J. Hydrol. Hydromech., 71, 2023, 2, 139–147
https://doi.org/10.2478/johh-2023-0010
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sciendo Journal of Hydrology and Hydromechanics Institute of Hydrology SAS

Influence of polycyclic aromatic hydrocarbons on water storage capacity of two lichens species

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Abstract: The wide variability in functional traits that enable the cosmopolitan distribution of lichens often includes the water storage capacity, S, of their thallus. Lichen S in forest canopies can be large enough to intercept and evaporate significant amounts of rainwater, contributing to the runoff-reduction ecosystem services provided by urban forests; however, S is likely influenced by the presence of air pollutants (polycyclic aromatic hydrocarbons, PAHs) in urban areas. PAHs, being both chemically hydrophobic and damaging to lichen thalli, are expected to reduce lichens' S and, thereby, limit their contribution to hydrologic ecoservices of urban forests. Hence, the relationship between PAH accumulation and rainwater uptake was examined for two lichen species, common in urban forests around the world – *Platismatia glauca* and *Pseudevernia furfuracea*. Samples were collected from an area of low air pollution and another area in a highly urbanized city centre with high air pollution exposure (Kraków, Poland). Lichen S was determined using laboratory-simulated rainfall. PAH bioaccumulation differed between species and among the samples from clean and polluted environments. After exposure to polluted air, the concentration of PAHs was higher in *P. glauca* than P. furfuracea. Samples from the non-urban setting, however, showed no differences between the two species. In the case of *P. glauca*, S decreased from 35.8% in samples from clean environment to 8.3% after six months of exposure in the urban setting. The respective S values for *P. furfuracea* were 25.4% and 12.4%. Results strongly suggest that PAH exposure reduces S in both lichen species.

The obtained results are important both in ecohydrology and microclimatology and are part of the research on the condition of urban forests.

Keywords: Ecohydrology; Simulated precipitation; PAH; Rainfall interception; Retention; Urban forests.

1 INTRODUCTION

Lichens are symbiotic organisms formed by algae (and/or cyanobacteria) and fungi. This symbiosis enables lichens' survival in diverse habitats, including those with extreme conditions like frequent desiccation (Łubek et al., 2018). During extended dry periods, the body of the lichen (the thallus) dehydrates relatively quickly as it has little control over its internal water content (i.e., it is poikilohydric). Once desiccated, the lichen deactivates its metabolic functions until water becomes available again. During periods of water availability, the thallus can quickly absorb water; increasing its mass by 300–3000% compared to their dry weight (Porada et al., 2018). Water that is absorbed by a lichen's thallus may come from condensation or precipitation sources (Gauslaa et al., 2021), and some portion of this moisture interacts with the environment – draining down leaves or barks – becoming enriched in solutes before reaching the lichen. Thus, when lichens absorb environmental waters, they also take up the various solutes (that can range from macronutrient ions to air pollutants) within those waters (Van Stan and Pypker, 2015). The rate at which lichen thalli absorb water and the thalli's water storage capacity are, therefore, significant traits that influence the organism's ecophysiological functions, the niche it may occupy on the surface of soils, rocks, or in tree canopies, and the ecosystem services the lichen community can provide.

When lichens reside within forest canopies (as epiphytic lichens), the water storage of their thalli can contribute significantly to the total water storage capacity (*S*) of the forest canopy and play an important role in maintaining a moist canopy microclimate (Mendieta-Leiva et al., 2020). This is due to lichens contributing significantly to the storage and evaporation (or "interception") of rainfall by forest canopies, reducing the amount and timing of rainfall that reaches the soil surface (Allen et al., 2020). Rainfall interception by forest canopies provides substantial ecoservices, having been estimated to save cities tens-to-hundreds of millions USD dollars per year (Nowak et al., 2020). Thus, investigation of factors controlling the patterns and timing of *S* in forest canopies is ecologically and economically merited.

