd *Dp*	-6.500	8	1.000
	-	Hg mb *Dp*	-0.909	8	0.805
Hg bo *Dp*	-	Hg dd *Dp*	-0.711	8	0.751

M1		M2	t	df	p
-	Hg wd	*Dp*	0.217	8	0.417

all tests, hypothesis is measurement one: M1 greater than measurement two: M2 (M1>M2); values in **bold** p are statistically significant values

240 Table 11 SM. Wilcoxon signed-rank test comparision of box method (bo) with the others

M1		M2	W	p
	-	Cd mb *Pl*	45.0	**0.004**
Cd bo *Pl*	-	Cd dd *Pl*	45.0	**0.005**
	-	Cd wd *Pl*	45.0	**0.005**
	-	Mn mb *Pl*	1.00	0.998
Mn bo *Pl*	-	Mn dd *Pl*	0.00	1.000
	-	Mn wd *Pl*	0.00	1.000
	-	Fe mb *Pl*	36.0	0.064
Fe bo *Pl*	-	Fe dd *Pl*	45.0	**0.002**
	-	Fe wd *Pl*	45.0	**0.002**
	-	Hg mb *Pl*	0.00	0.997
Hg bo *Pl*	-	Hg dd *Pl*	0.00	0.993
	-	Hg wd *Pl*	3.00	0.994
	-	Cu mb *Sp*	33.0	0.125
Cu bo *Sp*	-	Cu dd *Sp*	36.0	0.064
	-	Cu wd *Sp*	33.0	**0.021**
	-	Cd mb *Sp*	4.00	0.688
Cd bo *Sp*	-	Cd dd *Sp*	4.00	0.688
	-	Cd wd *Sp*	4.00	0.688
	-	Pb mb *Sp*	17.0	0.752
Pb bo *Sp*	-	Pb dd *Sp*	15.0	0.820
	-	Pb wd *Sp*	18.0	0.715
	-	Fe mb *Sp*	24.0	0.455
Fe bo *Sp*	-	Fe dd *Sp*	24.0	0.455
	-	Fe wd *Sp*	25.0	0.410
	-	Cd mb *Dp*	0.00	1.000
Cd bo *Dp*				
	-	Cd dd *Dp*	1.00	0.875

M1		M2	W	p
	-	Cd wd *Dp*	0.00	0.963
	-	Pb mb *Dp*	37.0	**0.049**
Pb bo *Dp*	-	Pb dd *Dp*	36.0	0.064
	-	Pb wd *Dp*	2900	0.248
	-	Mn mb *Dp*	2.00	0.996
Mn bo *Dp*	-	Mn dd *Dp*	3.00	0.994
	-	Mn wd *Dp*	0.00	1.000
	-	Fe mb *Dp*	17.0	0.752
Fe bo *Dp*	-	Fe dd *Dp*	32.0	0.150
	-	Fe wd *Dp*	36.0	0.064

all tests, hypothesis is measurement one: M1 greater than measurement two: M2 (M1>M2);); values in **bold** p are statistically significant values

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Article

Is Your Moss Alive during Active Biomonitoring Study?

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Abstract: Biomonitoring was proposed to assess the condition of living organisms or entire ecosystems with the use of bioindicators—species sensitive to specific pollutants. It is important that the bioindicator species remains alive for as long as possible while retaining the ability to react to the negative effects of pollution (elimination/neutralization of hazardous contaminants). The purpose of the study was to assess the survival of *Pleurozium schreberi* moss during exposure (moss-bag technique) based on the measurement of the concentration of elements (Ni, Cu, Zn, Cd, and Pb), chlorophyll content, and its fluorescence. The study was carried out using a CCM-300 portable chlorophyll content meter, portable fluorometer, UV-Vis spectrophotometer, and a flame atomic absorption spectrometer. As a result of the laboratory tests, no significant differences were found in the chlorophyll content in the gametophytes of mosses tested immediately after collection from the forest, compared to those drying at room temperature in the laboratory ($p = 0.175$ for Student's t-test results). Mosses exposed using the moss-bag technique of active biomonitoring were characterized by a drop in the chlorophyll content over 12 weeks (more than 50% and 60% for chlorophyll-*a* and chlorophyll-*b*, respectively). Chlorophyll content in mosses during exposure was correlated with actual photochemical efficiency (yield) of photosystem II (calculated value of Pearson's linear correlation coefficient was 0.94—there was a significant correlation between chlorophyll *a* and yield $p = 0.02$). The highest metal increases in mosses (*RAF* values) were observed for zinc, lead, and copper after the second and third month of exposure. The article demonstrates that the moss exposed in an urbanized area for a period of three months maintains the properties of good bioindicator of environmental quality.

Keywords: active biomonitoring; mosses; chlorophyll content; chlorophyll fluorescence; bioindicator

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1. Introduction

Bioindicators are indicator species that are used to assess environmental quality and changes occurring over time [1,2]. An example of a bioindicator used on a large scale in biomonitoring studies is moss that meets specific requirements as an indicator plant [3]. Mosses as bioindicators are often used in biomonitoring to assess air pollution by heavy metals [4]. It is necessary to bear in mind that biomonitoring uses living organisms, or parts thereof (tissues), in order to determine the conditions of the environment or the changes that have occurred in it under anthropopressure [5,6]. In biomonitoring with the use of moss to assess air pollution, two methods are distinguished [7]: one is passive biomonitoring that consists of the use of living organisms growing naturally on a given (test) site [8]; the other is a method of active biomonitoring, where living organisms are transferred from their natural habitats and exposed to pollutants on the test site [9]. Air quality biomonitoring that uses mosses is quite a common and frequently used method due to the properties of mosses, including the absence of a root system, uptake of nutrients via the entire body surface from dry and wet depositions, and the fact that the epidermis

is most often reduced, while ions deposited on moss surface have direct contact with the exchange points on walls of their cells [10]. An additional advantage of biomonitoring is the low cost and uncomplicated method of obtaining samples, as well as the possibility of complementing/competing with equipment-based monitoring by providing data, using the reaction of indicator species to existing or laboratory environmental conditions [11]. A disadvantage of biomonitoring is the absence of standardization of procedures and techniques used during tests [7,12]. A very important element of active biomonitoring is the sample preparation and all of the procedures occurring before the exposure [13,14]. Depending on the nature of a study, the exposure time of mosses ranges [15] from a few days [16] to even 12 months [17]. However, the question arises: can we say that, before the study and after such a long period of exposure, this material is still a living organism [18]? The measurement of the chlorophyll content is important in physiological and ecological studies of plants, as changes in its content are associated with various key life functions, including growth, photosynthetic capacity, production of metabolites, and responses to environmental stress in higher plants [19]. In the case of mosses, it has been proven that the morphological characteristics of the anatomical structure of mosses' leaves and the chlorophyll content are related to the photosynthetic activity [20]. The example of treating mosses with wood distillate indicates that the values for the chlorophyll content are related to the maximum quantum yield of primary photochemistry. Results presented in this work do not indicate different values between Fv/Fm and the chlorophyll content [21]. In *Sphagnum* mosses, a positive correlation between the chlorophyll content and the net photosynthesis has been recorded [22]. Environmental factors may affect directly the chlorophyll content in peat moss (temperature, light intensity) [23], and may influence the chlorophyll content in *Sphagna* [24]. Many works have determined the influence of heavy metal accumulation on the chlorophyll content in mosses [25,26]. However, there are no studies demonstrating that, before exposure and when exposed in active biomonitoring over a long period of time and to various types of stresses (e.g., heavy metals), mosses are still alive and thus can still be referred to as bioindicators. Theoretically, in a dry state, mosses are able to survive for a long period of time, even 14 years [27]. However, such an extended period of drought survival had not been confirmed later in the literature. Exposure to, for instance, PAH contamination for a certain period of time causes mosses to spend most of the exposure time in cryptobiosis [28], but there is little research regarding this issue. There is no evidence that, during exposure, moss is still a living organism and, as such, a bioindicator that meets the criteria for organisms intended for biomonitoring. There are also no works that focus on the fact that, before the exposure, moss is also a living organism.

The purpose of our study was to determine to what extent the environmental conditions before the exposure affected moss viability and sorption of heavy metals during active biomonitoring, and how a three-month exposure affects the chlorophyll content and its fluorescence measurements in moss, being a determinant of moss survival under exposure to environmental pollution.

2. Results

Figure 1 show the changes in the chlorophyll content and its fluorescence in the vertical profile of *P. schreberi* gametophyte.

Each of the 10 shoots was divided into 10 segments. In the above figure, we can see that there is no chlorophyll in the lower segments, or its concentration is very low (segments 1–4/5). In *P. schreberi*, the average chlorophyll content in segments 7–10 is very similar (219–254 mg/m^2), with the highest value recorded in segment 9 (465 mg/m^2). Actual yield of photosystem II was not determined in segments 1–4. In the higher parts, similar to chlorophyll content, the values of actual yield of photosystem II increase, where in segment 9 the highest mean (0.666) and median (0.679) were recorded. For further analyses, mosses were sampled from the higher segments (8–10), which, based on the

above results, proved to be the best sites for local measurements of the chlorophyll content and actual yield of photosystem II.

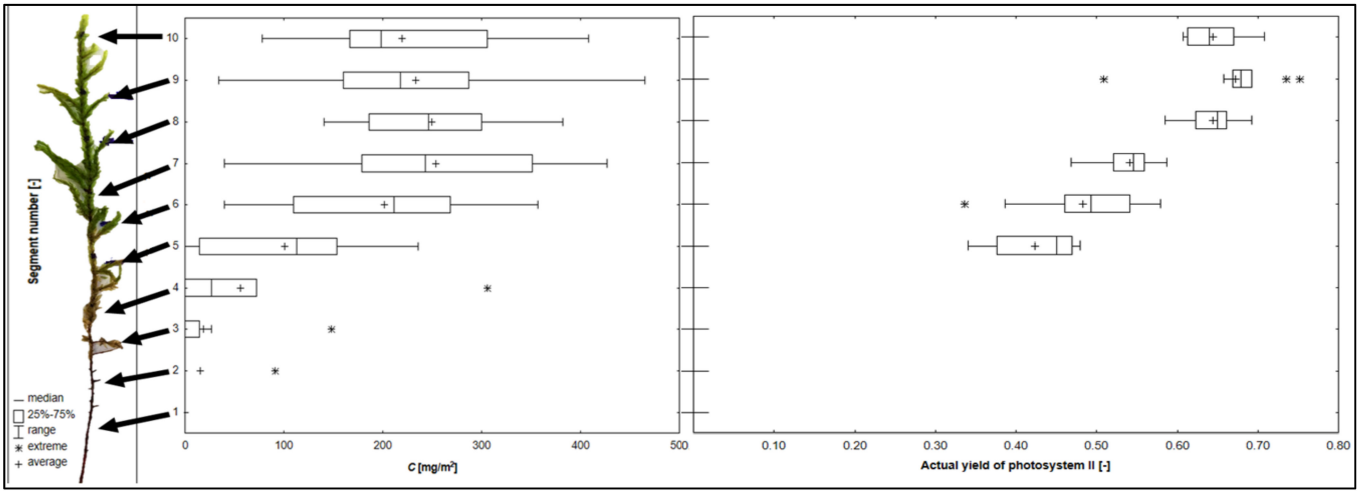

Figure 1. Changes in the chlorophyll content and actual yield of photosystem II in the vertical profile of moss gametophyte.

The experiment conditions/living conditions of mosses have a statistically significant influence on the chlorophyll content in mosses. The upper parts of shoots of mosses were collected for testing based on the results presented in Figure 1. The diagram in Figure 2 shows the spectra obtained from the analysis using the spectrophotometer.

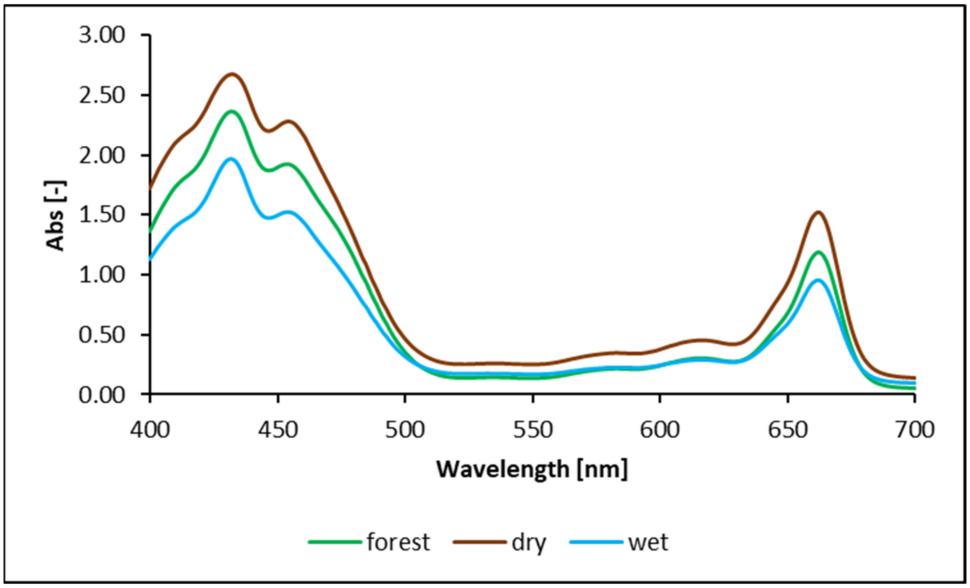

Figure 2. Photoabsorption spectra of pigment from the moss samples.

Figure 2 shows the spectra for mosses depending on environmental/laboratory conditions. The spectra are normalized to unity at 662 nm, and arranged at regular intervals of 1 nm. Based on the absorbance values from the diagram in Figure 2, the content of chlorophyll a and b was calculated in gametophytes of the mosses shown in Table 1 below.

Table 1. Chlorophyll *a* and *b* content [mg/L] and actual photochemical efficiency (yield) [-] in mosses depending on the living conditions.

		Forest	Dry	Wet
Chl-*a*	min-max	9.56–17.4	10.4–21.0	8.08–11.3
	average	12.7	16.1	10.1
	median	11.9	16.4	10.5
	SD	3.38	3.57	1.38
Chl-*b*	min-max	4.51–7.39	4.30–13.9	4.42–6.35
	average	5.49	8.16	5.25
	median	5.03	7.55	5.11
	SD	1.33	3.25	0.977
Yield	min-max	0.553–0.706	0.371–0.663	0.281–0.627
	average	0.647	0.532	0.503
	median	0.658	0.524	0.573
	SD	0.044	0.116	0.149

The results in Table 1 indicate variable concentrations of chlorophyll *a* and *b* in mosses. The content of this pigment in mosses depends on the living conditions to which they have been exposed. The lowest values according to the spectra shown in the diagram in Figure 2 refer to the mosses dried and sprayed with demineralized water (wet—mosses dried in the laboratory at room temperature and sprayed with deionized water one hour before the analysis). The mean and median values for dried samples (dry—mosses dried in the laboratory at room temperature after collecting them in the forest) are higher than for mosses collected directly from the environment (forest—mosses collected in the forest in situ, examined immediately after collection, fresh samples). Due to the influence of environmental/laboratory conditions on the decreasing chlorophyll *a* and *b* content, mosses can be classified as follows: dried mosses (dry) > mosses collected directly from the forest (forest) > mosses dried and sprayed with demineralized water (wet). For actual yield of photosystem II, the highest values were recorded for forest > dry > wet mosses, the same as for mean values. Nevertheless, the Mann–Whitney U test showed no differences between them, so forest = dry $\neq$ wet.

Study results of Table 2 below were analyzed using the Student's *t*-test, indicating statistically significant differences between the content of chlorophyll *a* and chlorophyll *b* in mosses, depending on their living conditions.

Table 2. Student's *t*-test results for chlorophyll *a* and *b* content in mosses.

		Forest	Dry	Wet
Chl-*a*	forest	-	0.175	0.196
	dry	0.175	-	<0.05
	wet	0.196	<0.05	-
Chl-*b*	forest	-	0.163	0.780
	dry	0.163	-	0.125
	wet	0.780	0.125	-

Results shown in Table 2 indicate that the experiment conditions/living conditions of mosses have a statistically significant influence on the chlorophyll content in mosses. Due to the absence of statistically significant differences between chlorophyll *a* and *b* content in the mosses collected directly from the forest, and in those dried in laboratory conditions, an experiment was carried out in which active mosses collected directly from the forest (forest) and the ones dried in the laboratory at room temperature (dry) were exposed for a period of three months as part of active biomonitoring. A comparison of the ability of fresh and dried mosses to accumulate heavy metals (Ni, Cu, Zn, Cd, and Pb) was carried out. The diagram below in Figure 3 shows *RAF* values for the exposed mosses.

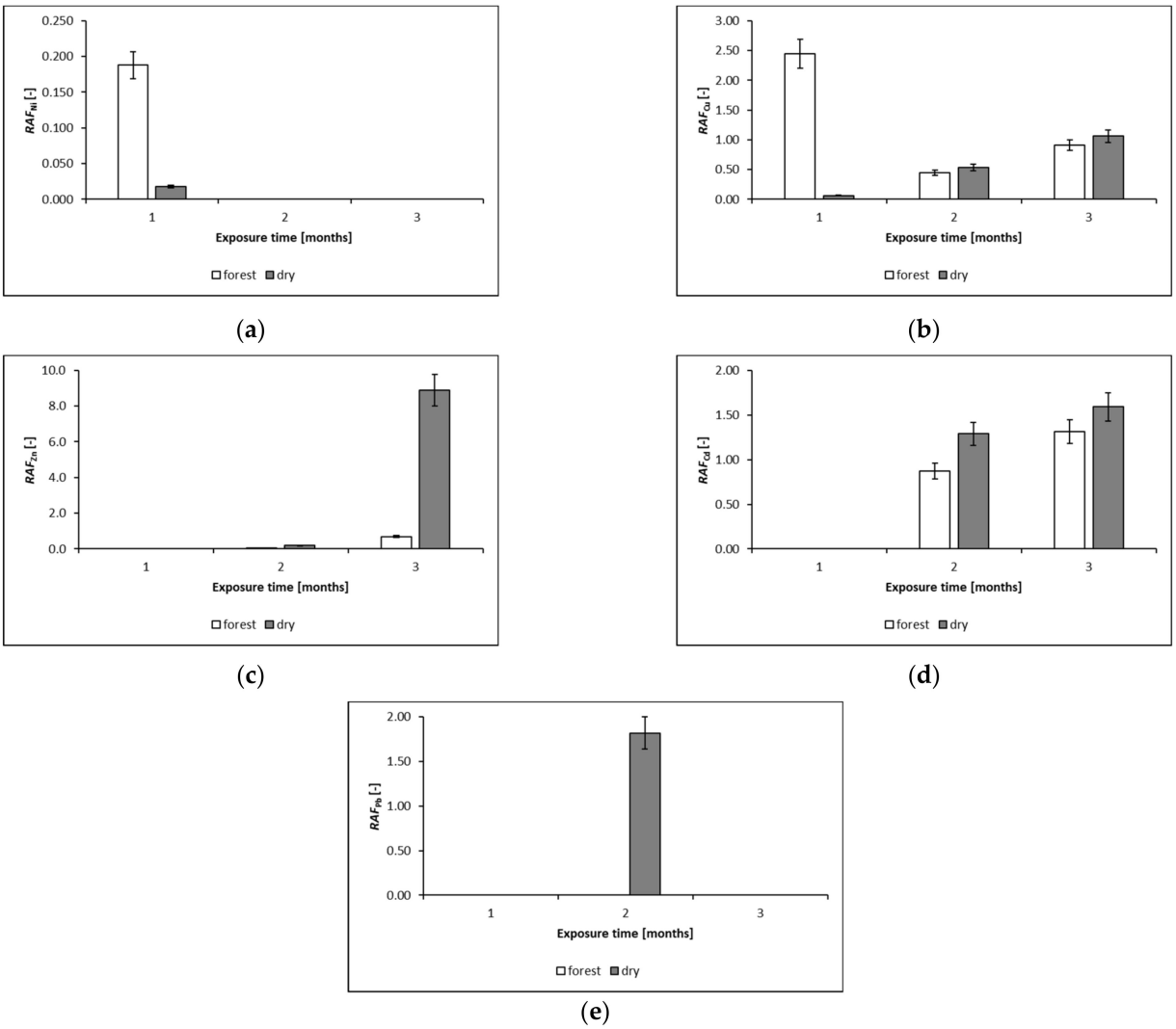

Figure 3. *RAF* values for: (**a**) nickel-, (**b**) copper-, (**c**) zinc-, (**d**) cadmium-, and (**e**) lead-exposed forest (fresh) and dried mosses. *RAF—Relative Accumulation Factors* (meaning and formula are given in Section 4.2).

The results presented in Figure 3 show that, for most of the analyzed elements, the relative metal accumulation occurs after 2–3 months, and is higher in mosses dried at room temperature: the conditions under which the mosses were before the exposure affect the level of the analyte accumulation. It has been shown that mosses as bioindicators (dried and still alive (Figure 2) of atmospheric aerosol contamination with heavy metals may accumulate analytes into their cells [29,30].

Demonstrating that dried mosses accumulate heavy metals better, and due to the lack of statistically significant differences in chlorophyll *a* and *b* content between moss samples collected directly from the forest and those dried at room temperature, moss samples dried at room temperature for up to 12 weeks of the experiment were used in order to check the influence of the exposure time and of the exposure to environmental stress caused by the presence of heavy metals in the air on the chlorophyll content and its fluorescence of their gametophytes, to verify moss as a bioindicator of pollutants as a function of time (Table 3).

Table 3. Average chlorophyll *a* and *b* content [mg/L] and actual photochemical efficiency (yield) [-] in mosses, depending on the time of their exposure.

	"0"	**1w**	**4w**	**8w**	**12w**
Chl-*a*	10.8	8.69	8.42	8.92	5.86
Chl-*b*	6.52	4.12	4.26	5.00	4.33
Yield II	0.532	0.332	0.350	0.354	0.240

"0"—mosses before exposure; 1w, 4w, 8w, 12w—chlorophyll content and fluorescence measurement after successive weeks of exposure.

As we can see in Table 3 above, the exposure of mosses to the environmental stress, such as pollution and changing weather conditions (drying), results in the chlorophyll content decreasing over time. The average chlorophyll *a* content dropped by 54.3%, whereas chlorophyll *b*—by 66.4% after 12 weeks of exposure. The calculated value of Pearson's linear correlation coefficient $r_{x,y}$ was 0.94—there was a significant correlation between chlorophyll *a* and yield (p = 0.02); the coefficient of determination R^2 was 0.89. For the correlation between chlorophyll *b* and yield $r_{x,y}$ was 0.89—there was a significant correlation (p = 0.04) with R^2 = 0.79.

3. Discussion

The structure of the body is not the only factor determining the chlorophyll concentration in the shoot of moss: it also depends on the degree of development and its age [31]. After all, the last, apical (topmost) segments represent a two-/three-year period of moss growth [32]. Other studies also confirm our results for the highest chlorophyll content in the upper parts of the shoot, as the photosynthetically active parts are mainly the upper 4 cm from the top, which constitute the living parts of the moss shoots in *Pleurozium schreberi* [33].

Optical meters are widely used to assess the chlorophyll content in the material in situ, and the measurement value varies within the species, as well as between species, due to the uneven distribution of chlorophyll in the gametophyte [34].

The diagram in Figure 2 shows a typical absorption spectrum of the green moss leaf extract containing a mixture of chlorophyll *a* and *b* [35,36]. Due to the thin leaf elements of the moss, the chlorophyll content in leaves is very low in to vascular plants [37]. We should note that, despite higher values for dry mosses, the results of the Student's *t*-test in Table 2 indicate that there are no statistically significant differences between mosses in situ and those dried at room temperature. This demonstrates that drying at room temperature does not adversely affect the chlorophyll content of mosses. The study of chlorophyll fluorescence is a valuable tool in ecophysiology, and fluorescence emission spectra are influenced by the chlorophyll content per leaf area [38]. In studies concerning moss sensitivity to prolonged simulated nitrogen deposition, small quantities of added N did not affect the chlorophyll fluorescence. Both the actual photochemical efficiency [Y (II)], as well as the maximum quantum yield (Fv/Fm) parameters were accompanied by specific quantities of chlorophyll. Therefore, the relevant chlorophyll concentration corresponded to the chlorophyll fluorescence (Figure 1 and Table 3), which was an indication of the viability of the moss species [39]. Other studies have proven that the potential rates of photosynthesis, at different depths, in layers of moss turf, were highly correlated with the chlorophyll content [40]. Therefore, a certain chlorophyll content with fluorescence measurements provides information about the vitality of the moss, as it is correlated with other values concerning its viability. The chlorophyll content is related to mosses "being alive", and is connected with its vital functions. Laboratory tests showed that a decreasing chlorophyll content with increasing Cu and Cr concentrations did not result in the maximum quantum yield PSII (Fv/Fm) changing significantly. The excess of Cu and Cr caused PSII photoinhibition; however, studies on ultrastructure and morphology of the chloroplast demonstrated that no major ultrastructural changes were observed: cells were still alive, even at the highest metal concentrations (100 µM) in solutions [41]. Other studies demonstrated that, with increasing metal concentrations and decreasing chlorophyll (as a reaction to the analyte), chlorophyll fluorescence parameters Fv/Fm

and PSII also dropped. Nevertheless, at such high concentrations as e.g., 50 µM Hg, *Eurhynchium eustegium* and *Taxiphyllum taxirameum* mosses still showed a low activity of vital functions. Moss damage caused by the analyte was not correlated with the metal accumulation level [42]. Therefore, it should be considered that the study of the chlorophyll content with fluorescence measurements reflects the viability of mosses and indicates that, during the study, mosses show characteristics of a living organism.

The heavy metal content of moss depends on such factors as contamination of the sampling site, integrity of the plasma membrane, or the type of element and its location in the cell fraction [43]. There are a number of other factors that affect the sorption of metals, but appropriate methods of moss preparation before their exposure seem to be very important [44,45]. Some metals, such as Cu, Zn, or Ni, are essential microelements for plants [46,47]. *P. schreberi* has a high capacity to accumulate especially Cu [48], hence the high *RAF* value in the first month of exposure in the moss samples collected from the forest compared to the control sample. The absence of an epidermis and cuticle makes the mosses capable of absorbing water and pollutants from precipitation that has accumulated on their surface due to dry deposition [49]. For the species under the study, the concave leaf blade causes the ionic forms of the elements dissolved in the precipitation to collect as a bottom pool in the leaf, which promotes bioaccumulation. The absorption of toxins (ectohydrity) in these parts of the leaf is due to the anatomical composition, owing to the absence of the upper protective layer: the drainage layer. Additionally, such features as leaf thickness, the spiral arrangement of the gametophyte leaves on the stem, or the shape of the leaf blade affect the intensity of bioaccumulation due to the easier possibility of and the large area of accumulation of heavy metals [50]. These elements are missing in dried samples, as partial damage resulting from drying [51] causes the analytes to deposit mainly on their surface over time (2–3 months). Furthermore, a lower initial analyte concentration in the control sample before exposure does not always result in a higher *RAF* value [52]. Calculated *RAF* values shown in the diagram in Figure 3 indicate that, for active biomonitoring studies, it is better to use dry moss samples that remain a living material when dried at room temperature. Studies confirm that living transplants generally do not accumulate more than dead material. Mosses have developed mechanisms of resistance to heavy metal contamination, such as cell wall binding of different non-toxic cations naturally occurring in the cells, cell wall thickening [53], chelating of heavy metals, or high reproductive potential [54]. Moss devitalization eliminates the metabolic contribution in the elemental intake. The oven-drying presented in the study did not significantly change the morphology or composition of moss elements [55]. Other studies confirm the thesis that drying mosses (105 °C) leading to their death did not inhibit their ability to accumulate trace elements. Drying caused damage to membranes and cell walls, thus the cell would become more permeable to heavy metals [56]. Another experiment also indicates that the devitalization of mosses does not inhibit their ability to accumulate pollutants [57]. However, according to our knowledge and the definition of biomonitoring [6], the use of dead, devitalized moss is contrary to the idea of biomonitoring, which is based on the use of living organisms. Therefore, relevant preparation techniques should be selected in order to enable the use of mosses that are still alive and able to accumulate pollutants to which they are exposed [58].

Active biomonitoring with the use of mosses focuses mainly on the determination and analysis of elements accumulated by mosses, and the indication of the sources of pollution emissions [59–62]. However, we should remember that even a short exposure causes damage to gametophyte cells [18,63], while exposure in heavily contaminated areas, or for a long time, may lead to the death of the biomonitor. After six weeks of exposure, a progressive cytoplasmic disorder was observed using a transmission electron microscope, which led to moss death [64]. Other studies have shown that, after six-week exposure, all of the moss samples were almost dead based on the chlorophyll content and fluorescence testing with TEM observations [63]. The results shown in Table 3 confirm that mosses are homochlorophyllous desiccation-tolerant plants [65,66]. The decrease in the chlorophyll

content is the result of the progressive moss contamination, and the exposure of mosses to other stress factors that cause their death. Most analytes deposit on the moss surface [54,67]. The deposition of pollutants on their surface also results from secondary enrichment of the atmospheric aerosol, with pollutants carried by the wind from the soil [68]. The fact that mosses maintain, for the period under study (three months during a period of changing weather conditions), their vitality and ability of continuous sorption of pollutants from the air may be associated with them entering the state of cryptobiosis: throughout their whole life cycle, they are able to vegetate in this way for a very long time [69,70]. However, the reference quoted in the introduction [28] is the first work in the literature on moss biomonitoring that concerns the possibility of moss survival in cryptobiosis in the context of changing vitality. Mosses exposed to PAHs and during the dehydration experiment maintained a minimal photochemical activity up to 80 min. There are no other publications in the literature concerning the moment of mosses' transition into cryptobiosis, and the fact of remaining in it during the exposure to contaminants.

4. Materials and Methods

4.1. Material

The species used for this study were the moss *Pleurozium schreberi* Mitten (Pl). They were collected/tested in situ between April and July 2020 from forests in Świętokrzyskie Voivodship, Poland. Moss samples were taken and prepared before exposure as part of active biomonitoring, in accordance with the guidelines [14,71]. Mosses were collected at least 5 m away from the canopy of the trees, so as to not be directly exposed to precipitation (only the green parts of moss were taken) [71].

4.2. Methods

The presented study was divided into separate experiments. During the first phase, the homogeneity of moss gametophytes in terms of their chlorophyll content was evaluated. The study was carried out using the CCM-300 portable chlorophyll content meter from Opti-Sciences, Inc. (Hudson, NH, USA). The analysis was completed in the vertical profile [in 10 sites—segments [72]—10 shoots of moss] (Figure 1). Each measurement was taken in 10 repetitions in order to determine the location on the gametophyte that had the highest chlorophyll content. Chlorophyll fluorescence of photosystem II (actual photochemical efficiency (yield)) was also measured through this method, using the modulated portable fluorometer (Opti-Sciences, Hudson, NH, USA) under ambient light [73]. Mosses were collected in the field, at noon time. Measurements were made with 10 replicates. Relative humidity ranged from 24 to 36%, and the temperature from 20 to 25 °C during the measurements. The data shown in the table are average values from these measurements (Table 1). These measurements were then used to select the appropriate fragment of the shoot of moss for analysis with a spectrophotometer.

In the first experiment, the influence of external conditions affecting mosses on the chlorophyll content in their gametophytes was analyzed. Mosses in situ (forest), mosses dried in the laboratory at room temperature (dry), and mosses dried in the laboratory at room temperature and sprayed with deionized water one hour before the analysis (wet) with a conductivity of 3.581 μS/cm were used in the study. The chlorophyll content in mosses was measured using a Cary 3500 UV-Vis Compact Peltier spectrophotometer from Agilent Technologies (Santa Clara, CA, USA). For analyses on the spectrophotometer, mosses (0.1 g) were divided into small pieces and ground in a porcelain mortar with 5 mL acetone (pure). The centrifuge (MPW-351RH, MPW Med. Instruments, Warsaw, Poland) was first cooled, and then samples were centrifuged in it (10 min, 10,000 rpm). Each measurement was performed in five repetitions. Chl *a* and chl *b* analytical standards (ChromaDex, Los Angeles, CA, USA, certified dye content > 97%) were purchased in liquid form. Based on the obtained absorbance values, the chlorophyll concentrations were calculated using the extinction coefficients and

equation [74] using a spectrophotometer at two wavelengths, 662 and 645 nm, for maximum absorption of chlorophyll-*a* and -*b*, respectively.

$$\text{Chlorophyll-}a = 11.75A_{662} - 2.35A_{645} \quad (1)$$

$$\text{Chlorophyll-}b = 18.61A_{645} - 3.96A_{662} \quad (2)$$

The second experiment was the three-month exposure as part of active biomonitoring of the mosses collected directly from the forest and dried in the laboratory at room temperature (dry). Then, 3 g of mosses were packed into nets, and exposed at a height of about 1.50–2.00 m above ground level. The mosses were exposed on the premises of the Institute of Environmental Engineering and Biotechnology, University of Opole, Opole, Poland, near a public road in use. Each month, samples were collected, and heavy metal concentrations were measured. The study was carried out to compare the ability of fresh and dried mosses to accumulate heavy metals (Ni, Cu, Zn, Cd, and Pb). In order to determine the heavy metals, each moss sample, with a mass of 1.000 ± 0.001 g dry mass (d.m.), was mineralized in a mixture of nitric acid (V) and hydrogen peroxide (HNO_3 65%: H_2O_2 37% = 10:6 mL) using a Speedwave Four Berghof, DE microwave oven. The mineralization process was carried out at a temperature of 180 °C. Heavy metals were determined using an atomic absorption flame spectrometer (F-AAS) type iCE 3500 (series 3000), manufactured by Thermo Scientific, USA.

In Table 4, the instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the spectrometer iCE 3500 are presented. The results were converted into 1 kg of sample. Calibration of the spectrometer was performed with a standard solution from ANALYTIKA Ltd. (CZ). The values of the highest concentrations of the models used for calibration (5 mg/dm^3 for Ni, Cu, Zn, Pb, and 2 mg/dm^3 for Cd) were approved as linear limits to signal dependence on concentration. Concentrations of metals were determined in solution after mineralization and dilution, and were filtered into volumetric flasks of 25 cm^3.

Table 4. The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the spectrometer iCE 3500 [mg/dm^3] [14,75].

Metal	*IDL*	*IQL*
Ni	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

In Table 5, concentrations of heavy metals in certified reference materials BCR-482 *lichen*, produced at the Institute for Reference Materials and Measurements, Belgium, are shown.

Table 5. Comparison of measured and certified concentrations in BCR-482 *lichen* [14].

Metal	BCR-482 *lichen*		AAS (n = 5)		*Dev.* **
	Concentration	Measurement Uncertainty	Average	±*SD* * of the Concentrations	
	[mg/kg d.m.]				[%]
Ni	2.47	0.07	2.16	0.32	−13.0
Cu	7.03	0.19	6.63	0.17	−5.70
Zn	101	2.20	95.1	2.30	−5.50
Cd	0.56	0.02	0.53	0.03	−5.30
Pb	40.9	1.40	38.2	1.00	−6.60

* *SD* standard deviation. ** relative difference between the measured (c_m) and certified (c_c) concentration $100\% \cdot (c_m - c_c)/c_c$.

The *RAF—Relative Accumulation Factors* was used to determine increases of concentrations of the analytes in the exposed mosses samples, as defined in [76]:

$$RAF = \frac{C_{i,1} - C_{i,0}}{C_{i,0}} \quad (3)$$

where: $C_{i,1}$ is the concentration of an analyte after exposure period [mg/kg d.m.], and $C_{i,0}$ is the concentration of an analyte before exposure period [mg/kg d.m].

In the last experiment, the chlorophyll content of dried moss samples (dry) exposed in active biomonitoring for 3 months was analyzed. The mosses were exposed in the car park of the Institute of Environmental Engineering and Biotechnology, University of Opole, Opole, Poland. Measurements using a Cary 3500 UV-Vis Compact Peltier spectrophotometer from Agilent Technologies (USA) were carried out at monthly intervals; measurements of fluorescence chlorophyll were also repeated in this experiment.

Microsoft Excel 2016 and STATISTICA ver. 13.3 software were used to process the data. Shapiro–Wilk's test was used to assess the normality of the variances. The Student's *t*-test and Mann–Whitney U test ($p < 0.05$) were also used to assess the statistical significance of the influence of conditions on the chlorophyll content in mosses. Correlation analysis was calculated to determine the statistical significance of the relationship between chlorophyll-a and chlorophyll-b content, and the actual photochemical efficiency (yield) of photosystem II.

5. Conclusions

In moss biomonitoring studies, it is often forgotten that, by definition, a bioindicator is a living organism, not a chemical adsorbent [77,78]. Therefore, an important role should be attributed to the fact that during the study, or at least before the exposure, moss should be a living matrix: it should be subjected only to the preparatory methods that will not lead to its death. Therefore, and according to the definition of biomonitoring, it is necessary to exclude devitalization that would make moss only a dead adsorbent of analytes.

Active biomonitoring should be carried out with the bioindicator that remains alive (according to its definition as a living organism), and moss exposure for three months, as shown by the presented results; despite causing a decrease in the chlorophyll content in the gametophytes, this does not lead to death of the bioindicator. This parameter was also related to its vital activity in the form of actual photochemical efficiency (yield) of photosystem II. It remains an organism able to accumulate contaminants due to the preserved viability. In the future, biomonitoring studies should pay attention to the control of moss viability during experiments and observations of the transition into the state of cryptobiosis.

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The influence of environmental conditions on the lifespan of mosses under long-term active biomonitoring

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ABSTRACT

Biomonitoring with living organisms is most often used in order to gather quick information about environmental quality – for example the level of heavy metals polluting the air. Experiments are not often performed when the bioindicator is exposed to pollutants over the long time (6 months and more) because this period required to obtain credible results and the risk not yet studied of physiological changes and of degeneration of the living material which would undoubtedly affect the biomonitoring results. The aim of study was to evaluate the physiological condition of *Pleurozium schreberi* moss species subjected to continuous exposure for a year, using the moss-bag method, under variable environmental conditions, including atmospheric aerosol contaminated by selected analytes: Cu, Zn, Cd, Hg, Pb determined by flame atomic absorption spectrometer. AMA apparatus was used to measure mercury. The survival of the moss during exposure was assessed based on the measurement of chlorophyll content. As a result of the research, it was found that the concentrations of metals in mosses increased depending on the exposure time and the certain element. It was also found that mosses, despite the long period of exposure to environmental factors and the decreasing content of chlorophyll, still revealed the features of a living organism during the study and can be used in long-term biomonitoring of the environment. It was also proposed to correct the period and duration of *P. schreberi* exposure to air pollution in relation to the previous literature and recommend the period of 3–6 months.

1. Introduction

Air pollution is a constant problem both on a global and local scale. However, the impact changes over the years (Godzik, 2020; Lequy et al., 2017). One of the ways to identify sources of pollution and monitor trends in atmospheric aerosol contamination is to use mosses as biomonitors (Gerdol et al., 2014; Kapusta et al., 2019; Mahapatra et al., 2019; Szczepaniak and Biziuk, 2003). There are ongoing attempts to standardize biomonitoring methods using mosses (Ares et al., 2012; Fernández et al., 2015) in order to optimize research and the possibility of correlating the results with instrumental analyses (Lazić et al., 2016; Vuković et al., 2013b). One of the more frequently described/studied parameters in the moss-bag method of active biomonitoring is the exposure time (Ares et al, 2012, 2014; Capozzi et al., 2016). This influences, among other things, the amount of accumulated heavy metals deposited from the air (Aničić et al., 2009a; Zinicovscaia et al., 2018), or the degree of viability of the species during the experiment (Capozzi et al., 2020; Tretiach et al., 2007).

The volume of analytes accumulated by the moss negatively affects the plant itself (Krzesłowska et al., 2009; Wells and Brown, 1995), e.g., affecting the chlorophyll content (Shakya et al., 2008), which may eventually lead to death (Adamo et al., 2007; Tretiach et al., 2007). It should be remembered that, during exposure, moss is not only an absorbent of chemical pollutants, but an organism which, as a bioindicator of environmental quality in relation to e.g., heavy metals, according to the definition should be alive (Boquete et al., 2017; Markert, 2008). One way to determine moss viability during a study is to measure chlorophyll content (Davies, 2007; Zhang et al., 2016), which is related to other parameters and vital functions (Carvalho et al., 2019; Vannini et al., 2020; Gaberščik and Martinčič, 1987; Hang, 2007; Shortlidge et al., 2017). Therefore, the control and measurement of chlorophyll content and related parameters seems to be fundamental to,

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and very important in biomonitoring (Capozzi et al., 2020; Świsłowski et al., 2021, 2020), especially when moss is exposed to adverse environmental conditions (Choudhury and Panda, 2005; Oliver et al., 2000; Sujetovienė and Galinytė, 2016). One example is the Antarctic moss *Grimmia antarctici* subjected to freezing stress, causing it to reduce the efficiency of photosystem II, which may be due to conformational changes in the protein-pigment complexes. However, reversible photoinhibition indicates the existence of processes that protect against such damage in low temperature environments (Lovelock et al., 1995). Photoperiodism/the effect of light is also essential as low light levels correlate with high chlorophyll contents and high rates of photosynthesis (McCall and Martin, 1991). In turn, specific calcium-permeable channels in the plasma membrane are responsible for the thermotolerance of *Physcomitrella patens* moss. A mild increase in temperature induces a moss response to the thermal shock, which is dependent upon the passage of calcium ions across the plasma membrane in advance (Saidi et al., 2009). In contrast, the drought-resistant *Grimmia laevigata* moss, in the absence of potential photodamage, can completely retain its chlorophyll throughout a dry period of at least 20 months (Alpert, 1988).

Research on active biomonitoring using the moss-bag technique is characterized by extremely long time spans (exposure time), as indicated by the literature; the exposure of mosses to air pollution can last, for example, several days (Cesa et al., 2014), four weeks (Demková et al., 2017; Tremper et al., 2004), two months (Culicov et al., 2016; Milićević et al., 2017), 12 weeks (Dmuchowski and Bytnerowicz, 2009; Giordano et al., 2009) or even six months (Aničić et al., 2009a; Salo, 2014; Sergeeva et al., 2021). There are few studies describing the exposure of mosses over a longer period (Ares et al., 2012; Saitanis et al., 2013). Due to the lack of studies in the literature dealing with the subject of long-term (6 months and more) active biomonitoring using the moss-bag method combined with the measurement of moss vitality parameters, it was considered as interesting, important and complementary to the existing research in this field.

The aim of the research was: (I) to analyse the influence of selected environmental factors on the viability of mosses during long-term exposure; (II) to determine the accumulation potential of *P. schreberi* mosses during continuous, active biomonitoring for a year, using the moss-bag method in relation to selected heavy metals and (III) to assess the influence of the contaminants on the content of chlorophyll in mosses.

2. Material and methods

2.1. Material

The species used for this study were the moss *P. schreberi*. They were collected in July 2019 from forests in the Świetokrzyskie Province, PL.

2.2. Methods

Moss samples were taken and pretreatment before exposure as part of active biomonitoring in accordance with the guideline (ICP Vegetation, 2020). Due to the significant impact of washing the samples (Adamo et al., 2008; Dołęgowska et al., 2017) the pre-exposure mosses were prepared in accordance with a previously-developed methodology specific for mosses (Świsłowski et al., 2021). It should be remembered that the time and method of storing mosses can have a negative impact on the results of the study (Dołęgowska and Migaszewski, 2020). Next, 4 g of mosses were packed into 12 nylon nets and displayed in flat bags (García-Seoane et al., 2019) at an altitude of 1.50–2.00 m from the ground for a period of 12 months (July 10, 2019–July 10, 2020). The samples exposed for the year were exposed to different types of contamination by heavy metals resulting, inter alia, from the heating season in Poland (November 2019–April 2020) and the non-heating periods (July–October 2019 and May–July 2020). The mosses were located in the centre of Opole (Opolskie Province, PL). One bag was removed each month and the concentration of heavy metals and chlorophyll content were measured. The result for a given month consists of 1–3 moss sub-samples.

In order to determine the heavy metals, each moss sample, with a mass of 1.000 ± 0.001 g dry mass (d.m.), was mineralised in a mixture of nitric acid (V) and hydrogen peroxide using a Speed wave Four Berghof, DE microwave oven. The mineralisation process was carried out at a temperature of 180 °C. Heavy metals (Cu, Zn, Cd and Pb) were determined using an atomic absorption flame spectrometer (F-AAS) type iCE 3500 (series 3000) made by Thermo Scientific, USA. Concentrations of metals were determined in solution after mineralisation and dilution and were filtered into volumetric flasks of 25 cm^3. The results were converted into 1 kg of sample. Calibration of the spectrometer was performed with a standard solution from ANALYTIKA Ltd. (CZ). The values of the highest concentrations of the models used for calibration (5 mg/dm^3 for Cu, Zn, Pb, 2 mg/dm^3 for Cd) were approved as linear limits to signal dependence on concentration. The concentration of mercury in the samples (0.04 g ± 0.001 g d.m.) was determined with AMA 254 mercury analysers from Altec Ltd., CZ. The results were in ppm. The *RAF - Relative Accumulation Factor* was used to determine increases in analyte concentrations in the exposed moss samples (Zinicovscaia et al., 2018).