Moreover, the storage of any forest canopy component, leaves, barks or epiphytes, can significantly vary with hydrometeorological and canopy structural variables, like rainfall intensity, tree species composition, age of the stand and air pollution (Klamerus-Iwan et al., 2018; Papierowska et al., 2018).

Common urban air pollutants include polycyclic aromatic hydrocarbons (PAHs) that, once deposited, can increase water repellency (i.e., hydrophobicity) of surfaces. PAHs were always present in the environment; however, due to urban development, their concentration in the atmosphere is rising (Klamerus-Iwan et al., 2018). Pollution in the form of particulate matter (PM_{10} and $PM_{2.5}$) and tar substances rich in PAHs, which settle on the thalli, can cause changes in hydrophobicity (Augusto et al., 2016). PAHs have hydrophobic properties and are non-polar and planar, which makes them particularly interesting in the context of the impact on the retention capacity of the organisms they cover. Therefore, it is of interest how PAHs modify the condition of lichens and thereby influence *S*. We hypothesize that the water uptake response and water storage capacity of lichen thalli will inversely relate to the PAH content of lichen thalli.

The present study fills a knowledge gap regarding hydrological properties of lichens resulting from various degrees of PAHs contamination, yielding insights into the effect of urban air pollution on forest canopy interception.

2 MATERIALS AND METHODS

Two lichen species were included in this study: *Platismatia glauca* (L.) W.L. Culb. & C.F. Culb. **(P)** and *Pseudevernia furfuracea* (L.) Zopf. **(E).** Samples of lichen thalli growing on spruce branches were collected from a nonurban setting, in the Borecka Forest (NE Poland 54.096305, 22.236309). Twenty samples of each species were analysed, of which ten were subject to hydrological analyses directly after collection (**nonurban**) and ten were transplanted to Cracow city centre for six months (April–September) and then analysed (**urban**) (Figure 1). These species were chosen due to their cosmopolitan occurrence across natural and urban areas. The city of Cracow was chosen as it is among the most heavily polluted areas in Europe (Lasota et al., 2021; Zarek et al., 2020). Both the geographical location of the city (valley area surrounded by high mountains) and compact urban development are limiting the air circulation. As a result, the concentration of PAHs is increasing at an alarming rate. Rather than testing the hypothesis through a comparative analysis of samples collected from different settings, this study employs a translocation experiment. We used this version of the experiment because the analysed lichens are not so common in large cities and we want to know how these epiphytes will react when we increase the level of pollution in the currently cleaner regions.



Fig. 1. a) Thalli of *Platismatia glauca*; b) Sample of *Pseudevernia furfuracea,* both after six months of exposition in an urban setting; c) natural area of lichen occurrence (nonurban); d) place in the city where lichens have been implemented (urban).

Samples of the study lichen species were collected from a forest with low air pollution (Kłos et al., 2018; Likus-Cieślik et al., 2020) and exposed to the contaminated environment of Cracow. During the exposure, branches with lichen thalli were attached to tree branches with a plastic band at the height of 1.5–1.7 m. All samples were analysed to determine the concentration of PAHs (Augusto et al., 2016).

Simulated rainfall experiments under laboratory conditions were employed to estimate values of thalli water storage capacity and uptake rates from these samples. Additionally, the contents of selected PAHs were measured in the study samples using gas chromatography-mass spectrometry (GC-MS).

2.1 Measurements of thalli interception and water storage capacity

Intercepted water was examined on lichen thalli separated from branches via forceps. The measurement cycle consisted of 20 runs where the thalli were saturated with distilled water through simulated rainfall. The experiment was done according to Klamerus-Iwan et al. (2018) with a calibrated sprinkler (Klamerus-Iwan et al., 2020). In each experimental run, lichen samples were wetted with constant doses of 10 g of water (P) over 0.03 m^2 of monitoring area, resulting in 0.33 mm $dose^{-1}$. After each dose the samples were weighed. The weight before and directly after wetting reflected the amount of water (W) intercepted by the thalli in each run. Maximum water storage capacity (S_{max}) was counted as the mean value from the last six measurements, where the amount of intercepted water was stable, despite the subsequent doses being added. S_{max} and interception (I) were calculated per unit of field biomass (bm), in order to compare the results from two species of different thalli sizes. The total applied mass of simulated rainfall was treated as 100% of rainfall. Retained water on the thalli was calculated as grams of gained water per gram of thalli [$g \cdot g^{-1}$].