The chlorophyll content in mosses was measured using a Cary 3500 UV–Vis Compact Peltier spectrophotometer from Agilent Technologies (USA). For analyses on the spectrophotometer, mosses (0.1 g) were divided into small pieces and ground in a porcelain mortar with 5 mL pure acetone. Centrifuge (MPW-351RH, MPW Med. Instruments, PL) was first cooled, and then samples were centrifuged in it (10 min, 10,000 rpm). Each measurement was made in 5 replicants. Chl *a* and chl *b* analytical standards (ChromaDex, USA, certified dye content >97%) were purchased in liquid form. Based on the obtained absorbance values, the chlorophyll contents were calculated using the extinction coefficients and equation (Lichtenthaler and Wellburn, 1983) using a spectrophotometer at two wavelengths, 662 and 645 nm, for maximum absorption of chlorophyll-*a* and -*b*, respectively. The results were converted into 1 kg of sample.

$$\text{Chlorophyll-}a = 11.75A_{662} - 2.35A_{645}$$

$$\text{Chlorophyll-}b = 18.61A_{645} - 3.96A_{662}$$

The response data have a gradient 0.3 SD units long, so linear method was applied, namely Principal Component Analysis (PCA). To show the explanatory power of environmental variables and moss time exposure on the moss viability traits, the interactive forward selection of explanatory variables was implemented. The log-transformation was conducted for standardizing the response variables and further computing was launched without down-weighting the rare cases. In order to test the axes significance, the Monte Carlo permutation test was executed with 499 runs, and only predictors with a significance of $p < 0.05$ were included in the RDA model. Canoco for Windows 5 was used for all ordinations (Smilauer and Leps, 2003; ter Braak and Šmilauer, 2002). STATISTICA ver. 13.3, MS Excel 2016 software were used to process the numerical data. Shapiro Wilk's test was used to assess the normality of the variances. Student's t-test ($p < 0.05$) were also used to assess the significance of statistical difference of heavy metal concentrations in specific months compared to the control sample. Spearman's rho test was used (Campbell et al., 1970) to analyse the correlations between chlorophyll content and heavy metal concentrations. A step-by-step selection of variables was also carried out to assess the impact of individual environmental conditions on the mosses' viability during the study. The source of data of environmental conditions was the Institute of Meteorology and Water Management (Institute of Meteorology and Water Management, n.d.).

Based on this work (Ares et al., 2012), which is a large literature review on the moss-bag technique, its continuation was made by

checking what moss exposure time is used in current research using this method. We used selected 50 published articles containing the term "moss bag technique" between 2012 and 2020, located through SciVerse SCOPUS and Springer. The results are given in Table in the supplementary material (SM).

3. Results

When analysing the results of the research, the impact on the viability of *P. schreberi* of selected factors was first assessed, including the duration of moss exposure time in relation to changing environmental conditions such as wind and temperature (Table 1, Fig. 1).

Based on the data in Table 1, it should be stated that the most important variable explaining the condition of the moss (metal accumulation, chlorophyll content) is temperature (so season of the year) that explains as much as 60% of the variability in the samples. However, the second most important factor is the time of exposure of moss. The youngest and the oldest samples have the greatest explanatory power. The third factor is the heating season, with an impact of 3%. The results of Principal Component Analysis (PCA) show the influence of environmental variables (temperature; heating season, wind) on the viability of mosses. The total variation is 47.81, and the described cumulative variation accounts for 99.73% of this. On the other hand, Fig. 1 shows that chlorophyll content is strongly correlated with the non-heating season and temperature, while high concentrations of metals clearly correlate with the heating season, and lead (Pb) itself mainly with the level of windiness.

During partitioning, the variables were divided into 3 groups and analysed after eliminating the relationships between them: group a: climatic factors (wind, precipitation, temperature, humidity); group b: moss exposure time; group c: pollution (heating season and metals). Fig. 2 shows a visualization of these groups.

Next, testing of these groups was performed as shown in Table 2.

The b + d + e + g group, i.e., those responsible for time exposure, is by far the most important. Thus, the chlorophyll content is significantly affected by the time of the moss exposure, many times more than by the environment and pollutants. The moss is alive, but its condition is determined mainly by the month of exposure, not the metal concentration itself. Climatic variables (group a+d + f + g) affect the condition of chlorophyll slightly more than contamination with analytes at the level determined in the research area - temperature is certainly the greatest influencer. Finally, with the result of F = 26.6 come the pollutants, where the most important variable is the lack of heating season, NG.

Heavy metal concentrations determined in *P. schreberi* moss samples after successive months of exposure are presented in the diagram in

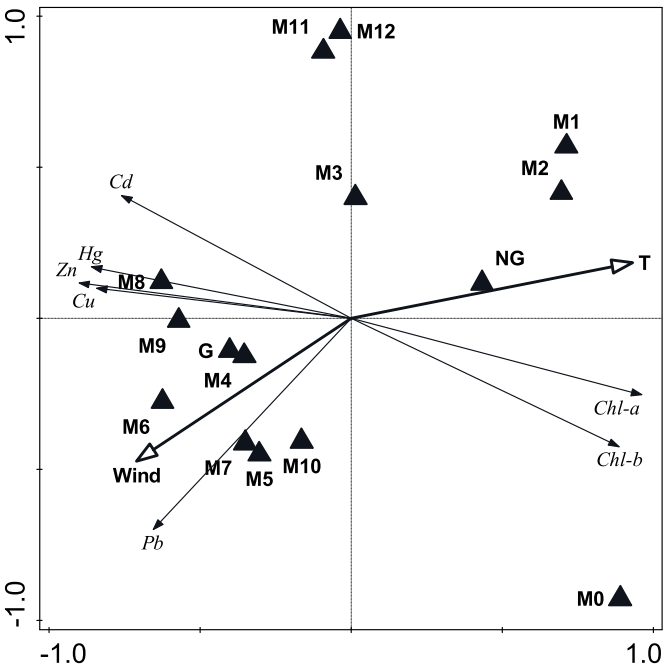

Fig. 1. The relationships between variables and samples. Explanation – see Table 1.

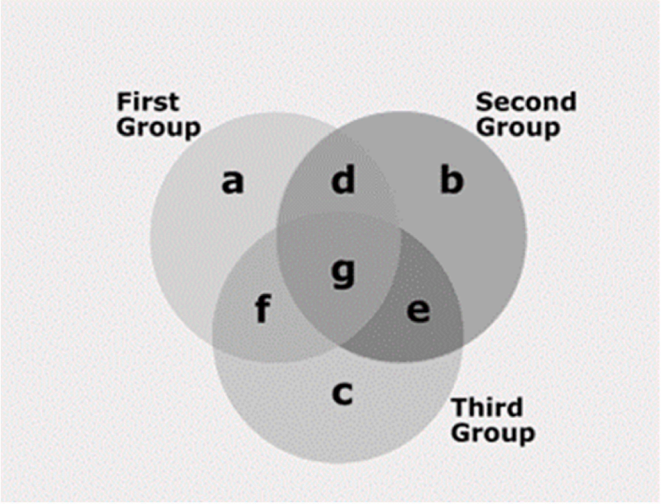

Fig. 2. Grouping of variables. First Group a: climatic factors (wind, precipitation, temperature, humidity); Second Group b: moss exposure time; Third Group c: pollution (heating season and metals).

Table 1
Results of analysis 'Interactive-forward-selection'.

Name	Explains [%]	Contribution [%]	pseudo-F	p
T	60.6	62.6	38.4	0.002
Time.M0	17.5	18.1	19.2	0.002
Time.M11	3.7	3.8	4.6	0.01
Time.M12	3.7	3.8	5.6	0.01
Season.G	3	3.1	5.4	0.004
Season.NG	3	3.1	5.4	0.004
Time.M7	2.4	2.5	5.2	0.006
Time.M4	2.3	2.4	6.4	0.012
Time.M5	1.7	1.8	6.2	0.006
Time.M10	0.8	0.8	3	0.064
Time.M6	0.6	0.6	2.4	0.106
Wind	0.4	0.4	1.8	0.192
Time.M3	0.2	0.2	0.9	0.344

T-temperature, Time.M0-moss control test, Time.M1-M12 – time of moss exposure after consecutive month of analysis from the first to the twelfth, Season.G - heating season (October–April), Season.NG - non-heating season (May–September).

Table 2
Variation Partitioning Results for Three Groups in Analysis 'Var-part-3 groups-Simple-effects-tested' Significance tests.

Tested Fraction	F	p
a+b + c + d + e + f + g	5294	0.002
a+b + d + e + f + g	11,667	0.002
a+c + d + e + f + g	63.5	0.002
b + c + d + e + f + g	5294	0.002
a+d + f + g	30.7	0.002
b + d + e + g	11,667	0.002
c + e + f + g	26.6	0.002

Figure in the supplementary material (SM).

The test results presented in the graphs in Fig. SM a) -d) in the SM file indicate changing air pollution depending on the element and the month of the study. In the case of copper, increasing changes in concentration can be noticed, where the highest concentration of this analyte was determined in the 9th month of the experiment (24.0 mg/kg d.m.). For the next 12 weeks, this concentration decreased. In the case of zinc, a similar trend can be observed as for Cu: increasing concentration up to a certain time (9 months) and then a slight decrease (last 3 months). In turn, the concentration of lead in the first months was below the concentration of the control sample, then it increased to its highest value in the 6th month of exposure (average - 78.4 mg/kg d.m.) and then also decreased to a concentration of 10.7 mg/kg d.m. In the case of cadmium, increases were observed in each month of the experiment. The last result for this element is 1.10 mg/kg d.m. Compared to the control sample with a value of 0.26 mg/kg d.m. Fig. SM d) shows the concentration of mercury in mosses. Hg accumulation is similar to that of Cu and Zn.

Table 3 shows the percentage changes in the content of the determined elements in successive months of exposure, comparing the results of the concentrations for a given month with the previous month.

The percentage changes in concentrations shown in Table 3 indicate that for all elements over a half-year exposure period, an increase in concentrations can be observed from month to month.

Table 4 below shows the levels of statistical significance of heavy metal concentrations in individual months of exposure in relation to its control sample, the coefficient of variation *CV* of the samples with the exposure time and the relative accumulation ratio *RAF*.

The results from Table 4 show that for most metals and successive months of the experiment, the statistical significance of *p* of the Student's t-test was most often at the level of <0.01, which indicates significant differences between the control sample and successive months of exposure, and thus also real increases in analyte concentrations (confirmed also by designated *RAF* values). The *CV* for copper was the lowest after the first four weeks of the experiment. The lowest *CV* values for zinc and cadmium were recorded in the 11th month and for lead it was the 10th month of exposure. It was not possible to determine the *CV* coefficient for the samples exposed until the last month of the experiment due to it being possible only to measure metals in a single sample, likewise with Hg - the amount of moss remaining for the analysis of this analyte allowed for a single measurement only. There is no strict correlation with the percentage variation in concentrations with respect to the exposure time. The highest increases were observed in the last months of exposure, with the *RAF*s for cadmium being high throughout the entire study period (values above 1.00), where the highest value was recorded in the last month. In turn, a larger *RAF*, but in the 11th month of the study, was determined for mercury.

Fig. 3 shows the changes in chlorophyll absorbance depending on the consecutive months of exposure of mosses to environmental stress.

As can be seen in Fig. 3, the absorbance values changed over the course of the experiment. There is no correlation regarding the impact of subsequent exposure periods on the decreasing absorbance values, because, for example, the ninth month sample (III/IV) is characterized by the lowest values among all in relation to the 10 month sample (IV/V) [represents the highest values]. In turn, the absorbance for the sample exposed for 11 months (V/VI) is almost the same as for the sample from the last four weeks (VI/VII). The characteristics of the discussed changes and differences are presented in Table 5, which presents the chlorophyll content -*a* and -*b* in mosses in individual months of the experiment.

As shown by the data in Table 5, after three months of exposure (IX/X), the chlorophyll content decreased by as much as 83% and 77.5% for chlorophyll-*a* and -*b*, respectively. In the following months of exposure, the content of chlorophyll decreased and increased alternately. The lowest loss of chlorophyll-*a* was recorded in the ninth month - III/IV (89.8%) and for chlorophyll-*b* in the 8th month - II/III (89.9%). After one-year of exposure (VI/VII), the moss samples had a chlorophyll content of 16.2% compared to the control sample. Throughout the experiment, despite a significant decrease in the photosynthetic dye, the content of chlorophyll-*a* and -*b* in mosses was determined. Comparing the concentration values of heavy metals with chlorophyll-*a* content during the studied period, the correlation (r_s) for only Zn and Hg is: 0.586 and −0.622 respectively. No other correlation was identified for the remaining analytes nor for chlorophyll-*b*.

4. Discussion

The influence of environmental factors has a significant impact on the life of plants (including mosses) (Van Gaalen et al., 2007). For *P. schreberi* moss, various experiments has revealed the significant influence of temperature on the growth performance (Jägerbrand et al., 2014). In the same species, exposure to variable (respectively low and high) temperatures resulted in a change in the Fv/Fm value as a response to stress (Jägerbrand and Kudo, 2016). Moreover, the photosynthetic apparatus of moss seems to be optimized for operation at moderately high temperatures, given that warming may even induce the activity of some enzymes involved in the production of chlorophyll (Perera-Castro et al., 2020; Rastogi et al., 2020). At the same time, it should be remembered that both the appropriate temperature and light intensity affect the photosynthesis rate in mosses (Haraguchi and Yamada, 2011; Tuba et al., 2012) and the seasonal variability of chlorophyll content may be related to the environmental requirements of a given species (Hyyryläinen et al., 2015). The dependencies of the influence of a given temperature on the viability of moss (including chlorophyll content) are also important in other plants (Janssen et al., 1992; Zhao et al., 2020). Recent reports indicate that, in order to perform photosynthesis in plants, apart from light, thermal energy is also needed (Zubik et al., 2020), which may confirm that temperature, correlating with the rate of photosynthesis, affects the chlorophyll content, which is also confirmed by the results of our research. It has also been proven that the aging of the plant (the age of its segment) negatively affects the rate of photosynthesis (Ross et al., 1998) and the correlation between the age of mosses, the vitality of their twigs and metal tolerance has been demonstrated (Wells and Brown, 1995). In our research, we find that, the effect of moss exposure time or the effect of its aging on the chlorophyll content, which is important in active biomonitoring exposure, in order to take into account how the moss was stored before exposure and for how long (Dołęgowska and Migaszewski, 2020).

The results of heavy metal concentrations (considering only the 3-month exposure time) obtained during the discussed studies are comparable to the results of the studies carried out in June–August 2020 (Swislowski et al., 2020). This indicates a similar level of air pollution in the city of Opole over the last two years, the repeatability of the pretreatment procedure before exposure (Adamo et al., 2008; Świsłowski

Table 3
Percentage changes in heavy metal concentrations (increases, falls) compared month to month [%].

Element/time	0/1	1/2	2/3	3/4	4/5	5/6	6/7	7/8	8/9	9/10	10/11	11/12
Cu	−0.13	14.2	19.1	1.27	20.8	19.4	14.6	−0.197	3.93	−2.76	−0.163	−8.35
Zn	0.53	23.3	17.2	9.32	12.8	10.8	−1.44	5.50	3.67	−1.08	−0.986	−8.61
Cd	128	8.65	4.3	1.30	14.2	2.58	−2.67	24.8	5.94	3.69	−20.9	30.2
Hg	48.2	16.3	14.0	23.5	30.2	11.8	7.10	9.02	12.7	−17.7	11.4	−13.7
Pb	−76.5	4.29	31.0	312	10.5	75.6	−23.1	−21.8	2.45	−1.45	−73.0	−25.9

Table 4
P significance levels in individual months, *CV* [%] and *RAF* [-] values for metals accumulated by *P. schreberi* moss.

Element	Cu			Zn			Cd			Hg		Pb		
Parameter/Time [months]	P	*CV*	*RAF*	p	*CV*	*RAF*	p	*CV*	*RAF*	p	*RAF*	p	*CV*	*RAF*
VII/VIII′19	0.99	2.90	n.a.	0.95	3.75	0.005	***	2.39	1.28	0.18	0.48	**	3.32	n.a
VIII/IX	0.08	4.53	0.14	*	0.87	0.24	***	6.87	1.48	0.09	0.72	**	23.1	n.a
IX/X	*	5.86	0.36	*	5.35	0.45	***	5.00	1.58	0.06	0.97	**	16.7	n.a
X/XI	*	4.85	0.38	**	7.61	0.59	***	5.72	1.62	*	1.43	0.16	20.7	0.32
XI/XII	**	7.73	0.66	**	5.11	0.79	***	2.96	1.99	*	2.16	*	12.2	0.46
XII/I′20	**	9.24	0.98	**	4.78	0.99	***	1.11	2.06	**	2.53	**	16.7	1.57
I/II	***	1.53	1.27	**	8.36	0.96	***	2.74	1.98	**	2.78	**	3.95	0.98
II/III	***	2.99	1.27	**	4.41	1.06	***	1.46	2.72	**	3.13	*	9.48	0.54
III/IV	**	10.2	1.36	**	6.05	1.14	***	2.76	2.94	**	3.65	*	6.59	0.58
IV/V	***	3.26	1.29	***	0.68	1.12	***	5.36	3.09	**	2.83	*	1.33	0.56
V/VI	**	6.79	1.29	***	0.03	1.10	***	0.96	2.23	**	3.26	0.07	2.49	n.a
VI/VII	**	n.d.	1.10	**	n.d.	0.92	***	n.d.	3.21	**	2.68	**	n.d.	n.a.

Statistical significance p at the level * <0.05, ** <0.01, *** <0.001; n.a. - no accumulation versus control (*RAF* not calculated); n.d. – no date.

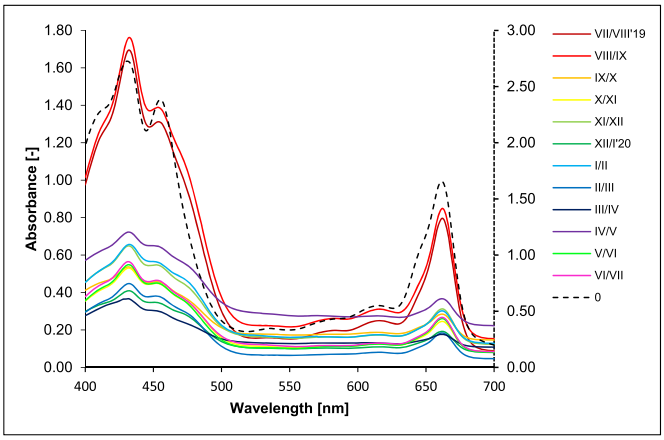

Fig. 3. Average absorbance values for the chlorophyll extracted from mosses over the course of the experiment; the dashed line symbolizes the absorbance for the control sample 0 (right OX axis); the remaining lines show the changes in absorbance in the following months of the experiment: VII/VIII '19 means 1 month of exposure.

et al., 2021) and the effectiveness and comparability of the moss-bag technique method (Aničić Urošević and Milićević, 2020). Urban space is generally a source of various pollutants; the concentration of lead in mosses may be caused by traffic or the influence of the heating season (low emission) on changes in the concentration of this analyte during the experiment (Capozzi et al., 2019; Świsłowski et al., 2020). The varied level of contamination of the Pb study area may also be influenced by distant point sources of pollution difficult to estimate, e.g., a cement plant that incinerates its waste on site, which may increase the emission of the analysed elements, transported on the wind over long distances. The range and intensity of the impact of line and point emission sources is influenced, among other things, by wind direction and strength (Kłos et al., 2011). The presence of Cu and Zn are a product of exhaust and non-exhaust traffic emissions, and road traffic (Iodice et al., 2016; Urošević et al., 2017). The concentrations of the majority of heavy metals, as assumed, showed linear increase over time, which is confirmed by other studies (Aničić et al., 2009b; Saitanis et al., 2013; Tremper et al., 2004). However, it should be taken into account that there may be some seasonal changes in the chemical composition of mosses (Klavina et al., 2018; H. Salo et al., 2016a; 2016b; Hanna Salo et al., 2016a; 2016b; Saxena and Arfeen, 2010), which results from the influence of meteorological conditions at the research site and leaching and/or volatilization processes (Ares et al., 2015; Čeburnis and Valiulis, 1999; Saitanis et al., 2013; Vuković et al., 2013a) and Fig. in SM). *RAF* values higher than 0.500 indicate insignificant elemental enrichment in the moss, and t values higher than 1.00 indicate significant elemental enrichment (Vuković et al., 2017). In our research, such *RAF* values for all elements were achieved after six months of exposure. For cadmium, significant increases in concentration were achieved throughout the entire experiment period. Two times higher *RAF* values for Cu and Zn for this moss species were obtained by conducting research in the same province, but close to the motorway, which indicates a higher level of land pollution and a significant impact from traffic (Konopka et al., 2019). Therefore, Opole is a relatively little polluted city, relative to *RAF* values of strictly industrial areas in Upper Silesia (Kosior et al., 2018).

As in the previous experiment (Swislowski et al., 2020), the content of chlorophyll decreased over time and there was no, or very weak correlation between the concentration of heavy metals with the chlorophyll content in mosses. This indicates the complexity and stronger influence of other factors on the content of chlorophyll in mosses, including generally-understood environmental stress, meteorological conditions, climatic factors and interactions with other airborne contaminants (Tremper et al., 2004; Urošević et al., 2017). In moss transplants, it was found that only when the threshold concentration of the analyte is reached, the level of chlorophyll decreases (Varela et al., 2013). In our research, a stable level of chlorophyll content was achieved after three months of exposure, which may be caused, for example, by the moss turning into a state of cryptobiosis (Capozzi et al., 2020) or acclimatization to environmental stress conditions. There are a number of publications that indicate the use of devitalized samples (Capozzi et al., 2016; Fernández et al., 2009; González et al., 2016), however, emphasis should be placed on the use and measurement of vital signs during biomonitoring studies, in other words, according to the definition of bioindicator and biomonitoring, about conducting research on living material (Kalaji et al., 2016; Markert, 2008; Swislowski et al., 2020; Tretiach et al., 2007). In this respect, it is important to use moss gametophytes that have not previously been stored for too long before exposure in the laboratory, because our research has shown that the length of moss exposure to environmental stress negatively affects the

Table 5
Chlorophyll content -*a* (Chl-*a*) i -*b* (Chl-*b*) [mg/kg] in mosses during the experiment.

	0	VII/VIII′19	VIII/IX	IX/X	X/XI	XI/XII	XII/I′20	I/II	II/III	III/IV	IV/V	V/VI	VI/VII	min-max	median	average	SD
Chl-*a*	851	421	446	142	126	159	97.5	153	95.4	87.1	181	134	137	87.1–446	140	182	121
Chl-*b*	631	213	250	142	96.4	132	84.6	134	64.0	96.9	201	99.4	103	64.0–250	117	135	57.6

0 – control sample; VII/VIII′19 - 1 month of exposure; SD – standard deviation.

chlorophyll content.

In the literature, as mentioned in the Introduction, the authors use different exposure times for different moss species: period of between 30 and 45 days (Ares et al., 2012; Limo et al., 2018), not shorter than 6 weeks (Capozzi et al., 2016), 8 weeks (Ares et al., 2014). The most frequently-used time for exposing mosses to various types of contamination is still the 4-week exposure suggested earlier in the literature (Table in the SM). Even such a short period allows different groups of pollutants to be determined, and reduction of the sample-to-sample variability also counts (Adamo et al., 2008; Arndt et al., 2014; Ayrault et al., 2006; Popovic et al., 2010; Świsłowski et al., 2021; Temple et al., 1981; Vuković et al., 2013a). However, it should be remembered that the timing and duration of the moss exposure should be considered in the interpretation of the results (Saitanis et al., 2013), as the moss-bag technique can also be used for long-term air quality monitoring not only in terms of a period of continuous exposure, but also by conducting research over the years to be able to assess and determine the influence of various factors on the concentration in mosses and to search for (changing) sources of pollution in a given area in order to assess the state of the environment (De Agostini et al., 2020; Demková et al., 2017; Dmuchowski and Bytnerowicz, 2009). However, only single studies have been carried out with regard to a continuous, annual period of exposure of mosses to pollution, and the control of their vital parameters constitutes an even smaller share of the studies. Our research shows, however, that long-term biomonitoring is possible while maintaining moss with the characteristics of a living organism.

Taking into account the above and taking into account such research parameters as: the latitude of the research area, the degree of contamination of the area (if it has been tested earlier), weather conditions, the bioindicator used and its bioaccumulation potential, for *P. schreberi* mosses the exposure time should not exceed half a year, because there are still increases in concentrations month to month (Table 3), but it should not be shorter than 12 weeks as indicated by the *RAF* values (Table 4). However, it should be remembered that the exposure time of mosses should reflect the actual state of the environment and should be comparable with the results of classical monitoring studies; therefore, integration and an attempt to correlate the results of these techniques is needed. For a better and more complete analysis of the state of the environment, we recommend collection of sub-samples over a longer period of time during active biomonitoring studies, which will allow better analysis of the contamination of a given area over subsequent months of the experiment. It is also advisable to control vital parameters to make sure that the performed biomonitoring tests are carried out with the use of a bioindicator and not only a natural pollutant sorbent.

5. Conclusion

In this study, heavy metals accumulated in *P. schreberi* moss bags were exposed to atmospheric aerosol for 12 months in the city of Opole. Prior to this annual survey, no data existed on the deposition of elements in the atmosphere in this area over such a long time. Environmental factors have a significant impact on the viability of mosses; therefore, an appropriate date/period of active biomonitoring studies should be taken into account. Mosses *P. schreberi* effectively accumulate air pollutants during an exposure period of one year and tolerate well different weather Polish climatic conditions - mosses exposed to environmental stress showed features of a living organism (chlorophyll content). The influence of temperature and exposure duration of mosses on their content of this photosynthetic pigment was demonstrated. Due to the constant presence of chlorophyll content in mosses and the increments of selected analytes during the exposure (to 6th month, see Table 3), *P. schreberi* moss can be used in long-term monitoring of the quality of the atmospheric aerosol in terms of heavy metal concentration. The exposure time is closely related to the degree of contamination of a given area, the sorption capacity of a specific species, its viability, and, for *P. schreberi* mosses under Polish climatic conditions, this should be three to six months.

Credit author statement

Paweł Świsłowski: Conceptualization, Methodology, Formal analysis, Investigation, Measurements, Writing - Original Draft, Visualization. **Arkadiusz Nowak**: Visualization, Validation, Writing - Review & Editing, Supervision. **Małgorzata Rajfur**: Measurements, Validation, Writing - Review & Editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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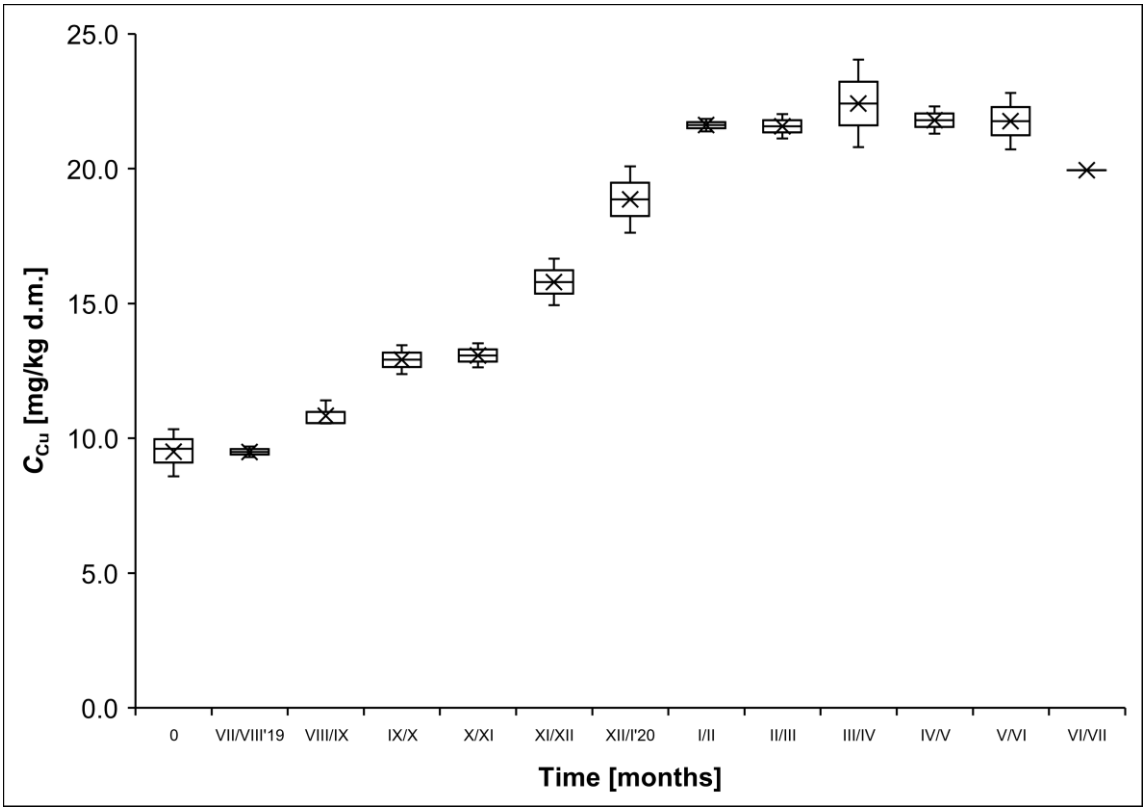
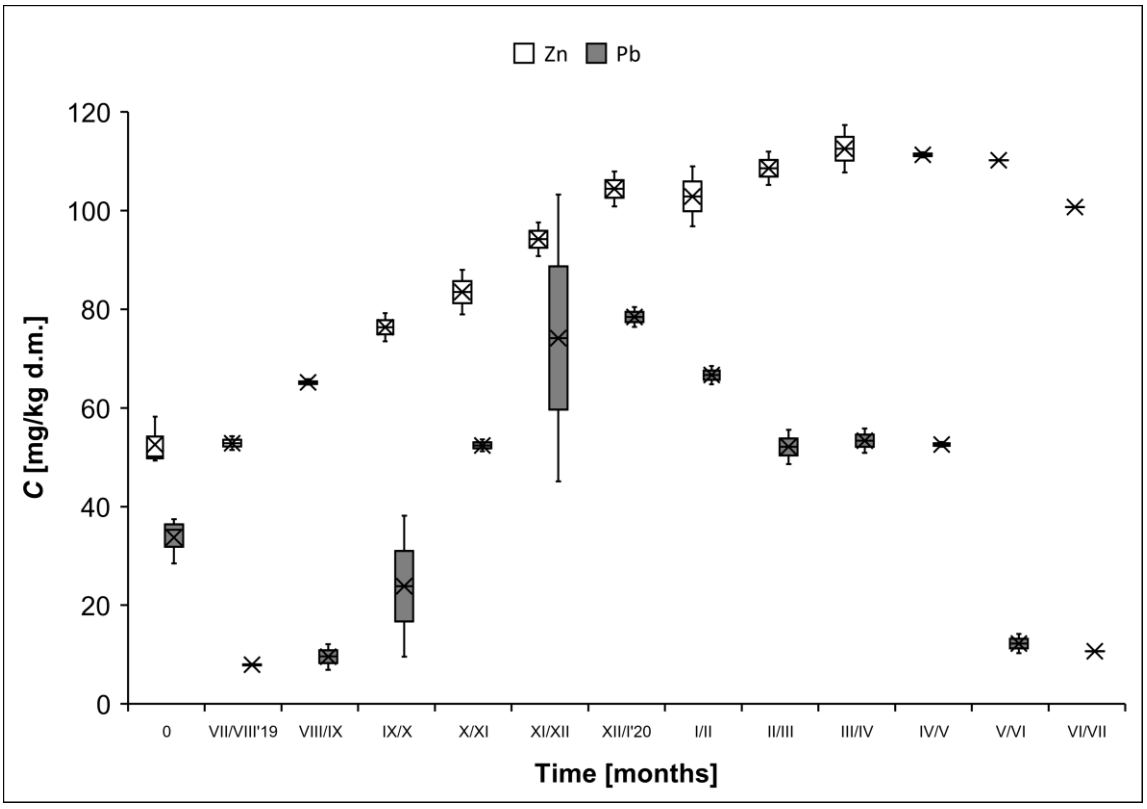
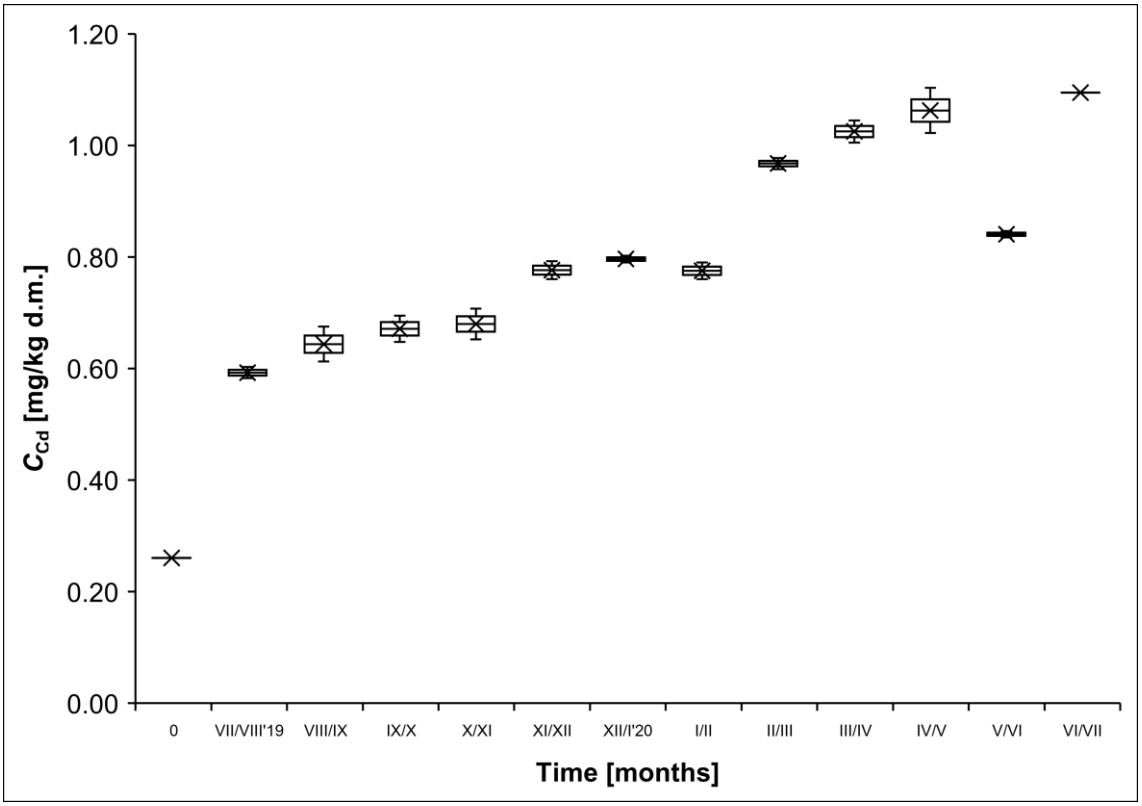
a)


25.0
20.0
15.0
10.0
5.0
0.0
C_{Cu} [mg/kg d.m.]
0
VII/VIII'19
VIII/IX
IX/X
X/XI
XI/XII
XII/I'20
I/II
II/III
III/IV
IV/V
V/VI
VI/VII
Time [months]

b)


Zn
Pb
120
100
80
60
40
20
0
C [mg/kg d.m.]
0
VII/VIII'19
VIII/IX
IX/X
X/XI
XI/XII
XII/I'20
I/II
II/III
III/IV
IV/V
V/VI
VI/VII
Time [months]

c)


1.20
1.00
0.80
0.60
0.40
0.20
0.00
C_{Cd} [mg/kg d.m.]
0
VII/VIII'19
VIII/IX
IX/X
X/XI
XI/XII
XII/I'20
I/II
II/III
III/IV
IV/V
V/VI
VI/VII
Time [months]

d)

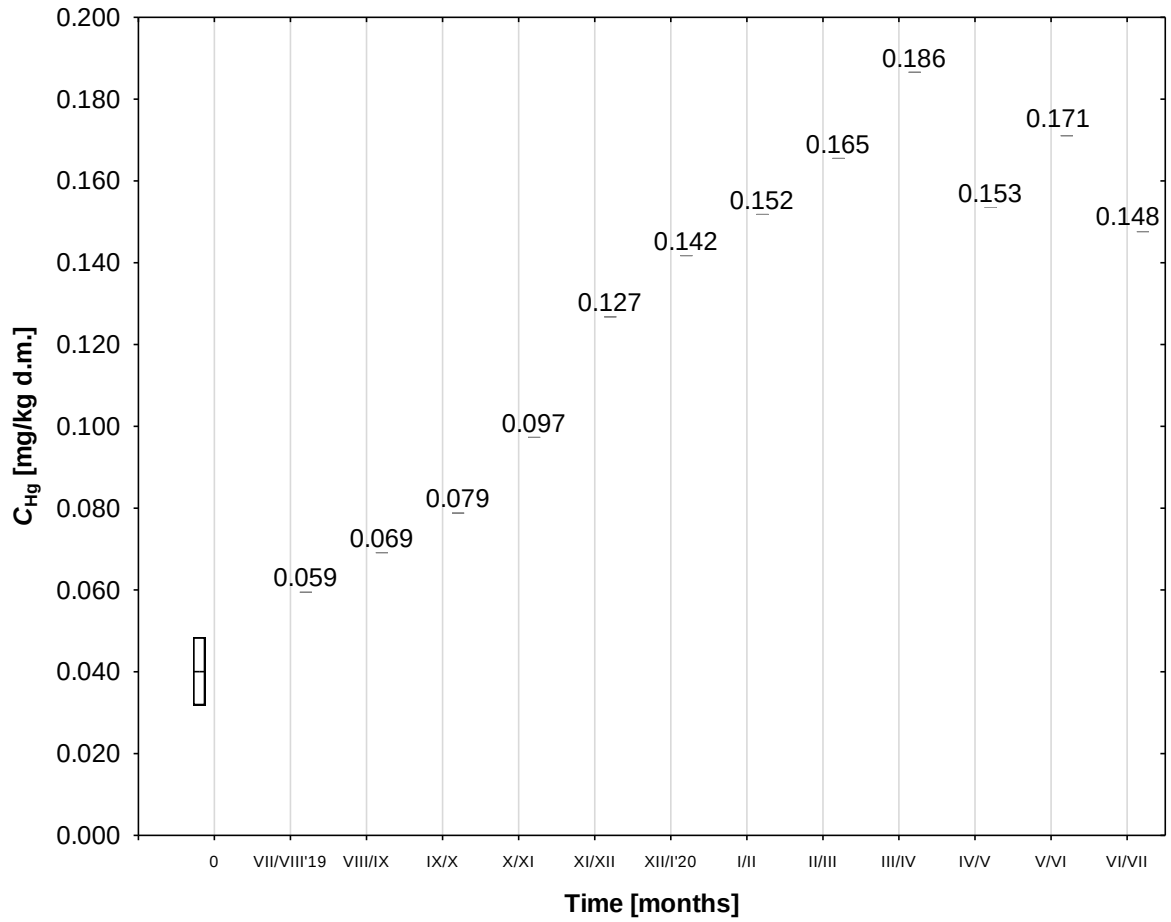


Figure SM. Changes in concentrations of heavy metals: a) copper, b) zinc and lead, c) cadmium, d) mercury during the experiment; 0 = control sample, VII / VIII'19 - 1 month of exposure; The "X" in the boxplot represents the mean value of the analyte concentration and — shows the median

Table SM

Analysis of the number of selected publications concerning the exposure time of mosses moss bag technique

Time of exposure **[number of publications]**	Authors
< 4 weeks **[10]**	(Arndt et al., 2017; Arndt and Planer-Friedrich, 2018; Calabrese et al., 2015; Capozzi et al., 2016; Cesa et al., 2015, 2014; Debén et al., 2016; Diviš et al., 2012; Herrmann et al., 2012; Urošević et al., 2017)
1 month (4 weeks) **[20]**	(Ares et al., 2014, 2015b, 2015a; Arndt et al., 2017; Arndt and Planer-Friedrich, 2018; Calabrese et al., 2015; Calabrese and D'Alessandro, 2015; Cesa et al., 2015; Cortis et al., 2016; Culicov et al., 2016; De Nicola et al., 2013; Demková et al., 2017; Demková and Bobul'ská, 2019; Gómez-Arroyo et al., 2020; Morales-Casa et al., 2019; Ndlovu et al., 2019; Shvetsova et al., 2019; Urošević et al., 2017; Yurukova et al., 2013; Zinicovscaia et al., 2018)
6 weeks **[5]**	(Capozzi et al., 2017, 2016; Demková et al., 2019, 2018; Urošević et al., 2017)

7 weeks **[2]**	(Motyka et al., 2013; Hanna Salo et al., 2016)
2 months (8 weeks) **[17]**	(Ares et al., 2014, 2015b, 2015a; Arndt et al., 2017; Calabrese et al., 2015; Culicov et al., 2016; Demková et al., 2020; Khiem et al., 2020; Milićević et al., 2017, 2020; Ndlovu et al., 2019; H. Salo et al., 2016; Shvetsova et al., 2019; Turgut et al., 2019; Urošević et al., 2017; Vuković et al., 2015; Zinicovscaia et al., 2018)
10 weeks **[6]**	(Lazić et al., 2016; van Laaten et al., 2020; Vingiani et al., 2015; Vuković et al., 2017, 2013a, 2013b)
3 months (12 weeks) **[12]**	(Ares et al., 2014, 2015b, 2015a; Arndt et al., 2017; Capozzi et al., 2016; Culicov et al., 2016; Madadzada et al., 2019; Ndlovu et al., 2019; Saitanis et al., 2013; Shvetsova et al., 2019; Yurukova et al., 2013; Zinicovscaia et al., 2018)
14 weeks **[1]**	(García-Seoane et al., 2019)
4 months **[6]**	(Ares et al., 2015b, 2015a; Culicov et al., 2016; Milićević et al., 2020, 2017; Zinicovscaia et al., 2018)
17 weeks **[1]**	(Giordano et al., 2013)
5 months **[4]**	(Ares et al., 2015a; Rogova et al., 2018; Yurukova et al., 2013; Zinicovscaia et al., 2018)
6 months **[6]**	(Ares et al., 2015a; Cortis et al., 2016; Lazo et al., 2013; Milićević et al., 2017; Saitanis et al., 2013; Salo, 2014)
7 months **[1]**	(Ares et al., 2015a)
8 months **[2]**	(Ares et al., 2015a; Cortis et al., 2016)
9 months **[2]**	(Ares et al., 2015a; Saitanis et al., 2013)
10 months **[1]**	(Ares et al., 2015a)
11 months **[1]**	(Ares et al., 2015a)
12 months (1 year) **[2]**	(Ares et al., 2015a; Saitanis et al., 2013)

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RESEARCH ARTICLE

Effects of tobacco smoke on indoor air quality: the use of mosses in biomonitoring

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Abstract

This research was carried out to assess the possibility of using *Pleurozium schreberi* mosses as bioindicators of atmospheric aerosol pollution in living quarters (kitchen and bedroom), with metals originating from tobacco smoke from various types of cigarettes: conventional cigarettes, e-cigarettes and heated tobacco products. The *moss-bag* method of active biomonitoring was used. The mosses were exposed in these indoor spaces for three months and, after the exposition period, their analytes – Ni, Cu, Zn, Cd and Pb – were determined using flame atomic absorption spectrometry *(F-AAS)*. Results were interpreted using the relative accumulation factors (*RAF*), coefficients of variation (*CV*) and the Wilcoxon test. As a result of the research, it was found that there were statistically significant differences in Zn and Cd concentrations in tobacco smoke from different types of cigarettes. The analyses showed that heated tobacco products contaminate indoor air with metals, similar to conventional cigarettes and e-cigarettes. It was demonstrated that the reliability of biomonitoring results was affected, for example, by the method of preparation of bioindicator samples, such as mosses.

Keywords *Pleurozium schreberi* mosses · Metals · Smoke · Active biomonitoring · Health

Introduction

The metal content of tobacco products may cause numerous diseases that pose health hazards to active and passive smokers [1, 2]. This is confirmed by studies on tobacco smoke carcinogenicity resulting from the analytes transfer into the lungs and the content of toxic metals, such as cadmium or lead [3]. Conventional cigarettes are also a source of such hazardous compounds as Polycyclic Aromatic Hydrocarbons (PAHs), to which children are exposed, for example, as confirmed by biomonitoring studies using human hair as biomarkers [4]. The sorption of metals into tobacco and its subsequent combustion in tobacco products may increase cancer risk [5]. Therefore, the harmfulness and toxicity of conventional cigarettes is undeniable.

However, this is also the case with e-cigarettes (E-C), which are battery-operated devices that deliver nicotine to the body while an aromatised solution (e-liquid flavour or cartridge) is heated. As a result of the combustion process, a typical, dense aerosol (mist) is generated, resembling the real combustion of nicotine in conventional cigarettes [6]. The interest in these products is growing because they are considered less harmful than conventional tobacco products, as promised by their manufacturers, or they provide the possibility of overcoming addiction more easily [7, 8]. This is partly true [9, 10]. Nevertheless, it is necessary to consider that e-cigarette users still face a high risk of exposure to environmental tobacco smoke (ETS).

Furthermore, passive smoking in this case (exposure to e-cigarette aerosol) is not entirely harmless [11]. Additives used in such products are equally hazardous, or at least their safety is questionable; we refer here to the chemical substances added to e-liquid flavours as aromatic compounds that ensure a certain taste or smell when using e-cigarettes. The absence of any developed and validated methods to

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detect aromatic compounds in these e-liquid flavours (of unknown composition and effect on human health) requires studies on the standardisation of analytical methods for their determination [12, 13].

Heated tobacco products (HTPs) are devices that heat-processed tobacco to 350 °C, but do not burn it completely. These are ignited with fire (such as carbon tip HTPs) or battery-operated devices that are characterised by the generation of small amounts of smoke (however, they are described as smokeless). These products are becoming more and more popular on the market [14], yet surveys show that respondents still know little about these devices [15]. It has been suggested that many studies carried out by companies that launch these products on the market or are related to the tobacco industry do not publish complete data on the harmfulness to health or report a significant reduction compared with the use of conventional cigarettes [16]. Other literature research also confirms the assumptions that the data on the harmfulness of HTPs originate mainly from the tobacco industry and suggest significantly fewer harmful effects than conventional cigarettes. Therefore, it is essential to carry out independent research in this area [17, 18]. Often, independent tests do not confirm the results obtained by the manufacturers.

In some cases, HTPs were more cytotoxic than e-cigarettes [17]. It is impossible to assess which of these devices (e-cigarettes or HTPs) are less harmful or make it easier to quit smoking [19]. The issue of the harmfulness of HTPs is controversial, and the data included in some publications are even mutually contradictory- medical publications accuse tobacco companies of misleading the public about the safety of HTPs [20]. This, however, contradicts the results of the positive biological effects of switching to HTPs and the reduction of the risk of harm from continuous smoking of conventional cigarettes [21]. On the other hand, ecotoxicity studies conclude that the consumption of HTPs may be a significant environmental problem in the future [22]. Other studies, however, consider HTPs to be less harmful than conventional cigarettes in terms of air pollution in public (indoor) spaces [23].

Biomonitoring is a biological method of environmental assessment. It uses plant and animal organisms to monitor the quality of water, air and soil. The most popular biomonitors include mosses, lichens, tree bark and tobacco [24–27]. Mosses are perceived as one of the main bioindicators of atmospheric aerosol pollution [28, 29]. The *moss-bag* method of active biomonitoring includes the exposure of biological material in highly contaminated areas and the subsequent analysis of changes occurring during the exposure [30]. The use of biomonitoring can supplement the conventional measurements that use various devices to monitor environmental quality [31].

Literature research in e-cigarettes and HTPs shows virtually no data on metal emissions of aerosol/smoke from such devices. They primarily discuss the content of nicotine or other chemical compounds, yet there are no data on concentrations of trace elements, such as metals [18, 32]. Therefore, we suppose that this is one of the first papers presenting the results of concentrations of metals contained in aerosol from e-cigarettes and HTPs, determined using biomonitoring. Mosses used in the *moss-bag* method of active biomonitoring act as biosensors in relation to the metals emitted with aerosols from the types of cigarettes discussed.

The purpose of the research was to evaluate the possibility of using *Pleurozium schreberi* mosses as biomonitors of atmospheric aerosol pollution in living quarters with selected metals originating from tobacco smoke/aerosol from various types of cigarettes. An attempt was also made to demonstrate that the method of preparation of moss samples affected the reliability of the biomonitoring results.

Material and Methods

Material

Pleurozium schreberi mosses, collected in a forest situated about 3 km from Stary Janów, Świętokrzyskie Province, 40 km north of Kielce (Poland), were used for the research. This species is commonly found in Europe and Poland, which is used as an air quality bioindicator in active biomonitoring [33, 34].

In the case of conventional cigarettes, one of the most popular cigarette brands globally was used.

An e-cigarette equipped with a heater of 1.4 Ω resistance was also used. The 'ice mint' e-liquid flavour with a nicotine content of 18 mg/cm^3 was selected for the studies. According to the information provided on the package, the e-liquid flavour contained propylene glycol, glycerine, nicotine, L-menthol, peppermint oil and diethyl malate.

In the case of HTPs, one of the leading products on the market in this line of business was used.

Methods

After collecting and transporting mosses to the laboratory, they were cleaned or had impurities removed (leaves, needles and soil particles) and dried at room temperature until dry mass (d.m.) was obtained (Method 1) [2]. The second method of preparation assumed the same cleaning procedure (Method 1) as well as the homogenisation of the material and conditioning in demineralised water before exposure (Method 2) [35]. This procedure aimed to reduce the initial metal concentration in the mosses [36] and increase the

Table 1 The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the spectrometer iCE 3500 [mg/L] [39]

Metal	*IDL*	*IQL*
Ni	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

sample homogeneity, thus leading to a drop in the coefficient of variation *(CV)* for the mosses.

The representative (average) samples of mosses with a mass of 5.00 ± 0.010 g, which included only the green part of the gametophyte, upper parts of the shoot, as the photosynthetically active parts- live and active tissues [37, 38], were placed in plastic net-bags and exposed in living quarters (in the kitchen and bedroom) for 3 months. Heavy metal concentrations in plastic net-bags themselves were below the limit of quantification of the analytical method used (Table 1).

Conventional cigarettes were smoked (ten cigarettes per day) in the kitchen where the moss samples were exposed (there was no cooking, washing, burning candles etc. in the kitchen, so there were no other sources of emissions). The 3-month experiment was followed by exposure to e-cigarettes (100 cm^3 e-liquid flavour). The duration of this experiment was also 3 months. During the subsequent 3 months, the experiment involved exposure to HTP (10 cigarettes per day), exhaling cigarette smoke/aerosol in the direction of the mosses. In contrast, the bedroom was a space that was potentially not exposed to the pollution from the cigarettes (the control sample). Following the exposure, the moss samples were taken to the laboratory and dried at a temperature of 303 K.

Each moss sample, with a mass of 1.000 ± 0.001 g d.m., was prepared in this way and mineralised in a mixture of nitric acid (V) and hydrogen peroxide (HNO_3 65%: H_2O_2 37% = 5:3) using a Speedwave Four Berghof, DE microwave oven. The mineralisation process was carried out at a temperature of 180 °C. Selected metals (Ni, Cu, Zn, Cd and Pb) were determined using an atomic absorption flame spectrometer (F-AAS) type iCE 3500 (series 3000) made by Thermo Scientific, USA.

Quality control

In Table 1, the instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the spectrometer iCE 3500 are presented. Calibration of the spectrometer was performed with a standard solution from ANALYTIKA Ltd. (CZ). The values of the highest concentrations of the models used for calibration (5 mg/dm^3 for Ni, Cu, Zn, Pb; 2 mg/dm^3 for Cd) were approved as linear limits to signal dependence on concentration. Concentrations of metals were determined in solution after mineralisation and dilution and were filtered into volumetric flasks of 20 cm^3.

In Table 2, concentrations of metals in certified reference materials BCR-482 *lichen*, produced at the Institute for Reference Materials and Measurements, Belgium, are shown.

In order to assess the relative differentiation of the results of the concentration levels (mg/kg d.m.) of analytes in the mosses, the concentration increases were calculated using the relative accumulation factor (*RAF*) [30]:

$$RAF = \frac{ci_1 - ci_0}{ci_0}$$

where:

$C_{i,0}$ – concentration of the analyte before the exposure period (mg/kg d.m.)
$C_{i,1}$ – concentration of the analyte after the exposure period (mg/kg d.m.)

The coefficient of variation (*CV*) was determined, which refers to the value of standard deviation *s* (absolute differentiation of the feature distribution) to the mean value of x_{av} [40]:

$$CV = \frac{s}{x_{av}} \cdot 100[\%]$$

Results and Discussion

In the first stage of the research, two methods of moss sample preparation were compared to obtain reliable results for active biomonitoring indoors. Tables 3, 4 and 5 and

Table 2 Comparison of measured and certified concentrations in BCR-482 *lichen*

Metal	BCR-482 *lichen*		AAS ($n = 3$)		
	Concentration [mg/kg d.m.]	Uncertainty	Average	±SD*	Dev.** [%]
Ni	2.47	0.07	2.16	0.32	−13.0
Cu	7.03	0.19	6.63	0.17	−5.70
Zn	101	2.20	95.1	2.30	−5.50
Cd	0.56	0.02	0.53	0.03	−5.30
Pb	40.9	1.40	38.2	1.00	−6.60

*Standard deviation

**Relative difference between the measured (c_z) and certified (c_c) concentration $100\% \bullet (c_z - c_c)/c_c$

n – number of samples

the diagram (Fig. 1) show the results of concentrations of selected metals determined in the mosses, *CVs* and *RAFs* as well as the metal concentrations determined in mosses prepared for the tests using the two methods and exposed in two living quarters (for smokeless HTP).

As can be seen, based on the determined coefficient of variation (*CV*), the mosses are not homogeneous material. This results from the variability in the elemental composition of the mosses as well as anthropogenic and natural factors [41]. In individual cases, it may be concluded that the application of the second moss preparation method resulted in reducing the *CV* value (Table 4). Figure 1 presents the comparison of the concentrations of analytes in the mosses exposed in two indoor spaces differing with respect to the level of pollution from smokeless HTP. The mosses were prepared using two methods. It should be emphasised that higher concentrations of metals, compared with the samples exposed in the bedroom, were determined in the mosses exposed in the kitchen where HTP was used. *RAF* values also support this (Table 5). However, it should be taken into account that a lower initial analyte concentration in the biomonitor before exposure does not always result in a higher *RAF* value [42] (Table 5). The analytes emitted during smoking in the kitchen through communication and ventilation routes are transported to other rooms such as a bedroom.

To statistically evaluate the effectiveness of the moss sample preparation methods compared, the Wilcoxon test (Table 6) was used to obtain reliable indoor active biomonitoring results.

Table 3 Concentrations of selected metals determined in the mosses from various types of cigarettes (mg/kg d.m)

Metal	Conventional cigarettes		E-cigarettes		Heated tobacco product			
	Kitchen (Method 1)	Bedroom (Method 1)	Kitchen (Method 2)	Bedroom (Method 2)	Kitchen (Method 1)	Bedroom (Method 1)	Kitchen (Method 2)	Bedroom (Method 2)
	($n=5$)	($n=5$)	($n=5$)	($n=4$)	($n=3$;repl. = 3)	($n=3$;repl. = 3)	($n=3$;repl. = 3)	($n=3$;repl. = 3)
Ni	6.44	6.51	8.31	n.d.	n.d.	n.d.	n.d	1.40
	(6.44)	(6.51)	(8.31)					(1.40)
Cu	8.11–10.4	7.92–15.8	9.18–13.8	8.44–10.5	7.03–8.55	6.67–8.37	7.97–10.9	6.99–9.35
	(8.99 ± 1.09)	(10.3 ± 3.42)	(10.7 ± 1.86)	(9.44 ± 0.794)	(7.58 ± 0.431)	(7.59 ± 0.497)	(8.99 ± 1.15)	(8.22 ± 0.658)
Zn	29.6–44.3	29.7–39.3	33.1–43.2	30.9–48.5	54.7–77.0	51.2–61.8	68.1–76.3	51.6–68.5
	(37.2 ± 6.66)	(33.7 ± 4.98)	(38.0 ± 4.19)	(38.6 ± 7.72)	(61.7 ± 6.44)	(56.8 ± 3.59)	(72.9 ± 3.14)	(63.0 ± 5.00)
Cd	n.d.	n.d.	n.d.	n.d.	0.573–0.615	0.585–1.13	0.590–1.23	0.558–0.685
					(0.598 ± 0.017)	(0.910 ± 0.160)	(0.717 ± 0.229)	(0.603 ± 0.046)
Pb	15.1–39.9	26.5	33.3	n.d.	2.19–26.0	2.90–25.6	10.5–38.0	8.90–25.9
	(27.5 ± 17.5)	(26.5)	(33.3)		(11.9 ± 7.96)	(10.8 ± 7.09)	(22.1 ± 9.64)	(14.6 ± 6.09)

Values in the range are min-max (average ± standard deviation)

n – number of samples

n.d. – not determined

repl. – replicant

Table 4 Comparison of *CVs* determined for metals found in mosses prepared for tests using the two methods and exposed in two indoor spaces differing with respect to the level of pollution levels from smokeless HTP

Metal	*CV* [%]			
	Kitchen (Method 1)	Kitchen (Method 2)	Bedroom (Method 1)	Bedroom (Method 2)
	($n=3$; repl. = 3)	($n=3$; repl. = 3)	($n=3$; repl. = 3)	($n=3$; repl. = 3)
Ni	n.d	n.d	n.d	n.d
Cu	5.68	12.8	6.56	8.00
Zn	10.4	4.31	6.32	7.94
Cd	2.79	32.0	17.6	7.60
Pb	66.9	2.64	65.6	41.6

n – number of samples

n.d. – not determined

repl. – replicant

Table 5 Comparison of *RAFs* determined for metals found in mosses prepared for tests using the two methods and exposed in two indoor spaces differing with respect to the level of pollution levels from smokeless HTP

Metal	*RAF* [−]			
	Kitchen (Method 1) ($n=3$; repl. = 3)	Kitchen (Method 2) ($n=3$; repl. = 3)	Bedroom (Method 1) ($n=3$; repl. = 3)	Bedroom (Method 2) ($n=3$; repl. = 3)
Ni	n.d	n.d	n.d	n.d
Cu	n.d	0.034	n.d	n.d
Zn	n.d	0.387	n.d	0.197
Cd	0.150	0.102	0.749	n.d
Pb	3.25	2.64	2.86	1.41

n – number of samples

n.d. – not determined

repl. – replicant

The data presented in Table 6 confirm that the appropriate method of preparation of the material influences the quality of the result, where statistically significant differences between methods occur. In the case of copper, zinc, cadmium and lead for the samples exposed in the kitchen, it can be observed that the lowest fluctuation of results was obtained when moss was prepared using Method 2. The thesis is that the method of moss sample preparation prior to their exposure in biomonitoring studies affects the homogeneity of the test material (Table 4), and the quality of the results (Table 5) was confirmed [35]. Sampling and sample preparation provide an object of study and revision of protocols used in biomonitoring [43].

The concentrations of the selected analytes accumulated in mosses, exposed in the kitchen where different types of cigarettes were smoked, were analysed in the second stage of the research. The results were compared with the data obtained for the control sample (the mosses exposed in an indoor space potentially not exposed to cigarette smoke – the bedroom).

Diagrams in Fig. 2 show the distribution of metal concentrations determined in the mosses exposed in two indoor spaces (kitchen and bedroom) differing with respect to the smoke/aerosol pollution level from different types of cigarettes (also Table 3). Higher concentrations were determined in the mosses exposed in the kitchen for most analytes.

The nickel concentration in the mosses exposed to conventional cigarette smoke was determined at 6.44–6.51 mg/kg d.m., and in the mosses exposed to e-cigarette aerosol at 8.31 mg/kg d.m., whereas in the mosses exposed to HTPs the Ni concentration was below the limit of quantification of the analytical method applied.

The copper concentrations in the mosses exposed to conventional cigarettes and HTPs in the kitchen were comparable. The concentration of this analyte in the mosses exposed to e-cigarette aerosol was much higher. The research confirms the hypothesis that the metal concentrations in the aerosol originating from e-cigarettes (e.g., copper and aluminium) are higher or comparable to the concentrations of the analytes found in conventional cigarette smoke. This may be due to the wear of e-cigarette parts during smoking [44].

No statistically significant differences were found in the Zn concentrations determined in the mosses exposed to conventional cigarette smoke and to e-cigarette aerosol. The content of this analyte in the mosses exposed to HTP smoke is twice as high as that of environmental tobacco smoke (ETS) originating from other cigarette types.

The cadmium concentration determined in the mosses exposed to conventional cigarette smoke and e-cigarette aerosol was below the limit of quantification of the analytical method applied (<0.520 mg/kg d.m.). However, the Cd concentration determined in the mosses exposed to HTP smoke was between 0.573 and 1.13 mg/kg d.m. The low cadmium concentrations in the moss samples exposed to conventional cigarette smoke may result from its limited transfer from the tobacco through an activated carbon filter and demonstrate the selective metal filtration in the filter during smoking [45].

E-cigarettes are a source of exposure to chemicals in the indoor environment. More and more evidence can be found that smoking e-cigarettes cause the deterioration of air quality due to releasing, for example, PAHs [46]. According to some sources, no significant increase in toxic elements (such as cadmium or arsenic) was observed in the aerosol released during e-cigarette smoking, which is also confirmed by our studies: the cadmium concentration was below the limit of quantification of the analytical method used. In the studies mentioned above, the concentrations of metals in e-liquid flavours were below acceptable standards [47], and the ultimate determination of harmfulness to human health requires further research [48]. In the studies presented, the concentrations of all metals determined in the sample of e-liquid flavours used were below the limit of quantification of the analytical method employed.

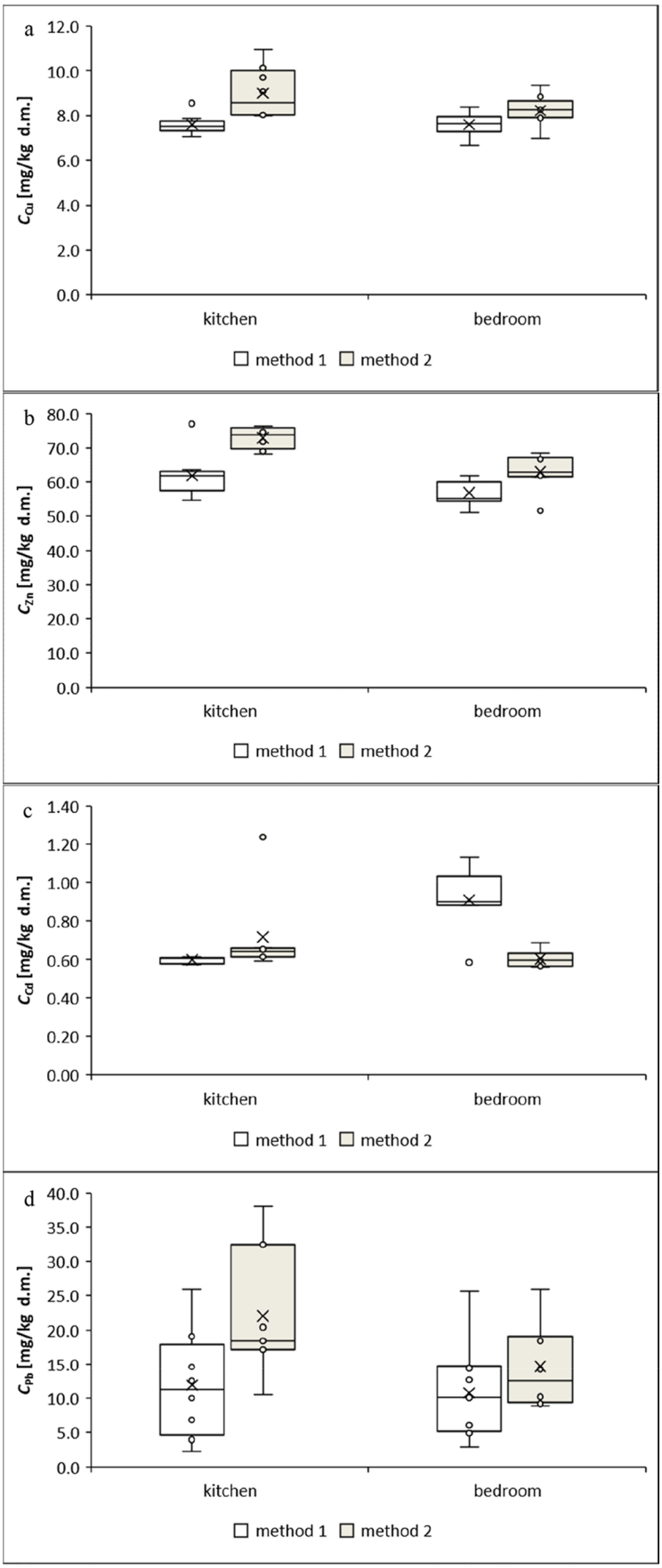

Fig. 1 Concentrations of selected metals: Cu (**a**), Zn (**b**), Cd (**c**), Pb (**d**) determined in the mosses exposed in two indoor spaces differing with respect to the level of pollution from smokeless HTP; X – average, —— – median, ° – outlier point

Table 6 The results of the Wilcoxon test for metals determined in moss samples depending on the method of their preparation for tests

		W	p	
Cu-B1	–	Cu-B2	9.000	0.129
Cu-K1	–	Cu-K2	0.000	0.008
Zn-B1	–	Zn-B2	6.000	0.055
Zn-K1	–	Zn-K2	1.000	0.016
Cd-B1	–	Cd-B2	20.00	0.063
Cd-K1	–	Cd-K2	0.000	0.031
Pb-B1	–	Pb-B2	2.000	0.023
Pb-K1	–	Pb-K2	0.000	0.031

W – test statistical value/the sum of the signed ranks

p – value: probability value/significance level alpha = 0.05

B1 – bedroom (Method 1), K1 – kitchen (Method 1); B2 – bedroom (Method 2), K2 – kitchen (Method 2)

It is visible that HTP smoke does contain analytes, as evidenced by the content in the moss samples (Figs. 1 and 2). As a result, everyone who remains in the vicinity of HTP smokers is exposed to ETS. Smokeless tobacco products (snuff and tobacco) were analysed for metals. The content of individual analytes in tobacco was as follows: beryllium – 0.031 ± 0.015 mg/kg; chromium – 1.37 ± 0.48 mg/kg; cobalt – 0.98 ± 0.64 mg/kg; nickel – 2.32 ± 1.63 mg/kg; arsenic – 0.19 ± 0.06 mg/kg; cadmium – 1.41 ± 0.56 mg/kg; barium – 114.4 ± 40.1 mg/kg; and lead – 0.55 ± 0.19 mg/kg. The authors of this paper emphasise that there is still insufficient knowledge about bioavailability, absorption, and the toxicological effects of toxic substances from smokeless tobacco [49].

The biomonitoring studies showed that HTPs contaminate indoor air with metals in a similar way to conventional cigarettes and e-cigarettes. Higher concentrations of zinc and cadmium can be observed in the mosses exposed to HTP smoke compared with the moss samples exposed to smoke/aerosol of the other two cigarette types (Fig. 2).

Conclusions

The location of the exposure sample is critical in biomonitoring studies. We have proven this by demonstrating that the mosses in the kitchen directly exposed to ETS accumulate more contaminants than the control samples exposed in the bedroom. The research also confirms that the proper preparation procedure for the biological material contributes to achieving statistically significant differences in the sorption of metals (Table 6). Special attention is paid to the sample preparation method in the *moss-bag* method of active biomonitoring. Our experiment confirms this based on *CVs*, *RAFs* and the Wilcoxon test. The preparation method in active biomonitoring

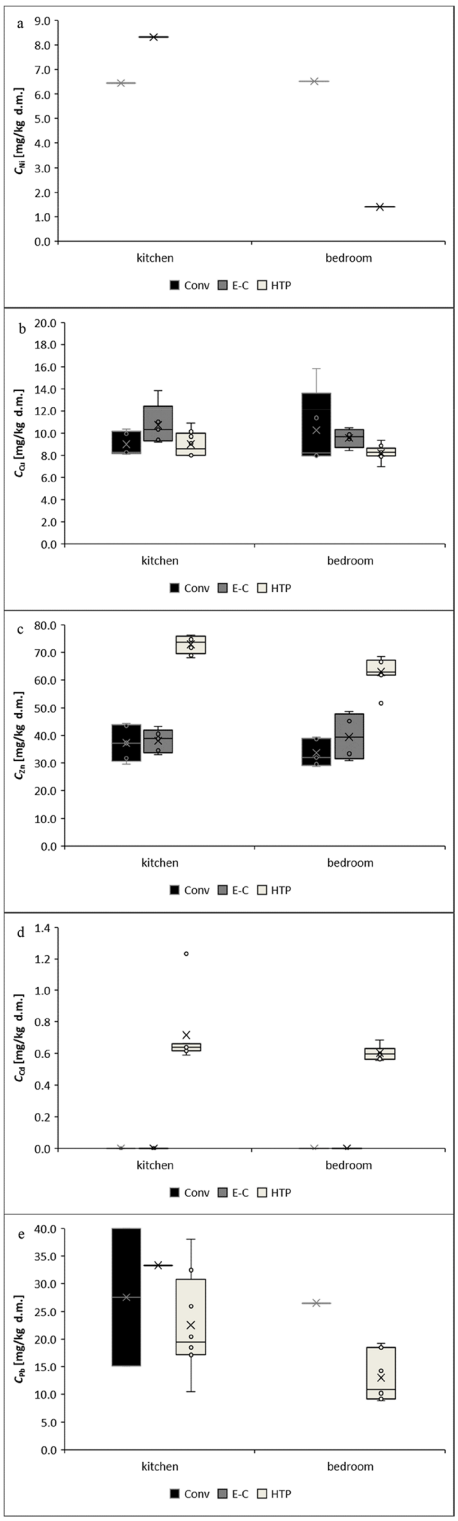

Fig. 2 Concentrations of selected metals: Ni (**a**), Cu (**b**), Zn (**c**), Cd (**d**), Pb (**e**) determined in the mosses exposed in two indoor spaces differing with respect to the level of smoke pollution from various types of cigarettes; Conv – conventional cigarettes (method 1), E-C – e-cigarettes (method 2), HTP – heated tobacco product (method 2); X – average,—— – median, ° – outlier point

using mosses affects the quality of results obtained, while the proper preparation of the samples results in the homogeneity of the material. Indeed, this is one of the first papers presenting results of biomonitoring tests that use mosses to evaluate air pollution with metals from different types of cigarettes.

The discussion regarding the reduced harmfulness of such products as e-cigarettes and HTPs is still ongoing, but our results indicate their negative effect on indoor air quality. The content of individual metals in aerosol (either from e-cigarettes or HTPs) could be higher when compared with conventional cigarette smoke. We have found that ETS originating from e-cigarettes and HTPs pollutes the atmospheric aerosol with metals in indoor spaces similarly. This makes human exposure to it possible to have a negative impact on health.

Authors' contributions Paweł Świsłowski: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Visualization;
Bogusław Śmiechowicz: Resources;
Małgorzata Rajfur: Validation, Resources, Writing - Review & Editing, Supervision, Project administration.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Conflit of Interest The authors declare that they have no conflict of interest.

Ethics approval The results presented do not encourage smoking cigarettes in any way. The research conducted first indicates the possibility of using mosses as a bioindicator of air pollution with heavy metals derived from tobacco smoke. The authors indicate that the currently promoted electronic cigarettes and heat tobacco products pollute the air with heavy metals indoors comparable to traditional cigarettes.

The study did not affect the personal well-being of the smoker. The study involved an addicted smoker (co-author of the publication), and his participation in the study was voluntary and in no way increased his addiction. The smoker's natural train was used to satisfy nicotine hunger in the research work.

Consent to participate The study involved an addicted smoker (co-author of the publication) and his participation in the study was voluntary and in no way increased his addiction. The smoker's natural train was used to satisfy nicotine hunger in the research work.

Consent for publication Not applicable.

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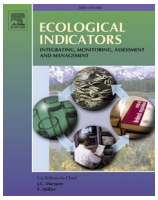

Original Articles

Mosses as a biomonitor to identify elements released into the air as a result of car workshop activities

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ABSTRACT

Human activity as a result of civilization development contributes to creating new sources of environmental pollution. Air pollution is one of the major problems because it affects the fauna and flora, and people themselves. There is a lack of public awareness of the level of atmospheric analytes pollution emitted from people's occupational and recreational (leisure) activities. A quick, cheap and easy way to investigate the state of the environment is to use organisms-biomonitors that indicate the level of environmental pollution. The study aimed to assess air pollution in an urban area using three moss species: *Sphagnum fallax*, *Pleurozium schreberi* and *Dicranum polysetum*. Mosses were exposed for 90 days, and the effects of traffic and car workshop activity on the increments in elemental concentrations were assessed using instrumental neutron activation analysis (INAA) and flame atomic absorption spectrometry (F-AAS). The actual quantum yield of Photo System II (PSII) photochemical was also analyzed to assess changes in moss vitality during the experiment. The results showed that the concentrations of individual elements in mosses and thus in the atmospheric aerosol depend on the site of exposure. The difference in analyte concentrations between outdoor and indoor environments depends on the type of element and its source. The mosses exposed in the workshop were good bioaccumulators of elements such as Al, Cr, Fe and Ba, whose concentrations were higher inside than outside and their emission sources related to the activities of the car workshop were defined. The mosses remained vital in the air during the exposure period, while they worked as a natural sorbent inside the workshop.

1. Introduction

Air pollution is one of the major problems of the 21st century. There is an increasing emphasis on the involvement of humans in this process and the impact of these activities on their health (Bakolis et al., 2020; Kermani et al., 2021; Li et al., 2017). Therefore, methods are sought that will inform about the quality of the environment in a way that is: fast, cheap and reliable. A complement to classical instrumental studies in monitoring atmospheric aerosol quality is the use of mosses as biomonitors of air pollution (Markert and Wünschmann, 2011; Ștefănuț et al., 2019; Vuković et al., 2014). Mosses sorb pollutants over their whole surface area and are therefore recognized as an effective method of biomonitoring the state of the environment in terms of, e.g., metal contamination (Kosior et al., 2018; Stihi et al., 2017; Yushin et al., 2020).

They are quite commonly used as biomonitors of air pollution in urban areas, where accumulated concentrations of analyzed elements are related to effects on human health (Ávila-Pérez et al., 2019; Chandra Bhan, 2019; Di Palma et al., 2017; Madadzada et al., 2019). Some of these studies concern the determination of air quality, e.g., indoors (Capozzi et al., 2019; Demková and Bobul'ská, 2019; Motyka et al., 2013; Rajfur et al., 2018), in garages, car parks (Demková et al., 2018; Vuković et al., 2014) or other semi-enclosed spaces (Goryainova et al., 2016; Lazić et al., 2016; Vuković et al., 2013; Zechmeister et al., 2006).

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As a bioindicator of air quality, i.e. a living organism, moss is not just a natural sorbent in these biomonitoring studies (Boquete et al., 2017; Markert, 2008). It is therefore essential to take into account the environmental conditions in which the moss is exposed (Sujetovienė and Galinytė, 2016; Świsłowski et al., 2021c), with a determination of chlorophyll content as an indicator of its vitality (Świsłowski et al., 2020; Zhang et al., 2016) which is related to other vital parameters (Carvalho et al., 2019; Vannini et al., 2020). Therefore, the measurement and control of vital parameters of mosses during surveys should not be excluded in any way (Capozzi et al., 2020), as the use of appropriate molecular markers could help in assessing the survival of mosses exposed to contamination (Cortis et al., 2016).

As previously mentioned, mosses are an excellent tool for detecting anthropogenic sources of air pollution (Makholm and Mladenoff, 2005; van Laaten et al., 2020). For example, *Sphagnum girgensohnii* and *Sphagnum papillosum* effectively reflect the seasonal variability of emerging air pollutants (Salo et al., 2016; Vuković et al., 2015a,b). On the other hand, *Sphagnum girgensohnii* and *Hypnum cupressiforme* proved to be effective monitors of elements present in vineyard sites influenced by chemical treatments related to their functioning and identifying additional points and line sources of pollution (Milićević et al., 2017). On the other hand, biomonitoring based on the moss-bag technique, together with classic measurement methods, complements the environmental quality monitoring system, as it allows to obtain additional data, as compared to if only one of these methods was used (Iodice et al., 2016; Ștefănuț et al., 2019).

Therefore, it was considered expedient to use them as biomonitors of air pollution in urban areas to identify elements released into the air due to car workshop activities. An element of novelty concerning other works in the field of active biomonitoring is simultaneous research on three moss species (possibility to compare accumulation properties- new knowledge in this field concerning rarely used species in active biomonitoring such as *Dicranum polysetum*), biomonitoring of two different spaces (outdoor and indoor of car workshop - determination of differences between the studied spaces) and connecting accumulated elements by mosses to their vitality by measuring their viability (chlorophyll fluorescence) - until now, biomonitoring studies have overlooked the essential element of using a living biomonitor during the study, this research draws attention to this.

The following hypotheses were verified: (I) the case of mosses suspended outside the workshop, the labeled elements will be identified with contamination resulting from traffic and combustion processes during the heating season, (II) samples located under the roof - elimination of wet deposition and therefore influence only contamination without the possibility of elements leaching from mosses and in case of samples inside the car workshop - contamination resulting from the work of the car workshop.

2. Material and methods

2.1. Material

Exposure of three moss species *Sphagnum fallax* (*Sp*), *Pleurozium schreberi* (*Pl*) and *Dicranum polysetum* (*Dp*) as part of active biomonitoring for three months (14 November 2020 – 14 February 2021) on property in Końskie (Świętokrzyskie Voivodeship, Poland).

2.2. Methods

Following the international guideline, moss samples were collected and prepared before exposure (ICP Vegetation, 2020). Minimum sample numbers (MSN) were calculated according to (Wosniok et al., 2020). It has been shown, in earlier studies, that prior to exposure in active biomonitoring, the preparation method is critical and fundamentally affects the initial elemental concentration in mosses. So, according to a previously developed methodology, mosses were prepared before exposure by conditioning in demineralized water (Świsłowski et al., 2021b). Mosses were hung at the height of about 2.00 m from the ground and samples were located outdoor in three points: by the street (beginning of the property) (S), then halfway along this area to the car workshop (H), under the roof (UR) of the car workshop; and inside of the car workshop (two points) on two walls - above the entrance and on the right (W1 and W2)- 45 samples: nine samples per point- three samples per species. Fig. 1 shows a visualization of the study area.

After 90 days of exposure, each moss sample, with a mass of 0.500 ± 0.001 g dry mass (d.m.), was mineralized in a mixture of nitric acid and hydrogen peroxide using a microwave oven (Berghof company, Germany) to determine Cu and Pb. In Table 1 in Supplementary Materials (SM), concentrations of metals in certified reference materials BCR-482 *lichen*, produced at the Institute for Reference Materials and Measurements, Belgium, are shown. The mineralization process was carried out at the temperature of 180 °C. These metals were determined using an atomic absorption flame spectrometer type iCE 3500 (Thermo Scientific, USA). Concentrations of metals were determined in solution after mineralization filtration and were diluted into volumetric flasks of 20 cm^3. Calibration of the spectrometer was performed with standard solutions (ANALYTIKA Ltd., Czech Republic). The values of the highest concentrations of the models used for calibration (5 mg/dm^3 for Cu and Pb) were approved as linear limits to signal dependence on concentration. The concentration of Hg in the moss samples (0.04 g ± 0.001 g d.m.) was determined with AMA 254 mercury analyzer (Altec Ltd., Czech Republic). For other elements, samples were subjected to neutron activation analysis at the IBR-2 reactor (Dubna, Russia). Two modes of samples irradiation were applied. The first one to determine the elements with short-lived isotopes (Mg, Al, Cl, Ca, V, Mn, and I) and the second for identification of elements with long-lived isotopes (Na, K, Sc, Cr, Fe, Co, Zn, As, Se, Br, Rb, Sr, Mo, Sb, Cs, Ba, La, Sm, Hf, Ta, Th and U). In the first mode samples were irradiated for 3 min at a neutron flux of 1.6×10^{12}n cm^{-2} s^{-1} and measured for 15 min directly after irradiation. In the second mode samples were irradiated for 4 days in the Cd-screen channel at a neutron flux of 3.31×10^{11}n cm^{-2} s^{-1} and then measured twice after 4 and 20 days of decay using HPGe detectors. Spectra processing and calculating metal concentration were done using Genie2000 and "Concentration" software. The quality control of the analytical measurements was provided using certified reference materials: NIST SRM 1549 - Non-Fat Milk Powder, NIST SRM 1566b - Oyster Tissue, NIST SRM 1633c- Coal fly ash, NIST SRM 2710a - Montana I Soil, IAEA SL3 - Lake Sediment, SDC-1 - Mica Schist, FFA-1 - Fine Fly Ash and BCR-723 - Road dust, which were irradiated under the same experimental conditions as the samples. Good agreement between experimental results and certified values was obtained (see Table 2 SM).

Chlorophyll fluorescence of photosystem II, actual photochemical efficiency (yield) (Loriaux et al., 2013) was measured using the modulated portable fluorometer (Opti-Sciences, USA) under ambient light condition (Šraj Kržič and Gaberščik, 2005). Mosses for the study were collected in autumn and actual photochemical efficiency (yield) was measured in the morning (Węgrzyn et al., 2021).

2.3. Data analysis

MS Excel 2016, STATISTICA ver. 13.3, and JASP 0.10.2 software were used to process and present the data. For computation and methodological details for MSN counting was used (Wosniok et al., 2019). Shapiro Wilk's test was used to assess the normality. Differences in concentration of the examined elements between species and locations were evaluated by the Student *t*-test and Wilcoxon test at a p-value of 0.05.

3. Results

As a first step, the distribution of elements concentrations was analyzed according to the species of moss exposed and the location of its

Fig. 1. Location of measurement points. Individual letters are used to indicate the moss exposure sites described in section *2.2 Methods*. The black color marks the main road's marking running alongside all properties. The green color indicates the area of the selected property. The grey color indicates the buildings: the first is the mechanic's house and the second is the workshop where there were two measuring points (W1, W2).

exposure. Part of the results is shown in Fig. 2. Other results are presented in Figs. 1-3 SM.

In Fig. 2. it is shown the concentrations for two selected elements, Zn and Br. In addition to the variation in concentrations and the variation for a given species, there is also variation in concentration depending on where the samples are exposed. For zinc and bromine, their highest concentrations were determined at point "H" - half of the property. Similar relationships, with the highest concentrations noted for this measurement point, were observed for arsenic, antimony, lanthanum and samarium, among others (Figs. 1-3 in SM). This relationship confirms the influence of traffic emissions on metal deposition in the transport route (road) vicinity. The wind transports elements over long distances, so analyte concentrations are not always highest at the emitter itself (Kłos et al., 2009). *Relative Accumulation Factor* (*RAF*) values higher than half indicate a slight enrichment of elements in the moss and values higher than one indicate a significant enrichment of elements (Vuković et al., 2017). Minor enrichment will include elements such as Br, Sb and La for all moss species. *RAF* increments >1 were noted for: Al, Cl, V, Cr, Fe, Br, Ba and Cu. This analysis also indicates characteristic points (measurement locations) with the highest increases in

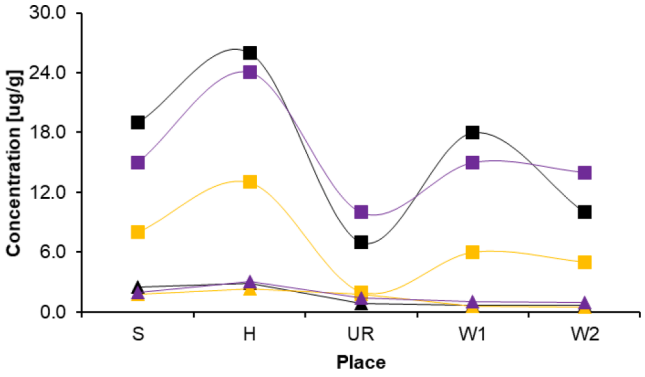

Fig. 2. Zn and Br concentrations accumulated in mosses exposed on the property. Black indicates *Sp* species, yellow *Pl* species and violet *Dp* species. Squares represent zinc concentrations and triangles indicate bromine concentrations.

analyte concentrations (Table 3 SM). The MSN results presented in Table 4 SM indicate that for most cases, the selected number of samples was adequate to assess elemental deposition in mosses during their exposure. Underestimation of sample number occurred only for the point outside under the workshop roof (UR) for all moss species.

The second stage of the analyses comprised a cross-species comparison of mosses, taking into account the variation in concentrations of the element concerned, divided into samples exposed outside and inside the workshop. A partial comparison is shown in Fig. 3.

Fig. 3 summarizes selected elements whose concentrations were higher inside the workshop (W1 and W2) during exposure compared to samples exposed outside (combination of points: S, H, UR). The results indicate that the elements whose emission is caused by the activity of the car workshop accumulate in mosses in concentrations higher than those present in atmospheric aerosol outside. In addition, it can be noted that the species of moss taken for exposure is essential. So, for example, the aluminium concentrations in the workshop are significantly higher for *S. fallax* and *D. polysetum* than *P. schreberi* (statistically significant difference of Student's *t*-test in concentrations between *S. fallax* and *P. schreberi* and also *D. polysetum* and *P. schreberi* is 0.034 and 0.009, respectively). This species (*P. schreberi*) is a better biomonitor of chromium (together with *D. polysetum*) concerning the sorption capacity of *S. fallax*. The element that was also better accumulated by mosses in the workshop compared to outdoor samples was hafnium, whose mean concentrations after exposure were 0.032, 0.015 and 0.007 μg/g for *S. fallax*, *P. schreberi* and *D. polysetum*, respectively (graphs for the other elements are shown in Fig. 4 in SM).

Another parameter that depends on exposure conditions and moss species is their vitality, the changes of which are shown in Fig. 4.

The actual quantum yield of the mosses was dependent on the species and the location of their exposure. Samples exposed directly outdoors had a significant difference in vitality compared to mosses exposed at the UR point as well as those in the workshop. Relative to the control ('0'), the decrease in moss vitality after exposure only at point 'S' was 32.5%, 64.9% and 40.2% for *S. fallax*, *P. schreberi* and *D. polysetum,* respectively. Mosses exposed at points inside the workshop were characterized by decreases below the cut-off value, where they should only be considered as dead natural sorbents after a three-month exposure.

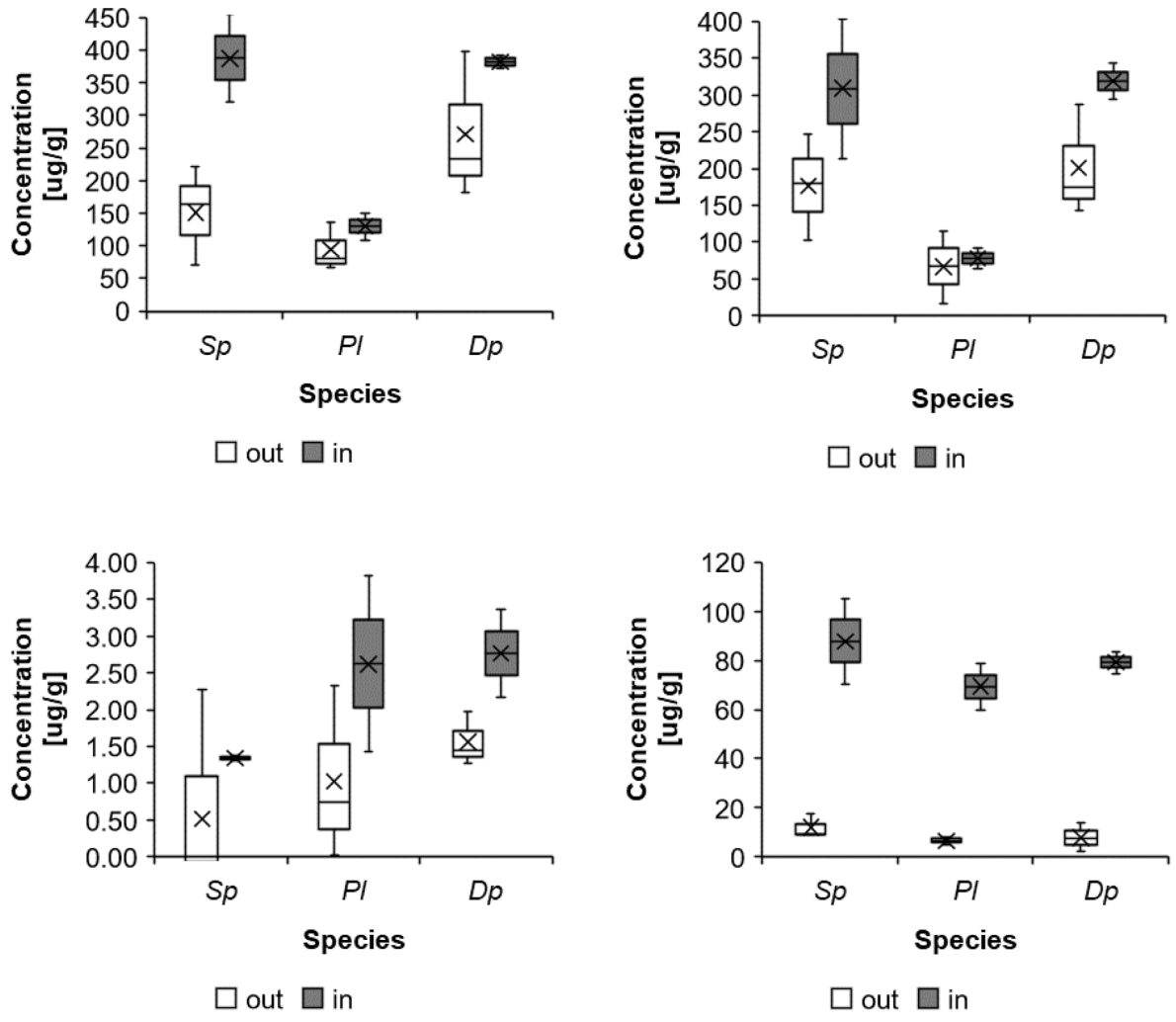

Fig. 3. Variation in concentrations of a) aluminium, b) chromium, c) iron, d) barium in mosses after exposure by species and site of exposure: outdoor and indoor.

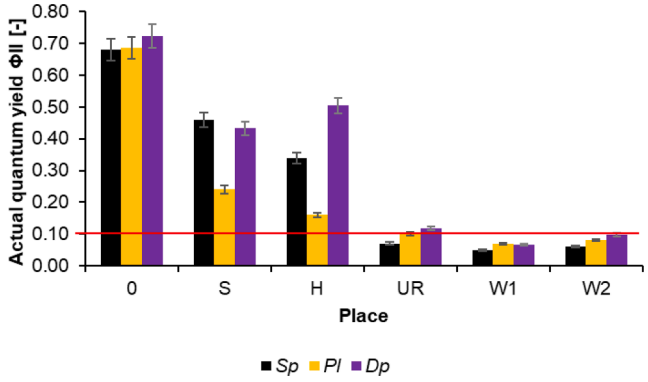

Fig. 4. Changes in actual quantum yield before '0′ exposure and after exposure at measurement points according to moss species. The box means the mean concentration (n = 5), the whisker represents the standard deviation. The "0″ point represents the measurement in the forest before the mosses were collected. The red line indicates the cut-off value for vitality (Lichtenthaler et al., 2005).