The result was calculated as grams of water, according to the following formula:

$$I\ [g\ g^{-1}] = W\ [g] / bm\ [g] \quad (1)$$

I [$g\ g^{-1}$] – actual interception after each water dose (10 g);
I [%] – actual interception after each water dose converted into percentages of total rainfall (P), according to the formula (2);
W – difference in weight between the sample after and before wetting (g) – the amount of water adsorbed by the sample (g);
bm – fresh biomass of lichen sample (g).
P – each 10 g dose of water, in 20 runs

$$I\ [\%] = (I\ [g\ g^{-1}] / P\ [g] / bm\ [g]) * 100\ \% \quad (2)$$

The simulated rainfall was conducted separately for lichen samples from nonurban setting in Borecka Forest and those exposed to the urban setting in Cracow.

2.2 Determination of polycyclic aromatic compounds

The determination of polycyclic aromatic compounds (20 compounds) in the samples was conducted according to previous study (Klamerus-Iwan et al., 2020). The sample preparation method was based on the PAH extraction with acetonitrile supported by salt partitioning process and followed by dispersive solid phase extraction (d-SPE) with the use of PSA sorbent. The sample extracts, preconcentrated in DLLME step, were then analysed using gas chromatography-mass spectrometry.

As the PAHs do not occur in the atmosphere separately, further analyses of water storage were conducted on the basis of total concentration of the compounds (SUM_PAH) [µg/kg]. SUM_PAH is the sum of the concentrations of the compounds: Naphthalene (NP); 2-methylnaphthalene (MeNap2); 1-methylnaphthalene (MeNap1); Acenaptylene (ACY); Acenaphthene (ACE); Fluorene (FL); Phenathrene (PHE); Anthracene (ANT); Fluoranthene (FLA); Pyrene (PYR); Benzo[a]anthracene (BaA); Chrysene (CHR); Benzo(b)fluoranthene (BbF); Benzo(k)fluoranthene (BkF); Benzo[a]fluoranthene (BaF); Indeno[c,d]pyrene (IcdP); Dibenzo[a,h]anthracene (DahA); Benzo[g,h,i]perylene (BghiP).

2.3 Statistical analyses

We analysed the differences between lichen species with regard to interception (I) and the maximum water storage capacity (S_{max}) – for lichens collected from the nonurban setting (from Borecka forest), and for lichens exposed to the urban setting of Cracow. Student's t-test series for independent trials were used to compare interception, aromatic hydrocarbon content, and maximum water retention capacity for two lichen species from the nonurban and urban settings.

Effects of setting, dose of water, lichen species and their combinations on I and S_{max} were tested using multiple ANOVA, after checking the assumptions. If the data were not normally distributed, the log-transformation was performed.

Regression analysis was used to determine the effect of PAH content on S_{max} for two species of lichens, from the nonurban and urban settings. The analyses were performed using the Statistica 12.0 (2020) and R (R Core Team (2022)) software applications.