4. Discussion

4.1. Biomonitoring of selected areas

The use of mosses in biomonitoring of air pollution in urban areas provides a cheap and effective tool to detect point sources of pollution (Messager et al., 2021). They are also used to assess the impact of linear sources of traffic emissions (Zechmeister et al., 2006). Concentrations of selected metals will have a distribution dependent on the location of the samples from the emission source (Naszradi et al., 2004). In our study, some elements had concentrations higher in the middle of the property (Fig. 2) than directly next to the road (e.g., Sr, V for *S. fallax* or Sb, Mn, Zn for *P. schreberi* or Br for *D. polysetum*- compare with Figs. 1-3 SM). This is also indicated by other studies that the highest concentrations deposited in *P. schreberi* mosses are observed at some distance from the road edge (Radziemska et al., 2019), however, it is important to consider the strength and direction of the wind in such a case (Kłos et al., 2009). It does not change the fact, however, that the presence of concentrations of such elements as Al, V, Fe, Cr or Sb are indicators of air pollution in urban areas originating, among others, from road traffic (Shvetsova et al., 2019) and Al, Zn, Cr may be emitted as a result of brake wear, tire wear (Pant and Harrison, 2013). *RAF* values determined for our moss samples indicate in most cases, insignificant increases and those elements with *RAF* > 1 are few (compare Table 3 SM). Differences in

elemental concentrations in mosses are due to their different responses to their effects and to differences in the morphology of individual species (Sergeeva et al., 2021b, 2021a). In our own previous studies, when analyzing the *RAF* value, previous values were not high (Konopka et al., 2019; Świsłowski et al., 2020, Świsłowski et al., 2021e, Świsłowski et al., 2021d, Świsłowski et al., 2021c) compared to sites with increments of more than 50 in the Arctic mining area (Opekunova et al., 2021) or more than 100 at unreclaimed mine workings in a post-mining area (Demková et al., 2020). Such high values are related to the accumulation of elements by mosses exposed in characteristic selected emission sources (Chaligava et al., 2021).

4.2. Effect of moss exposure method on concentrations of accumulated elements

The concentration of elements in mosses also depends, among other things, on the conditions (season) or the way they are exposed (Dharmasiri and Deeyamulla, 2013; Hu et al., 2018; Rogova et al., 2018). For our results, this is visible for a specific UR measurement point (Fig. 2 and compared with Figs. 1-3 SM). Dry deposition is an essential pathway for accumulating airborne metals in mosses (Sun et al., 2009). However, mosses uptake substances through both dry and wet deposition (Lazo et al., 2019), therefore, in the study, uncovered moss bags have higher concentrations than covered moss bags (Arndt et al., 2014). Our use of moss cover under the roof significantly reduces the number of accumulated elements due to the elimination of wet deposition- Wilcoxon test result $p < 0.001$. It is also confirmed by literature studies (Aničić et al., 2009a; Aničić Urošević et al., 2022). The minimum number of samples exposed at this point calculated according to (Wosniok et al., 2020) is an underestimate. Due to the reduction of wet deposition by exposure at such a site, more samples would need to be prepared to properly assess air pollution at this point. The use of the moss-bag technique in the uncovered version in active biomonitoring is the subject of continuous standardization and optimization of the moss exposure method (Ares et al., 2014, 2012; Sorrentino et al., 2021).

4.3. Identification of sources of elements accumulated in mosses

Mosses in active biomonitoring are used to monitor outdoor air pollution in cities (Rivera et al., 2011). However, it is more important to compare the outdoor with the indoor environment, focusing on looking for indoor sources of pollution (Capozzi et al., 2019; Ndong Ba et al., 2019). This is also indicated by the results of our study comparing the concentration of elements in both environments (Fig. 3 and Fig. 4 SM). According to the interview with the mechanic in the surveyed workshop, the source of indoor air pollution was supposed to be activities such as heating the workshop in winter with bottled gas, smoking, and welding. In addition, the workshop carried out the routine, standard activities associated with its business: repair, cosmetics and care of vehicles (including painting and varnishing of cars, maintenance of car floors, application of polishes). In addition, the source of pollution was to be the car exhaust itself, emitted during the work of the engines of the cars coming into and going out of the workshop.

For lichens exposed in one workshop in Nigeria, concentrations of, among others, Cu, Fe, Zn, Pb (Odiwe et al., 2014). The workshop activities in general, were identified as sources of their concentrations. Similarly, the same metals were also determined in bark samples taken from trees near the workshops (Odiwe et al., 2018). In the case of our study with mosses, the high Al-concentrations, higher than outside (Fig. 3), are to be attributed to the use of a filling putty with aluminum dust or a fine-grained filling putty with aluminum plates. Higher concentrations of chromium in mosses in the workshop than outside are the result of brake wear, for example (Suvarapu and Baek, 2017), it also provides a source of traffic pollutant emission (Vuković et al., 2016). Chromium together with vanadium mean vehicle exhaust emission (Aničić et al., 2009b). Due to its anti-corrosive properties, chromium is used as an external coating for steel components (Zhang et al., 2019). Iron in turn is an element characteristic for industry, traffic or oil burning (Shvetsova et al., 2019). It is also blown in by the wind from soil dust as well as being a component of paints (Madadzada et al., 2019). The combustion reaction of a mixture of iron oxide and powdered aluminium, is used in MIG-welding (Dasch and D'Arcy, 2008). The *RAF* values obtained for barium (Table 3 SM) are comparable to those obtained for mosses exposed for 10 weeks in canyon streets in Belgrade urban area (Vuković et al., 2013). Barium is an industry indicator (Capozzi et al., 2016), and on anthropogenic traffic-borne element (Arndt et al., 2017). Our results show the difference between dry and wet moss bags (out: S, H *versus* indoor samples) in relation to research (Aničić et al., 2009c). This indicates its characteristic emission source, e. g. diesel engine exhaust (Kravchenko et al., 2014). A study of active biomonitoring of moss around petrol stations has shown that concentrations of metals in the air around them (Fe, Zn, Mn), come not from fuel combustion but from the car body, which is mechanically damaged during fueling (Demková et al., 2017a). The determination of such an element as hafnium indicates the influence of anthropogenic activities; however, due to the low concentrations obtained, also for the elements: Sc, La, Sm, Th or U in relation to the literature studies, where the obtained results are two–three orders higher (Pratas et al., 2017; Sergeeva et al., 2021a) should be considered as irrelevant. The determination of such low concentrations of the above-mentioned elements is due to the efficiency of the NAA method, which fully demonstrates this capability for a large number of elements with the lowest possible error (Culicov et al., 2005).

To our knowledge, the moss-bag technique has not been used in a car workshop to determine sources and levels of elemental emissions; the results will be compared to studies carried out in garages, where it is potentially a similar type of the area to a workshop. Emissions of elements such as Ba and Zn are related to the wear and tear of vehicle brakes and tires and, together with Cu, will come from brake dust (Vuković et al., 2014). Car exhaust was not a source of lead in the exposed mosses and in the garages, the traffic influence was responsible for the lead concentration (Deljanin et al., 2015). The same factor was also responsible for enriching the mosses with elements such as Cr, Cu, Fe or Ni (Vuković et al., 2015a,b). For the study in street canyons with public garages, it was generally determined that exhaust emissions, tyre abrasion together with the movement of road dust caused entrapped Al, Ba, Co, Cr, Cu, Fe, Ni, Pb Zn in moss-bags of *S. girgensohnii* (Lazić et al., 2016). The source of Al, Fe, Ni and Cr was corrosion of the car body confirmed by statistics. In turn, emissions of Cd, Ba, Co, Cu, Mn and Zn are due to fuel combustion or tyre wear (Demková et al., 2018). Thus, rapid urbanization and an increase in the number of vehicles means that the concentrations of elements analyzed by the authors in the literature are related to human activity (Suvarapu and Baek, 2017). The difference in elemental concentrations between the outdoor and the workshop (Fig. 3 and Fig. 4 SM) is mainly due to the specific exposure conditions of the mosses indoors (Zechmeister et al., 2020). Poor airflow and thus stagnation, together with poor dust resuspension, will account for these differences (Vuković et al., 2014).

4.4. Lifespan of mosses during the study

A large part of the pollutant load is accumulated in mosses through wet deposition (Paliulis and Blagnytė, 2010; Rogova et al., 2021). It has been shown that irrigation of mosses during exposure can improve the ability to accumulate certain elements (Aničić et al., 2009b). By monitoring indoor air pollution from cigarette smoke, this approach makes moss a good bioaccumulator of elements such as Pb, Zn and Hg (Rajfur et al., 2018). Periods of drought during exposure cause a decrease in biological activity in mosses (Amblard-Gross et al., 2002). There are significant differences between dry and living mosses' accumulation properties towards elements (Astel et al., 2008). Therefore, attention should be paid to the measurement of moss vitality during

biomonitoring studies (Capozzi et al., 2020; Świsłowski et al., 2021d). For example, mosses accumulating chromium, when exposed to its salts, caused a significant decrease in photosynthetic efficiency and obvious cell death (Chen et al., 2018). Exposure to air pollution leads to a loss of Cl, K and Rb, which means damage to the moss cell membrane (Zinicovscaia et al., 2018). Concentrations of other nutritional elements such as Ca and Mg in mosses are also characterized by a relationship inversely proportional to the metal concentration (Markert and Weckert, 1993). The actual photochemical yield we determined varied significantly from one measurement point to another. Those points located outdoors, where mosses could draw water from rainfall and had access to natural sunlight, survived better than those under the roof (Fig. 4).

On the other hand, mosses exposed inside the workshop were deprived of water in any form (they were not sprayed), exposure to artificial light from lamps (no windows) resulted in the loss of vitality after three months of exposure. The cut-off set value is 0.1 (Laisk et al., 2014; Lichtenthaler et al., 2005), below which mosses at points W1 and W2 should be considered as a natural sorbent of contaminants only and not as a living bioindicator. A better picture of the physiological and metabolic processes changes would be provided by monitoring these parameters, not after the whole exposure period but measuring them after the following months (Świsłowski et al., 2021c).

4.5. Use of selected moss species in air biomonitoring

P. schreberi can be used as a biomonitor for such rare elements as Pd, Pt, and Rh (Suoranta et al., 2016). The results of our research cited above, also indicate its usefulness in active biomonitoring studies, also confirmed by other authors (Demková et al., 2017b; Mahapatra et al., 2019; Zawadzki et al., 2014). Mosses of the genus *Dicranum* have been used as biomonitors in passive biomonitoring (Gorelova et al., 2016; Mahapatra et al., 2019). It is used successfully as an indicator of air pollution by elements in urban areas (Ojiodu et al., 2018). It is not widely used as a biomonitor in active biomonitoring (Świsłowski et al., 2021a). As our results indicate, it is a good bioaccumulator of elements such as V, Mn and Rb, but significant increases in *RAF* for this species were noted for Cr and Ba (Table 3 SM). *Sphagnum* mosses collected from peatlands mostly isolated from anthropogenic polluting sources can be used to identify distant emitters of analytes and the distribution of trace elements in the environment (Meyer et al., 2015). *Sphagnum* moss species are recommended as the most suitable for active biomonitoring due to their large surface area and a number of ion exchange sites (Aničić et al., 2009a). *Sphagnum* moss bags have been successfully characterized elements from aviation aircraft emissions (Turgut et al., 2019). *Sphagnum girgensohnii* has a high or very high relative accumulation (Culicov et al., 2005). However, these mosses tend to dry out and their effectiveness in retaining elements is therefore dependent on environmental conditions (De Agostini et al., 2020; Świsłowski et al., 2021c). In order to obtain the most accurate results from such biomonitoring studies, different species should be used as bioaccumulators for the different elements (Demková et al., 2018).

4.6. Negative effects of selected elements on the human body

It is undeniable that the use of plants indoors generally improves comfort and positively impacts people and their environment (Moya et al., 2019). However, the activity of such a workshop, together with the activities inside (e.g., use of the heating system, use of solvents, paints, smoking, car exhaust) are also a source of such compounds as: benzene, carbon monoxide, formaldehyde, naphthalene, nitrogen dioxide or polycyclic aromatic hydrocarbons, which are hazardous to human health and life (Vardoulakis et al., 2020; WHO, 2010). As part of active biomonitoring, mosses exposed in different environments showed changes in physical appearance and growth rates as influenced by air quality (Yatim and Azman, 2021). Metals accumulated by plants indicate concentrations that threaten human health; similar values were obtained in our study, e.g., for Cr (Ghoma et al., 2022). The examples given here and the studies mentioned above also indicate that, apart from the risk of exposure to the elements concerned, working in such an environment is hazardous for the mechanic's health. The activities, operations and processes associated with the functioning of the workshop generate emissions of elements whose concentrations will have a toxic effect on humans.

5. Conclusion

To the best of our knowledge, the moss-bag technique has not yet been used to assess air pollution in the indoor environment of a car workshop. The results of the conducted experiment and their analysis positively verified the assumed research hypotheses. Elements were identified whose emissions inside the workshop are directly related to its activity and the action declared by the mechanic. Each moss species used proved to be a good biomonitor for individual elements, with *S. fallax* being the best under the given outdoor and indoor environmental conditions. During the twelve-week exposure, mosses exposed outdoors were bioindicators, but exposure indoors meant that mosses were only a passive natural sorbent, indicating that they needed to be irrigated and sprayed during indoor air quality monitoring. In the future, biomonitoring research on sources of air pollution emissions and their control inside should be intensified, as the concentrations of elements determined by us indicate their potential negative impact on human health and life.

CRediT authorship contribution statement

Paweł Świsłowski: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Visualization. **Konstantin Vergel:** Methodology, Validation. **Inga Zinicovscaia:** Methodology, Validation, Writing – review & editing, Supervision, Project administration. **Małgorzata Rajfur:** Formal analysis, Methodology, Validation, Writing – review & editing, Supervision, Project administration. **Maria Wacławek:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.108849.

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Supplementary Materials (SM)

Mosses as a biomonitor to identify elements released into the air as a result of car workshop activities

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Table 1 SM. Comparison of measured and certified concentrations of Cu and Pb in BCR-482 *lichen* for F-AAS

Metal	BCR-482 *lichen*		F-AAS (n = 3)		
	Concentration	Uncertainty	Average	±SD*	Dev.**
	[mg/kg d.m.]				[%]
Cu	7.03	0.19	6.63	0.17	-5.70
Pb	40.9	1.40	38.2	1.00	-6.60

* standard deviation

** relative difference between the measured (c_z) and certified (c_c) concentration $100\% \cdot (c_z - c_c)/c_c$

n – number of samples

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Table 2 SM. Certified and experimental values (mean ± SD) for the used reference materials for INNA

Element	Reference material	Experimental value	Certified value
		[µg/g]	
Na	SL3	6520 ± 300	6690 ± 180
Mg	1549	1190 ± 35	1200 ± 30
Al	528	29813 ± 3200	29806 ± 625
Cl	1566b	4942 ± 170	5140 ± 100
Ca	2710a	9626 ± 530	9640 ± 450
V	SDC1	94 ± 10	102 ± 12
Mn	SDC1	865 ± 65	880 ± 60
I	1549	3.27 ± 0.09	3.38 ± 0.03
K	SL3	8445 ± 700	8740 ± 550
Sc	FFA-1	24.9 ± 1.1	24.2 ± 0.8
Cr	FAA-1	164 ± 8.3	156 ± 7.2
Co	723	29.3 ± 1.7	29.8 ± 1.2
Fe	FAA-1	49334 ± 2910	48900 ± 1418
Zn	FFA-1	590 ± 40	569 ± 55
As	FFA-1	53.8 ± 2.0	53.6 ± 2.7
Se	1633c	13.9 ± 1.4	13.9 ± 0.5
Br	SL3	5.5 ± 0.04	5.6 ± 0.04
Rb	723	74.8 ± 7.4	75 ± 5.0
Sr	723	242 ± 24	254 ± 19
Mo	723	42 ± 3.0	40 ± 2.5
Sb	723	27.9 ± 1.7	28.2 ± 2.3
Cs	FFA-1	46 ± 2.0	48 ± 2.4
Ba	723	461 ± 46	460 ± 38
La	FFA-1	60.4±3.1	60.7±4.0
Sm	SL3	3.79 ± 0.1	3.83 ± 0.3
Hf	FAA-1	6.0 ± 0.3	6.0 ± 0.4
Ta	SL3	0.66 ± 0.05	0.7 ± 0.03
Th	723	4.9 ± 0.12	4.8 ± 0.48
U	1633c	9.35 ± 0.63	9.25 ± 0.45

Table 3 SM. Mean *RAF* values in mosses

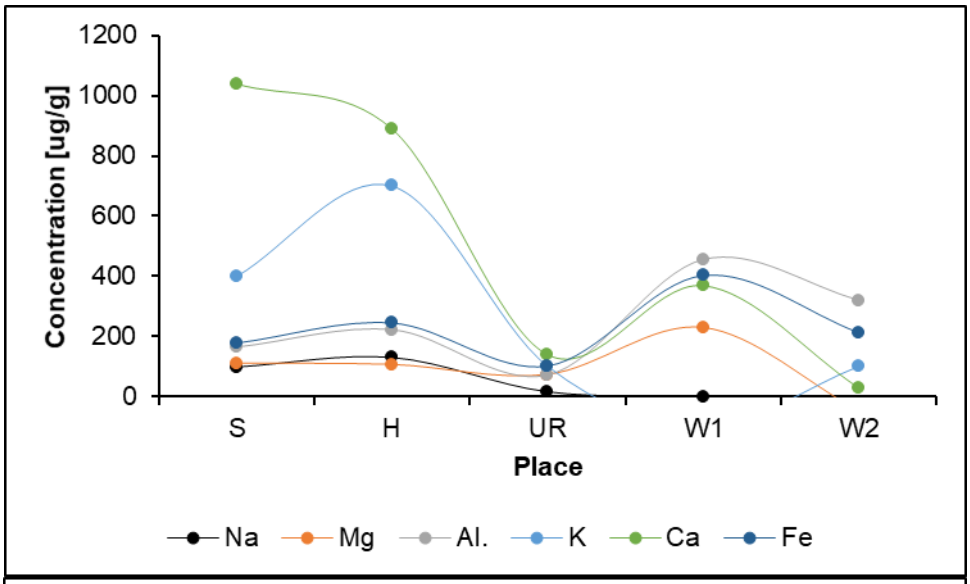
Spec.	Element/ Place	Na	Mg	Al.	Cl	K	Ca	Sc	V	Cr	Mn	Fe	Co	Zn	As	Se	Br	Rb	Sr	Mo	Sb	I	Cs	Ba	La	Sm	Hf	Ta	Th	U	Cu	Pb	Hg
Sp	S	0.39	0.10	0.43	0.08	0.03	0.37	0.64	0.74	*	0.18	0.64	0.27	0.36	0.41	0.39	0.68	*	0.48	0.34	0.49	0.06	*	0.31	0.70	0.45	0.04	0.36	0.47	0.19	0.08	2.96	0.59
	H	0.51	0.10	0.58	0.13	0.06	0.32	0.59	1.68	1.12	0.19	0.88	0.39	0.49	0.68	0.39	0.78	0.02	0.67	0.18	0.97	0.46	0.02	0.58	0.76	0.82	0.25	0.81	0.67	0.10	0.10	1.39	0.60
	UR	0.06	0.07	0.18	0.12	0.01	0.05	0.07	0.81	*	0.43	0.37	*	0.13	0.09	0.16	0.24	*	0.23	0.24	0.16	0.41	*	0.31	0.38	0.05	*	0.35	0.13	0.33	*	0.22	0.07
	W1	0.004	0.21	1.19	*	*	0.13	0.33	1.19	0.65	0.06	1.45	0.21	0.34	0.16	0.13	0.19	*	0.28	0.16	0.73	*	*	3.55	0.58	0.14	0.69	0.14	0.18	0.07	0.19	0.00	0.07
	W2	*	*	0.84	0.11	0.01	0.01	0.16	0.90	0.67	0.19	0.77	0.18	0.19	0.23	*	0.19	*	0.21	0.11	0.62	0.33	*	2.37	0.60	0.08	0.25	0.23	*	*	6.03	0.00	*
Pl	S	*	*	0.17	0.45	*	0.06	0.00	0.02	0.02	0.02	0.04	*	0.12	0.39	0.52	0.60	*	0.27	*	0.39	*	*	0.15	0.08	0.22	0.12	0.11	0.12	0.05	0.49	1.36	0.50
	H	*	0.09	0.28	0.28	*	0.12	0.28	*	2.37	0.10	0.25	*	0.19	0.47	0.24	0.81	*	0.15	*	0.77	0.47	*	0.20	0.24	0.31	0.12	0.38	0.23	0.32	0.36	0.48	0.49
	UR	*	0.00	0.14	0.50	*	*	0.05	*	0.76	0.01	0.14	*	0.03	0.28	0.18	0.60	*	0.02	*	0.22	0.22	*	0.24	0.13	0.22	0.04	0.23	0.29	0.39	0.29	1.55	0.58
	W1	*	*	0.23	*	*	*	*	*	1.45	*	0.19	*	0.09	0.23	0.07	0.20	*	0.11	*	0.45	*	*	2.39	0.02	*	0.25	0.06	*	0.03	0.38	0.32	0.58
	W2	*	0.13	0.31	*	*	*	*	*	3.90	*	0.14	*	0.07	0.16	0.18	0.16	0.02	0.09	0.26	0.55	*	*	1.82	*	0.03	0.18	0.00	*	*	0.28	0.51	0.51
Dp	S	0.26	0.37	0.46	0.50	*	0.31	0.29	0.29	1.01	0.14	0.28	0.14	0.21	0.05	*	0.68	*	0.08	*	0.58	0.01	*	0.07	0.38	0.44	*	0.36	0.36	0.05	0.14	*	0.06
	H	0.67	0.56	0.79	0.66	0.18	0.27	0.43	1.13	1.38	0.24	0.55	0.34	0.33	0.32	*	1.07	0.12	0.19	0.005	0.75	0.50	0.02	0.48	0.64	0.59	0.20	0.77	0.43	0.02	0.13	*	0.11
	UR	0.35	0.06	0.36	1.08	0.16	0.17	0.11	0.33	0.89	0.12	0.34	0.11	0.14	0.18	0.09	0.49	0.12	0.43	0.37	0.11	0.16	0.10	0.27	0.18	0.18	*	0.44	0.20	0.02	0.01	*	0.17
	W1	0.18	0.18	0.77	0.43	0.00	0.17	0.09	0.37	1.52	0.13	0.66	0.32	0.21	0.00	0.21	0.37	*	0.20	0.10	0.36	0.11	*	2.64	0.41	0.14	0.11	0.20	0.04	*	0.12	*	*
	W2	0.11	0.22	0.73	0.11	0.05	*	0.04	0.29	2.36	*	0.57	0.16	0.19	0.05	0.21	0.33	0.03	0.03	0.19	0.46	*	0.03	2.96	0.26	0.24	0.01	0.56	0.25	*	*	*	*

* negative values of *RAF* - no increments; yellow indicates slight increments: *RAF* > 0.50 and red indicates significant increments in mosses: *RAF* > 1.00

Table 4 SM. Element-specific minimum sample numbers (MSN) and actual sample sizes (*n*) calculated for the study area for elements with *RAF* > 1.00 using (Wosniok et al., 2020)

n = 27 Place/Site	Species/ Element	*Sp*	*Pl*	*Dp*
S	Al.	26	20	21
	V	21	26	21
	Cr	20	40	22
	Fe	19	17	19
	Br	20	16	36
	Ba	33	27	26
	Pb	28	15	26
H	Al.	25	37	13
	V	26	24	17
	Cr	31	22	19
	Fe	22	25	18
	Br	26	22	27
	Ba	20	18	25
	Pb	21	20	22
UR	Al.	31	28	31
	V	31	26	34
	Cr	34	31	32
	Fe	35	38	32
	Br	32	29	35
	Ba	39	30	31
	Pb	30	35	27
W1	Al.	19	20	15
	V	14	15	24
	Cr	17	14	18
	Fe	22	15	37
	Br	13	11	14
	Ba	12	15	19
	Pb	28	26	20
W2	Al.	21	19	17
	V	16	14	21
	Cr	17	13	19
	Fe	14	22	14
	Br	22	19	20
	Ba	17	20	19
	Pb	27	30	18

red numbers = MSN exceeded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800
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W1
W2
Place
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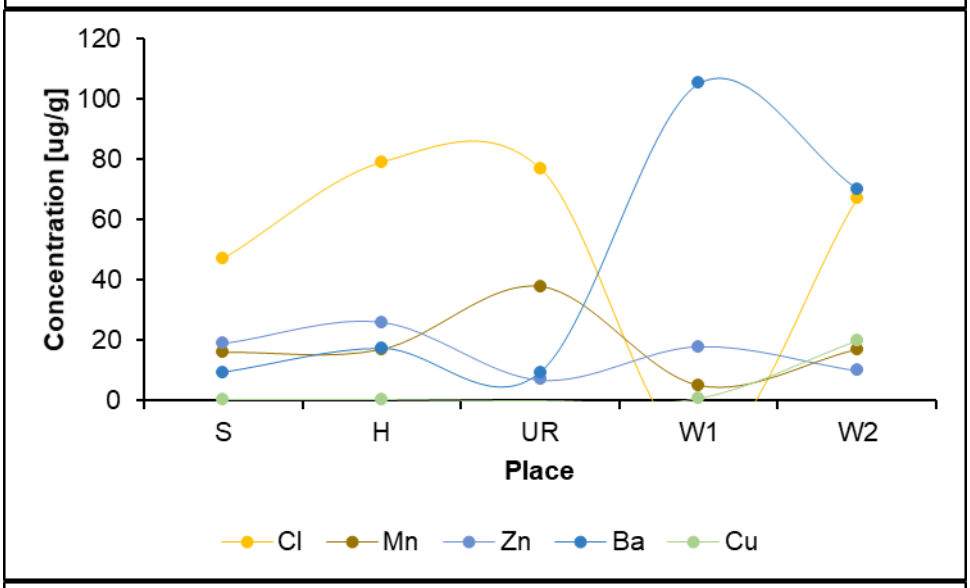
Al.
K
Ca
Fe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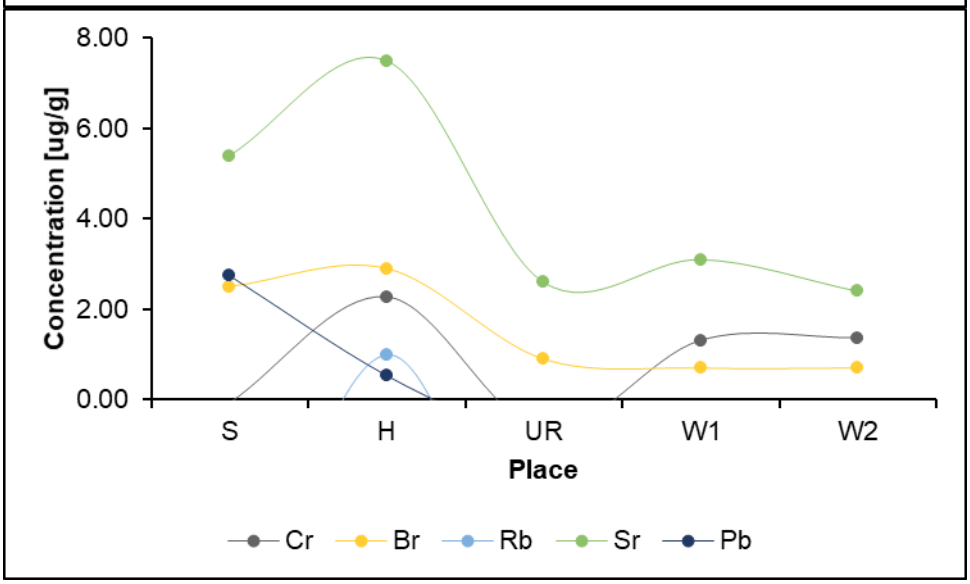
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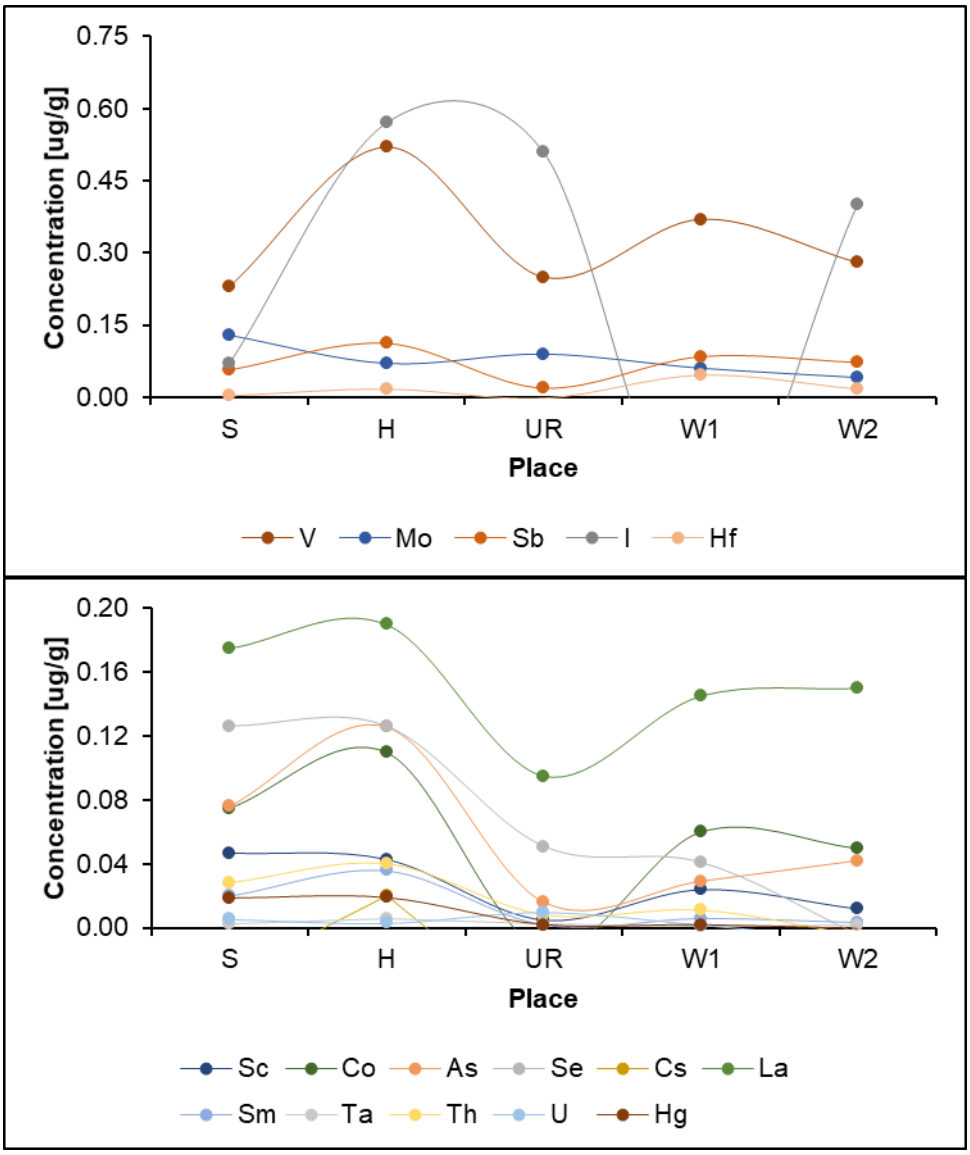


Fig. 1 SM. Concentrations of individual elements accumulated in mosses of *Sp*. species depending on the place of their exposure

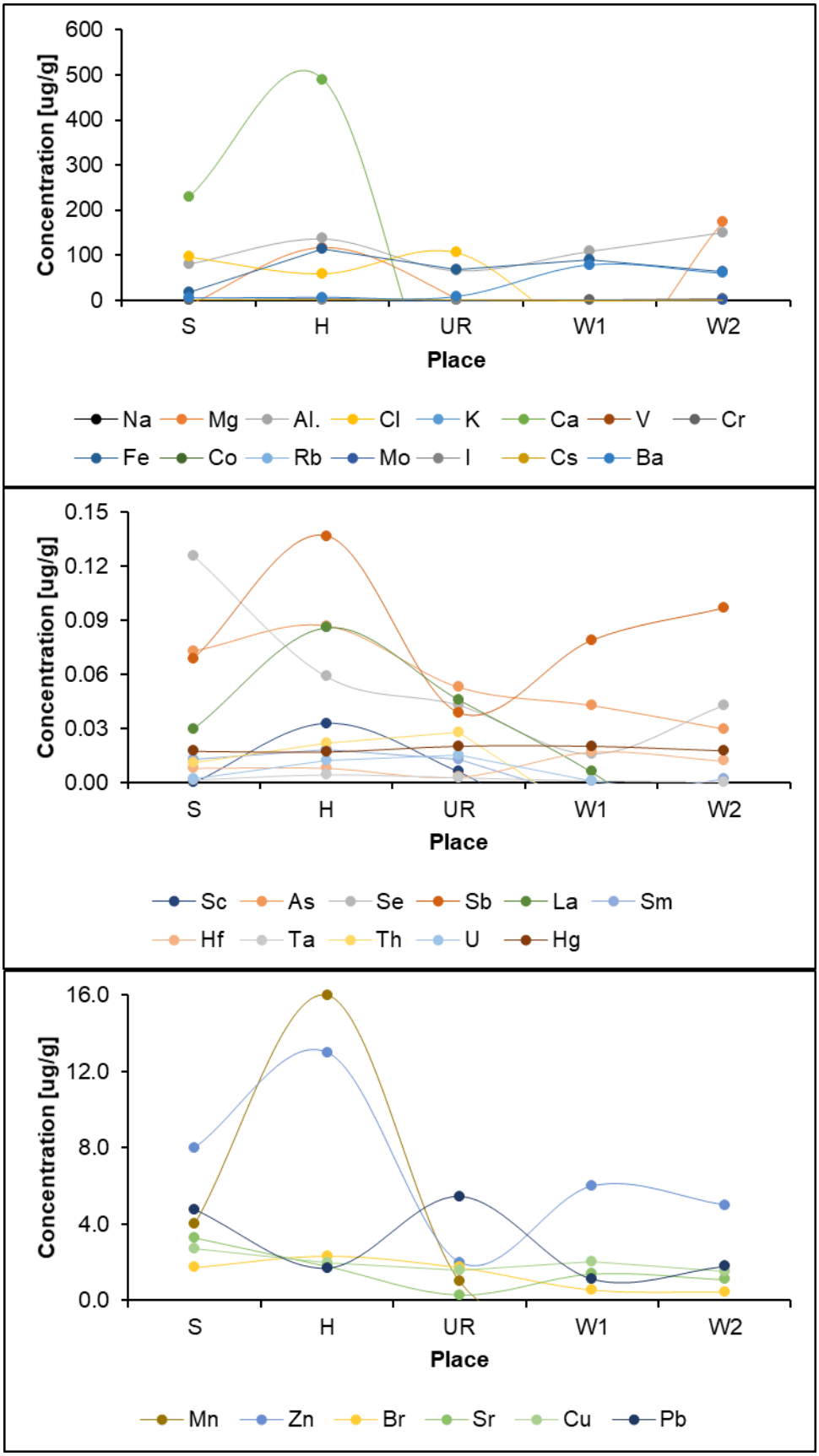
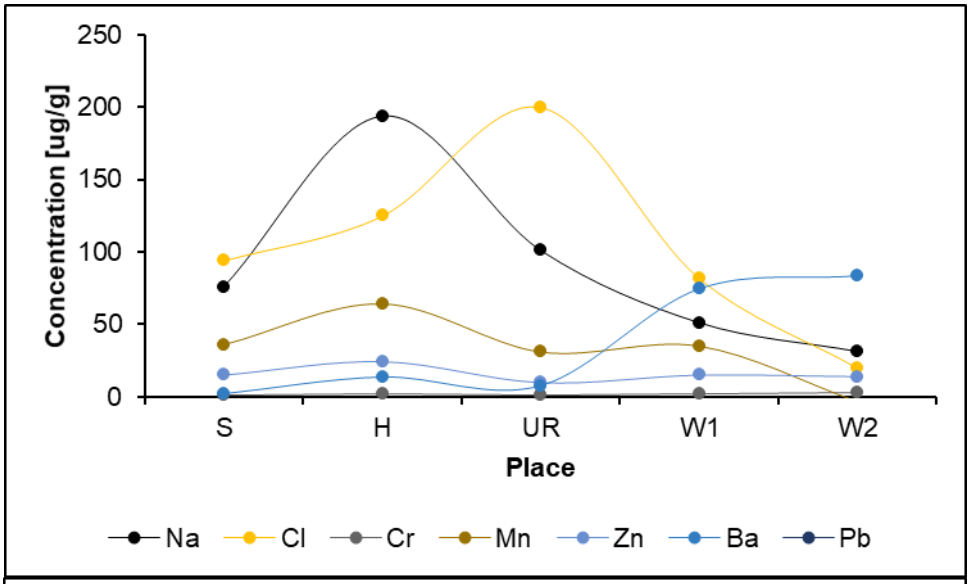


Fig. 2 SM. Concentrations of individual elements accumulated in mosses of *Pl.* species depending on the place of their exposure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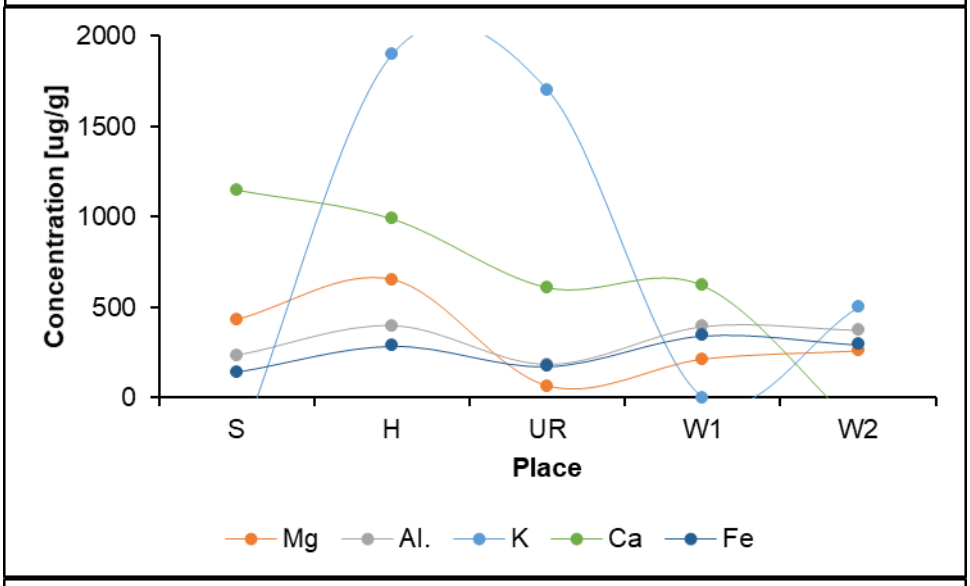
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K
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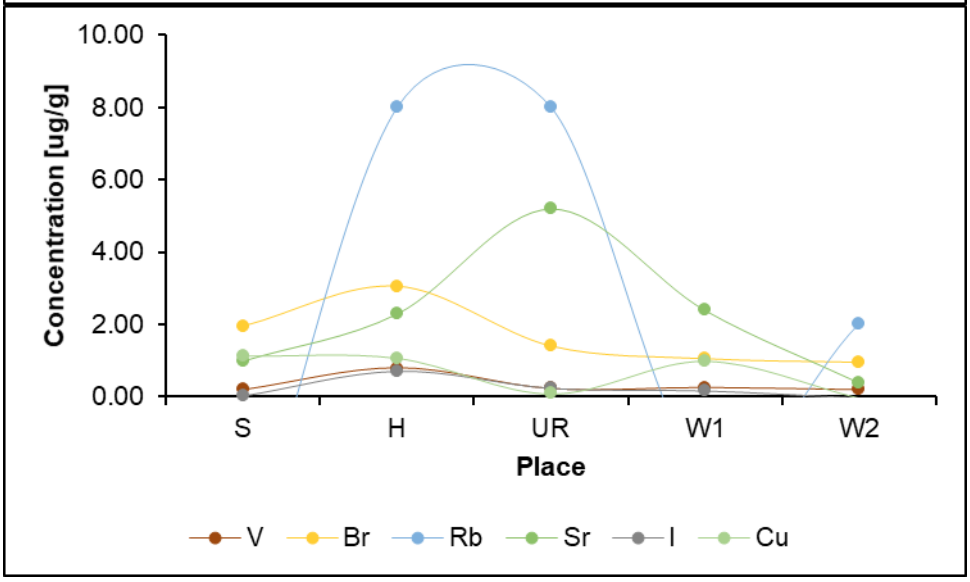
Fe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W2
Place
V
Br
Rb
Sr
I
Cu

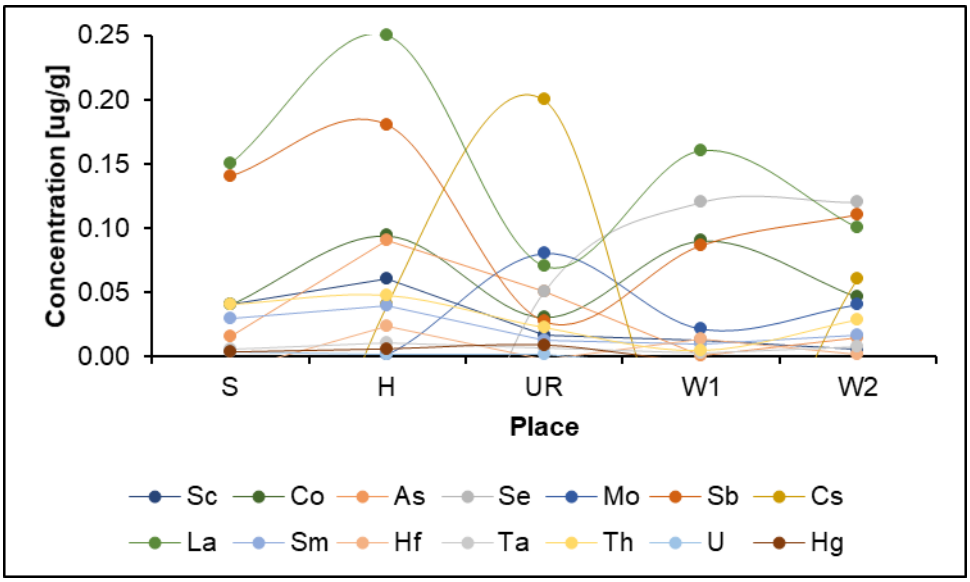
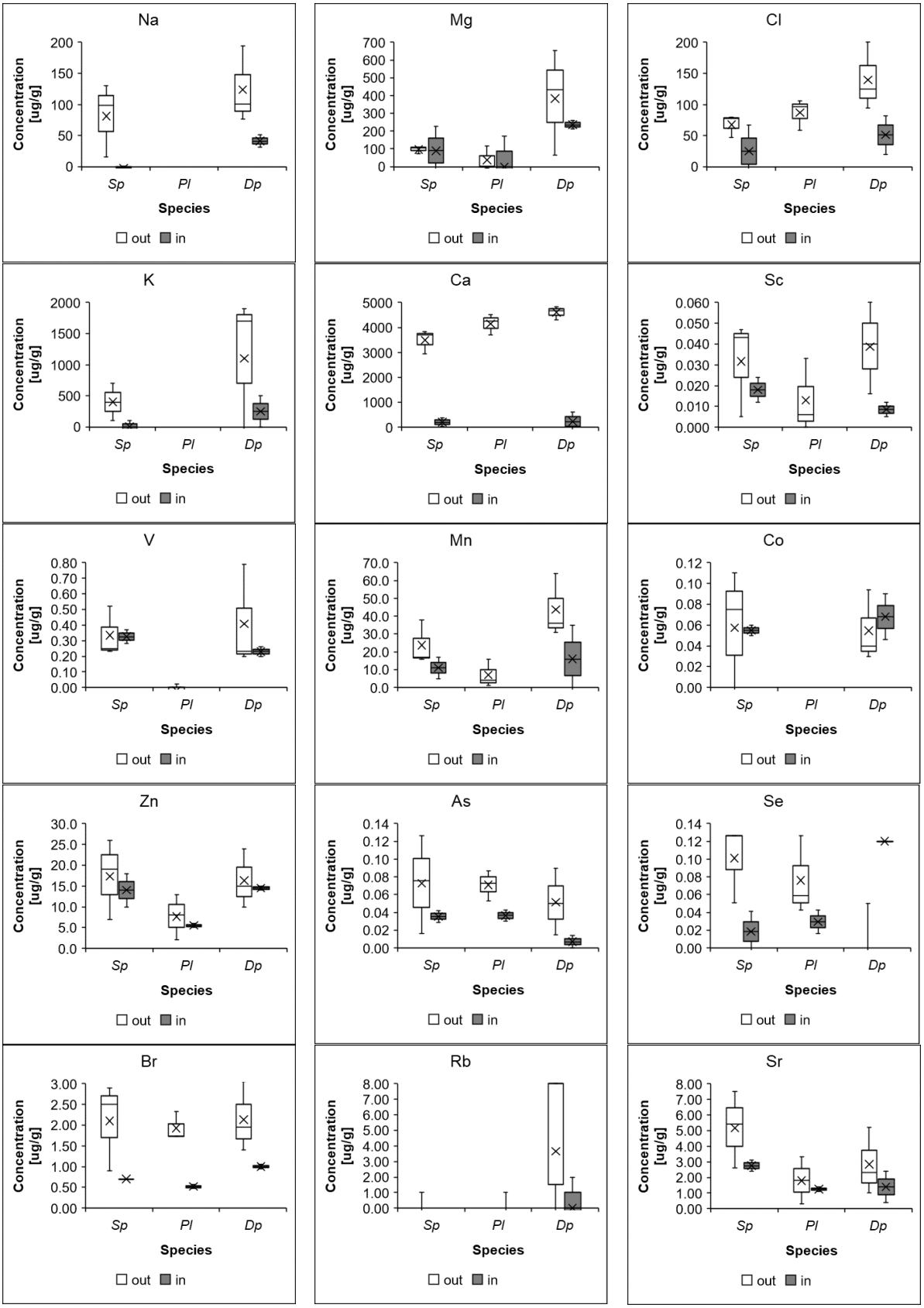


Fig. 3 SM. Concentrations of individual elements accumulated in mosses of *Dp*. species depending on the place of their exposure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K
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Sc
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Mn
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Zn
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Se
Br
Rb
Sr
Concentration [ug/g]
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Pl
Dp
Species
out
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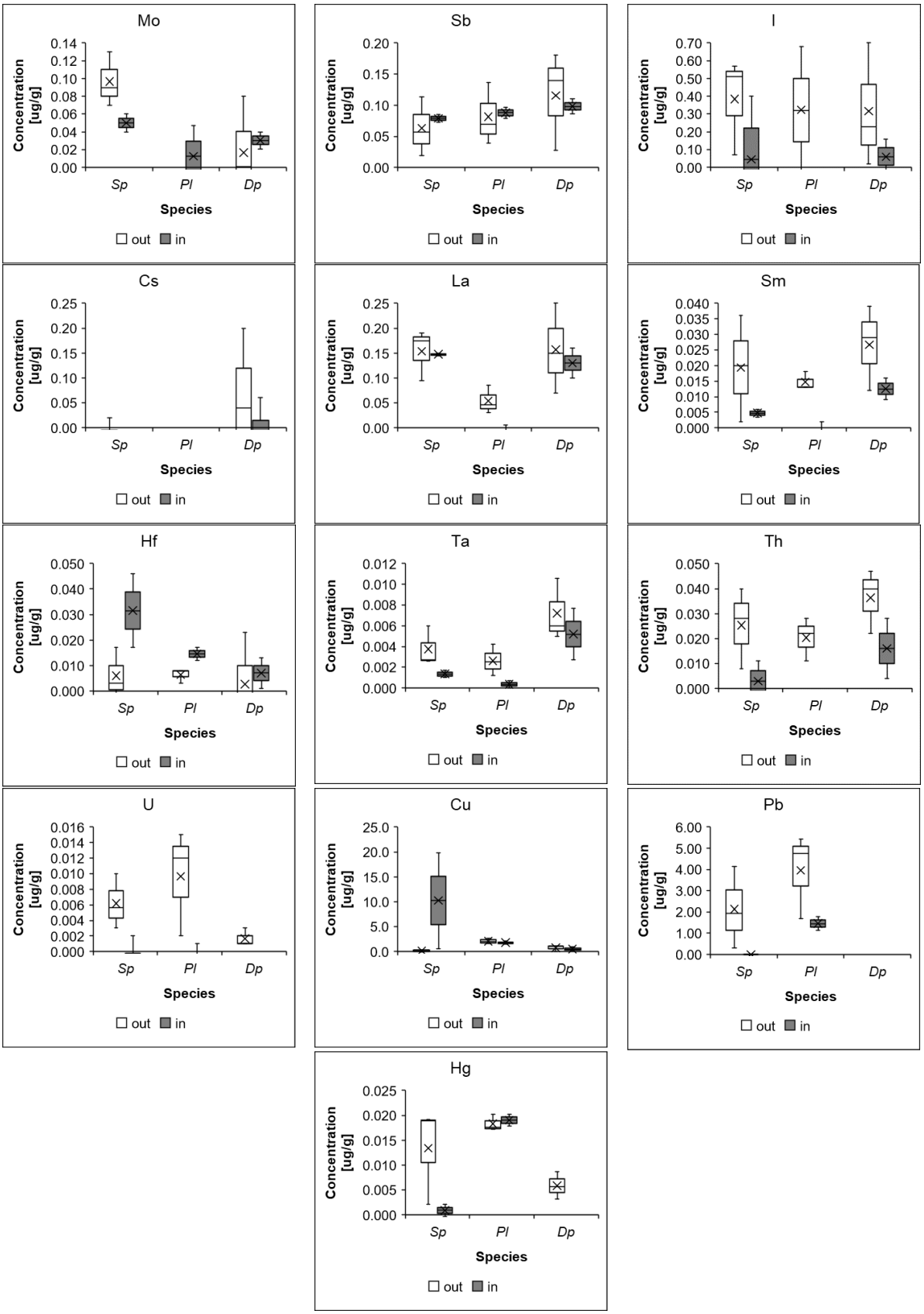

Fig. 4 SM. Variability of concentrations of analysed elements by species and site of exposure: outside and inside the workshop

atmosphere

MDPI

Article

Air Quality during New Year's Eve: A Biomonitoring Study with Moss

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Abstract: Mosses are one of the best bioindicators in the assessment of atmospheric aerosol pollution by heavy metals. Studies using mosses allow both short- and long-term air quality monitoring. The increasing contamination of the environment (including air) is causing a search for new, cheap and effective methods of monitoring its condition. Once such method is the use of mosses in active biomonitoring. The aim of the study was to assess the atmospheric aerosol pollution with selected heavy metals (Ni, Cu, Zn, Cd, Hg and Pb) from the smoke of fireworks used during New Year's Eve in the years 2019/2020 and 2020/2021. In studies a biomonitoring moss-bag method with moss *Pleurozium schreberi* (Willd. *ex* Brid.) Mitt. genus *Pleurozium* was used. The research was conducted in the town Prószków (5 km in south direction from Opole, opolskie voivodship, Poland). The moss was exposed 14 days before 31 December (from 17 to 30 of December), on New Year's Eve (31 December and 1 January) and 2 weeks after the New Year (from 2–15 January). Higher concentrations of analysed elements were determined in samples exposed during New Year's Eve. Increases in concentrations were demonstrated by analysis of the Relative Accumulation Factor (RAF). The results indicate that the use of fireworks during New Year's Eve causes an increase in air pollution with heavy metals. In addition, it was shown that the COVID-19 induced restrictions during New Year's Eve 2020 resulted in a reduction of heavy metal content in moss samples and thus in lower atmospheric aerosol pollution with these analytes. The study confirmed moss usefulness in monitoring of atmospheric aerosol pollution from point sources.

Keywords: air pollution; moss monitoring; bioindicator; heavy metals; fireworks

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1. Introduction

Air quality in Poland is one of the most important research topics concerning atmospheric aerosol pollution [1–3]. Special attention is given to air pollution by heavy metals and particulate matter (PM) in areas of high exposure and smog [4–8]. The main causes of poor air quality in Poland include: quality of heating systems used in households [8–11] and emissions generated by vehicular traffic [8,10,11].

Among the different types of pollutants involved in the overall atmospheric aerosol contamination, short-lasting but active point sources of pollution should also be considered [12,13], which include fireworks shows [14]. This is one of the most unusual sources of air pollution, which has been investigated quite frequently and on a large scale in the literature [15–17] which allows the identification of specific pollutants related only to the burning of fireworks [18,19]. Although the show associated with the New Year celebrations only takes place once a year, it has a very significant impact on the air quality. The environmental pollution burden is enormous in terms of sudden increases in pollution by selected heavy metals, PM of various fractions and also negative health effects [18,20].

People's activity related to celebrating the New Year in such a way causes that, along with the increase of social awareness of the bad condition of the environment (including the atmosphere), people ''run away'' from polluted places and move, if only for a short

time, to places where the air quality is better [21]. This choice is made possible by increasingly better ways of predicting and forecasting air pollution, based for example on various types of air pollution indices (APIs) [22]. Such methods are also used in the air pollution mathematical model for comparison with moss biomonitoring results [23]. This biological method of environmental assessment is becoming increasingly competitive with instrumental methods for measuring environmental pollution [24], but due to its lack of referenceability in relation to classical measurement methods, it is an object of continuous standardisation and review [25], which does not prevent it from being included in the national system for assessing environmental quality and monitoring pollution [26].

For active biomonitoring studies using mosses, however, it is crucial to use a biomonitor whose (research) value matches its definition [27]. Moss as a biological indicator of air quality is not only a chemical adsorbent [28], but above all a living organism [29], so it is important to control and measure vital parameters during the experiment [30]. Research to date indicates that moss as a biomonitor of air pollution, e.g., heavy metals, is most often used for short exposure periods. Such studies were performed for a minimum of one day [31], several days [32,33] or a week of time [34]. This is mainly related to the fast feedback time about the air condition at the study site [25]. So far, only a laboratory experiment was encountered in the literature [35], in which moss was used as a biomonitor of air quality analytes from fireworks being fired. In the second case, the authors only suspected contamination of the biomonitors due to fireworks being fired [36]. It was used as a space to use in an environmental the use of a moss bioindicator to assess the concentrations of pollutants emitted by fireworks as they are set off on New Year's Eve under field conditions.

The aim of this study was to measure the concentrations of heavy metals accumulated by the moss *Pleurozium schreberi* in a town during the fireworks show on New Year's Eve at the turn of 2 consecutive years. The moss species *Pleurozium schreberi* has also been shown to be a sensitive biomonitor of point sources of pollution.

2. Materials and Methods

2.1. Materials

The species used for this study was the moss *Pleurozium schreberi*. It was collected in December 2019 and December 2020 from forests in the Świetokrzyskie voivodship, PL.

2.2. Methods

Moss samples were taken and pretreated before exposure in accordance with the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation (ICP Vegetation) program guidelines for moss species, field sampling and cleaning and storing of moss samples [37]. Before exposure, the mosses were conditioned in demineralised water in accordance with a previously-developed methodology specific for mosses [38]. Then 3 g of mosses (3 samples per period) were packed into nets and exposed at a height of about 1.50–2.00 m from ground level. Mosses were exposed during several study periods: 2 weeks before New Year's Eve (B) [from 17 to 30 of December], before New Year's Eve and during New Year's Eve (B_NYE) [from 17 to 1 January], only on New Year's Eve for 2 days (NYE) [31 December and 1st January], during New Year's Eve and 2 weeks after (NYE_Af) [from 31 December to 15 January], only 2 weeks after New Year's Eve (Af) [from 2–15 January], for the whole experimental period—1 month: from mid-December to mid-January (Al.) [from 17 December to 15 January]. The survey was conducted in 2019/2020 and 2020/2021 in the same periods. Mosses were exposed in the city town of Prószków (15 km south of Opole, opolskie voivodship, PL). Each time, samples were collected and heavy metal concentrations were determined. Photographs of the mosses and exposure site are shown in Figure 1.

(**a**) (**b**)

Figure 1. Photos of mosses: (**a**) *Pleurozium schreberi* close-up, (**b**) moss exposure.

To determine the heavy metals (Ni, Cu, Zn, Cd and Pb), each moss sample, with a mass of 1.000 ± 0.001 g dry mass (d.m.), was mineralised in a mixture of HNO_3 and H_2O_2 using a Speedwave Four microwave oven (Berghof, Eningen, Germany). The mineralisation process was carried out at a temperature of 180 °C. Heavy metals (Ni, Cu, Zn, Cd and Pb) were determined using an atomic absorption flame spectrometer (F-AAS) type iCE 3500 (Thermo Scientific, Grand Island, NY, USA). Concentrations of metals were determined in solution after mineralisation and dilution and were filtered into volumetric flasks of 25 cm^3. The results were converted into 1 kg of sample of dry mass (mg/kg d.m.). The concentration of mercury in the samples (0.04 g ± 0.001 g d.m.) was determined with AMA 254 mercury analyzer (Altec Ltd., Prague, Czech Republic). The results were in mg/kg d.m. Measurements were made in 3–5 replicates (control, samples).

The RAF—Relative Accumulation Factor was used to determine increases of concentrations of the analytes in the exposed mosses samples [39].

Chlorophyll fluorescence of photosystem II (PSII) was also measured (but only in the second year of the study) using the modulated portable fluorometer (Opti-Sciences, Hudson, NH, USA). Actual photochemical efficiency (yield) was measured under ambient light [40]. Mosses were collected in the field after each term of exposure separately. In 10 (control) and 15 replicates (samples) actual photochemical efficiency (yield) were determined.

2.3. Quality Control

Table 1 presents the instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) of the iCE 3500 spectrometer which was used for heavy metals (Ni, Cu, Zn, Cd and Pb) detection. Calibration of the spectrometer was performed with a standard solution (ANALYTIKA Ltd., Prague, Czech Republic). The values of the highest concentrations of the models used for calibration (2 mg/dm^3 for Cd, 5 mg/dm^3 for Ni, Cu, Zn and Pb) were approved as linear limits to signal dependence on concentration. For mercury, the instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) of the apparatus are 0.003 ng (0.03 µg Hg/dm^3) and 0.01 ng (0.1 µg Hg/dm^3) in the test sample, respectively. Calibration of the apparatus was performed with a standard solution (ANALYTIKA Ltd., Prague, Czech Republic) [41].

Table 2 shows the concentrations of heavy metals in certified reference materials BCR-482 *lichen*, produced at the Institute for Reference Materials and Measurements, Belgium [42].

Table 1. The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the iCE 3500 (mg/dm^3) spectrometer [43].

Metal	*IDL*	*IQL*
Ni	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

Table 2. Comparison of measured and certified concentrations in BCR-482 *lichen* [42].

Metal	BCR-482 *Lichen*		AAS (n = 5)		*Dev.* **
	Concentration	Measurement Uncertainty	Average	±*SD* * of the Concentrations	
	[mg/kg dm]				[%]
Ni	2.47	0.07	2.16	0.32	−13.0
Cu	7.03	0.19	6.63	0.17	−5.70
Zn	100.6	2.20	95.1	2.30	−5.50
Cd	0.56	0.02	0.53	0.03	−5.30
Pb	40.9	1.40	38.2	1.00	−6.60

* standard deviation. ** relative difference between the measured (c_m) and certified (c_c) concentration 100% $(c_m - c_c)/c_c$.

2.4. Statistical Analysis of Data

For metals' deposition intensity comparison Kruskal-Wallis and pairwise Wilcoxon tests were used [44]. Computations were carried out in R language version 4.1.0 [45].

3. Results

Table 3 shows the statistical parameters of heavy metal concentrations in moss samples.

Table 3. Statistical parameters of metals' concentration (mg/kg dm) pooled for all periods of moss exposition. *min* minimal concentration, q_l lower quartile, *ME* median, *mean* arithmetic mean, q_u upper quartile, *max* maximum, *SD* standard deviation, and *n* number of moss samples.

	Ni	Cu	Zn	Cd	Pb	Hg
min	<1.25	5.48	38.2	<0.325	2.50	0.0201
q_l	<1.25	7.13	48.6	<0.325	3.04	0.0357
ME	<1.25	7.66	50.5	<0.325	3.50	0.0406
mean	1.45	10.9	57.7	0.390	7.09	0.0394
q_u	<1.25	11.8	58.5	0.473	9.80	0.0443
max	4.62	25.0	118	0.608	23.4	0.0561
SD	0.71	5.92	16.9	0.110	6.06	0.0081
n	39	43	43	43	43	43

At least up to the upper quartile Ni content in moss samples was lower than the instrumental quantification limit. Similarly, in most of the samples Cd concentration was lower than the *IQL*.

The presented metal concentrations in mosses are comparable to those obtained in the woods of Turawa commune in opolskie voivodship [46], although they are low in relation to industrial sites of Upper Silesia [47].

Figure 2a–d shows a comparison of heavy metal concentrations accumulated by mosses over two exposure periods: mid December 2019/mid January 2020 and mid December 2020/mid January 2021. The mean concentrations of determined heavy metals (mg/kg dm) naturally accumulated in mosses exposed during the first year of the study were for: Ni—<1.25, Cu—5.85, Zn—53.5, Cd—<0.325, Hg—0.038, Pb—7.85. and in mosses exposed in the second year of the study were respectively: Ni—<1.25, Cu—6.42, Zn—42.8, Cd—<0.325, Hg—0.019, Pb—2.12.

In Figure 2, it can be seen that heavy metal accumulation by mosses was observed during both study periods. Elemental concentrations from 2019/2020 are higher than for the second study period. For copper, zinc, lead and mercury the values for the "NYE" in 2019/2020 period only were 20.9, 90.7, 19.2, 0.04 mg/kg d.m., respectively, though during the same period in 2020/2021 they were half as high. For nickel and cadmium, depending on the survey year and exposure period, the concentration of these metals in some moss samples was below the limit of quantification of the analytical method used. Additionally, the values for Cu, Zn and Pb for 2019/2020 reach their highest values only during the 2-day

"NYE" exposure [31 December and 1 January]. In the following year, the concentrations of these elements are more homogeneous across the different periods of moss exposure.

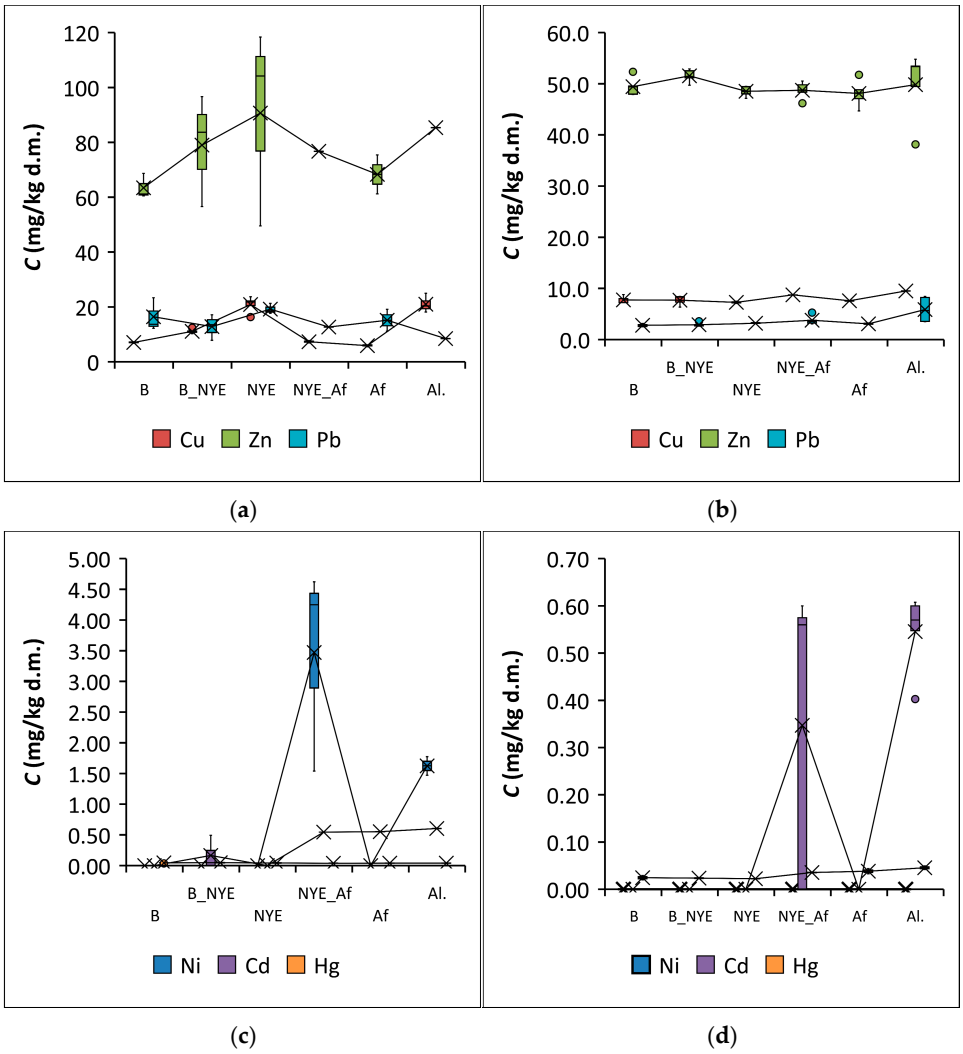
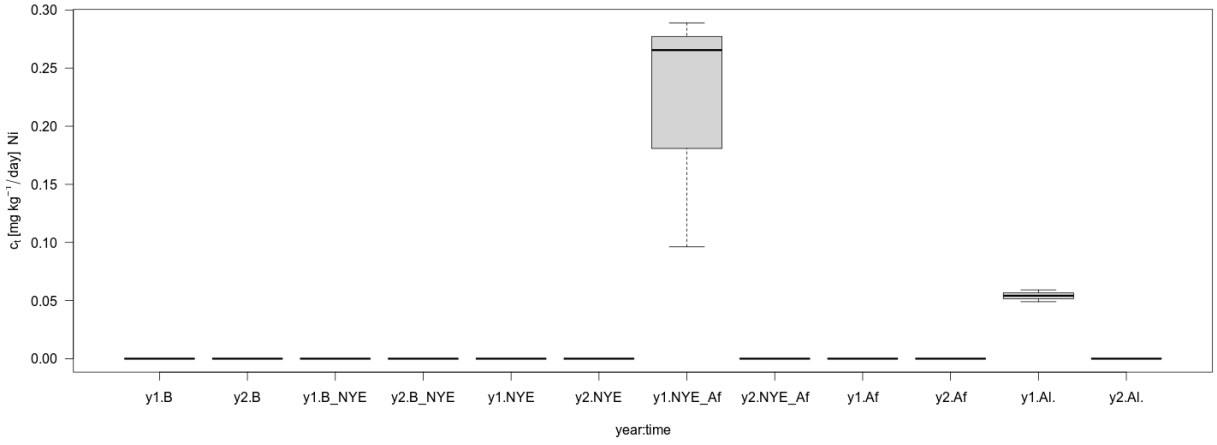


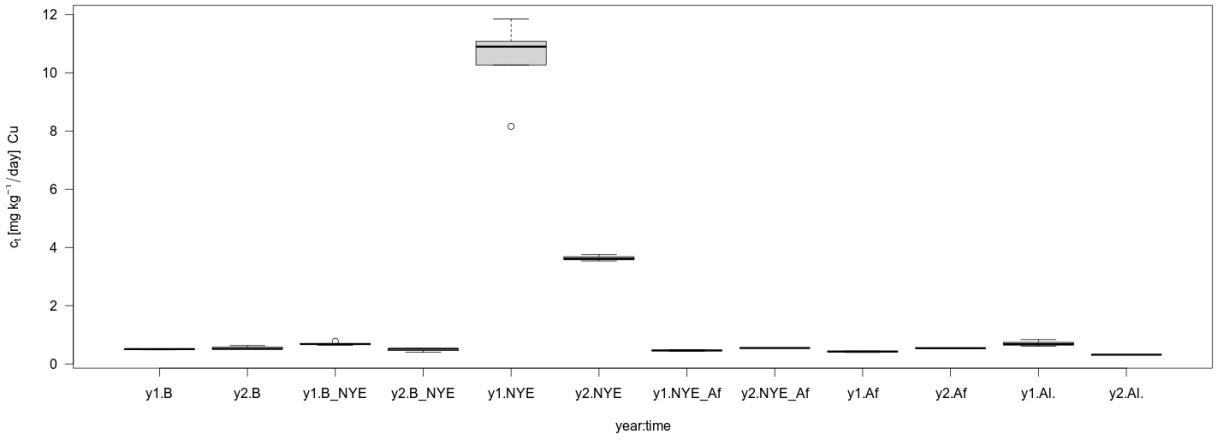
Figure 2. Heavy metal concentrations in moss samples (mg/kg dm): (**a**) Cu, Zn, and Pb concentrations in 2019/2020; (**b**) Cu, Zn, and Pb concentrations in 2020/2021; (**c**) Ni, Cd, and Hg concentrations in 2019/2020; (**d**) Ni, Cd, and Hg concentrations in 2020/2021. "X" in the bar represents the mean value connected by a line and "–" represents the median, "whiskers" at the bottom and top represent min-max values, "circles" represent extreme values.

The effect of meteo conditions on heavy metal concentrations in moss samples was also analyzed. The source of data was the Institute of Meteorology and Water Management [48]. During 1st half of both exposure periods an irregular decrease in mean air temperature T was observed. Starting from NYE the mean temperatures did not exhibit a clear trend of changes. At NYE 2019/2020 and 2020/2021 the difference in T was 5.1 °C. It should be noticed that T at 2019/2020 was positive, and negative at 2020/2021. Time patterns of relative air humidity H_r changes were different during exposure periods. The humidity difference at the first and second NYE was 23.5%. In a period from 30 December to 2 January no precipitation was observed at both NYE. Additionally, a few days before and after this period precipitation was poor. During exposure periods wind speed v was in the range 1.0–5.8 m/s. At the two NYE the wind speed difference was 2.6 m/s. In Figure S1 in the supplementary material parameters describing weather conditions during the moss samples exposure are presented. For moss samples duration of deposition was different. To estimate an intensity of the deposition, a ratio c_t of chemical elements' concentration

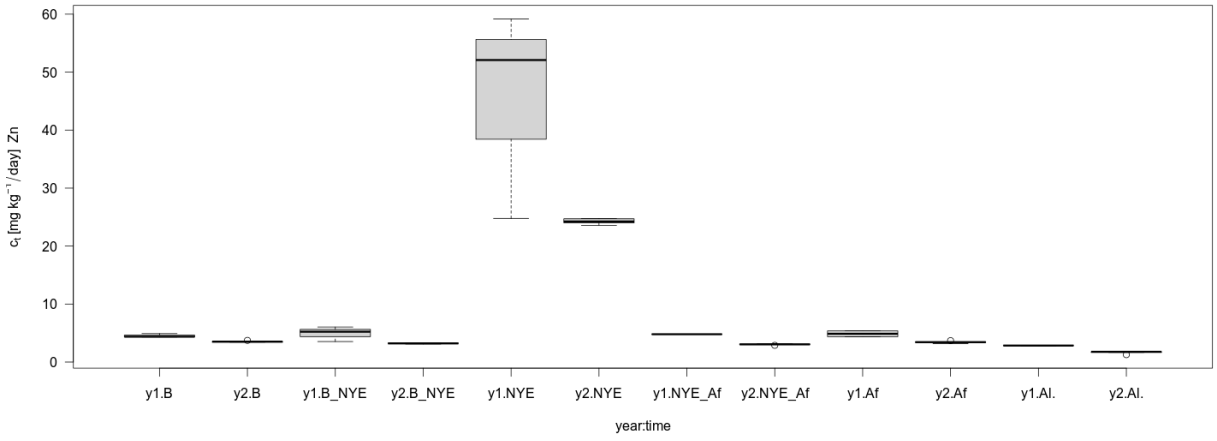
and the deposition time t was calculated. In Figure 3. boxplots of the calculated c_t are presented. For the metals studied, clear differences in deposition intensities are observed.



(**a**)

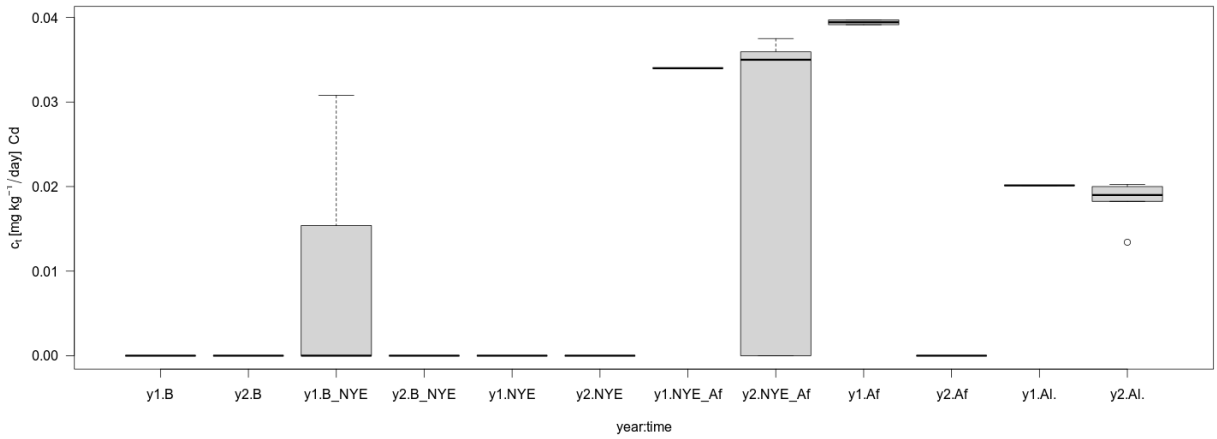


(**b**)

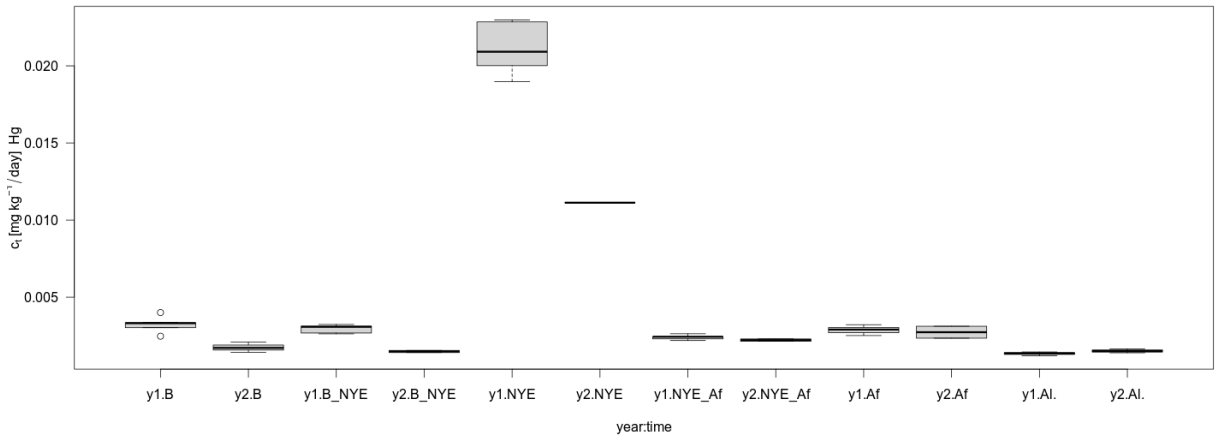


(**c**)

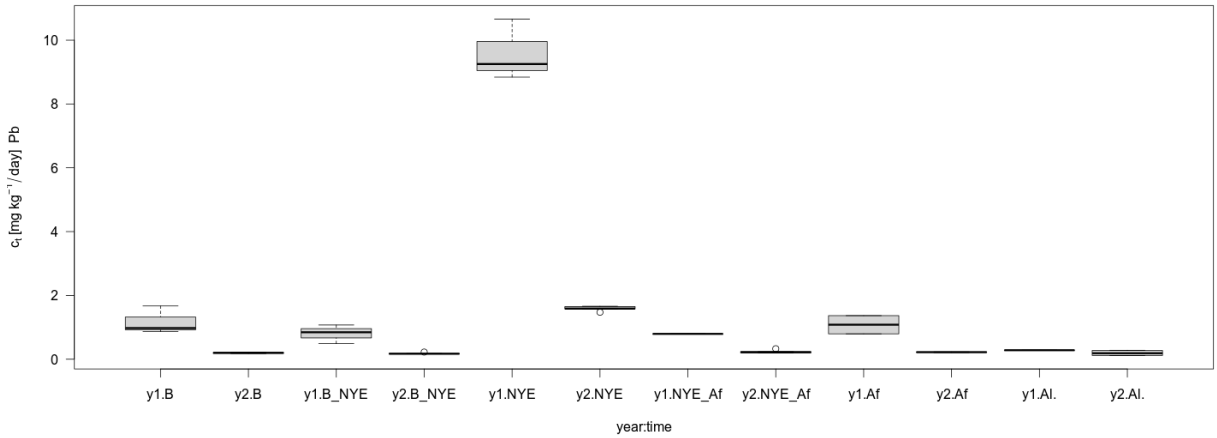
Figure 3. *Cont*.



(**d**)



(**e**)



(**f**)

Figure 3. Concentrations of heavy metals in mosses samples (mg kg^{-1}/day): (**a**) Ni, (**b**) Cu, (**c**) Zn, (**d**) Cd, (**e**) Hg and (**f**) Pb depending on the exposure time (y1, y2—period 2019/2020 and 2020/2021 respectively).

To test uniformity of central values in the grouped c_t the Kruskal-Wallis test was used. In calculations the group data were pooled over years 2019–2020. The *p*-values calculated were 6.4×10^{-5} (Ni), 4.8×10^{-5} (Cu), 4.9×10^{-6} (Zn), 1.0×10^{-3} (Cd), 1.7×10^{-3} (Pb), and 2.7×10^{-5} (Hg). For comparisons of median c_t in pairs of individual periods the pairwise Wilcoxon test was used. For *p*-values multiple testing Bonferroni correction was applied. A critical *p*-value = 0.05 was applied in the test result interpretation.

In Table 4 the *p*-values calculated in Wilcoxon test for intensity of Ni deposition during the periods studied are presented. Numerous *IQL* results disables comparison of some groups. Pairs of groups which cannot be compared are marked using "x" symbol. For Cu, Zn, Cd, Pd and Hg, results are included in Tables S1–S5 in the supplementary material).

Table 4. The *p*-values calculated in Wilcoxon test for intensity of Ni deposition during the periods studied.

	B	**B_NYE**	**NYE**	**NYE_Af**	**Af**
B_NYE	x	–	–	–	–
NYE	x	x	–	–	–
NYE_Af	0.093	0.093	0.093	–	–
Af	x	x	x	0.146	–
Al.	0.093	0.093	0.093	1.000	0.146

x — pairs of groups which cannot be compared. – — no comparison.

In all pairs of the periods the null hypothesis cannot be rejected. This conclusion is apparently inconsistent with observation on Figure 3a where c_l for NYE_Af and y1 clearly dominates all other periods. But in contrast, c_t in NYE_Af and y2 is considerably lower than that during y1, and as a result an effect of NYE_Af become statistically insignificant.

For Cu, Zn, Cd, Pb and Hg deposition intensity in group NYE and depositions in the remaining groups all *p*-values were lower than the critical one. This result indicates statistically significant difference between metal deposition intensities during NYE and during the other periods.

In Figure 3, it can be seen that metal concentrations vary considerably between the study periods when relating them to exposure time (number of days). For Cu, Zn, Hg and Pb, concentrations in 2019/2020 for the NYE period significantly exceed the corresponding period in the following year. In the next step of the analysis of the results, the increments in the concentrations of heavy metals accumulated in the mosses were determined. Table 5 shows a summary of the *RAF* values for both study periods for each element separately.

Table 5. Mean *RAF* values for the metals analysed (*n.d.*: no data).

Metal	**2019/2020**						**2020/2021**					
	B	**B_NYE**	**NYE**	**NYE_Af**	**Af**	**Al.**	**B**	**B_NYE**	**NYE**	**NYE_Af**	**Af**	**Al.**
Ni	*n.d.*	*n.d.*	*n.d.*	1.78	*n.d.*	0.30	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Cu	0.21	0.90	2.57	0.25	0.01	2.59	0.21	0.20	0.13	0.36	0.18	0.48
Zn	0.19	0.48	0.70	0.43	0.28	0.60	0.15	0.20	0.13	0.14	0.12	0.16
Cd	*n.d.*	*n.d.*	*n.d.*	0.03	0.05	0.14	*n.d.*	*n.d.*	*n.d.*	0.78	*n.d.*	0.68
Hg	0.19	0.24	0.11	0.01	0.05	0.06	0.27	0.23	0.16	0.85	0.99	1.37
Pb	1.09	0.64	1.44	0.62	0.93	0.08	0.32	0.37	0.50	0.78	0.46	1.77

Due to the fact that in some of the moss samples the concentration of nickel and cadmium was below the limit of quantification of the analytical method used, no *RAF* increments were noted (*n.d.*). For the other elements, high *RAF* values were observed for copper, zinc and lead, especially during the 2019/2020 period. In the 'NYE' period itself, *RAF* values are higher or comparable to other periods where exposure has been longer. The exceptions are cadmium and mercury, where increases are higher after New Year's Eve and in 2020/2021.

To assess the condition of the mosses during the exposure period, chlorophyll fluorescence was measured (Table 6).

Table 6. Chlorophyll fluorescence of mosses samples [-].

Parameter	Fs	Fms	Y(II)	Ft
Exposure Period	**Average ± *SD***			
control	113 ± 27.7	158 ± 49.1	0.273 ± 0.07	107 ± 26.1
B	125 ± 25.0	144 ± 30.7	0.125 ± 0.03	124 ± 24.9
B_NYE	128 ± 16.8	264 ± 96.3	0.509 ± 0.11	117 ± 16.1
NYE	137 ± 28.8	214 ± 77.9	0.332 ± 0.11	133 ± 25.5
NYE_Af	107 ± 10.4	139 ± 14.0	0.224 ± 0.08	105 ± 10.1
Af	112 ± 20.2	138 ± 26.8	0.184 ± 0.07	110 ± 19.9
Al.	115 ± 39.1	161 ± 74.5	0.257 ± 0.11	115 ± 36.3

Fs: Fluorescence under steady state; Fms: Maximum fluorescence at steady state; Y(II): Quantum Photosynthetic Yield of Photo System (II); Ft: Instantaneous fluorescence.

The chlorophyll fluorescence values varied between the study periods. The lowest value was measured in the period before New Year's Eve and the highest value was observed during the "B_NYE" exposure. Since this period the Photosynthetic Yield of Photo System (II) has decreased by about 64% to "Af". The sample exposed for the whole time was not statistically significantly different from the control.

4. Discussion

Concentrations of the heavy metals Ni, Cu, Zn, Cd, Pb and Hg, accumulated by *Pleurozium schreberi* mosses samples differ from each other within and between study periods of a given year. The concentration of individual elements is influenced by the moss species used and their accumulation capacity, exposure time and other environmental factors (interactions among elements, temperature, humidity) [49–51]. The changes in concentrations between the two study periods are also due to the global lockdown situation caused by the coronavirus pandemic restrictions. In the literature, one can find results indicating that air pollution has decreased significantly in this period in comparison to the values previously reported [52,53]. The impact of the pandemic on air pollution in terms of biological monitoring has so far been described by only one known publication, where is indicated that concentrations of selected metals accumulated by the moss *Pleurozium schreberi*, as part of passive biomonitoring studies, decreased by lockdown [54]. Our study confirms the above statements that heavy metal concentrations during the "NYE" period and in 2020/2021 were lower compared to the same period a year earlier (see Figure 3). This was influenced by national restrictions related to the limited possibility of overcrowding and participation in mass events celebrating the New Year, which had an impact on the reduced emissions from fireworks fired. According to the literature, air concentrations of heavy metals such as Cu, Zn and Pb are related to their emission during the fireworks burning [17,18,20,55–57] as was also confirmed by this research. During moss exposure for only 2 days (NYE period) [31 December and 1 January], concentrations of these elements were characterised by values comparable or higher to periods of longer exposure to moss contamination with these analytes (see also Figure 3). In our opinion, the results presented here represent the first environmental experiment using the moss-bag technique to determine the concentrations of heavy metals accumulated by mosses during New Year's Eve (due to fireworks burning), together with the impact of the pandemic on air pollution at this time using active moss biomonitoring.

RAF values higher than 1.00 indicate significant elemental enrichment [58]. Such values were recorded for copper and lead in 2019/2020 and the impact of increases in these elements was related to the fireworks being fired. In contrast, high increments for nickel and mercury may be related to other air pollutants sources such as local, low level emissions [59].

The actual quantum efficiency of PSII photochemistry in the light measures the fraction of the absorbed light energy that is actually being used to drive photochemistry at

PSII [60]. Its value is influenced by a number of factors, such as a change in metal concentration [61,62] and a positive effect on the increase in fluorescence yield over the course of the experiment may have been due to variable hydration [63]. The dependence of chlorophyll fluorescence yield variability on water content has been reported previously for *Pleurozium* moss [64] and our actual quantum yield values of PSII photochemistry were comparable with the results obtained by other authors for this species [65,66]. Due to the association of chlorophyll fluorescence with other vital parameters (chlorophyll content, osmotic indices, antioxidant enzyme activities) [67], we know that biomonitoring studies have been conducted on a living air quality bioindicator [68].

5. Conclusions

The results of the biomonitoring study indicate a variable air quality in the town of Prószków during New Year's Eve on the example of a two-year study. Increased concentrations of copper, zinc and lead only during the 2-day "NYE" exposure confirm the effect of fired fireworks on the accumulation of these elements in the moss *Pleurozium schreberi*. This demonstrates the good accumulation capacity of this species for use during short periods of exposure to air pollution. Further research should focus on finding the best moss species for the determination of heavy metals in air from fireworks smoke.

Deposition of metals on moss during and after fireworks show could be affected by weather conditions. During the periods studied, the temperature, humidity, and wind speed were similar but not the same. A common feature at both NYE was no precipitation.

Actually the results of our research did not provide feasible information to estimate an influence of weather conditions on metals' deposition after fireworks show. Valid conclusions could be drawn from repeated observations. This assertion implies continuation of the research with extension to other than NYE celebrations.

In the literature, the use of classical methods for determination of metals concentration in the air during fireworks show has been already described, but our study confirms the possibility of using bioindicators in air quality monitoring as a complementary method. Biomonitoring studies using the moss-bag method for the determination of other pollutants from fireworks shows should be continued.

In addition, the positive impact of the 2020/2021 lockdown on air pollution from emissions of fireworks launched was demonstrated, which was confirmed by determining lower concentrations of selected heavy metals accumulated by mosses. The practice of welcoming the New Year should change the accepted way of celebrating to one that does not cause deterioration of air quality and further pollution emissions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/atmos12080975/s1, Figure S1: The parameters describing weather conditions during the moss samples exposure. The parameters are daily means of temperature T (in subplot a), relative humidity H_r (subplot b), precipitation h (subplot c) and wind speed v (subplot d), Table S1: The p-values calculated in Wilcoxon test for intensity of Cu deposition during the periods studied, Table S2: The p-values calculated in Wilcoxon test for intensity of Zn deposition during the periods studied, Table S3: The p-values calculated in Wilcoxon test for intensity of Cd deposition during the periods studied, Table S4: The p-values calculated in Wilcoxon test for intensity of Pb deposition during the periods studied, Table S5: The p-values calculated in Wilcoxon test for intensity of Hg deposition during the periods studied.

Author Contributions: Conceptualization, P.Ś.; methodology, P.Ś. and M.R.; validation, M.R. and Z.Z.; formal analysis, P.Ś. and Z.Z.; investigation, P.Ś.; measurements, P.Ś. and M.R.; writing—original draft preparation, P.Ś.; writing—review and editing, M.R. and Z.Z.; visualization, P.Ś. and Z.Z.; supervision, M.R.; project administration, M.R. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials:

Air Quality during New Year's Eve: A Biomonitoring Study with Moss

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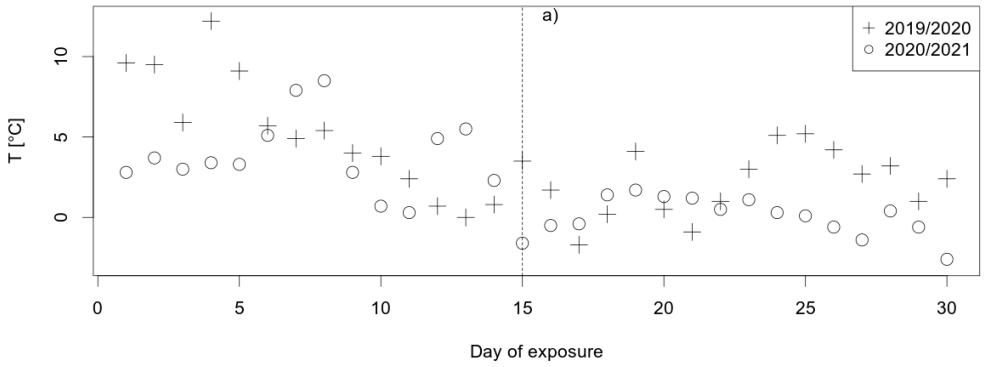
a)
2019/2020
2020/2021
T [°C]
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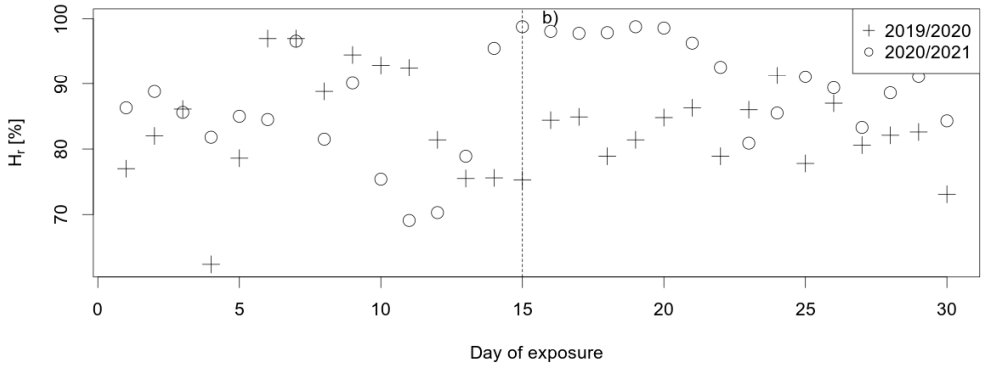
Day of exposure


b)
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2020/2021
H_r [%]
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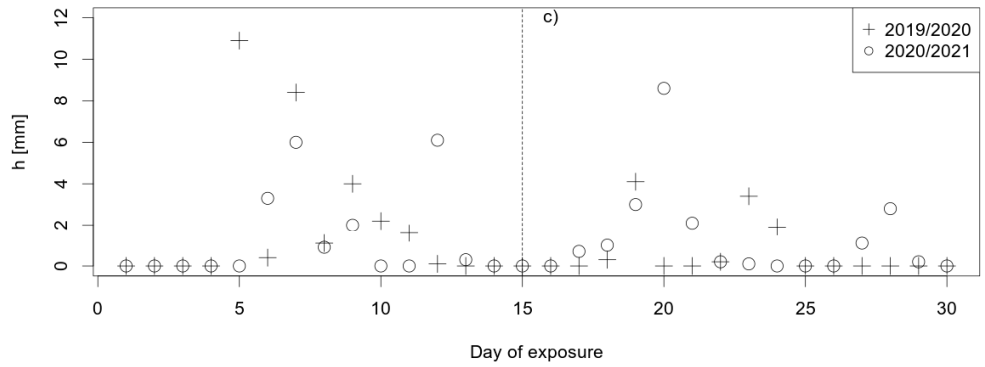
Day of exposure


c)
2019/2020
2020/2021
h [mm]
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4
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10
12
0
5
10
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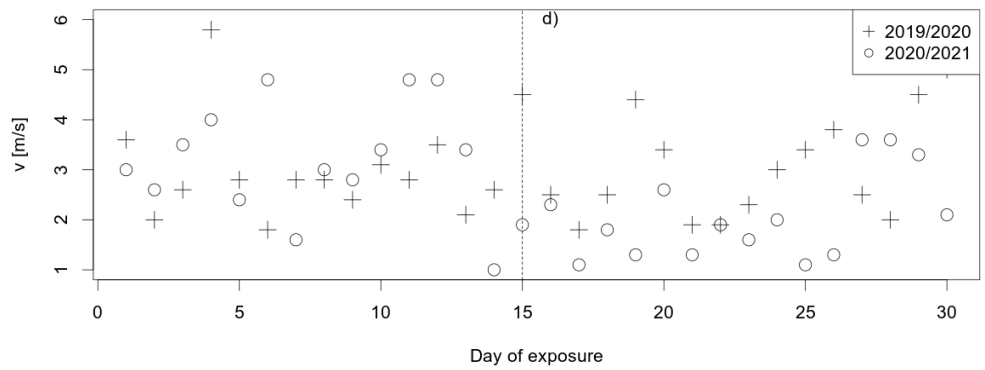
25
30
Day of exposure


d)
2019/2020
2020/2021
v [m/s]
1
2
3
4
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6
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10
15
20
25
30
Day of exposure

Figure S1. The parameters describing weather conditions during the moss samples exposure. The parameters are daily means of temperature *T* (in subplot a), relative humidity *Hr* (subplot b), precipitation *h* (subplot c) and wind speed *v* (subplot d).