3 RESULTS

3.1 Lichen S_{max} in clean and polluted environments

Water uptake patterns and S_{max} for both studied lichens differed between the nonurban and urban settings (Figure 2). The amount of simulated rainfall pulses required to saturate the thalli of the study species increased by 3–4 doses for samples collected from the urban setting, especially for *P. furfuracea*. The S_{max} for both study species was higher for samples collected in the nonurban setting, compared to the samples translocated to the urban setting (Figure 2). Mean S_{max} with standard deviation was greatest for *P. glauca* samples collected in the Borecka Forest, 3.07 ± 0.77 g g^{-1}, and samples from the same species produced the lowest mean S_{max}, 0.65 ± 0.23 g g^{-1}, after exposure to the urban setting of Cracow. These differences in S_{max} between settings for *P. glauca* resulted in a decrease from 35.8% to 8.3% of relative interception and were statistically significant ($t = 12.65$; $p < 0.001$). Values of S_{max} for *P. furfuracea* also decreased significantly, from 2.10 ± 0.59 g g^{-1} (nonurban) to 0.80 ± 0.42 g g^{-1} (urban) ($t = 5.36$; $p < 0.001$), effectively halving the relative interception from 25.4% to 12.4%. There were no significant differences between study species, however, when both environments were considered together ($t = 0.90$; $p = 0.374$; $d = 0.38$) (Figure 2; Appendix A).

Multiple analysis of variance (ANOVA) was preformed to examine the effect of the setting (nonurban vs. urban), species (*P. furfuracea v. P. glauca*), the number of water doses (from 1 to 25) and their interactions on the interception. The model explained 98% of the variance ($F = 632.14$; $p < 0.0001$) and the p-values for each model component is given in Table 1. It showed a significant multivariate effect for each of the variables and their interactions in relation to the fraction of intercepted simulated precipitation pulses (Apendix B; Apendix C).

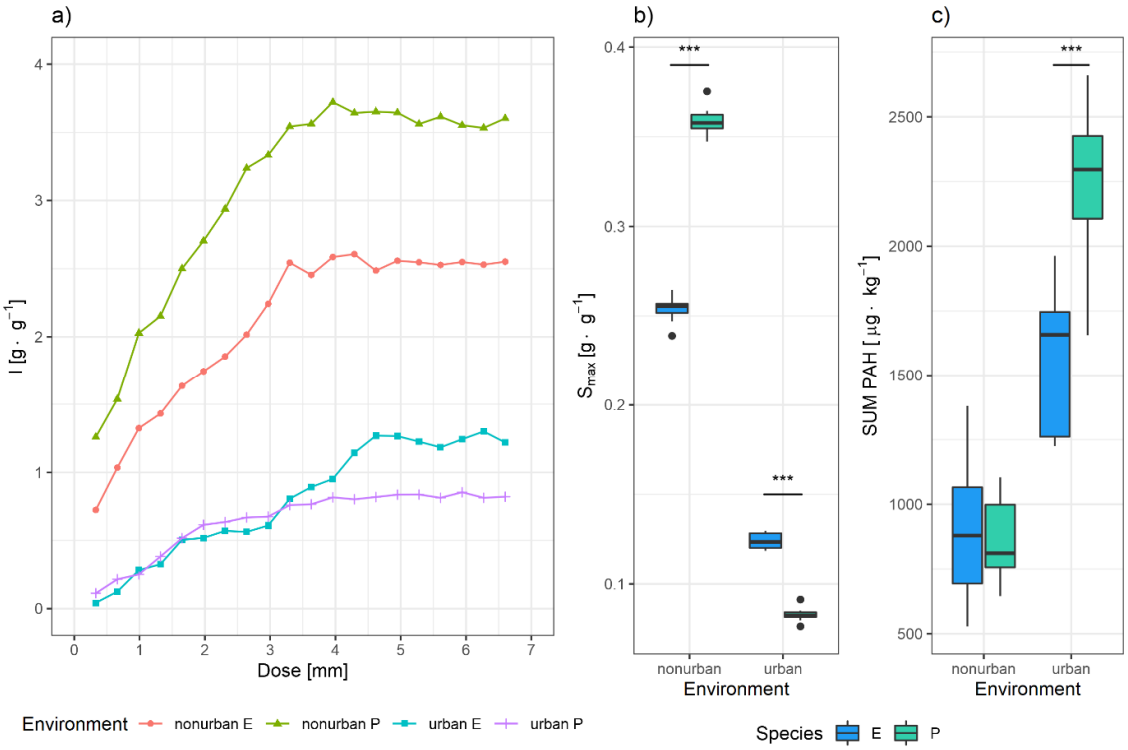


Fig. 2. a) Average change in water storage capacity of two lichen species from the nonurban and urban setting; *I* [%] – actual interception after each water dose (10 g) **b)** S_{max} values for two lichen species in two different settings; **c)** Concentration of PAHs in two lichen species thalli in Borecka Forest (nonurban) and in Cracow city centre (urban); E – *Pseudevernia furfuracea*; P – *Platismatia glauca*; black line inside of the box represents median, box represents interquartile range, whiskers represent minimum and maximum, black points represent outliers. *** - $p < 0.000$ according to t test.

Table 1. Results of multiple ANOVA, showing F-statistics and p-values for responses of actual interception (*I* [%]) to setting, species of lichen, dose of water and combined effects.

Factor	F	p
Setting	34117.6	0.0000
Species	1652.0	0.0000
Dose	486.8	0.0000
Setting * Species	3107.2	0.0000
Setting * Dose	72.9	0.0000
Species * Dose	6.5	0.0000
Setting * Species * Dose	16.1	0.0000

3.2 Lichen PAHs concentration in clean and polluted environments

The concentration of PAHs in lichen thalli significantly differed between settings (nonurban v. urban: $t = 9.06$; $p < 0.001$; $d = 2.87$) (Figure 3). The sum of PAHs in the samples collected from the nonurban setting was significantly lower than observed in samples exposed to the urban setting. However, the differences between the two tested lichen species in this respect were not significant ($t = 1.70$; $p = 0.098$; $d = 0.54$). Although the samples exposed in the city centre had higher concentrations of PAHs in *P. glauca*, 2257.75 ± 300.47 µg kg^{-1}, compared to *P. furfuracea*, 1562.12 ± 279.71 µg kg^{-1}, the samples collected from the nonurban setting were similar for both species, 898.49 ± 273.85 µg kg^{-1} for *P. furfuracea* ~ 863.55 ± 176.53 µg kg^{-1} ($t = 0.34$; $p = 0.738$; $d = 0.15$).

3.3 Interaction of PAHs and lichen S_{max}

The results of a multiple ANOVA (Table 2) show that the setting, species and their combined effect had a significant impact on S_{max} and the sum of PAHs in lichen thalli.

Linear regression analysis indicates that the SUM_PAH value affected S_{max} in all samples ($F = 91.64$; $p < 0.05$; $R^2 = 0.71$). The relationship was strongly inverse ($t = –9.57$; $p < 0.001$; $\beta = –0.84$), with lichen thalli containing higher PAH concentrations had lower S_{max}. This relationship was strongest for *P. glauca* (nonurban: $t = –3.01$; $p < 0.05$; $\beta = –0.73$ and urban: $t = –3.14$; $p < 0.05$; $\beta = –0.74$). However, for *P. furfuracea*, the influence of PAH content was not statistically significant between settings (nonurban: $t = –1.93$; $p = 0.089$; $\beta = –0.56$ and urban: $t = –1.59$; $p = 0.151$; $\beta = –0.49$) (Figure 3; Table 3; Apendix D).

4 DISCUSSION

Lichens living in forest canopies can play important roles in the way these canopies store and evaporate rainwater (Mendieta-Leiva et al., 2020). As evaporation fluxes strongly depend on the saturation state of forest canopy surfaces, water storage capacity (S_{max}) is a central variable in estimating rainfall interception (Keim and Link, 2018; Klamerus-Iwan et al., 2020; Rutter et al., 1971). Epiphytic lichens can often store the greatest rainwater per unit mass of all canopy components; thus, the intra- and interspecific variation in lichen S_{max} may strongly modulate the forest canopy water balance. Our results suggest that S_{max} of two lichen species with a cosmopolitan distribution can be reduced with increasing PAH concentrations in urban settings. This result extends our knowledge of how anthropogenic activities can