The abbreviations are explained in main text of article in 2.2. *Methods.*

Table S1. The p-values calculated in Wilcoxon test for intensity of Cu deposition during the periods studied.

	B	**B_NYE**	**NYE**	**NYE_Af**	**Af**
B_NYE	0.422	-	-	-	-
NYE	0.002	0.002	-	-	-
NYE_Af	0.440	0.639	0.010	-	-
Af	1.000	0.601	0.005	1.000	-
Al.	0.889	1.000	0.010	0.974	0.769

Table S2. The p-values calculated in Wilcoxon test for intensity of Zn deposition during the periods studied.

	B	**B_NYE**	**NYE**	**NYE_Af**	**Af**
B_NYE	1.000	-	-	-	-
NYE	0.002	0.002	-	-	-
NYE_Af	0.440	0.639	0.010	-	-
Af	1.000	1.000	0.005	0.524	-
Al.	0.010	0.010	0.010	0.032	0.017

Table S3. The p-values calculated in Wilcoxon test for intensity of Cd deposition during the periods studied.

	B	**B_NYE**	**NYE**	**NYE_Af**	**Af**
B_NYE	1.000	-	-	-	-
NYE	0.000	1.000	-	-	-
NYE_Af	0.171	0.408	0.171	-	-
Af	1.000	1.000	1.000	1.000	-
Al.	0.011	0.222	0.011	1.000	1.000

Table S4. The p-values calculated in Wilcoxon test for intensity of Pb deposition during the periods studied.

	B	**B_NYE**	**NYE**	**NYE_Af**	**Af**
B_NYE	1.000	-	-	-	-
NYE	0.044	0.002	-	-	-
NYE_Af	1.000	1.000	0.010	-	-
Af	1.000	1.000	0.005	1.000	-
Al.	1.000	1.000	0.010	1.000	1.000

Table S5. The p-values calculated in Wilcoxon test for intensity of Hg deposition during the periods studied.

	B	**B_NYE**	**NYE**	**NYE_Af**	**Af**
B_NYE	1.000	-	-	-	-
NYE	0.010	0.017	-	-	-
NYE_Af	1.000	1.000	0.017	-	-
Af	1.000	1.000	0.017	0.105	-
Al.	0.016	0.089	0.010	0.005	0.005

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Article

Is Active Moss Biomonitoring Comparable to Air Filter Standard Sampling?

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Abstract: Recently, significant attention has been paid to air quality awareness and its impact on human health, especially in urban agglomerations. Many types of dust samplers for air quality monitoring are used by governmental environmental monitoring agencies. However, these techniques are associated with high costs; as a consequence, biological methods such as active moss biomonitoring are being developed. The main disadvantages of such techniques are the lack of standardization of the preparation procedures and the lack of reliable comparisons of results with data from instrumental analyses. Our study aimed to compare the results obtained from active biomonitoring with the use of three moss species: *Pleurozium schreberi*, *Sphagnum fallax* and *Dicranum polysetum*. Samples were exposed via the moss-bag technique to measure the concentrations of analytes (Mn, Fe, Cu, Zn, Cd, Hg and Pb) which had accumulated among the total suspended particulates (TSP) collected from the filters of a dust collector in the city of Opole (Opole voivodeship, Poland). With regard to the physicochemical and biological traits of the mosses, their assessed lifetime and actual photochemical efficiency (yield) following exposure were meagre, which may have been related to the change of environment and their exposure to pollutants. When comparing the results obtained by the two methods used to monitor air pollution, the biomonitoring method was found to be incompletely consistent with the reference method. Biological monitoring using mosses must be carefully considered depending on the monitoring objectives, the required level of sensitivity and quality of measurement and the type of pollutant.

Keywords: mosses; total suspended particulate (TSP); heavy metals; biomonitoring

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1. Introduction

Heavy metals in street dust originate from anthropogenic pollution [1]. This contamination contributes to air pollution and increasing concentrations of various fractions of particulate matter (PM) [2] as well as different levels of total suspended particles (TSP) [3,4]. Air pollution in urban areas leads to adverse health effects [5], so the scale of air quality research is increasing [6–8], leading to the growth and intensification of human biomonitoring [9–11].

In addition to classical air quality assessments and monitoring methods [12–15], other approaches are increasingly being used [16], with modeling, biota sampling and ecological indicators or green infrastructure being the most widespread [17–20]. One example is lichens [21] or mosses [22,23] for monitoring atmospheric aerosol quality. Plants are used extensively in environmental biomonitoring of PM pollution [24,25], and tree leaves have been employed in a national system for long-term biomonitoring of heavy metals

in the air. The Romanian Ministry of Environment has implemented this system as a complementary tool to the National Air Quality Monitoring Network [26]. The same authors also incorporated the moss-bag technique into long-term monitoring of heavy metals in the air to further develop the BioMonRo monitoring tool [27]. In turn, the combined use of the moss-bag technique and emission inventories appears to be an effective approach for quantifying pollutants, and could be a part of a project to develop and improve air quality modelling [28].

In general, the number of studies in which biological methods are used to assess air pollution is increasing, but the proper preparation of biological materials and the measurement method should be taken into consideration [29]. Not many researches have undertaken direct comparisons between the results from active moss biomonitoring with those obtained from dust samplers [30] in order to integrate these methods in assessments of the viability of the aforementioned bioindicators [31]. This is compatible with the definition of biomonitoring and research in this field [32]. So far, comparisons have been made for passive biomonitoring of TSP [33,34]. Therefore, in this research, the challenge of comparing the results of active moss biomonitoring with instrumental measurements was addressed. TSP was chosen because dust of different fractions can be deposited on mosses [29,35].

In this work, for the first time to our knowledge, an attempt was made to correlate biomonitoring results with results from air monitoring. We have tried to verify the research hypothesis that concentrations of heavy metals accumulated in mosses are correlated to those in TSP dust deposited in filters. We expect to provide evidence supporting this hypothesis by several means, i.e., by: (I) evaluating metal concentration changes in TSP and mosses during exposition; (II) comparing TSP and elemental moss composition during exposition; (III) evaluating the relationships among metal concentrations; and (IV) controlling moss survival during exposure.

2. Materials and Methods

The moss species used for this study were *Pleurozium schreberi* (Pl), *Sphagnum fallax* (Sp) and *Dicranum polysetum* (Dp). They were collected in October 2020 from forests in the Swietokrzyskie Voivodship in southeastern Poland.

Moss samples were taken and prepared before exposure as part of active biomonitoring in accordance with the relevant guidelines [36]. According to a previously developed methodology, mosses were prepared before exposure [37]. Moss samples (27 bags, 3 g each) were hung on the viewing terrace of the building of the Institute of Environmental Engineering and Biotechnology of the University of Opole (Opole, PL). During the winter season, mosses were exposed for three months (27 October 2020–27 January 2021). After each month of exposure, three bags of each species were collected (1 month = 9 samples). At the same time, TSP were collected on QM-A quartz filters (Whatman, 47-mm diameter). The sampling time was 24 h, from noon to noon of the next day. TSP filters were changed every day for three months (i.e., a total of 81 filters). The airflow of the PNS3D15/LVS3D dust collector was 2.3 m^3/h, in accordance with the standard procedure [38]. The concentrations of Mn, Fe, Cu, Zn, Cd, Hg and Pb in the filters before exposure were below the limit of quantification of the analytical method used.

After exposure, each moss sample, with a dry mass (d.m.) of 1.000 $\pm$ 0.001 g, and each filter were mineralized in a mixture of nitric acid and hydrogen peroxide using a Speedwave Four microwave oven (Berghof, DE) to determine the heavy metal contents. Anthropogenic emitters are the source of these analytes in the study area. The mineralization process was carried out at a temperature of 180 °C. For filters, this process was carried out at 220 °C, and was performed twice to ensure complete digestion of all dust samples, according to a method described in [39]. Heavy metals were quantified using an atomic absorption flame spectrometer type iCE 3500 (Thermo Scientific, Grand Island, NY, USA). Concentrations of metals were evaluated in solution after mineralization and filtration, and were diluted into volumetric flasks of 25 cm^3. Calibration of the spectrometer

was performed with standard solutions (ANALYTIKA Ltd., Prague, Czech Republic). The values of the highest concentrations of the models used for calibration (10 mg/dm^3 for Fe, 7.5 mg/dm^3 for Mn, 5 mg/dm^3 for Cu, Zn, Pb, 2 mg/dm^3 for Cd) were approved as linear limits to signal dependence on concentration. The concentration of Hg in the samples (0.04 g ± 0.001 g d.m.) was determined with an AMA 254 mercury analyzer (Altec Ltd., Prague, Czech Republic).

Table 1 presents the instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) of the iCE 3500 spectrometer. Table 2 shows the concentrations of heavy metals in certified reference materials, i.e., BCR-482 *lichen*, produced at the Institute for Reference Materials and Measurements, Belgium.

Table 1. The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) of the iCE 3500 (mg/L) spectrometer [40].

Metal	*IDL*	*IQL*
Mn	0.0016	0.020
Fe	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

Table 2. Comparison of measured and certified concentrations in BCR-482 *lichen* [41].

	BCR-482 *lichen*		**AAS (n = 5)**		***Dev.*** **
Metal	**Concentration**	**Measurement Uncertainty**	**Average**	**±*SD* * of the Concentrations**	
		[mg/kg d.m.]			**[%]**
Mn	33.0	0.50	31.7	0.68	−3.90
Fe	804	160	771	154	−4.10
Cu	7.03	0.19	6.63	0.17	−5.70
Zn	100.6	2.20	95.1	2.30	−5.50
Cd	0.56	0.02	0.53	0.03	−5.30
Pb	40.9	1.40	38.2	1.00	−6.60

* standard deviation. ** relative difference between the measured (c_m) and certified (c_c) concentration 100% $(c_m - c_c)/c_c$.

The chlorophyll fluorescence of photosystem II and actual photochemical efficiency (yield) were measured using a modulated portable fluorometer (Opti-Sciences, Hudson, NH, USA) under ambient light conditions [42].

Comparisons of the metal concentrations in the mosses during the periods studied with the TSP sample composition were carried out in a multistep process. The first required adjustments of the time scales of the moss exposition and TSP collection. Since the moss samples had been exposed for one, two and three months, their compositions could not be compared with the metal contents in the daily TSP samples. To overcome this problem, the masses of the relevant components were calculated for each measurement day. Given the mass of the TSP sample and the metal concentrations, the mass of a given element was calculated. A monthly sum of TSP and metal masses was used for cumulated composition calculations.

The second problem was related to the incomparability of moss and TSP compositions. Besides the determined metals, both types of materials also contained many other components. The predominance of organic compounds in the mosses and mineral ones in TSP was expected. To compare the metal contents in mosses and TSP, the appropriate

subcompositions were considered [43,44]. Concentrations x_d in a d subcomposition could be derived from D compositions x_D ($D > d$) using the formula:

$$x_d = \frac{x_D}{\sum_{i=1}^{d} x_{D,i}} \quad (1)$$

where x_D and x_d are vectors of the concentrations, respectively, in terms of composition and subcomposition. The components used in subcomposition formation were numbered from 1 to d.

A problem related to numerous data being below the detection limit occurred. However, individual observations lower than the detection limit (*BDL*) would not affect conclusions resulting from the data elaboration. Nevertheless, plentiful *BDL*s might have significantly affected the sum of the calculated metal abundances. To overcome this problem, data imputation for *BDL*s was applied. For this purpose, computations were conducted in R language version 4.1.0 [45]; the *multLN* function in the R language and the *zCompositions* library were used [46].

The third problem arose from the discontinuity of the data collection. The TSP collection was corrupted by incidental breaks in sample collections (Figure 1). To supplement the data, a temporary liner trend was considered. Since temporal changes in the metal concentrations were low during the period studied, linear interpolation was sufficient to describe concentration changes over time. The missing data for breaks were calculated using the estimated course of concentration changes throughout the sampling period.

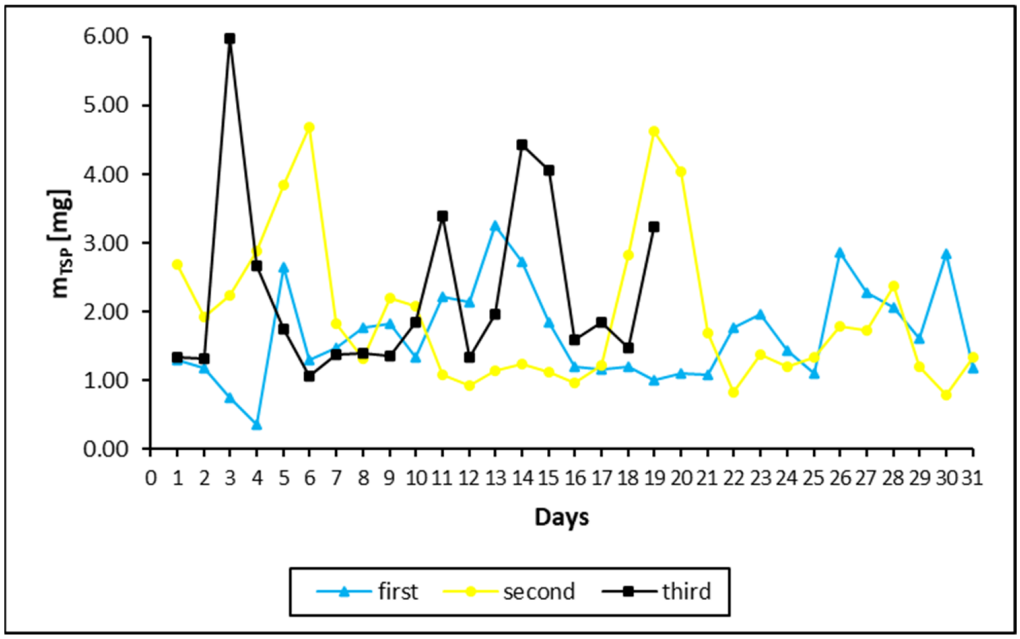


Figure 1. Daily TSP mass changes during the first (blue triangles), second (yellow dots) and third months (black squares) of sample collections in filters. The differences in the duration and daily sampling of the TSP filters were due to breaks associated with the Christmas and New Year holidays and a technical fault with the dust collector pump lasting 14 days.

The contents of elements Mn, Fe, Cu, Zn, Cd, Hg, and Pb in subcomposition (i = {1,..., 7}) for TSP and moss were calculated. In this way, metal concentrations in the material could be determined independently of other component abundances, facilitating comparisons of the results from TSP and moss.

Despite different materials and collection periods, the procedures applied in the data elaboration process enabled the comparison of moss and TSP compositions. As a result, valid conclusions about the material compositions could be drawn.

3. Results

The daily mass increments of TSP in the filters are shown in Figure 1. As shown, there was considerable variation in the amount of deposited dust over time, and there were differences from one month to another. The average daily weight of TSP intake was 0.0016, 0.0020 and 0.0023 g for the first, second and third months, respectively.

The variable daily amount of dust deposited on the filters was also reflected in the concentrations of heavy metals detected therein, as shown in Table 3.

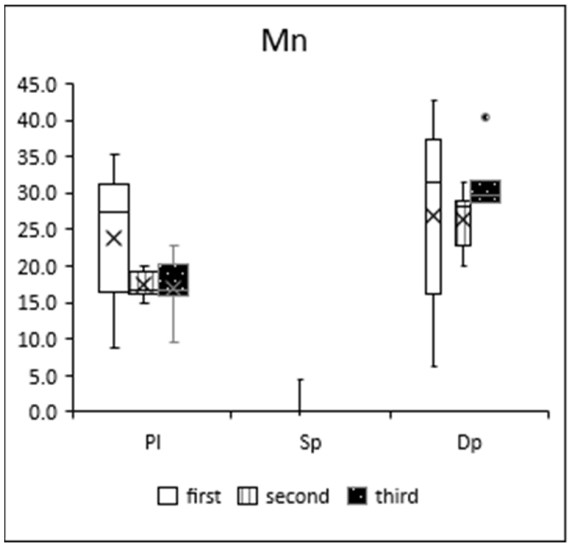
Table 3. Monthly element abundances (ng m^{-3}) detected in TSP filter samples.

	Mn	Fe	Cu	Zn	Cd	Hg	Pb
1st month							
min	64.1	6305	202	951	21.7	67.5	124
max	473	29,664	586	4433	21.7	88.8	6049
median	182	11,036	327	2091	21.7	81.6	2312
average	189	12,033	352	2215	21.7	79.3	2216
SD	81.8	4613	115	804	-	10.8	1442
n	31	31	31	31	1	3	31
2nd month							
min	93.5	6335	182	1066	8.70	49.2	443
max	661	29,712	584	6545	207	69.2	5499
median	213	10,138	320	2240	117	68.7	1892
average	252	11,163	357	2634	103	62.4	2065
SD	117	4801	112	1454	57.8	11.4	1159
n	31	31	31	31	31	3	31
3rd month							
min	8.70	3774	196	691	4.35	74.5	234
max	247	19,722	834	7184	59.8	81.0	8963
median	96.7	7575	358	1245	35.3	78.5	1011
average	110	8953	432	2453	34.4	78.0	1651
SD	82.7	3855	183	2003	20.4	3.29	2049
n	15	19	19	19	8	3	19

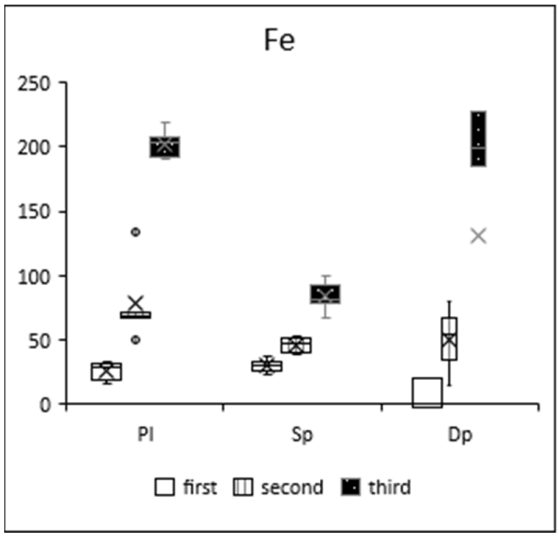
In general, the order of element abundances in TSP samples was: Fe > Pb > Zn > Cu > Mn > Hg > Cd for first month; Fe > Zn > Pb > Cu > Mn > Cd > Hg for the second; and Fe > Zn > Pb > Cu > Mn > Hg > Cd for the third.

The results of the moss-bag technique showed increases in heavy metal concentrations in three moss species depending on exposure time and element, as shown in Figure 2. The values shown are the increases, i.e., the relative concentrations, which are the differences between the concentrations measured in the moss after exposure (C_{af}) and those in the control sample before exposure (C_{be}): ($C_{af} - C_{be}$).

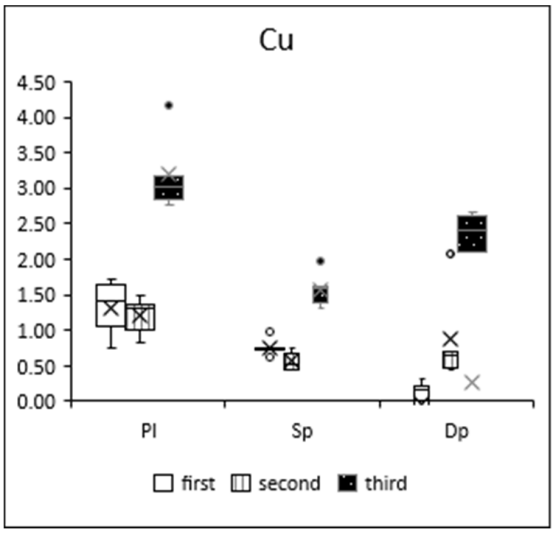
The results shown in Figure 2 indicate changes in element concentrations over time. Increases in these metals can be observed from month to month; for most elements and moss species, the greatest increases in concentrations were observed after three months of exposure (iron, copper, zinc, cadmium, mercury).



(**a**)



(**b**)



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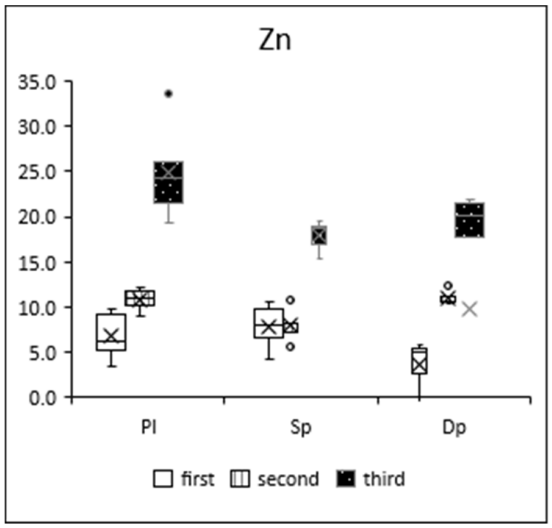
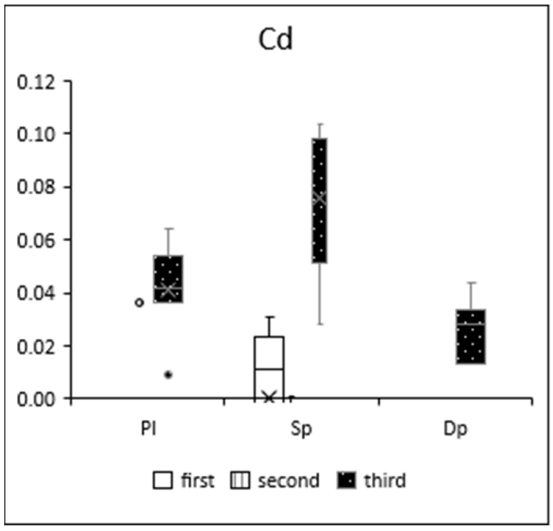
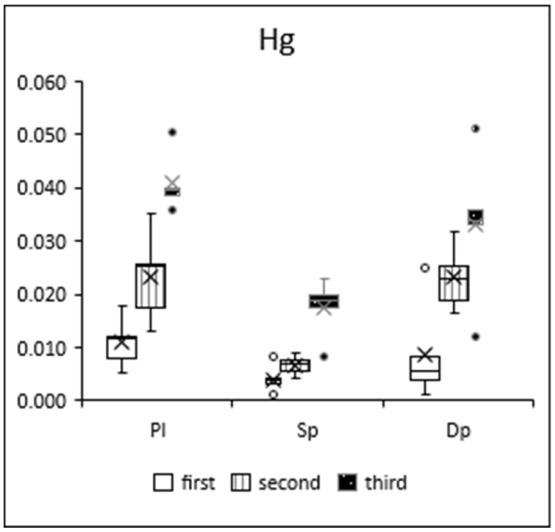


(**d**)

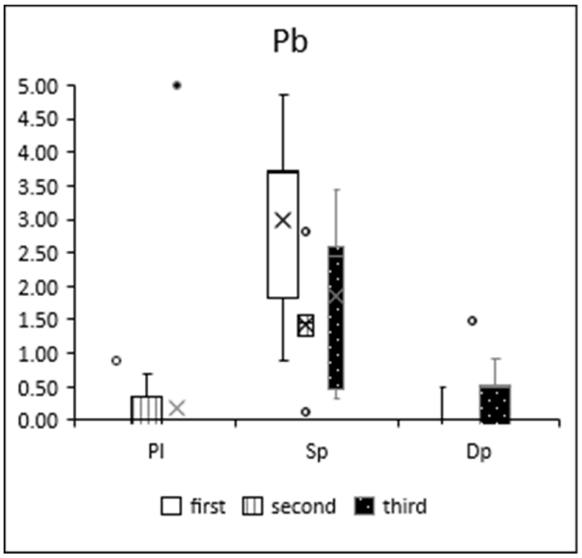


(**e**)



(**f**)

Figure 2. *Cont*.



(**g**)

Figure 2. Elemental concentrations (mg/kg d.m.) of (**a**) manganese, (**b**) iron, (**c**) copper, (**d**) zinc, (**e**) cadmium, (**f**) mercury and (**g**) lead in the mosses after the first (white), second (white with stripes) and third (black with dots) month of exposure. Elemental concentrations determined in mosses prior to exposure are presented in Supplementary Materials, Table S1.

A linear model was constructed to describe changes in metal concentrations with respect to exposition duration and moss species. The model is described by the symbolic expression [47]:

$$\log(c) \sim (\textit{moss species}) * (\textit{exposition duration}) \quad (2)$$

The values of structural parameters β_i, their standard errors SE_β, and p-values for the null hypothesis $H_{0,i}$: $\beta_{pop,i} = 0$ ($\beta_{pop,i}$ is the *i*-th ($i = \{0,1\}$) structural parameter in the general data population) were calculated. For the β_0 and β_1 parameters and differences $\beta_{\{0,1\}}(\{Pl,Sp\} - \beta_{\{0,1\}}(Dp))$, the 95 % confidence intervals *CI* (in the range from 2.5% to 97.5%) were calculated. The results are presented in Table S2 in the Supplementary Materials.

The computation results led to the following conclusions. For Mn, Fe and Hg, statistically significant differences in the starting concentrations described by β_0 in the moss species were found. In comparison to *D. polysetum*, the Mn concentration was higher in *P. schreberi* and lower in *S. fallax*. In *P. schreberi* and *S. fallax*, the Fe and Hg concentrations were lower than in *D. polysetum*. For Cd and Pb, the differences between β_0 parameters for *D. polysetum*–*P. schreberi* and *D. polysetum*–*S. fallax* were statistically insignificant. The difference in Cu concentration for *D. polysetum* and *P. schreberi* was statistically insignificant, but the concentration in *S. fallax* was significantly lower than that in *D. polysetum*. A similar effect as that for Cu was observed for Zn, but the concentration in *S. fallax* was higher than in *P. schreberi* and *D. polysetum*.

Changes in the metal concentration over time are described by slope β_1. Concerning moss species, β_1 indicates an increase in metal concentration during exposition ($\beta_{1,pop} > 0$) or no changes ($\beta_{1,pop} = 0$). No rinse effect on the metal concentration ($\beta_{1,pop} < 0$) was noticed. No statistically significant changes in Mn and Pb concentrations during the study period in the moss species were observed. The increase in Cu, Cd and Hg concentrations was not related to the moss species. The difference in the accumulation rate of Fe and Zn in *D. polysetum* and *P. schreberi* was statistically insignificant. In *S. fallax*, a smaller increase in metal concentrations was observed.

The composition of metals accumulated in mosses and filters was compared in the next step. For the subcomposition comprising Mn, Fe, Cu, Zn, and Pb, [43] the distances between points representing the metal contents in the moss samples and TSP were calculated. In the dendrogram (Figure 3), the structure of the distances is shown. To determine the structure

of the clusters, a complete linkage method was used. Two main clusters representing moss and TSP were observed. Within the moss cluster, three subclusters can be recognized. One of them presents *D. polysetum*, independent of the exposure period. The remaining clusters represent the other moss species, which were not uniquely assigned to groups.

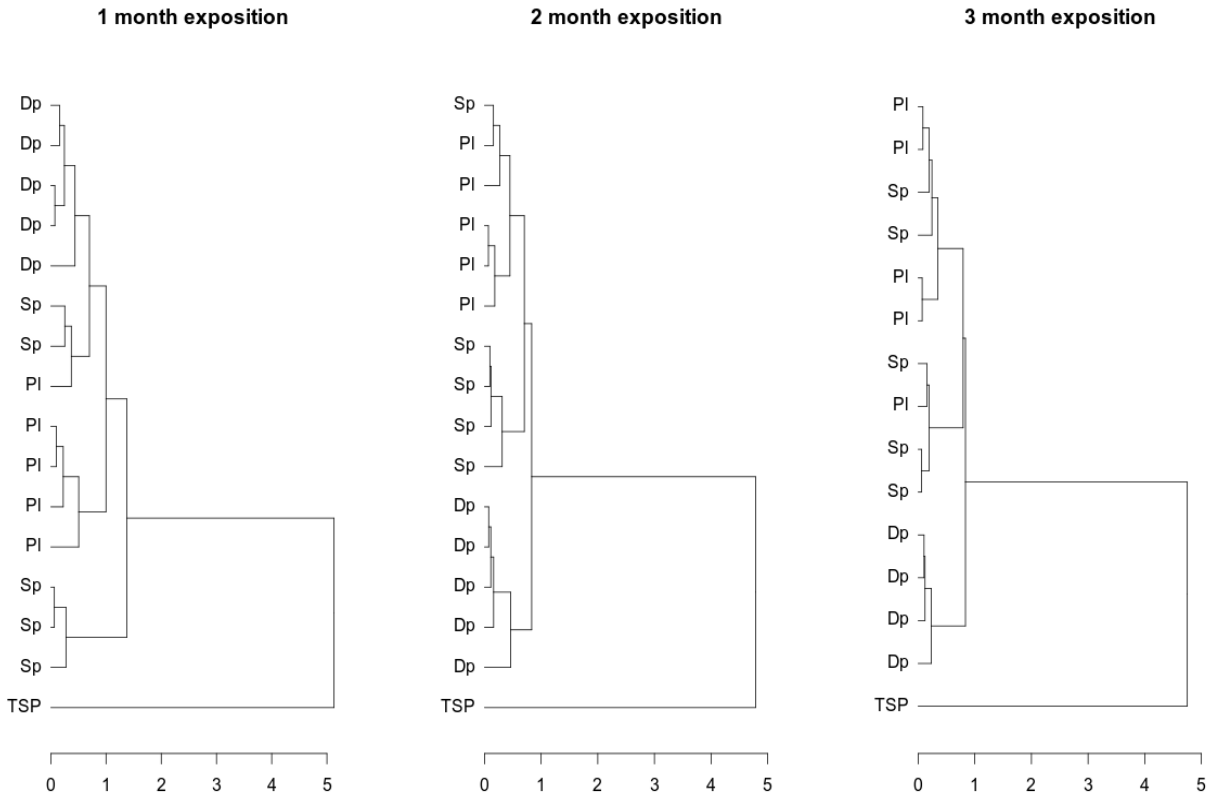


Figure 3. Cluster analysis of heavy metals in three moss species and in filter TSP.

The proportionality of metal concentrations in the materials was studied. To assess concentration covariabilty variance, logarithmized concentration ratio t was calculated. The t parameter was calculated with the formula:

$$t_{jl} = \mathrm{var}\left(ln\frac{c_j}{c_l}\right) \quad (3)$$

The value of the t parameter was low, revealing a common trend in concentration changes, i.e., an increase in c_j was followed by an increase in c_l. To estimate the covariability in the concentration of the metal pairs, t_{jl} (j,l = {Mn, Fe, Cu, Zn, Pb}) were calculated according to a method described in [48]. The results are presented in Table S3 in the Supplementary Materials. For Fe and Pb (first month of exposition), a low t_{jl} value indicating concentration change tendency was observed. For Pb and Zn concentrations in the second and third months of exposition, a similar trend was observed. The standard increase in Pb and Zn concentrations could be assigned to low emissions during the heating season [49,50].

The influence of heavy metal pollution and environmental conditions causes significant variability in the lifespan of mosses, as shown in Figure 4.

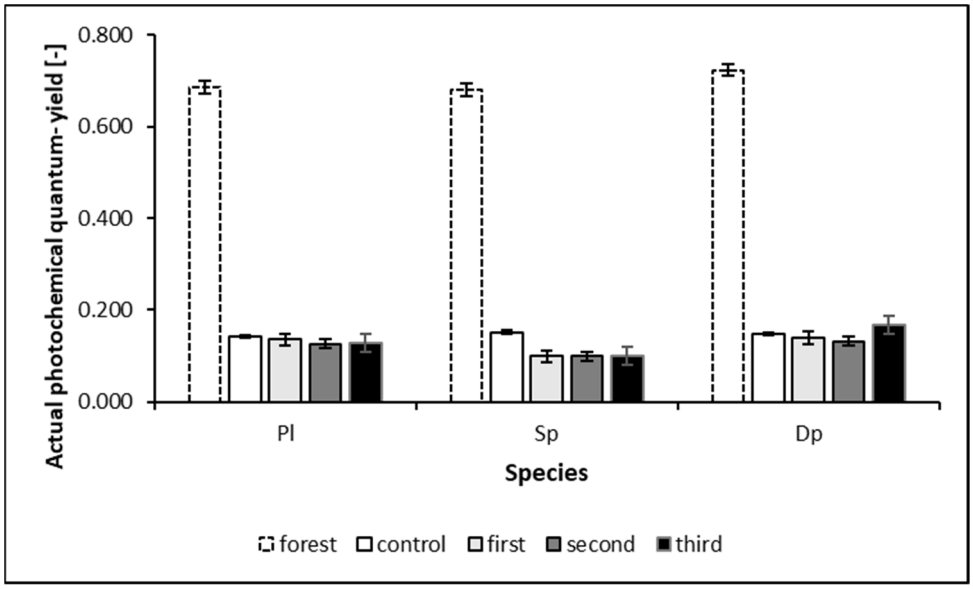


Figure 4. Changes in actual photochemical quantum yield (PQY) with duration of exposure (whiskers indicate a standard deviation).

The graph in Figure 4 indicates that all species are susceptible to environmental change. Their vitality decreases dramatically when they experience unfavorable variable meteorological-environmental conditions and air pollution in autumn and winter. During the experiment, the exposed mosses significantly decreased their photosystem II activity. The rate of accumulation of elements depends on environmental conditions [51]; in the same way, the result of a photosynthetic activity measurement is dependent on the conditions under which it is performed [52]. Sudden changes in environmental conditions and (associated with it) stress cause the moss condition to deteriorate [53,54].

A multiple regression model was used to assess the effect of metal concentrations and exposure time on the life span, as measured by POY (Table 3 SM). In this model, the POY variable describes a specific metal concentration and exposure time. Calculations were performed separately for each of the moss species studied. The results indicated that the viability of the mosses decreased over successive months of exposure. This was indicated by the negative value of the structural parameter describing changes in POY during successive exposure periods ($p < 0.05$ is marked in yellow in Table 3 SM). With exposure time, viability decreased for *D. polysetum* in terms of iron concentration. For *S. fallax*, this trend was observed with Mn, Cd and Pb. For *P. schreberi*, on the other hand, viability decreased over time in terms of Fe, Cu, Zn, Cd and Hg concentrations. When considering separately the effect of changes in element concentrations over time after the calculated coefficient of variation (mean of three months), the results indicated that low concentration variation generally did not affect moss viability. Concentration variability higher than 10% resulted in increased viability with increasing iron concentrations for *D. polysetum* and increasing lead and mercury concentrations for *P. schreberi*.

4. Discussion

The sorption of pollutants by mosses has already been discussed many times [55,56], but it is essential to take into account the mechanism of this process, especially when considering active forms and bioaccumulation [57–62]. However, depending on the testing method, their context and purpose must be taken into account [63]. The elemental concentrations shown in Figure 2 indicate a cumulative trend over time. This observation was consistent with previous literature studies, in which the concentrations of some elements (Fe, Zn, Cd and Hg) in *Sphagnum girgensohnii* increased continuously (linear accumulation trend) with exposure time [64]. The concentration of elements in mosses was influenced by the time of exposure; during the heating season, it was observed that moss samples

were particularly enriched in Cu or Zn [65]. The moss-bag technique with *Sphagnum junghuhnianum* also confirmed higher concentrations of elements in winter than in summer; additionally, for Cu, Pb and Zn, it showed that the source was traffic pollution [66]. The same sources can be attributed to the mosses exposed in this study. In the city center during the winter, combined with the previously mentioned low emissions, an effect was noted on the enrichment of heavy metals in mosses [50].

In our opinion, moss vitality measurements should not be excluded during experiments [31]. Despite the high proportion of analyses using devitalized mosses [67–70], we still believe that this approach is inadequate, according to the definition of biomonitoring and bioindicators, i.e., living indicator organisms [71–74]. Otherwise, we treat moss only as a chemical adsorbent, i.e., a natural sorbent [75–77] that has nothing to do with biomonitoring.

Despite significant damage to moss tissue and cell integrity after exposure, they are able to efficiently accumulate airborne trace elements [78]. However, heavy metal concentrations were not a determinant factor of moss vitality (Table S4) [79]. Suitable sample preparation prior to exposure homogenizes the sample material, as indicated by the low variability in metal concentrations (*CV*) [37]. In most cases, the small percentage of variation in the concentrations of metallic elements accumulated by the moss (less than 10%) did not adversely affect its lifespan. In contrast, Pb and Hg values higher than those observed (10%) represented a positive change in the vitality of *P. schreberi*, as did Fe values for *D. polysetum*. This supports the conclusion that elemental concentrations in moss after exposure are independent of the vitality of the organism [80]. Most elements (Fe, Cu, Zn, Cd and Hg) showed a cumulative trend in the moss with the length of exposure for the three species. Time of exposure (together with the accompanying variable environmental conditions) negatively affected the vitality of the mosses according to our analyses [81]. We recommended using a single species to monitor atmospheric pollution because different species of mosses have different accumulation capacities [82]. We found *D. polysetum* to have the highest accumulation capacity for regional monitoring of the atmosphere [83], but our study did not confirm this. In the case of our study, *P. schreberi*, which is used for active and passive biomonitoring studies, proved to be the best [84,85]. Other metals did not show such a trend; this was attributed to the influence of precipitation, which may have washed the metals away [86] (among other factors). The monthly rainfall was 35.6 mm, 18.6 mm and 37.4 mm respectively, for the studied exposure months [87]. In previous studies, we have shown how important it is to consider the influence of environmental conditions on the accumulation of heavy metals by mosses [88,89].

The anatomical and structural features of the plant influence which and how much PM they capture [90,91]. Although mosses capture mainly fine particles (<2.5 μm), the results from Pseudoscleropodium *purum* moss indicated that particles entrapped by mosses represent different fractions, and the amount of PM was strongly related to the concentration of metallic elements [92]. Other studies have confirmed that *Hypnum cupressiforme* entraps a prevalence of potentially inhalable or breathable particles (≤PM10), where the smallest particle classes were predominant [93]. The need to compare biomonitoring results with other methods is also stressed for TSP [35,94]. Hence, in our study, we decided to investigate dust in the whole TSP fraction, and not only selected PM.

In the first case, a comparison of biomonitoring studies with an automatic device using *H. cupressiforme* mosses and cellulose filters yielded different results: cellulose filters showed the lowest accumulation ability [95]. The interception and accumulation of airborne particles in exposed moss bags occur through different mechanisms than those involved in the PM10 collection by automatic devices [96]. A correlation analysis between PM10 API (Air Pollution Index) in the air and depositions of S, Pb, Cu, Zn in the moss bags showed a significant correlation with the concentration of Cu [74]. In contrast, in an Austrian experiment, toxic elements in mosses correlated well with data on overall air pollution obtained by the Index of Atmospheric Purity (IAP) method [97]. Other studies in the field of passive biomonitoring have indicated that bryophytes are suitable for the verification of

air pollution in mathematical models of PM10 due to their ability to capture the long-term deposition of pollutants [98]. In another case, it was shown that this moss bag technique (using *S girgensohnii*) could be a valuable tool to verify model performance; both methods showed the same trend [99]. However, in most of the works cited, studies referred to PM10 in the dust as well as in moss [30]. We find it particularly difficult to understand how, for the latter case, heavy metals were quantified only in PM10 in relation to mosses. The authors concluded that there was no statistically significant difference between the two methods (*S. girgensohnii* moss bags and PM10 samples); however, we cannot find any statistical analysis confirming this [30].

The examples cited above indicate that different fractions of dust (mainly fine) are deposited in plants. Comparing elemental concentrations in PM10 deposited on filters with elemental concentrations in mosses where there are different fractions yielded inconsistent results in terms of pollutants from different fractions. From our point of view, we think this is the wrong approach, so we decided to collect TSP in filters (and quantify the heavy metals therein), and we treated mosses the same way (they also collected different fractions, including TSP). In the future, more attention should be paid to research on the dust fractions that are deposited in mosses (depending on the species); only then should they be compared to the corresponding PM fractions deposited on filters (this applies to both biomonitoring methods). Therefore, biomonitoring studies should be compared in-house, with considerable attention being paid to how contamination affects the viability of the bioindicator. This method shows the form of accumulation of contaminants (in our case, heavy metals) and their effects on mosses. We recommend continuing research into this phenomenon and standardizing further procedures associated with the moss-bag technique.

5. Conclusions

In our study, we tested three moss species, i.e., *P. schreberi*, *S. fallax* and *D. polysetum*, with the objective of verifying the hypothesis that the concentrations of heavy metals accumulated in mosses are proportional to those in TSP dust deposited in the filter.

We found that the most abundant elemental components in the collected TSP particles were Fe, Pb and Zn, whereas lower concentrations of Mn, Hg and Cd were detected.

Concentration changes over time were related to the moss species and the element in question. Excluding Cd and Pb, the initial metal concentrations were related to the moss species.

No prevailing rinse phenomenon was observed. No statistically significant changes in Mn and Pb concentrations by moss species were observed. An increase in Cu, Cd, and Hg concentrations was not related to the moss species. In *S. fallax*, a smaller increase in the metal concentrations was revealed.

Moss species are sensitive to environmental changes. Their vitality decreased when exposed to unfavorable meteorological and environmental conditions or air pollution. Despite the significantly decreased activity in photosystem II, the exposed moss was still able to accumulate TSP components from its surroundings.

The clusters observed in dendrograms for moss composition were distinct from TSP composition clusters. This observation led us to conclude that the elemental compositions of moss and TSP are significantly different. One factor influencing the biological activity of the moss is its affinity for chemical compounds; this brought about differences between TSP and moss composition.

A common trend in terms of changes in Pb and Zn concentrations indicated low emission sources as the main origin of these metals in the TSP.

The present research indicates that the results obtained by the two methods (active biomonitoring and deposited dust on the filter) have different applications. Mosses accumulate bioavailable forms of metals and are affected by many external factors during exposure (thus changing their degree of vitality); therefore, the results were different from those obtained with an automatic device.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph19084706/s1, Table S1: Mean metal concentration in mosses before exposure (mg/kg d.m.),Table S2: Structural parameters (Estimate) of the linear model describing changes in element (Element) concentration in plant in relation to time of exposure, Table S3: Estimates of co-variability type in pairs of elements' concentrations during periods of expositions, Table S4: Structural parameters (Estimate) of the linear model describing changes in photochemical quantum yield (POY) in relation to element's concentration and the plant species (Species).

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Table S1. Mean metal concentration in mosses before exposure (mg/kg d.m.).

	Mn	**Fe**	**Cu**	**Zn**	**Cd**	**Hg**	**Pb**
Pl	212	173	7.07	52.2	0.51	0.031	6.65
Sp	151	128	3.59	30.1	0.53	0.036	2.97
Dp	168	306	8.49	32.8	0.54	0.049	7.41
n	5	5	5	5	5	5	5

Table S2. Structural parameters (Estimate) of the linear model describing changes in element (Element) concentration in plant in relation to time of exposure.