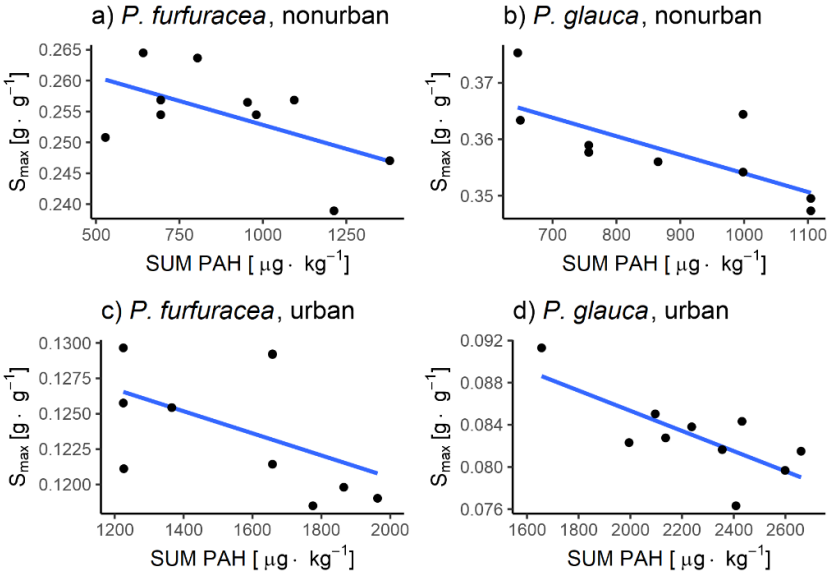


Fig. 3. Impact of PAHs concentration on the maximum water storage capacity of lichens in different environments; S_{max} – maximum water storage capacity; SUM_PAH – total concentration of polycyclic aromatic compounds.

Table 2. Results of multiple ANOVA, showing F-statistics and p-values for responses of S_{max} and SUM PAH to setting, species of lichen and combined effect.

	S_{max}		SUM PAH	
	F	p	F	p
Setting	10608.6	0.000	154.2	0.0000
Species	255.3	0.000	15.9	0.0000
Setting*Species	1354.3	0.000	19.4	0.0000

influence water adhesion to lichens and provides data to develop models describing changes in S_{max} due to air pollution (Klamerus-Iwan et al., 2020). The results improve our understanding of the response of common lichen species' to different levels of PAHs contained in lichen thalli in forests and city centres.

The two studied lichen species differed in their maximum water storage capacity and PAH absorption. These interspecific differences may be a result of these lichens having different tolerances to air pollution. For example, *P. furfuracea* can inhabit places where the content of sulphur dioxide in the air episodically reaches 50–60 µg/m^3 (Bielczyk, 2001). *Platismatia glauca*, on the other hand, is rarely found in places with this level of air pollution and, when found, its thalli are usually degenerated (Bielczyk, 2001; Hawskworth and Rose, 1970). The results show that the pollution-sensitive study species, *P. glauca*, can retain more rainwater than the less-sensitive study species, *P. furfuracea* in nonurban conditions. This difference is not confined to the value of S_{max}, but may also be related to the time of rainfall required to achieve saturation, as suggested by the saturation curves. However, both lichen species' water storage and uptake rates decreased, and became similar after exposure to elevated PAH deposition in the urban environment. We observe that the reduction in water storage capacity is more distinct in the case of more pollution-vulnerable species. As lichens do not have differentiated tissues, precipitation and pollutants may be absorbed by the thallus over its entire surface, disturbing the biochemical processes in thallus cells (Osyczka et al., 2018).