Description	Parameter	Element	Estimate	Std. Error	t.stat.	p-value	CI 2.5%	CI 97.5%
Concentration in Dp for t=0	Intercept for Dp	Mn	2.28	0.02	100	0.000	2.23	2.33
Concentration difference Pl-Dp for t=0	Specie Pl	Mn	0.10	0.03	3.06	0.004	0.03	0.16
Concentration difference Sp-Dp for t=0	Specie Sp	Mn	-0.18	0.03	-5.72	0.000	-0.25	-0.12
Slope for concentration changes in Dp	Time slope for Dp	Mn	0.01	0.01	0.61	0.548	-0.02	0.03
Difference in the slopes' of concentration changes Pl-Dp	Diff. in Pl time slope	Mn	-0.01	0.02	-0.85	0.400	-0.04	0.02
Difference in the slopes' of concentration changes Sp-Dp	Diff. in Sp time slope	Mn	0.00	0.02	-0.17	0.863	-0.03	0.03
Concentration in Dp for t=0	Intercept for Dp	Fe	5.31	0.06	81.7	0.000	5.17	5.44
Concentration difference Pl-Dp for t=0	Specie Pl	Fe	-0.36	0.09	-3.98	0.000	-0.55	-0.18
Concentration difference Sp-Dp for t=0	Specie Sp	Fe	-0.41	0.09	-4.50	0.000	-0.59	-0.22
Slope for concentration changes in Dp	Time slope for Dp	Fe	0.32	0.03	10.2	0.000	0.25	0.38
Difference in the slopes' of concentration changes Pl-Dp	Diff. in Pl time slope	Fe	0.00	0.04	0.03	0.973	-0.09	0.09
Difference in the slopes' of concentration changes Sp-Dp	Diff. in Sp time slope	Fe	-0.17	0.04	-3.95	0.000	-0.26	-0.08
Concentration in Dp for t=0	Intercept for Dp	Cu	2.00	0.05	42.1	0.000	1.90	2.09
Concentration difference Pl-Dp for t=0	Specie Pl	Cu	-0.01	0.07	-0.15	0.883	-0.14	0.12
Concentration difference Sp-Dp for t=0	Specie Sp	Cu	-0.65	0.07	-9.86	0.000	-0.79	-0.52
Slope for concentration changes in Dp	Time slope for Dp	Cu	0.13	0.02	5.62	0.000	0.08	0.17
Difference in the slopes' of concentration changes Pl-Dp	Diff. in Pl time slope	Cu	-0.03	0.03	-0.87	0.392	-0.09	0.04
Difference in the slopes' of concentration changes Sp-Dp	Diff. in Sp time slope	Cu	-0.04	0.03	-1.36	0.183	-0.11	0.02
Concentration in Dp for t=0	Intercept for Dp	Zn	3.40	0.04	77.6	0.000	3.31	3.49
Concentration difference Pl-Dp for t=0	Specie Pl	Zn	0.52	0.06	8.48	0.000	0.40	0.64
Concentration difference Sp-Dp for t=0	Specie Sp	Zn	0.08	0.06	1.25	0.219	-0.05	0.20
Slope for concentration changes in Dp	Time slope for Dp	Zn	0.19	0.02	9.06	0.000	0.15	0.23
Difference in the slopes' of concentration changes Pl-Dp	Diff. in Pl time slope	Zn	-0.06	0.03	-1.95	0.058	-0.11	0.00
Difference in the slopes' of concentration changes Sp-Dp	Diff. in Sp time slope	Zn	-0.07	0.03	-2.49	0.017	-0.13	-0.01
Concentration in Dp for t=0	Intercept for Dp	Cd	-0.82	0.04	-20.2	0.000	-0.90	-0.73
Concentration difference Pl-Dp for t=0	Specie Pl	Cd	-0.03	0.06	-0.45	0.655	-0.14	0.09
Concentration difference Sp-Dp for t=0	Specie Sp	Cd	0.08	0.06	1.44	0.159	-0.03	0.20
Slope for concentration changes in Dp	Time slope for Dp	Cd	0.09	0.02	4.44	0.000	0.05	0.13
Difference in the slopes' of concentration changes Pl-Dp	Diff. in Pl time slope	Cd	-0.01	0.03	-0.36	0.719	-0.06	0.04
Difference in the slopes' of concentration changes Sp-Dp	Diff. in Sp time slope	Cd	-0.02	0.03	-0.74	0.464	-0.07	0.03
Concentration in Dp for t=0	Intercept for Dp	Hg	-3.02	0.08	-38.1	0.000	-3.18	-2.86
Concentration difference Pl-Dp for t=0	Specie Pl	Hg	-0.44	0.11	-3.93	0.000	-0.67	-0.21
Concentration difference Sp-Dp for t=0	Species Sp	Hg	-0.36	0.11	-3.17	0.003	-0.58	-0.13
Slope for concentration changes in Dp	Time slope for Dp	Hg	0.18	0.04	4.78	0.000	0.10	0.25
Difference in the slopes' of concentration changes Pl-Dp	Diff. in Pl time slope	Hg	0.10	0.05	1.87	0.069	-0.01	0.20
Difference in the slopes' of concentration changes Sp-Dp	Diff. in Sp time slope	Hg	-0.03	0.05	-0.63	0.534	-0.14	0.07
Concentration in Dp for t=0	Intercept for Dp	Pb	1.70	0.17	9.69	0.000	1.34	2.05
Concentration difference Pl-Dp for t=0	Specie Pl	Pb	-0.19	0.24	-0.78	0.440	-0.69	0.30
Concentration difference Sp-Dp for t=0	Specie Sp	Pb	0.11	0.24	0.43	0.670	-0.39	0.60
Slope for concentration changes in Dp	Time slope for Dp	Pb	0.11	0.08	1.34	0.189	-0.06	0.28
Difference in the slopes' of concentration changes Pl-Dp	Diff. in Pl time slope	Pb	0.01	0.12	0.10	0.918	-0.22	0.25
Difference in the slopes' of concentration changes Sp-Dp	Diff. in Sp time slope	Pb	-0.22	0.12	-1.91	0.064	-0.45	0.01

Standard Errors (Std. Error), t statistics (t.stat.), p-values (p-value) for H0: Estimate=0, and limits (CI 2.5%, CI 97.5%) of the 95% confidence interval are shown

Table S3. Estimates of co-variability type in pairs of elements' concentrations during periods of expositions.

First month

variable1	variable2	lower quant.	t	upper quant.	Co-variation
Mn	Fe	0.891	1.261	1.231	negative
Mn	Cu	0.913	0.916	1.094	random
Mn	Zn	0.818	1.241	1.276	random
Mn	Pb	0.963	2.560	2.523	negative
Fe	Cu	0.024	0.062	0.062	random
Fe	Zn	0.060	0.135	0.155	random
Fe	Pb	0.331	0.309	0.564	positive
Cu	Zn	0.058	0.109	0.120	random
Cu	Pb	0.371	0.521	0.510	negative
Zn	Pb	0.352	0.354	0.690	random

Second month

variable1	variable2	lower quant.	t	upper quant.	Co-variation
Mn	Fe	0.810	1.091	1.066	negative
Mn	Cu	0.783	0.893	1.050	random
Mn	Zn	0.755	1.102	1.152	random
Mn	Pb	1.043	2.450	2.463	random
Fe	Cu	0.021	0.037	0.064	random
Fe	Zn	0.041	0.102	0.109	random
Fe	Pb	0.334	0.348	0.514	random
Cu	Zn	0.041	0.097	0.109	random
Cu	Pb	0.345	0.456	0.529	random
Zn	Pb	0.328	0.323	0.611	positive

Third month

variable1	variable2	lower quant.	t	upper quant.	Co-variation
Mn	Fe	0.834	1.028	1.012	negative
Mn	Cu	0.822	0.923	0.960	random
Mn	Zn	0.691	1.138	1.207	random
Mn	Pb	1.055	2.599	2.599	random
Fe	Cu	0.009	0.019	0.025	random
Fe	Zn	0.067	0.137	0.134	negative
Fe	Pb	0.418	0.437	0.560	random
Cu	Zn	0.070	0.078	0.111	random
Cu	Pb	0.448	0.467	0.547	random
Zn	Pb	0.407	0.384	0.796	positive

Variable1 and variable2 indicate elements' name, t is variance of concentrations' log ratios, lower quant. and upper quant. are respectively 2.5% and 97.5% quantiles of the t parameter distribution, and Co-variation announces type of character of co-variability (increase in variable1 concentration followed by increase in variable2 concentration is marked by yellow background)

Table S4. Structural parameters (Estimate) of the linear model describing changes in photochemical quantum yield (POY) in relation to element's concentration and the plant species (Species).

	Element	Species	Estimate	Std. Error	t.stat.	p-value	*CV* [%]
Intercept	Mn	Pl	0.080	0.077	1.04	0.303	
Time slope	Mn	Pl	-0.006	0.004	-1.54	0.128	
Concentration slope	Mn	Pl	0.000	0.000	0.78	0.440	2.59
Intercept	Mn	Sp	0.075	0.029	2.55	0.013	
Time slope	Mn	Sp	-0.007	0.004	-2.09	0.040	
Concentration slope	Mn	Sp	0.000	0.000	1.45	0.150	6.69
Intercept	Mn	Dp	0.149	0.062	2.41	0.019	
Time slope	Mn	Dp	0.009	0.006	1.63	0.109	
Concentration slope	Mn	Dp	0.000	0.000	-0.31	0.759	4.30
Intercept	Fe	Pl	0.112	0.016	6.82	0.000	
Time slope	Fe	Pl	-0.019	0.008	-2.37	0.021	
Concentration slope	Fe	Pl	0.000	0.000	1.87	0.067	6.71
Intercept	Fe	Sp	0.097	0.046	2.10	0.039	
Time slope	Fe	Sp	-0.011	0.010	-1.18	0.242	
Concentration slope	Fe	Sp	0.000	0.000	0.42	0.676	4.46
Intercept	Fe	Dp	0.061	0.013	4.70	0.000	
Time slope	Fe	Dp	-0.019	0.006	-3.13	0.003	
Concentration slope	Fe	Dp	0.000	0.000	6.07	0.000	11.9
Intercept	Cu	Pl	0.086	0.041	2.09	0.041	
Time slope	Cu	Pl	-0.011	0.005	-2.01	0.049	
Concentration slope	Cu	Pl	0.007	0.005	1.33	0.187	4.55
Intercept	Cu	Sp	0.139	0.036	3.82	0.000	
Time slope	Cu	Sp	-0.005	0.005	-1.09	0.279	
Concentration slope	Cu	Sp	-0.006	0.009	-0.63	0.529	3.83
Intercept	Cu	Dp	0.025	0.055	0.45	0.657	
Time slope	Cu	Dp	-0.004	0.008	-0.46	0.648	
Concentration slope	Cu	Dp	0.014	0.007	1.92	0.060	4.66
Intercept	Zn	Pl	0.049	0.052	0.95	0.348	
Time slope	Zn	Pl	-0.018	0.008	-2.28	0.026	
Concentration slope	Zn	Pl	0.002	0.001	1.75	0.084	4.55
Intercept	Zn	Sp	0.133	0.033	3.99	0.000	
Time slope	Zn	Sp	-0.005	0.006	-0.87	0.384	
Concentration slope	Zn	Sp	-0.001	0.001	-0.53	0.600	5.02
Intercept	Zn	Dp	0.043	0.052	0.83	0.412	
Time slope	Zn	Dp	-0.013	0.013	-0.95	0.345	
Concentration slope	Zn	Dp	0.003	0.002	1.71	0.093	4.37
Intercept	Cd	Pl	0.046	0.054	0.85	0.397	
Time slope	Cd	Pl	-0.010	0.005	-2.18	0.033	
Concentration slope	Cd	Pl	0.204	0.117	1.74	0.087	4.41
Intercept	Cd	Sp	0.086	0.039	2.24	0.028	
Time slope	Cd	Sp	-0.009	0.004	-2.27	0.026	
Concentration slope	Cd	Sp	0.059	0.075	0.79	0.435	5.58
Intercept	Cd	Dp	0.049	0.062	0.79	0.431	
Time slope	Cd	Dp	0.005	0.006	0.86	0.395	
Concentration slope	Cd	Dp	0.169	0.127	1.33	0.188	3.30
Intercept	Hg	Pl	0.109	0.015	7.13	0.000	
Time slope	Hg	Pl	-0.020	0.007	-2.72	0.008	
Concentration slope	Hg	Pl	1.089	0.485	2.24	0.028	11.7
Intercept	Hg	Sp	0.095	0.031	3.11	0.003	
Time slope	Hg	Sp	-0.011	0.006	-1.83	0.071	
Concentration slope	Hg	Sp	0.624	0.872	0.72	0.476	7.05
Intercept	Hg	Dp	0.134	0.025	5.37	0.000	
Time slope	Hg	Dp	0.009	0.008	1.19	0.241	
Concentration slope	Hg	Dp	-0.076	0.502	-0.15	0.880	13.9
Intercept	Pb	Pl	0.107	0.013	8.54	0.000	
Time slope	Pb	Pl	-0.007	0.004	-1.97	0.054	
Concentration slope	Pb	Pl	0.006	0.002	3.11	0.003	30.5
Intercept	Pb	Sp	0.137	0.013	10.42	0.000	
Time slope	Pb	Sp	-0.008	0.004	-2.29	0.025	
Concentration slope	Pb	Sp	-0.004	0.002	-1.82	0.073	25.7
Intercept	Pb	Dp	0.111	0.023	4.77	0.000	
Time slope	Pb	Dp	0.008	0.005	1.48	0.143	
Concentration slope	Pb	Dp	0.003	0.003	0.87	0.388	14.8

Standard Errors (Std. Error), t statistics (t.stat), p-values (p-value) for H0: Estimate=0, and coefficient of variation (CV) are shown

OŚWIADCZENIA WSPÓŁAUTORÓW ODNOŚNIE ICH UDZIAŁU W POWSTAWANIU PUBLIKACJI PRZEDSTAWIONYCH DO OCENY W PRZEWODZIE DOKTORSKIM

Opole, 13.01.2023 r.

Oświadczenie doktoranta o procentowym wkładzie autorskim w artykuł naukowy wraz z opisem tego wkładu

Bioaccumulation of Trace Elements from Aqueous Solutions by Selected Terrestrial Moss Species

(tytuł publikacji naukowej)

Biology 2022;11:1692. https://doi.org/10.3390/biology11121692.

(czasopismo)

ON.1. Udział procentowy doktoranta oraz współautorów w artykuł naukowy.

Lp.	Nazwisko i imię współautora	Afiliacja	Udział procentowy (%)	Potwierdzam prawdziwość danych – czytelny podpis współautora
1.	Świsłowski Paweł	Uniwersytet Opolski	70	Świsłowski
2.	Nowak Arkadiusz	PAN Ogród Botaniczny w Powsinie	10	Arkadiusz Nowak
3.	Wacławek Stanisław	Uniwersytet Techniczny w Libercu	5	
4.	Silvestri Daniele	Uniwersytet Techniczny w Libercu	5	Daniele Silvestri
5.	Rajfur Małgorzata	Uniwersytet Opolski	10	Rajfur M.

Opis wkładu doktoranta:

Opracowanie koncepcji artykułu (sformułowanie celów i zadań badawczych), opracowanie metodologii, przeprowadzenie badań terenowych i laboratoryjnych, analiza formalna (zarządzanie danymi, walidacja wyników), wizualizacja danych, przygotowanie oryginalnego projektu publikacji.

Opole, 13.01.2023 r.

Oświadczenie doktoranta o procentowym wkładzie autorskim w artykuł naukowy wraz z opisem tego wkładu

The influence of preparation methodology on the concentration of heavy metals in Pleurozium schreberi moss samples prior to use in active biomonitoring studies

(tytuł publikacji naukowej)

Environmental Science and Pollution Research 2021;28(8):10068-10076. https://doi.org/10.1007/s11356-020-11484-7.

(czasopismo)

ON.2. Udział procentowy doktoranta oraz współautorów w artykuł naukowy.

Lp.	Nazwisko i imię współautora	Afiliacja	Udział procentowy (%)	Potwierdzam prawdziwość danych – czytelny podpis współautora
1.	Świsłowski Paweł	Uniwersytet Opolski	70	Świsłowski
2.	Kosior Grzegorz	Uniwersytet Opolski	10	Kosior
3.	Rajfur Małgorzata	Uniwersytet Opolski	20	Rajfur M.

Opis wkładu doktoranta:

Opracowanie koncepcji artykułu (sformułowanie celów i zadań badawczych), opracowanie metodologii, przeprowadzenie badań terenowych i laboratoryjnych, analiza formalna (zarządzanie danymi, walidacja wyników, analiza statystyczna), wizualizacja danych, przygotowanie oryginalnego projektu publikacji.

Opole, 13.01.2023 r.

Oświadczenie doktoranta o procentowym wkładzie autorskim w artykuł naukowy wraz z opisem tego wkładu

Comparison of exposure techniques and vitality assessment in active biomonitoring for suitability in assessing atmospheric aerosol heavy metal pollution

(tytuł publikacji naukowej)

Environmental Toxicology and Chemistry 2022;41(6):1429-1438. https://doi.org/10.1002/etc.5321.

(czasopismo)

ON.3. Udział procentowy doktoranta oraz współautorów w artykuł naukowy.

Lp.	Nazwisko i imię współautora	Afiliacja	Udział procentowy (%)	Potwierdzam prawdziwość danych – czytelny podpis współautora
1.	Świsłowski Paweł	Uniwersytet Opolski	80	Świsłowski
2.	Nowak Arkadiusz	PAN Ogród Botaniczny w Powsinie	10	Arkadiusz Nowak
3.	Rajfur Małgorzata	Uniwersytet Opolski	10	Rajfur M.

Opis wkładu doktoranta:

Opracowanie koncepcji artykułu (sformułowanie celów i zadań badawczych), opracowanie metodologii, przeprowadzenie badań terenowych i laboratoryjnych, analiza formalna (zarządzanie danymi, walidacja wyników, analiza statystyczna), wizualizacja danych, przygotowanie oryginalnego projektu publikacji.

Opole, 13.01.2023 r.

Oświadczenie doktoranta o procentowym wkładzie autorskim w artykuł naukowy wraz z opisem tego wkładu

Is Your Moss Alive during Active Biomonitoring Study?

(tytuł publikacji naukowej)

Plants 2021;10(11):2389. https://doi.org/10.3390/plants10112389.

(czasopismo)

ON.4. Udział procentowy doktoranta oraz współautorów w artykuł naukowy.

Lp.	Nazwisko i imię współautora	Afiliacja	Udział procentowy (%)	Potwierdzam prawdziwość danych – czytelny podpis współautora
1.	Świsłowski Paweł	Uniwersytet Opolski	70	Świsłowski
2.	Nowak Arkadiusz	PAN Ogród Botaniczny w Powsinie	15	A. Nowak
3.	Rajfur Małgorzata	Uniwersytet Opolski	15	Rajfur M.

Opis wkładu doktoranta:

Opracowanie koncepcji artykułu (sformułowanie celów i zadań badawczych), opracowanie metodologii, przeprowadzenie badań terenowych i laboratoryjnych, analiza formalna (zarządzanie danymi, walidacja wyników, analiza statystyczna), wizualizacja danych, przygotowanie oryginalnego projektu publikacji.

Opole, 13.01.2023 r.

Oświadczenie doktoranta o procentowym wkładzie autorskim w artykuł naukowy wraz z opisem tego wkładu

The influence of environmental conditions on the lifespan of mosses under long-term active biomonitoring

(tytuł publikacji naukowej)

Atmospheric Pollution Research 2021;12(10):101203.
https://doi.org/10.1016/j.apr.2021.101203.

(czasopismo)

ON.5. Udział procentowy doktoranta oraz współautorów w artykuł naukowy.

Lp.	Nazwisko i imię współautora	Afiliacja	Udział procentowy (%)	Potwierdzam prawdziwość danych – czytelny podpis współautora
1.	Świsłowski Paweł	Uniwersytet Opolski	70	Świsłowski
2.	Nowak Arkadiusz	PAN Ogród Botaniczny w Powsinie	15	Arkadiusz Nowak
3.	Rajfur Małgorzata	Uniwersytet Opolski	15	Rajfur M.

Opis wkładu doktoranta:

Opracowanie koncepcji artykułu (sformułowanie celów i zadań badawczych), opracowanie metodologii, przeprowadzenie badań terenowych i laboratoryjnych (pomiary), analiza formalna (zarządzanie danymi, walidacja wyników, analiza statystyczna), wizualizacja danych, przygotowanie oryginalnego projektu publikacji.

Opole, 13.01.2023 r.

Oświadczenie doktoranta o procentowym wkładzie autorskim w artykuł naukowy wraz z opisem tego wkładu

Effects of tobacco smoke on indoor air quality: the use of mosses in biomonitoring

(tytuł publikacji naukowej)

Journal of Environmental Health Science and Engineering 2022;20:485-493.

https://doi.org/10.1007/s40201-022-00794-2.

(czasopismo)

ON.6. Udział procentowy doktoranta oraz współautorów w artykuł naukowy.

Lp.	Nazwisko i imię współautora	Afiliacja	Udział procentowy (%)	Potwierdzam prawdziwość danych – czytelny podpis współautora
1.	Świsłowski Paweł	Uniwersytet Opolski	70	Świsłowski
2.	Śmiechowicz Bogusław	ATMOTERM S.A.	15	Śmiechowicz
3.	Rajfur Małgorzata	Uniwersytet Opolski	15	Rajfur dl.

Opis wkładu doktoranta:

Opracowanie koncepcji artykułu (sformułowanie celów i zadań badawczych), opracowanie metodologii, przeprowadzenie badań terenowych i laboratoryjnych, analiza formalna (zarządzanie danymi, walidacja wyników, analiza statystyczna), wizualizacja danych, przygotowanie oryginalnego projektu publikacji.

Opole, 13.01.2023 r.

Oświadczenie doktoranta o procentowym wkładzie autorskim w artykuł naukowy wraz z opisem tego wkładu

Mosses as a biomonitor to identify elements released into the air as a result of car workshop activities

(tytuł publikacji naukowej)

Ecological Indicators 2022;138:108849. https://doi.org/10.1016/j.ecolind.2022.108849.

(czasopismo)

ON.7. Udział procentowy doktoranta oraz współautorów w artykuł naukowy.

Lp.	Nazwisko i imię współautora	Afiliacja	Udział procentowy (%)	Potwierdzam prawdziwość danych – czytelny podpis współautora
1.	Świsłowski Paweł	Uniwersytet Opolski	75	Świsłowski
2.	Vergel Konstantin	Zjednoczony Instytut Badań Jądrowych (Dubna)	5	Kbeg
3.	Zinicovscaia Inga	Zjednoczony Instytut Badań Jądrowych (Dubna)	5	
4.	Rajfur Małgorzata	Uniwersytet Opolski	10	Rajfur M.
5.	Wacławek Maria	Uniwersytet Opolski	5	Maria Wacławek

Opis wkładu doktoranta:

Opracowanie koncepcji artykułu (sformułowanie celów i zadań badawczych), opracowanie metodologii, przeprowadzenie badań terenowych i laboratoryjnych, analiza formalna (zarządzanie danymi, walidacja wyników, analiza statystyczna), wizualizacja danych, przygotowanie oryginalnego projektu publikacji.

Opole, 13.01.2023 r.

Oświadczenie doktoranta o procentowym wkładzie autorskim w artykuł naukowy wraz z opisem tego wkładu

Air Quality during New Year's Eve: A Biomonitoring Study with Moss

(tytuł publikacji naukowej)

Atmosphere 2021;12(8):975. https://doi.org/10.3390/atmos12080975.

(czasopismo)

ON.8. Udział procentowy doktoranta oraz współautorów w artykuł naukowy.

Lp.	Nazwisko i imię współautora	Afiliacja	Udział procentowy (%)	Potwierdzam prawdziwość danych – czytelny podpis współautora
1.	Świsłowski Paweł	Uniwersytet Opolski	80	Świsłowski
2.	Ziembik Zbigniew	Uniwersytet Opolski	10	Ziembik
3.	Rajfur Małgorzata	Uniwersytet Opolski	10	Rajfur

Opis wkładu doktoranta:

Opracowanie koncepcji artykułu (sformułowanie celów i zadań badawczych), opracowanie metodologii, przeprowadzenie badań terenowych i laboratoryjnych (pomiary), analiza formalna (zarządzanie danymi, walidacja wyników), wizualizacja danych, przygotowanie oryginalnego projektu publikacji.

Opole, 13.01.2023 r.

Oświadczenie doktoranta o procentowym wkładzie autorskim w artykuł naukowy wraz z opisem tego wkładu

Is active moss biomonitoring comparable to air filter standard sampling?

(tytuł publikacji naukowej)

International Journal of Environmental Research and Public Health 2022;19(8):4706..

https://doi.org/10.3390/ijerph19084706.

(czasopismo)

ON.9. Udział procentowy doktoranta oraz współautorów w artykuł naukowy.

Lp.	Nazwisko i imię współautora	Afiliacja	Udział procentowy (%)	Potwierdzam prawdziwość danych – czytelny podpis współautora
1.	Świsłowski Paweł	Uniwersytet Opolski	70	Świsłowski
2.	Nowak Arkadiusz	PAN Ogród Botaniczny w Powsinie	5	Arkadiusz Nowak
3.	Wacławek Stanisław	Uniwersytet Techniczny w Libercu	10	
4.	Ziembik Zbigniew	Uniwersytet Opolski	10	
5.	Rajfur Małgorzata	Uniwersytet Opolski	5	Rajfur M.

Opis wkładu doktoranta:

Opracowanie koncepcji artykułu (sformułowanie celów i zadań badawczych), opracowanie metodologii, przeprowadzenie badań terenowych i laboratoryjnych, analiza formalna (zarządzanie danymi, walidacja wyników), wizualizacja danych, przygotowanie oryginalnego projektu publikacji.

WYKAZ DOROBKU NAUKOWEGO

Spis treści

WYKAZ DOROBKU NAUKOWEGO PRZED WSTĄPIENIEM DO SZKOŁY DOKTORSKIEJ UNIWERSYTETU OPOLSKIEGO (przed rokiem akademickim 2019/2020)

Przebieg kariery naukowej:

- 2013-2017: 3,5-letnie studia inżynierskie na kierunku Odnawialne Źródła Energii (Uniwersytet Opolski, Wydział Przyrodniczo-Techniczny, Samodzielna Katedra Inżynierii Procesowej).

- 2017/2018: 1,5-letnie studia magisterskie na kierunku Biotechnologia (Uniwersytet Opolski, Wydział Przyrodniczo-Techniczny, Samodzielna Katedra Biotechnologii i Biologii Molekularnej).

- 2018/2019: 1,5-letnie studia magisterskie na kierunku Odnawialne Źródła Energii (Uniwersytet Opolski, Wydział Przyrodniczo-Techniczny, Instytut Inżynierii Środowiska i Biotechnologii).

- 2018/2019: studia III stopnia doktoranckie na kierunku Biologia (Uniwersytet Opolski, Wydział Przyrodniczo-Techniczny, Instytut Biologii).

I) Rozdziały w monografiach (9):

L.p.	Wyszczególnienie	Punkty*
1.	Kalinichenko A., **Świsłowski P**.: *Zasada zrównoważonego rozwoju w NSRO 2007-2013 i SRK 2007-2015* [w:] Materiali mižnarodnoi-naukovo praktičnoi konferencii "Aktual'ni problemi ta perspektivi rozvitku obliku, analoizu ta kontrolu v social'no-orientovanij sistemi upravlinnâ pidpriemstvom", styczeń 2015, Połtawa [UA], PDAA, s. 6-13.	5
2.	**Świsłowski P**., Kalinichenko A.: *Analiza matematyczna efektywności procesów uzyskania biogazu z odpadów rolniczych* [w:] Pikoń K., Bogacka M. (red.): Współczesne problemy energetyki II. Praca zbiorowa, czerwiec 2015, Gliwice, Mastermedia, s. 81-90, ISBN 9788393725588.	5
3.	**Świsłowski P**., Kalinichenko A.: *Biogaz w rolnictwie* [w:] Materiali XII soričnogo miždisciplinarnogo seminaru: Students'ki raboti za naukovou tematikou kafedri ekonomičnoi kibernetiki ta informacijnih tehnologij (XII annual interdisciplinary seminar „Students work on a scientific topic of economic cybernetics and information technologies"), listopad 2015, Połtawa [UA], PDAA, s. 51-55.	5
4.	**Świsłowski P**., Kalinichenko A.: *Przegląd współczesnych, światowych technologii (rozwiązań, patentów) produkcji biogazu* [w:] Gawdzik A. (red.): Monografia: Wybrane zagadnienia szeroko pojętej inżynierii procesowej. T. 2, grudzień 2015, Opole, Wydawnictwo i Drukarnia Świętego Krzyża, s. 63-78, ISBN 9788373424999.	5
5.	**Świsłowski P**., Kalinichenko A.: *Polskie rozwiązania i technologie wytwarzania biogazu* [w:] Ratuszny P., Suszanowicz D. (red.): Odnawialne źródła energii - teoria i praktyka, 2016, Opole, Wydawnictwo Świętego Krzyża, s. 25-42, ISBN 978-83-7342-548-4.	5
6.	**Świsłowski P**., Kalinichenko A.: *Uprawa Palczatki Gerarda do celów energetycznych* [w:] Materìali students'koï naukovoï konferencìï, 26-27 kvitâ 2017 r, kwiecień 2017, vol. 1, Połtawa [UA], RVV PDAA, s. 253-254.	5
7.	**Świsłowski P**.: *Analiza i ocena pracy nowego systemu fotowoltaicznego w Uniwersytecie Opolskim* [w:] Dyjakon A., Krzyś A. (red.): Monografia naukowa pokonferencyjna XXII Międzynarodowa Konferencja Studenckich Kół Naukowych Uniwersytet Przyrodniczy we Wrocławiu, 25-26 maja 2017 r., Wrocław. Problematyka nauk przyrodniczych i technicznych – Tom 1, maj 2017, Wrocław, DSS UPWR, str. 190-202, ISBN 978-83-948516-0-6.	5
8.	Dębska L., **Świsłowski P**., Kalinichenko A.: *Szkodniki i choroby kukurydzy* (s. 36-39), Materiali XIV soričnogo miždisciplinarnogo seminaru: Students'ki raboti za naukovou tematikou kafedri ekonomičnoi kibernetiki ta informacijnih tehnologij (XIV annual interdisciplinary seminar „Students work on a scientific topic of economic cybernetics and information technologies"), listopad 2017, Połtawa [UA], PDAA.	5
9.	**Świsłowski P**., Dębska L., Kalinichenko A.: *Porównanie roślin energetycznych – róży wielokwiatowej i konopi siewnych* (s. 89-94), Materiali XIV soričnogo miždisciplinarnogo seminaru: Students'ki raboti za naukovou tematikou kafedri ekonomičnoi kibernetiki ta informacijnih tehnologij (XIV annual interdisciplinary seminar „Students work on a scientific topic of economic cybernetics and information technologies"), listopad 2017, Połtawa [UA], PDAA.	5

* Punktacja monografii naukowych, Rozporządzenie MNiSW z dnia 17.10.2007 r.

II) Publikacje opublikowane w czasopismach o zasięgu krajowym i międzynarodowym (17):

L.p.	Wyszczególnienie	Punkty	*IF*
1.	**Świsłowski P**., Rajfur M., Kłos A.: *Biomonitoring aktywny rzeki Czarnej Koneckiej (woj. świętokrzyskie) z wykorzystaniem glonów Palmaria palmata*. Proc. ECOpole 2015;9(1):339-345. DOI: 10.2429/proc.2015.9(1)043.	9	-
2.	**Świsłowski P**., Marciniak M., Rajfur M.: *Zastosowanie badań biomonitoringowych do oceny zanieczyszczenia metalami ciężkimi*	9	-

	wybranych ekosystemów. Proc. ECOpole 2016;10(1):251-266. DOI: 10.2429/proc.2016.10(1)033.		
3.	Fortalska E., **Świsłowski P**., Rajfur M.: *Ocena właściwości sorpcyjnych Hottonia palustris L.* Proc. ECOpole 2016;10(1):117-126. DOI: 10.2429/proc.2016.10(1)015.	9	-
4.	**Świsłowski P**., Rajfur M., Rodziewicz T.: *Assessment of effectiveness of the photovoltaic system installed on the roof of the University of Opole building*. Ecol. Chem. Eng. A 2017;24(1):7-18. DOI: 10.2428/ecea.2017.24(1)1.	11	-
5.	**Świsłowski P**., Marciniak M., Rajfur M.: *Wpływ warunków prowadzenia eksperymentu na wyniki badań biomonitoringowych z zastosowaniem mchów*. Proc. ECOpole 2017;11(1):313-323. DOI: 10.2429/proc.2017.11(1)033.	9	-
6.	**Świsłowski P**., Dębska L., Kalinichenko A.: *Charakterystyka palczatki Gerarda (Andropogon gerardi) jako rośliny energetycznej*. Zagadnienia Doradztwa Rolniczego 2017;3(89):90-99.	10	-
7.	Dębska L., **Świsłowski P**., Kalinichenko A.: *Odnawialne źródła energii w zrównoważonym rozwoju* [w:] Kuzior A. (red.): Etyka biznesu i zrównoważony rozwój. Interdyscyplinarne studia teoretyczno-empiryczne nr 2: Wokół podstawowych zagadnień współczesności". 2017, Zabrze, Śląskie Centrum Etyki Biznesu i Zrównoważonego Rozwoju, s. 9-16, ISBN 978-83-61975-58-8.	0	-
8.	**Świsłowski P**., Rajfur M.: *Biokumulacja pierwiastków w grzybach wielkoowocnikowych – przegląd wybranej literatury*. Proc. ECOpole 2017;11(2):591-599. DOI: 10.2429/proc.2017.11(2)067.	9	-
9.	Kaczmarek K., **Świsłowski P**., Rajfur M.: *Biomonitoring aktywny z zastosowaniem mchów w pobliżu Miasteczka Śląskiego*. Proc. ECOpole 2017;11(2):507-516. DOI: 10.2429/proc.2017.11(2)055.	9	-
10.	Nabrdalik M., **Świsłowski P**.: *Ocena mikrobiologiczna niepasteryzowanych soków owocowych i warzywnych*. Proc. ECOpole 2017;11(2):541-551. DOI: 10.2429/proc.2017.11(2)062.	9	-
11.	Kłos A., Ziembik Z., Rajfur M., Dołhańczuk-Śródka A., Bochenek Z., Bjerke J.W., Tømmervik H., Zagajewski B., Ziółkowski D., Jerz D., Zielińska M., Krems P., Godyń P., Marciniak M., **Świsłowski P**.: *Using moss and lichens in biomonitoring of heavy-metal contamination of forest areas in southern and north-eastern Poland.* Sci. Total. Environ. 2018;627:438–449. DOI: 10.1016/j.scitotenv.2018.01.211.	40	4,900
12.	Rajfur M., **Świsłowski P**., Nowainski F., Śmiechowicz B.: *Mosses as biomonitor of air pollution with analytes originating from tobacco smoke*. Chemistry-Didactics-Ecology-Metrology 2018;23(1-2):127-136. DOI: 10.1515/cdem-2018-0008.	9	-
13.	Rajfur M., **Świsłowski P**., Dębska L.: *Kora brzozy brodawkowatej jako biomonitor zanieczyszczenie powietrza metalami ciężkimi*. Proc. ECOpole 2018;12(1):237-246. DOI: 10.2429/proc.2018.12(1)028.	9	-
14.	**Świsłowski P**., Dębska L., Rajfur M., Rodziewicz T.: *Roczna wydajność instalacji PV zamontowanej na dachu budynku dydaktycznego Uniwersytetu Opolskiego*. Proc. ECOpole 2018;12(1):253-263. DOI: 10.2429/proc.2018.12(1)032.	9	-
15.	**Świsłowski P**., Rajfur M.: *Mushrooms as biomonitors of heavy metals contamination in forest areas*. Ecol. Chem. Eng. S. 2018;25(4):557-568. DOI:10.1515/eces-2018-0037.	15	0,700
16.	Pańczyk M., **Świsłowski P**., Rajfur M.: *Ocena jednorodności zanieczyszczenia kory drzew liściastych metalami ciężkimi*. Proc. ECOpole 2018;12(2)539-549. DOI: 10.2429/proc.2018.12(2)054.	9	-
17.	Pieczka M., **Świsłowski P**., Rajfur M.: *Zanieczyszczenie metalami ciężkimi Matricaria chamomilla L. i Plantago lanceolata L*. Proc. ECOpole 2019;13(1):135-143. DOI: 10.2429/proc.2019.13(1)014.	5	-

* Ujednolicony wykaz czasopism punktowanych obowiązujący w roku wydania publikacji

** *Impact Factor* w roku wydania publikacji

III) Wygłoszenie referatów (17) i posterów (13) na międzynarodowych (14) i krajowych (13) konferencjach naukowych:

L.p.	Wyszczególnienie
1.	**Świsłowski P**., Rajfur M., Kłos A.: *Biomonitoring aktywny z wykorzystaniem glonów morskich Palmaria palmata.* **Referat** na krajowej konferencji XIX Metrologia-Ekologia-Dydaktyka (MED'14), Bělá pod Pradědem (CZ), 19-21.06.2014 r.
2.	**Świsłowski P**., Rajfur M., Kłos A.: *Active biomonitoring of the surface waters using marine algae Palmaria palmata*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'14, Jarnołtówek, 15-18.10.2014 r.
3.	**Świsłowski P**., Kalinichenko A.: *Modelowanie procesów uzyskania biogazu z odpadów rolniczych*. **Referat** na międzynarodowej konferencji II Konferencji Ochrony Środowiska i Energetyki dla młodych pracowników, Gliwice, 12.12.2014 r.
4.	**Świsłowski P**., Rajfur M., Kłos A.: *Wpływ temperatury na sorpcję cynku w wybranych organizmach roślinnych*. **Referat** na międzynarodowej konferencji XX Międzynarodowej Konferencji Studenckich Kół Naukowych i XXXII Sejmiku SKN, Wrocław, 14-15.05.2015 r.
5.	**Świsłowski P**., Rajfur M., Kłos A.: *Wpływ temperatury na sorpcję wybranych analitów w glonach Palmaria palmata*. **Referat** na krajowej konferencji XX Metrologia-Ekologia-Dydaktyka (MED'15), Bělá pod Pradědem (CZ), 04-06.06.2015 r.
6.	**Świsłowski P**., Rajfur M., Kłos A.: *Sources of heavy metal contamination of Janow water reservoir (Swietokrzyskie Province)*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'15, Jarnołtówek, 14-17.10.2015 r.
7.	**Świsłowski P**., Kalinichenko A.: *Polskie rozwiązania i technologie wytwarzania biogazu*. **Poster** na krajowej konferencji VII Ogólnopolski Festiwal Ekoenergetyki, Opole, 15-18.10.2015 r.
8.	**Świsłowski P**., Rajfur M., Kłos A.: *Biomonitoring of contamination of Janow water reservoir with heavy metals (Swietokrzyskie Province)*. **Poster** na międzynarodowej konferencji III międzynarodowej konferencji naukowo-technicznej „Pure water. Fundamental, applied and industrial aspects", Kijów [UA], 28-30.10.2015 r.
9.	**Świsłowski P**., Rajfur M., Kłos A.: *Zastosowanie bioty w monitoringu zbiornika wodnego Janów (woj. świętokrzyskie)*. **Poster** na międzynarodowej konferencji XXI Międzynarodowej Konferencji Studenckich Kół Naukowych i XXXIII Sejmiku SKN, Wrocław, 19-20.05.2016 r.
10.	**Świsłowski P**., Rajfur M., Kłos A.: *Wpływ warunków prowadzenia badań biomonitoringowych z wykorzystaniem mchów na ich wynik.* **Referat** na krajowej konferencji XXI Metrologia-Ekologia-Dydaktyka (MED'16), Bělá pod Pradědem (CZ), 26-28.05.2016 r.
11.	**Świsłowski P**., Zielińska M., Rajfur M., Kłos A.: *Heavy metal contamination of mosses and soil in forests of the south and north-eastern Poland*. **Referat** na międzynarodowej konferencji 15th Workshop on Progress in Trace Metal Speciation for Environmental Analytical Chemistry, Gdańsk, 04-07.09.2016 r.
12.	**Świsłowski P**., Rajfur M., Kłos A.: *The influence of conditions of the biomonitoring study using mosses on its results*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'16, Zakopane, 05-08.10.2016 r. b) **Świsłowski P**., Marciniak M., Rajfur M.: *Sorption of copper and zinc by Hottonia palustris L.*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'16, Zakopane, 05-08.10.2016 r.
13.	**Świsłowski P**., Rajfur M., Kłos A.: *Biomonitoring aktywny zbiornika wodnego Janów (woj. świętokrzyskie) z wykorzystaniem glonów morskich Palmaria palmata*. **Poster** na międzynarodowej konferencji IV międzynarodowej konferencji naukowo-technicznej „Pure water. Fundamental, applied and industrial aspects", Kijów [UA], 26-28.10.2016 r. b) **Świsłowski P**., Marciniak M., Rajfur M.: *Ocena właściwości sorpcyjnych Hottonia palustris L*. **Poster** na międzynarodowej konferencji IV międzynarodowej konferencji naukowo-technicznej „Pure water. Fundamental, applied and industrial aspects", Kijów [UA], 26-28.10.2016 r.
14.	**Świsłowski P**., Rajfur M.: *Biomonitoring aktywny z określeniem warunków prowadzenia eksperymentu z zastosowaniem mchów*. **Referat** na krajowej konferencji IV Ogólnopolskiej Konferencji Młodych Naukowców „Przyroda - Las - Technologia", Poznań, 16-17.02.2017 r.

15.	**Świsłowski P**., Rajfur M., Rodziewicz T.: *Analiza i ocena pracy nowego systemu fotowoltaicznego w Uniwersytecie Opolskim*. **Referat** na międzynarodowej konferencji XXII Międzynarodowej Konferencji Studenckich Kół Naukowych i XXXIV Sejmiku SKN, Wrocław, 25-26.05.2017 r.
16.	**Świsłowski P**., Rajfur M.: *Mushroom as biomonitors of heavy metals contamination in forest areas*. **Poster** na międzynarodowej konferencji VIII International Scientific Conference „Toxic substances in the environment", Kraków, 14-15.09.2017 r.
17.	**Świsłowski P**., Rajfur M., Rodziewicz T.: *Photovoltacis in the Independent Chair of Biotechnology and Molecular Biology*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'17, Polanica Zdrój, 04-07.10.2017 r.
18.	**Świsłowski P**., Rajfur M.: *Analiza i ocena dwóch lat pracy systemu fotowoltaicznego zamontowanego na dachu budynku Uniwersytetu Opolskiego*. **Referat** na krajowej konferencji III Ogólnopolskiej konferencji naukowej „Odnawialne źródła energii - teoria i praktyka" w ramach IX Festiwalu Ekoenergetyki, Opole, 22-23.11.2017 r.
19.	**Świsłowski P**., Rajfur M.: *Biomonitoring pasywny z wykorzystaniem grzybów jadalnych*. **Referat** na krajowej konferencji V Ogólnopolskiej Konferencji Młodych Naukowców „Przyroda - Las - Technologia", Poznań, 02.03.2018 r.
20.	**Świsłowski P**., Rajfur M.: *Biomonitoring terenów leśnych z wykorzystaniem grzybów jadalnych.* **Referat** na krajowej konferencji XXIII Metrologia-Ekologia-Dydaktyka (MED'18), Zlate Hory [CZ], 31.05-02.06.2018 r.
21.	a) **Świsłowski P**., Rajfur M.: *Validation of mosses sample preparation methodology for active biomonitoring*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'18, Polanica Zdrój, 10-13.10.2018 r. b) Rajfur M., **Świsłowski P**.: *Betula pendula bark as a biomonitor of atmospheric aerosol contamination with heavy metals*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'18, Polanica Zdrój, 10-13.10.2018 r. c) **Świsłowski P**., Rajfur M., Rodziewicz T., Ząbkowska-Wacławek M.: *Photovoltaics in the Institute of Biotechnology at University of Opole*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'18, Polanica Zdrój, 10-13.10.2018 r.
22.	**Świsłowski P**., Rajfur M.: *Influence of mosses sample preparation methodology on the coefficient of variation (CV)*. **Referat** na międzynarodowej konferencji 9th International Conference on „Climate Change and Environmental (Bio) Engineering", Budapeszt [HU], 22-24.11.2018 r.
23.	**Świsłowski P**., Rajfur M.: *Biomonitoring terenów leśnych z wykorzystaniem kory brzozy brodawkowatej*. **Referat** na krajowej konferencji XI Interdyscyplinarnej Konferencji Naukowej TYGIEL 2019 „Interdyscyplinarność kluczem do rozwoju", Lublin, 23-24.03.2019 r.
24.	**Świsłowski P**., Rajfur M.: *Wpływ metodyki przygotowania próbek mchów w badaniach biomonitoringowych*. **Referat** na krajowej konferencji VI Ogólnopolskiej Konferencji Młodych Naukowców „Przyroda - Las - Technologia", Poznań, 05.04.2019 r.
25.	**Świsłowski P**., Rajfur M.: *Grzyby jadalne w biomonitoringu pasywnym terenów leśnych*. **Referat** na krajowej konferencji MycoRise Up! Młodzi w Mykologii, Spała, 12-13.04.2019 r.
26.	Rajfur M., **Świsłowski P**.: *Fitokumulacja metali ciężkich w ziołach*. **Referat** na krajowej konferencji III Konferencji Doktorantów Nauk Przyrodniczych, Gdańsk, 25-28.06.2019 r.
27.	**Świsłowski P**., Rajfur M.: *Zanieczyszczenie kory drzew liściastych metalami ciężkimi*. **Referat** na krajowej konferencji Ogólnokrajowej Konferencji Naukowej „Młody Naukowiec Część VII - Nauki interdyscyplinarne, Kraków (on-line), 12-13.09.2019 r.

opublikowane 23 streszczenia z w/w wystąpień

IV) Kierowanie międzynarodowymi i krajowymi projektami badawczymi oraz udział w takich projektach (4):

1.	**Wykonawca** (2014-2016) grantu finansowanego ze środków Narodowego Centrum Badań i Rozwoju w ramach Programu Badawczego Funduszy Norweskich pt.: *Ecosystem stress from the combined effects of winter climate change and air pollution – how do the impacts differ between biomes?*, Pol-Nor/198571/83/2013.
2.	**Kierownik projektu, główny wykonawca, wykonawca** (czerwiec-październik 2015) samodzielne badania w ramach działalności Koła Naukowego Biotechnologów, na mocy Decyzji Rady Wykonawczej Forum Studenckiego Ruchu Naukowego nr 6/3/2015 z dnia

	11 maja 2015 r. o przyznaniu środków finansowych; badania pt.: *Badania zanieczyszczenia powietrza, gleby oraz wody wokół wybranych użytkowych zbiorników wodnych*.
3.	**Wykonawca** (2018-2020) projektu INTERREG VA, CZ.11.3.119/0.0/0.0/16_022/0001150, pt.: *Współpraca UO i UHK zwiększająca możliwości absolwentów na transgranicznym rynku pracy*.
4.	**Wykonawca** [asystent] (2019-2022) w grancie finansowanym ze środków Naukowego Centrum Badań i Rozwoju, pt.: *Innowacyjna metoda poprawy jakości wody w wielofunkcyjnych zbiornikach retencyjnych (ZBIORTUR)*, umowa nr BIOSTRATEG3/343733/15/NCBR/2018.

V) Międzynarodowe i krajowe nagrody za działalność naukową (10):

1.	(2014-2018) **stypendium** Rektora Uniwersytetu Opolskiego dla najlepszych studentów za wysokie wyniki w nauce.
2.	2015, 2017 **stypendium** Marszałka Województwa Opolskiego.
3.	2015, 2017 **stypendium** Ministra Nauki i Szkolnictwa Wyższego za wybitne osiągnięcia.
4.	19-20.05.2016 r., **I miejsce w sesji posterowej** Sekcji Biologii i Hodowli Zwierząt na XXI Międzynarodowej Konferencji Studenckich Kół Naukowych we Wrocławiu na Uniwersytecie Przyrodniczym za poster: „*Zastosowanie bioty w monitoringu zbiornika wodnego Janów (woj. świętokrzyskie)*".
5.	25-26.05.2017 r., **I miejsce w sekcji referatowej** na XXII Międzynarodowej Konferencji Studenckich Kół Naukowych i XXXIV Sejmiku SKN we Wrocławiu na Uniwersytecie Przyrodniczym za referat: *Analiza i ocena pracy nowego systemu fotowoltaicznego w Uniwersytecie Opolskim*.
6.	styczeń 2018 r. **wyróżnienie za dyplom inżyniera**.
7.	2018 **stypendium** Prezydenta Miasta Opola dla studentów.
8.	10-13.10.2018 r., **wyróżnienie** na Central European Conference ECOpole'18 w Polanicy Zdrój; Forum Młodych Naukowców za poster: *Betula pendula bark as a biomonitor of atmospheric aerosol contamination with heavy metals*.
9.	2018/2019 **stypendia doktoranckie**: dla najlepszych doktorantów, doktoranckie, doktoranckie z dotacji podmiotowej na dofinansowanie zadań projakościowych.
10.	2018/2019 **stypendium** doktoranckie Prezydenta Miasta Opola.

VI) Działalność organizacyjna (14):

1.	od 2014 roku **członek** Koła Naukowego Biotechnologów.
2.	**pokaz naukowy** przygotowany dla dzieci z Przedszkola Publicznego nr 6 w Opolu, Opole, 23.06.2014 r.
3.	od października 2014 **organizacja** międzynarodowej konferencji ECOpole
4.	**warsztaty naukowe** KN Biotechnologów zorganizowane dla Zespołu Szkół w Gogolinie, Gogolin, 20.03.2015 r.
5.	**pokaz doświadczeń biotechnologicznych** zaprezentowanych podczas Festynu Rodzinnego w PSP nr 5 w Opolu, Opole 11.06.2016 r.
6.	**pokaz doświadczeń biotechnologicznych i chemicznych** zaprezentowanych w Zespole Szkół Sportowych nr 1 w Krapkowicach, Krapkowice, 22.05.2017 r.
7.	**pokaz doświadczeń biotechnologicznych** zaprezentowanych podczas Festynu Rodzinnego w PSP nr 5 w Opolu, Opole, 10.06.2017 r.
8.	**prace nad wizualizacją wniosku projektowego** Centrum Strategii i Bezpieczeństwa Energetycznego UO- projekt Dąbrowa, 23.06.2017 r.
9.	**pokaz doświadczeń chemicznych** zaprezentowanych dla uczniów Publicznej Sportowej Szkoły Podstawowej nr 5 w Krapkowicach, Krapkowice, 27.10.2017 r.
10.	**pokaz chemiczny** dla uczniów klasy IVa z Publicznej Szkoły Podstawowej nr 1 w Brzegu, Opole, 11.04.2018 r.
11.	**pokaz doświadczeń biotechnologicznych i chemicznych** dla Zespołu Szkół Budowlanych im. Papieża Jana Pawła II w Opolu, Opole, 13.12.2018 r.
12.	**wykłady oraz warsztaty edukacyjne** dla uczniów z Publicznej Szkoły Podstawowej nr 1 w Brzegu, Opole, 26.03.2019 r.
13.	**prezentacja osiągnięć naukowo-dydaktycznych** Instytutu Biotechnologii na Opolskim Pikniku Naukowym w ramach XVII Opolskiego Festiwalu Nauki, Opole, 02.06.2019 r.

14.	**pokazy chemiczne** w Dniu Otwartym Nauk Ścisłych, Przyrodniczych i Medycznych na zajęciach pt.: *Kosmetyki od kuchni* w ramach XVII Opolskiego Festiwalu Nauki, Opole, 05.06.2019 r.

VII) Szkolenia i staże (8):

1.	**szkolenie** poświęcone obsłudze inwertera układu instalacji fotowoltaicznej oraz automatyki zabezpieczająco – sterującej dla układu instalacji fotowoltaicznej, Opole, 10.06.2015 r.
2.	**szkolenie** pt.: *Ochrona środowiska przed hałasem* zorganizowane przez firmę ProSilence, Opole, 23.01.2016 r.
3.	**seminarium** pt.: *Trendy analityczne i nowe rozwiązania w analizach wielopierwiastkowych i przygotowaniu próbek* poświęcone technikom ASA, ICP, ICP-MS i przygotowaniu próbek zorganizowane przez firmę Spectro-Lab, Wrocław, 31.05.2016 r.
4.	**staż naukowy** w firmie Novavis Spółka Akcyjna, Warszawa, sierpień 2017 r.
5.	**szkolenie** pt.: *Zapewnienie jakości wyników badań w akredytowanym laboratorium badawczym na podstawie normy ISO 17025* zorganizowane przez firmę qualLab, Opole, 06.04.2018 r.
6.	**szkolenie** z zakresu budowy i obsługi aparatury badawczej: mineralizatora mikrofalowego, absorpcyjnego spektrometru atomowego ASA, analizatora rtęci AMA, Opole, 14.08.2018 r.
7.	**staż naukowy**, Hradec Kralove [CZ], 02-28.09.2018 r.
8.	**szkolenie** z obsługi programu AutoCAD, Opole, 20.01.2019 r.

WYKAZ DOROBKU NAUKOWEGO, PO WSTĄPIENIU DO SZKOŁY DOKTORSKIEJ UNIWERSYTETU OPOLSKIEGO (od roku akademickiego 2019/2020; w tym po ocenie Komisji Śródokresowej 09.07.2021 r.)

Przebieg kariery naukowej:

- 2019/2020: Szkoła Doktorska Uniwersytetu Opolskiego (nauki biologiczne, Instytut Biologii).

Zbiór opublikowanych i powiązanych tematycznie artykułów naukowych zgodnie z art. 187 ust. 1 z dnia 20 lipca 2018 r. Ustawy Prawo o szkolnictwie wyższym i nauce jako rozprawa doktorska (9)

L.p.	Wyszczególnienie	Punkty	*IF*
1.	**Świsłowski P**., Nowak A., Wacławek S., Silvestri D., Rajfur M.: *Bioaccumulation of Trace Elements from Aqueous Solutions by Selected Terrestrial Moss Species*. Biology 2022;11(12):1692. DOI: 10.3390/biology11121692.	100	5,168
2.	**Świsłowski P**., Kosior G., Rajfur M.: *The influence of preparation methodology on the concentration of heavy metals in Pleurozium schreberi moss samples prior to use in active biomonitoring studies.* Environ. Sci. Pollut. Res 2021;28(8):10068-10076. DOI: 10.1007/s11356-020-11484-7.	100	3,056
3.	**Świsłowski P**., Śmiechowicz B., Rajfur M.: *Effects of tobacco smoke on indoor air quality: the use of mosses in biomonitoring*. J. Environ. Health Sci. 2022. DOI: 10.1007/s40201-022-00794-2.	100	2,130
4.	**Świsłowski P**., Nowak A., Rajfur M.*: Is Your Moss Alive during Active Biomonitoring Study?* Plants 2021;10(11):2389. DOI: 10.3390/plants10112389.	70	3,935
5.	**Świsłowski P**., Nowak A., Rajfur M.*: The influence of environmental conditions on the lifespan of mosses under long-term active biomonitoring*. Atmos. Pollut. Res 2021;12(10):101203. DOI: 10.1016/j.apr.2021.101203.	70	4,352
6.	**Świsłowski P**., Nowak A., Rajfur M.*: Comparison of exposure techniques and vitality assessment in active biomonitoring for suitability in assessing atmospheric aerosol heavy metal pollution*. Environ. Toxicol. Chem. 2022;41(6):1429-1438. DOI: 10.1002/etc.5321.	100	3,742
7.	**Świsłowski P**., Ziembik Z., Rajfur M.: *Air Quality during New Year's Eve: A Biomonitoring Study with Moss*. Atmosphere 2021;12(8):975. DOI: 10.3390/atmos12080975.	70	2,686
8.	**Świsłowski P**., Vergel K., Zinicovscaia I., Rajfur M., Wacławek M.: *Mosses as a biomonitor to identify elements released into the air as a result of car workshop activities*. Ecol. Indic 2022;138:108849. DOI: 10.1016/j.ecolind.2022.108849	140	4,958
9.	**Świsłowski P**., Nowak A., Wacławek S., Ziembik Z., Rajfur M.: *Is active moss biomonitoring comparable to air filter standard sampling?* Int. J. Environ. Res. Public Health 2022;19(8):4706. DOI: 10.3390/ijerph19084706	140	3,390

II) Publikacje opublikowane w czasopismach o zasięgu krajowym i międzynarodowym (13):

L.p.	Wyszczególnienie	Punkty	*IF*
1.	Konopka Z., **Świsłowski P**., Rajfur M.: *Biomonitoring of atmospheric aerosol with the use of Apis mellifera and Pleurozium schreberi*. Chemistry-Didactics-Ecology-Metrology 2019;24(1-2):107-116. DOI: 10.2478/cdem-2019-0009.	20	-
2.	**Świsłowski P**., Banach E., Rajfur M.: *Passive biomonitoring of influence of the communication traffic on deposition the pollution near the motorway*. Ecol. Chem. Eng. A 2019;26(1-2):113-125. DOI: 10.2428/ecea.2019.26(1-2)14.	20	-
3.	**Świsłowski P**., Dołhańczuk-Śródka A., Rajfur M.: *Bibliometric analysis of European publications between 2001 and 2016 on concentrations of selected elements in mushrooms.* Environ. Sci. Pollut. Res 2020;27:22235-22250. DOI: 10.1007/s11356-020-08693-5.	100	3,056
4.	**Świsłowski P**., Kříž J., Rajfur M.: *The use of bark in biomonitoring heavy metal pollution of forest areas on the example of selected areas in Poland*. Ecol. Chem. Eng. S. 2020;27(2):1-16. DOI: 10.2478/eces-2020-0013.	40	1,488
5.	**Świsłowski P**., Rajfur M., Wacławek M.: *Influence of heavy metal concentration on chlorophyll content in Pleurozium schreberi mosses.* Ecol. Chem. Eng. S 2020;27(4):591-601. DOI: 10.2478/eces-2020-0037.	40	1,488

6.	Rodziewicz T., Rajfur M., Teneta J., **Świsłowski P**., Wacławek M.: *Modelling and analysis of the influence of solar spectrum on the efficiency of photovoltaic modules.* Energy Rep. 2021;7:565–574. DOI: 10.1016/j.egyr.2021.01.013.	100	3,595
7.	Słonina N., **Świsłowski P**., Rajfur M.: *Passive and active biomonitoring of atmospheric aerosol with the use of mosses*. Ecol. Chem. Eng. S 2021;28(2):161-170. DOI: 10.2478/eces-2021-0012.	40	1,488
8.	Stojanowska A., Mach T., Olszowski T., Bihałowicz J., Górka M., Rybak J., Rajfur M., **Świsłowski P**.: *Air Pollution Research Based on Spider Web and Parallel Continuous Particulate Monitoring—A Comparison Study Coupled with Identification of Sources*. Minerals 2021;11(8):812. DOI: 10.3390/min11080812.	100	2,644
9.	**Świsłowski P**., Hrabák P., Wacławek S., Liskova K., Antos V., Rajfur M., Ząbkowska-Wacławek M.: *The Application of Active Biomonitoring with the Use of Mosses to Identify Polycyclic Aromatic Hydrocarbons in an Atmospheric Aerosol*. Molecules 2021;26(23):7258.	140	4,412
10.	Michalak A., **Świsłowski P**., Rajfur M.: *The Assessment of Heavy Metal Contamination of the Cultivated Soils in the Odra River Floodplain*. CDEM 2021;26(1-2):55-64. DOI: 10.2478/cdem-2021-0004.	20	-
11.	Budzyńska-Lipka W., **Świsłowski P**., Rajfur M.: *Biological Monitoring Using Lichens as a Source of Information About Contamination of Mountain with Heavy Metals*. Ecol. Chem. Eng. S 2022;29(2):155-168. DOI: 10.2478/eces-2022-0012.	40	1,663
12.	Respondek Z., Jerz D., **Świsłowski P**., Rajfur M.: *Active Biomonitoring of Heavy Metal Concentrations in Aquatic Environment Using Mosses and Algae*. Water 2022;14(20):3335. DOI: 10.3390/w14203335.	100	3,530
13.	Wacławek M., **Świsłowski P**., Rajfur M.: *The biological monitoring as a source of information on environmental pollution with heavy metals*. CDEM 2022;27(1-2):53-78. DOI: 10.2478/cdem-2022-0006.	20	-

Liczba cytowań publikacji według baz Web of Science (WoS) i Scopus:
Liczba cytowań (WoS) = 176; Liczba cytowań z wyłączeniem autocytowań (WoS) = 110 (stan na 18.03.2023 r..).

Liczba cytowań (Scopus) = 173; Liczba cytowań z wyłączeniem autocytowań (Scopus) = 119 (stan na 18.03.2023 r.).

Indeks Hirscha według baz Web of Science (WoS) i Scopus:
h-index (WoS) = 8; *h*-index (Scopus) = 7 (stan na 18.03.2023 r.).

III) Wygłoszenie referatów (18) i posterów (19) na międzynarodowych (12) i krajowych (13) konferencjach naukowych:

1.	a) **Świsłowski P**., Kłos A., Rajfur M., Dołhańczuk-Śródka A., Ziembik Z., Wierzba S., Piechaczek-Wereszczyńska M., Jerz D., Wiatkowski M.: *Pollution of the Large Turawa Lake*. **Referat** na międzynarodowej konferencji Central European Conference ECOpole'19, Polanica Zdrój, 09-12.10.2019 r. b) Rajfur M., Konopka Z., **Świsłowski P**.: *Atmospheric aerosol pollution on the heavy metal content in honey bees and honey*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'19, Polanica Zdrój, 09-12.10.2019 r. c) Rodziewicz T., Rajfur M., **Świsłowski P**., Zaremba A., Wacławek M.: *Air mass – methods of determination and properties*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'19, Polanica Zdrój, 09-12.10.2019 r. d) Rodziewicz T., Rajfur M., **Świsłowski P**., Zaremba A., Wacławek M.: *Estimation of spectral incompatibility error for PV measurements in outdoor conditions*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'19, Polanica Zdrój, 09-12.10.2019 r.
2.	**Świsłowski P**., Rajfur M.: *Algae – a source of information on contamination of surface waters with heavy metals*. **Poster** na międzynarodowej konferencji VI International

	Scientific and Technical Conference: „Pure water. Fundamental, applied and industrial aspects", Kijów (on-line) [UA], 14-15.11.2019 r.
3.	**Świsłowski P.**, Rajfur M.: *Ocena zanieczyszczenia aerozolu atmosferycznego terenów miejskich z wykorzystaniem wybranych biomonitorów*. **Referat** na krajowej konferencji Ogólnokrajowej Konferencji Naukowej „Młody Naukowiec Część VIII - Nauki interdyscyplinarne", Kraków (on-line), 25-27.11.2019 r.
4.	**Świsłowski P.**, Rajfur M.: *Rośliny zielarskie jako przykłady metalofitów*. **Referat** na krajowej konferencji Ogólnopolskiej Przyrodniczej Konferencji Naukowej „Mater Naturae" – osiągnięcia, wyzwania i problemy nauk przyrodniczych, Lublin, 14.12.2019 r.
5.	**Świsłowski P.**, Rajfur M.: *Biomonitoring w ocenie zanieczyszczenia środowiska metalami ciężkimi*. **Referat** na krajowej konferencji III edycja Wirtualnej Konferencji Młodych Przyrodników – Zima 2019, on-line, 30.12.2019-05.01.2020 r.
6.	**Świsłowski P.**, Rajfur M.: *Chemizm mchów w metodologicznych badaniach biomonitoringu aktywnego*. **Referat** na krajowej konferencji II Seminarium Młodych Naukowców pt. „Nowoczesne Trendy i Wyzwania w Inżynierii Chemicznej", Poznań, 13-14.01.2020 r.
7.	**Świsłowski P.**, Rajfur M.: *Zanieczyszczenie aerozolu atmosferycznego metalami ciężkimi pochodzącymi z dymu wystrzeliwanych fajerwerków – biomonitoring aktywny z wykorzystaniem mchów*. **Referat** na krajowej konferencji Wirtualna Konferencja Młodych Przyrodników – Wiosna 2020, on-line, 30.03-05.04.2020 r.
8.	**Świsłowski P.**, Rajfur M.: *Wszystko poszło z dymem… czyli jak bawimy się w Sylwestra – biomonitoring aktywny z wykorzystaniem mchów*. **Referat** na krajowej konferencji Ogólnokrajowa Konferencja Interdyscyplinarna „OMNIBUS cz. II", on-line, 03-04.04.2020 r.
9.	**Świsłowski P.**, Śmiechowicz B., Rajfur M.: *Wykorzystanie mchów do oceny zanieczyszczenia powietrza metalami ciężkimi uwalnianymi podczas palenia papierosów*. **Referat** na krajowej konferencji II Ogólnopolska konferencja naukowa: „Ochrona środowiska – rozwiązania i perspektywy", Lublin (on-line), 04.09.2020 r.
10.	**Świsłowski P.**, Rajfur M.: *Bark as a biomonitor of forest contamination in north-eastern and southern Poland*. **Referat** na międzynarodowej konferencji IV międzynarodowa konferencja Natural resources of border areas under a changing climate, Słupsk (on-line), 24-25.09.2020 r.
11.	a) **Świsłowski P.**, Nowak A., Rajfur M.: *Moss as bioindicator of environmental quality – measurement of chlorophyll content*. **Referat** na międzynarodowej konferencji Central European Conference ECOpole'20, Tomaszowice (koła Krakowa), 07-11.10.2020 r. b) **Świsłowski P.**, Śmiechowicz B., Rajfur M.: *Bryomonitoring of air pollution with elements from cigarette smoke*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'20, Tomaszowice (koła Krakowa), 07-11.10.2020 r. c) Rodziewicz T., Rajfur M., Teneta J., **Świsłowski P.**, Zaremba A., Wacławek M. *Application of SOLARSPECTRUM to modeling and analysis of the impact of solar spectrum on the efficiency of solar modules*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'20, Tomaszowice (koła Krakowa), 07-11.10.2020 r. d) Rodziewicz T., Rajfur M., Teneta J., **Świsłowski P.**, Wacławek M.: *SOLARSPECTRUM – description and validation of the solar radiation spectrum synthesis model*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'20, Tomaszowice (koła Krakowa), 07-11.10.2020 r. e) Štěpánek V., **Świsłowski P.**, Kříž J., Dołhańczuk-Śródka A., Hubálovský Š., Rajfur M., Král P., Radocha K., Vojta M., Wierzba S., Janecki D., Hyšplerová L., Wahlig W., Studnička F., Eminger S., Hyšpler R., Němec R., Halamek M., Ziembik Z., Kłos A., Wacławek M., Lyčka A.: *Mathematical modeling, measurement and biomonitoring of environmental microparticle pollutions; CZ-PL cross-border universities education and curricular practice*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole'20, Tomaszowice (koła Krakowa), 07-11.10.2020 r.
12.	**Świsłowski P.**, Rajfur M.: *Bryomonitoring w ocenie zanieczyszczenia aerozolu atmosferycznego pierwiastkami zawartymi w dymie papierosowym*. **Referat** na krajowej konferencji II Ogólnopolska Konferencja Naukowa „Nauki przyrodnicze na rzecz człowieka i środowiska", on-line, 27.10.2020 r.
13.	**Świsłowski P.**, Rajfur M.: *Pomiar zawartości chlorofilu w mchach wykorzystywanych w badaniach biomonitoringowych*. **Referat** na krajowej konferencji Ogólnopolska

	Konferencja Naukowa „Perspektywy wykorzystania roślin w nauce i przemyśle”, on-line, 26.11.2020 r.
14.	**Świsłowski P**., Rajfur M.: *Możliwość prowadzenia długoterminowego biomonitoringu aktywnego jakości aerozolu atmosferycznego z wykorzystaniem mchu Pleurozium schreberi*. **Referat** na krajowej konferencji Ogólnopolskiej Konferencji Młodych Naukowców: „Biologia, Chemia i Środowisko - Spojrzenie Młodych Naukowców - Edycja II”, Kraków (on-line), 24-25.04.2021 r.
15.	**Świsłowski P**., Rajfur M.: *Porównanie sposobów ekspozycji mchów w biomonitoringu aktywnym do oceny skażenia powietrza pierwiastkami śladowymi*. **Referat** na krajowej konferencji III Ogólnopolskiej Konferencji „Środowisko przyrodnicze jako obszar badań”, Poznań (on-line), 27.05.2021 r.
16.	**Świslowski P**., Rajfur M.: *Porównanie dwóch metod ekspozycji mchów w biologicznym monitoringu jakości aerozolu atmosferycznego*. **Poster** na krajowej konferencji Ogólnopolskiej Konferencji Młodych Naukowców za pośrednictwem Internetu nt. Dokonania naukowe doktorantów, edycja IX Wielka Sesja Posterowa, Kraków (on-line), 26.06.2021 r.
17.	a) **Świsłowski P**., Rajfur M., Wacławek M.: *Influence of heavy metal concentration on chlorophyll content in Pleurozium schreberi mosses*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole’21, Tomaszowice (koła Krakowa), 13-16.10.2021 r. b) Michalak A., **Świsłowski P**., Rajfur M.: *Assessment of heavy metal contamination of the cultivated soils of the Odra river flood*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole’21, Tomaszowice (koła Krakowa), 13-16.10.2021 r. c) Słonina N., **Świsłowski P**., Rajfur M.: *Passive and active biomonitoring of atmospheric aerosol with the use of mosses*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole’21, Tomaszowice (koła Krakowa), 13-16.10.2021 r. d) Rodziewicz T., Rajfur M., **Świsłowski P**., Zaremba A., Wacławek M.: *Effect of spectral irradiance distribution on performance of various modules PV. Simulation results with experiment*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole’21, Tomaszowice (koła Krakowa), 13-16.10.2021 r. e) Rodziewicz T., Rajfur M., **Świsłowski P**., Zaremba A., Wacławek M.: *Use of SPECTRAL2 procedures to study the influence of the solar spectrum distribution on the properties of solar modules*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole’21, Tomaszowice (koła Krakowa), 13-16.10.2021 r.
18.	Wacławek M., **Świsłowski P**., Rajfur M.: *The biological monitoring as a source of information on environmental pollution with heavy metals*. **Referat** na międzynarodowej konferencji 7th International Conference on Water Resource and Environment (WRE 2021), on-line, 04.11.2021 r.
19.	Michalak A., **Świsłowski P**., Rajfur M.: *Ocena zanieczyszczenia metalami ciężkimi gleb uprawnych terenów zalewowych rzeki Odry*. **Poster** na międzynarodowej konferencji VII International Scientific and Technical Conference: „Pure water. Fundamental, applied and industrial aspects”, Kijów (on-line) [UA], 25-26.11.2021 r.
20.	**Świsłowski P**., Rajfur M., Ziembik Z., Wacławek M.: *Mosses as a biomonitor of air pollution with heavy metals from point source of pollution*. **Poster** na 35th Task Force Meeting of the ICP Vegetation, on-line, 21-23.02.2022 r.
21.	Wacławek M, Świsłowski P, Rajfur M.: *Biomonitoring of environmental pollution by heavy metals*. **Poster** na międzynarodowej konferencji XI International Scientific-Technical Conference “Advance in Petroleum and Gas Industry and Petrochemistry” (APGIP-11), Lwów (on-line) [UA], 16-20.05.2022 r.
22.	**Świsłowski P**., Nowak A., Rajfur M., Wacławek M.: *Application of moss-bag technique in daily life*. **Referat** na 9th International Workshop on Biomonitoring of Atmospheric Pollution (BIOMAP 9), Neapol on-line [IT], 03-05.10.2022 r.
23.	a) **Świsłowski P**., Nowak A., Rajfur M., Wacławek M.: *The moss bag technique in everyday life*. **Referat** na międzynarodowej konferencji Central European Conference ECOpole’22, Tomaszowice (koła Krakowa), 19-22.10.2022 r. b) Respondek Z., Jerz D., **Świsłowski P**., Rajfur M.: *Active biomonitoring of heavy metal concentrations in aquatic environment using bioindicators*. **Poster** na międzynarodowej konferencji Central European Conference ECOpole’22, Tomaszowice (koła Krakowa), 19-22.10.2022 r.

24.	**Świsłowski P**., Rajfur M.: *Możliwość prowadzenia długoterminowego biomonitoringu aktywnego jakości aerozolu atmosferycznego z wykorzystaniem mchu Pleurozium schreberi*. **Referat** na krajowej konferencji Analiza Zagadnienia, Analiza Wyników – Wystąpienie Młodego Naukowca Edycja V, on-line, 28-29.01.2023 r.
25.	**Świsłowski P**., Nowak A., Wacławek S., Silvestri D., Rajfur M.: *Bioaccumulation of trace elements from aqueous solutions by selected terrestrial moss species*. **Poster** na 36th Task Force Meeting of the ICP Vegetation, on-line, 13-15.02.2023 r.

opublikowane 23 streszczeń z w/w wystąpień

IV) Kierowanie międzynarodowymi i krajowymi projektami badawczymi oraz udział w takich projektach (3):

1.	**Wykonawca** (2018-2020) projektu INTERREG VA, CZ.11.3.119/0.0/0.0/16_022/0001150, pt.: *Współpraca UO i UHK zwiększająca możliwości absolwentów na transgranicznym rynku pracy*.
2.	**Wykonawca** [asystent] (2019-2022) w grancie finansowanym ze środków Naukowego Centrum Badań i Rozwoju, pt.: *Innowacyjna metoda poprawy jakości wody w wielofunkcyjnych zbiornikach retencyjnych (ZBIORTUR)*, umowa nr BIOSTRATEG3/343733/15/NCBR/2018.
3.	**Wykonawca** (2020-2021) w grancie finansowanym przez JINR (Frank Laboratory of Neutron Physics of JINR, Dubna, Rosja), 03-4-1104-2019/2021, pt.: *Air pollution study in Poland using nuclear and related analytical techniques* № 75/32/2020, umowa nr 75 z dnia 03.02.2020 r.

V) Międzynarodowe i krajowe nagrody za działalność naukową (10):

1.	09-12.10.2019 r., **wyróżnienie** na Central European Conference ECOpole'19 w Polanicy Zdrój; Forum Młodych Naukowców za referat: *Pollution of the Large Turawa Lake*.
2.	2019/2020 i 2020/2021 **stypendia doktoranckie**: dla najlepszych doktorantów, doktoranckie, doktoranckie z dotacji podmiotowej na dofinansowanie zadań projakościowych.
3.	2019/2020 **Nagroda Rektora** dla uczestników studiów doktoranckich i słuchaczy szkoły doktorskiej za działalność na rzecz Uniwersytetu Opolskiego oraz środowiska doktoranckiego
4.	07-11.10.2020 r., **wyróżnienie** na Central European Conference ECOpole'20 w Tomaszowicach (koło Krakowa); Forum Młodych Naukowców za referat: *Moss as bioindicator of environmental quality – measurement of chlorophyll content*.
5.	24-25.04.2021 r. **wyróżnienie** na krajowej konferencji Ogólnopolskiej Konferencji Młodych Naukowców: „Biologia, Chemia i Środowisko - Spojrzenie Młodych Naukowców - Edycja II", Kraków (on-line) za referat: *Możliwość prowadzenia długoterminowego biomonitoringu aktywnego jakości aerozolu atmosferycznego z wykorzystaniem mchu Pleurozium schreberi*.
6.	2020/2021 **Nagroda Rektora** dla uczestników studiów doktoranckich i słuchaczy szkoły doktorskiej za działalność na rzecz Uniwersytetu Opolskiego oraz środowiska doktoranckiego.
7.	13-16.10.2021 r., **wyróżnienie** na Central European Conference ECOpole'21 w Tomaszowicach (koło Krakowa); Forum Młodych Naukowców za poster: *Influence of heavy metal concentration on chlorophyll content in Pleurozium schreberi mosses*.
8.	2021/2022 **Nagroda Rektora** dla uczestników studiów doktoranckich i słuchaczy szkoły doktorskiej za działalność na rzecz Uniwersytetu Opolskiego oraz środowiska doktoranckiego.
9.	04.08.2022 r. **stypendium** Doktoranckie Prezydenta Miasta Opola
10.	19-22.10.2022 r., **wyróżnienie** na Central European Conference ECOpole'22 w Tomaszowicach (koło Krakowa) za referat: *The moss bag technique in everyday life*

VI) Działalność organizacyjna (20):

1.	od października 2019 roku **członek** Komitetu Organizacyjnego międzynarodowej konferencji ECOpole.
2.	od grudnia 2019 roku **członek** Rady Programowej Szkoły Doktorskiej Uniwersytetu Opolskiego
3.	**pokaz doświadczeń** pt.: *Nauka wokół nas* w ramach Nocy Biologów, Opole, 10.01.2020 r.
4.	od 14.02.2020 r. **przewodniczący** Samorządu Doktorantów Uniwersytetu Opolskiego (2020-2022).
5.	od 17.02.2020 r. **elektor** Uczelnianego Kolegium Elektorów (2020-2024).
6.	od października 2021 r. (nadanie 20.12.2021 r.) **współopiekun** Koła Naukowego Biotechnologów, Sekcji Biomonitoringu.
7.	**przewodniczący** Komisji Stypendialnej dla Doktorantów na rok akademicki 2020/2021 oraz 2021/2022
8.	**pokaz doświadczeń** pt.: *Nauka wokół nas vol. 2* w ramach XVIII Opolskiego Festiwalu Nauki, Opole, 03.11.2020 r.
9.	**prezentacja** pt.: *Monitoring biologiczny zanieczyszczenia powietrza analitami pochodzącymi z dymu tytoniowego* w ramach Dnia Otwartego dyscypliny Inżynieria środowiska, górnictwo i energetyka w ramach XVIII Opolskiego Festiwalu Nauki, Opole, 03.12.2020 r.
10.	od 30.11.2020 r **członek** Uczelnianej Komisji Dyscyplinarnej dla Doktorantów (2020-2024)
11.	**koordynator** wydarzenia Noc Biologów 2021 z ramienia Instytutu Inżynierii Środowiska i Biotechnologii, Opole, 10.12.2020 r.
12.	**pokaz doświadczeń** pt.: *Nauka wokół nas* oraz *Włosy ludzkie jako bioczujnik zanieczyszczenia środowiska rtęcią* w ramach Nocy Biologów, Opole, 08.01.2021 r.
13.	od 2020 roku **sekretarz** czasopisma Chemia-Dydaktyka-Ekologia-Metrologia.
14.	od 27.01.2021 r. **członek** Towarzystwa Chemii i Inżynierii Ekologicznej a od 11.12.2021 r. **członek** Komisji Rewizyjnej tegoż Towarzystwa
15.	**współorganizacja** Światowego Dnia Wody 2021, organizowanego przez Przedsiębiorstwo Wodociągów i Kanalizacji „WiK" w Opolu, prezentacja pt.: *Glony Palmaria palmata jako bioczujnik zanieczyszczenia metalami ciężkimi wód powierzchniowych*, Opole (on-line), 22.03.2021 r.
16.	**referat** pt.: *Jakie zanieczyszczenia wdychamy – mchy prawdę Ci powiedzą* w ramach Szkoły Letniej UO zorganizowanej przez Szkołę Doktorską Uniwersytetu Opolskiego, Opole, 17.09.2021 r.
17.	od 28.10.2021 r. **członek** Polskiego Towarzystwa Inżynierii Ekologicznej – Oddział Opolski.
18.	a) **wykład** pt.: *Biologia w filozofii i projektach klocków LEGO®* w ramach Nocy Biologów, Opole, 14.01.2022 r. b) **wykład** pt.: *Mchy – źródło informacji o zanieczyszczeniu powietrza* w ramach Nocy Biologów, Opole, 14.01.2022 r. c) **pokaz** pt.: *Ocena zanieczyszczenia rtęcią ludzkich włosów* w ramach Nocy Biologów, Opole, 14.01.2022 r.
19.	a) **pokaz** pt.: *Bioróżnorodność mchów i ich zastosowanie w monitorowaniu jakości powietrza* na 19 Opolskim Pikniku Naukowym w ramach XIX Opolskiego Festiwalu Nauki, Opole, 07.05.2022 r. b) **pokaz** pt.: *Mchy prawdę Ci powiedzą jakie zanieczyszczenia wdychamy* na Nocy Nauki w ramach XIX Opolskiego Festiwalu Nauki, Opole, 13.05.2022 r.
20.	a) **pokaz** pt.: *Zanieczyszczenie ludzkich włosów rtęcią- ocena* w ramach Nocy Biologów, Opole, 13.01.2023 r. b) **wykład z pokazem** pt.: *Ocena zanieczyszczenia powietrza z wykorzystaniem mchów* w ramach Nocy Biologów, Opole, 13.01.2023 r.

VII) Szkolenia i staże (23):

1.	**staż naukowy** w firmie ATMOTERM, Opole, 03.02.-02.03.2020 r.
2.	**webinarium** pt.: *Podstawy i możliwości techniki ICP-MS* zorganizowane przez firmę Perlan, on-line, 19.05.2020 r.

3.	**webinarium** pt.: *Podstawy i możliwości techniki ICP-OES* zorganizowane przez firmę Perlan, on-line, 28.05.2020 r.
4.	**webinarium** pt.: *Trace Elemental Analysis Educational Week: Training Course on Sample Preparation and Introduction, Instrument Technology and Troubleshooting Hints and Tips* zorganizowane przez firmę Thermo Scientific Sales Support Team, on-line, 20-24.07.2020 r.
5.	**szkolenie** pt.: *Dydaktyka cyfrowa z MS Teams czyli jak pracować ze studentami w formule online* zorganizowane przez firmę Instytut Innowacyjnej Edukacji sp. z o. o., on-line, 18.09.2020 r.
6.	**webinarium** pt.: *iCAP TQe ICP-MS: For samples where single quadrupole performance isn't enough* zorganizowane przez firmę ThermoFisher Scientific, on-line, 04.11.2020 r.
7.	**webinarium** pt.: *Techniki emisyjne w oznaczeniach pierwiastków* zorganizowane przez firmę Perlan, on-line, 15.12.2020 r.
8.	**webinarium** pt.: *Dobry początek w wyszukiwaniu literatury* zorganizowane przez firmę BrightTALK, on-line, 11.03.2021 r.
9.	**webinarium** pt.: *Twoi znajomi i Ty w bazie Scopus* zorganizowane przez firmę BrightTALK, on-line, 16.03.2021 r.
10.	**warsztaty** pt.: *Bryologia praktyczna*, Bohušov [CZ], 31.05-04.06.2021 r.
11.	**webinarium** pt.: *Badania pozostałości środków ochrony roślin w żywności i środowisku – praktyczne wykorzystanie technik GC-MS/MS i LC-MS/MS* zorganizowane przez firmę Perlan, on-line, 06.07.2021 r.
12.	**staż** (STT) w ramach Programu Erasmus+ w Uniwersytecie Technicznym w Libercu [CZ], 02-06.08.2021 r.
13.	**szkolenie** pt.: *Podstawowa obsługa Zooma* zorganizowanym przez firmę Acnet sp. z o. o. - Referral Partner Zoom, on-line, 17.08.2021 r.
14.	**warsztaty** pt.: *Biologia obszarów nadmorskich* na Półwyspie Helskim w ramach projektu: „Innowacje edukacyjne nakierowane na zwiększenie szans zatrudnienia absolwentów w ekoturystyce"; INTERREG CZ.11.3.119/0.0/0.0/16_022/0001153, 30.08-08.09.2021 r.
15.	**webinarium** pt.: *ICP-OES Educational Webinar* zorganizowane przez firmę ThermoFisher Scientific, on-line, 03.11.2021 r.
16.	**webinarium** pt.: *ICP-MS Educational Webinar* zorganizowane przez firmę ThermoFisher Scientific, on-line, 10.11.2021 r.
17.	**warsztaty** pt.: *Early Career Researchers – Challenges, Support Measures and Strategies within the FORTHEM Alliance* zorganizowanym przez University of Mainz i University of Latvia, FIT FORTHEM, on-line, 16.11.2021 r.
18.	**webinarium** pt.: Triple quadrupole *ICP-MS Educational Webinar* zorganizowane przez firmę ThermoFisher Scientific, on-line, 17.11.2021 r.
19.	**webinarium** pt.: a) *How to choose the best elemental analysis technique for your application* oraz b) *How can you ensure the instrument you choose will meet your lab's specific needs?* zorganizowane przez firmę Agilent Technologies Inc, on-line, 13.03.2022 r.
20.	**szkolenie** pt.: *Podstawy pracy ze spektrometrami mas typu potrójny kwadrupol sprzężonymi z chromatografami gazowymi* zorganizowane przez firmę Perlan, MS Teams (on-line), 06.05.2022 r.
21.	**warsztaty** mykologiczne pt.: *Nieznany świat grzybów* zorganizowane przez Stobrawski Park Krajobrazowego w Ładzy, 07.10.2022 r.
22.	**webinarium** pt.: *Triple quadrupole ICP-MS Educational Webinar* oraz *ICP-MS Educational Webinar* zorganizowane przez firmę ThermoFisher Scientific, on-line, 27.10.2022 r.
23.	**szkolenie** pt.: *Budowanie realistycznych rzeźb z klocków LEGO* zorganizowane przez firmę Domestika (on-line), 01.01.2023 r.

VIII) Recenzje publikacji w czasopismach międzynarodowych (26):

1.	18-19.04.2020 r. recenzja artykułu *The effect of inspiratory muscle training and royal jelly supplementatıon on male reproductive hormones* (AERJ-2020-038) dla czasopisma African Educational Research Journal.
2.	17.08.2020 r. recenzja artykułu *Bibliometric analysis of researches on digital citizenship in web of science database* (AERJ-2020-131) dla czasopisma African Educational Research Journal.

3.	01.09.2020 r. recenzja artykułu *Ecological Risk Assessment of Heavy Metal Contamination in Mangrove Forest Sediment of Gulf of Khambhat Region, West Coast of India* (SNAS-D-20-07528) dla czasopisma SN Applied Sciences.
4.	15.09.2020 r. recenzja artykułu *Modified Cellulose Cigarette Filters Applied for Metals Removal* (ao-2020-043928) dla czasopisma ACS Omega.
5.	29.11.2020 r. recenzja artykułu *Bio monitoring of lead, zinc, copper, cadmium and iron heavy metals in soil and plant using atomic absorption spectroscopy in Najran area, Saudi Arabia* (ECES-00178-2020-01) dla czasopisma Ecological Chemistry and Engineering S.
6.	04.01.2021 r. recenzja artykułu *STUDY OF THE EFFECT OF HEAVY INDUSTRY ON AIR POLLUTION BY ACTIVE MOSS BIOMONITORING IN DONETSK REGION (UKRAINE)* (AECT-D-20-00767) dla czasopisma Archives of Environmental Contamination and Toxicology.
7.	19.01.2021 r. recenzja artykułu *Bibliometric analysis of nanoscale zerovalent iron particles research for environmental remediation from 2000 to 2019* (ESPR-D-20-15509) dla czasopisma Environmental Science and Pollution Research.
8.	07.03.2021 r. recenzja artykułu *MULTIPLE BIOMONITORING TESTS FOR ENVIRONMENTAL ASSESSMENTS* (ECES-00194-2021-01) dla czasopisma Ecological Chemistry and Engineering S.
9.	10.04.2021 r. recenzja artykułu *First attempt of major and trace elements atmospheric deposition study in Azerbaijan based on moss technique, using neutron activation analysis* (ECES-00201-2021-01) dla czasopisma Ecological Chemistry and Engineering S.
10.	18.09.2021 r. recenzja artykułu *Benzo(a)pyrene in PM10 - Air Monitoring Results in Poland* (ECES-00225-2021-01) dla czasopisma Ecological Chemistry and Engineering S.
11.	06.11.2021 r. recenzja artykułu *Determination and statistical analysis of atmospheric deposition of heavy metals in Kosovo. A moss survey* (AECT-D-21-00514) dla czasopisma Archives of Environmental Contamination and Toxicology.
12.	22.01.2022 r. recenzja artykułu *Global Assessment of Air Pollution Indices of Trees and Shrubs for biomonitoring and green belt development – A tabulated review* (ESPR-D-21-15096) dla czasopisma Environmental Science and Pollution Research.
13.	20.02.2022 r. recenzja artykułu *Analysis of Heavy Metals and Pesticides in Vegetables (Food Quality)* (ECES-00249-2021-01) dla czasopisma Ecological Chemistry and Engineering S.
14.	06.03.2022 r. recenzja artykułu Leaching of heavy metals from contaminated sediments stabilised by Portland fly ash cement CEM II / A-V and slag Bremen (ECES-00255-2022-01) dla czasopisma Ecological Chemistry and Engineering S.
15.	06.03.2022 r. recenzja artykułu *The case of Ailanthus altissima (Mill.) used in the monitoring of heavy metals caused by traffic in Ankara, Turkey* (BCAB-D-22-00413) dla czasopisma Biomass Conversion and Biorefinery.
16.	19.03.2022 r. recenzja artykułu *The analysis of parameters affecting indoor air pollution and noise levels under the applied theory of covariance functions* (ECES-00236-2021-01) dla Ecological Chemistry and Engineering S.
17.	03.04.2022 r. recenzja artykułu *Global Ambient Air Quality Monitoring – Can Mosses Help? A Systematic Meta-analysis of Practices and Advances* (ENVI-D-22-01167) dla czasopisma Environment, Development and Sustainability.
18.	26.05.2022 r. recenzja artykułu *Phytoextraction of heavy metals from a decommissioned tannery waste disposal area by pioneer herbaceous plants* (ECES-00258-2022-01) dla czasopisma Ecological Chemistry and Engineering S.
19.	12.06.2022 r. recenzja artykułu *Moss Biomonitoring of Air Pollution with Potentially Toxic Elements in the Kumanovo Region, North Macedonia* (JESHA-2022-0218) dla czasopisma Journal of Environmental Science and Health, Part A
20.	28.08.2022 r. recenzja artykułu *Study of atmospheric deposition of Tl, Hg, and As, in the Kosovo area by mosses as biomonitors* (GCHE-2022-0151) dla czasopisma Chemistry and Ecology.
21.	10.09.2022 r. recenzja artykułu *Moss biomonitoring for Co, Cr, and Ni contamination in a national study of atmospheric deposition in Albania* (AECT-D-22-00443) dla czasopisma Archives of Environmental Contamination and Toxicology.
22.	11.09.2022 r. recenzja artykułu *Biomonitoring, bioaccumulation and bioenergy aspects of moss: A critical review* (Submission ID d0eae939-8161-43fc-895d-689b8f54047f) dla czasopisma Environmental Monitoring and Assessment.

23.	01.01.2023 r. recenzja artykułu *Biomonitoring of polyaromatic hydrocarbons (PAHs) by Murraya paniculata (L.) Jack in South Kolkata, West Bengal, India: Spatial and temporal variations* dla czasopisma Environmental Geochemistry and Health.
24.	06.03.2023 r. recenzja artykułu *Impact assessment of urbanization on vegetation net primary productivity: A case study of the core development area in Central Plains urban agglomeration, China* (ER-22-9513) dla Environmental Research.
25.	10.03.2023 r. recenzja artykułu *Fish muscle mercury concentration and bioaccumulation fluctuate year-round – insights from cyprinid and percid fishes in a humic boreal lake* (ER-23-2182) dla Environmental Research.
26.	12.03.2023 r. recenzja artykułu *Change of Cr concentration from past to present in areas with elevated air pollution* (JEST-D-23-00405) dla International Journal of Environmental Science and Technology.