There are additional air pollutants present in urban settings beyond PAHs and, thus, PAHs in this study may be considered a proxy for overall air pollution. Indeed, the interactions between a mixture of pollutants in atmospheric environments are complex and can have a synergistic (negative) effect on the organisms (Sicard et al., 2016). For example, the vitality of lichens can be easily affected by sulphur and/or nitrogen compounds that cause oxidative stress and or direct degradation of chlorophyll (Likus-Cieślik et al., 2020; Popek et al., 2013). Thus, the effect of our translocation study may be due to a mixture of air pollutants that include PAHs. However, if quantifiable, PAH monitoring may be seen as a proxy for the general impact of air pollution. Given the PAH proxy indicator for air pollution impacts, the differences between species in terms of the amount of accumulated PAHs is noteworthy. These differences may be related to the differing thallus morphology of the study species. *Platismatia glauca* represents leaf-like, flat form of thallus, markedly undulating at the ends of the segments. The segments are wider than those of *P. furfuracea*. This particular shape creates better conditions for the retention of rainwater as well as atmospheric pollutants, coming into contact with lichen's surface over a larger area (Agnan et al., 2017; Cecconi et al., 2019). *Pseudevernia furfuracea* has a bushy form that tends to develop a circular shape and attaches to the substrate in fewer places. The laciniae are much more fragmented, subdivided into smaller components, and additionally they are slightly convex, which may facilitate the flow of water from the thallus.

We note that the effect of total PAH accumulation on S_{max} for the same species and the same treatment is relatively weak (and not significant for *Pseudevernia furfuracea*: see Fig. 3), suggesting a potential indirect relationship between species-specific responses to translocation. Epiphytic lichens have various adaptive mechanisms to cope with the substantial microhabitat variability inherent to residing on bark surfaces (Porada and Giordani, 2022). For example, the species may have altered their thallus structure in response to the translocation experiment, because of factors like: exposure to a stressful environment; substantial changes in light, rainfall, or temperature conditions; or the cutting of the host branches. Another aspect to consider is that living thalli exposed to contaminants may experience varying degrees of physiological impairment, depending on the site-specific environmental conditions and the initial health status of

Table 3. Results of regression analysis of the impact of PAHs concentration on the maximum water storage capacity.

S_{max}		B	SE	β	t	p	R^2	F
Total	(Const)	41.07	2.35		17.45	***	0.71	91.64***
	SUM PAH	–0.01	0.00	–0.84	–9.57	***		
Forest E	(Const)	26.83	0.75		35.73	***	0.32	3.73
	SUM PAH	0.00	0.00	–0.56	–1.93	0.089		
Forest P	(Const)	38.69	0.96		40.13	***	0.53	9.04*
	SUM PAH	0.00	0.00	–0.73	–3.01	0.017*		
City E	(Const)	13.61	0.78		17.49	***	0.24	2.54
	SUM PAH	0.00	0.00	–0.49	–1.59	0.151		
City P	(Const)	10.45	0.70		14.95	***	0.55	9.71*
	SUM PAH	0.00	0.00	–0.74	–3.12	0.014*		

S_{max} – maximum water storage capacity; B – unstandardised coefficient, SE – standard error, β – standardised coefficient, p – statistical significance, R^2 – coefficient of determination, F – analysis of variance coefficient, * – $p < 0.05$, *** – $p < 0.001$; SUM_PAH – total concentration of polycyclic aromatic compounds; P – *Platismatia glauca*; E – *Pseudevernia furfuracea.*

each lichen (Piccotto and Tretiach, 2010; Piccotto et al., 2011). The lichens reside on tree barks, where changes due to air pollution may also affect the water stored within the bark and the quantity and quality of water that drains down the bark (stemflow) (Porada and Giordani, 2021). This is especially important in dry climates (Giordani et al., 2019; Porada et al., 2013), whereas the lichens' presence is important for the global carbon cycle (Fortuna and Tretiach, 2018; Sicard et al., 2021).

It is also possible that the lichen samples may have been selected for a certain, narrow range of S_{max} in the original forested setting, thereby compensating for the varying degree of negative effect of PAHs content on growth (also possible evidenced by the weak regression). Due to the short-term exposure to higher PAH levels in Cracow, it may be reasonable to hypothesize that the growth or thallus structure might have been damaged, or even consistently reduced, consistently for all samples by a similar amount. If this were the case, variation in the "new" PAH content (due to exposure in the urban environment) would simply reflects the original variability in the lichen samples from the forested setting, plus a more or less constant addition, which caused a uniform decrease in S_{max} per species. Finally, as the host branches were cut for the transplantation experiment, it may have changed the branch properties in meaningful ways. Given that the experimental setup required the attachment of branches by tape, this (in conjunction with the aforementioned changes in microclimate) may have affected the water storage component of the bark and its moisture variability (including the disruption of stemflow compared to the natural condition) – which may have impacted the lichen morphology (Porada and Giordani, 2021).

The impact of air pollution on plant ecosystems has been demonstrated in numerous studies over the past decades. The role of trees in minimising health risk of air pollutants and improving thermal comfort in cities is evident (Klamerus-Iwan et al., 2020; Stark, 2017). Water retention by lichens also affects the amount of precipitation stored in the canopy and reaching the soil (Nascimbene et al., 2020). This is important from the hydrological point of view (Crockford and Richardson, 2000; Sadeghi et al., 2016) regarding the water cycle in the atmosphere. The present study supplies the missing element between air pollution that is rich in PAHs and the ecohydrological properties of particular lichens species by pointing to the amount of water that can be retained in tree crowns containing epiphytic lichens. Pollution levels of PAHs may thus be included in current urban and forest hydrological models to improve their reliability.

Acknowledgement. Article was financed by a subvention from the Ministry of Science and Higher Education of the Republic of Poland for the University of Agriculture in Krakow (SUB/040014/D019) and by the Jan Kochanowski University in Kielce (Project no. 638).

Author Contributions. Conceptualization, AK-I. and R.K.; methodology, A.K-I., R.K, A.S-R.; validation A.K-I.; formal analysis, A.K-I, E.S-O.; investigation, A.K-I, E.S-O.; writing—original draft preparation, A.K-I, R.K., J.P., E.S-O., A.S-R., D.K., P.G.., P.P., J.V.S; writing—review and editing, A.K-I.; visualization, A.K-I, J.P., D.K.; All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest. The authors declare no conflict of interest.

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Received 22 December 2022
Accepted 13 March 2023

APPENDIX

Appendix A. Results of Student's *t*-tests for independent samples – comparisons of interception of the two species in two environments.

I [%]	Clean	Polluted	t	p	d	*I* [%]	E	P	t	p	d
	M (SD)	M (SD)					M (SD)	M (SD)			
1	9.91 (3.10)	0.77 (0.47)	13.03	***	4.12	1	3.83 (3.59)	6.86 (6.00)	1.93	0.06	0.61
2	12.87 (2.74)	1.70 (0.58)	17.83	***	5.64	2	5.80 (4.74)	8.77 (6.80)	1.6	0.117	0.51
3	16.76 (3.76)	2.67 (0.38)	16.68	***	5.28	3	8.03 (5.42)	11.39 (9.14)	1.41	0.165	0.45
4	17.92 (3.90)	3.54 (0.48)	16.38	***	5.18	4	8.78 (5.73)	12.67 (9.14)	1.61	0.115	0.51
5	20.68 (4.91)	5.10 (0.44)	14.12	***	4.46	5	10.70 (5.87)	15.08 (10.36)	1.65	0.108	0.52
6	22.25 (5.13)	5.67 (0.71)	14.33	***	4.53	6	11.32 (6.37)	16.60 (10.78)	1.88	0.067	0.6
7	23.97 (5.90)	6.02 (0.73)	13.51	***	4.27	7	12.12 (6.67)	17.87 (11.96)	1.88	0.068	0.59
8	26.27 (6.76)	6.15 (0.74)	13.22	***	4.18	8	12.89 (7.65)	19.53 (13.32)	1.93	0.061	0.61
9	27.87 (5.99)	6.42 (0.55)	15.95	***	5.04	9	14.26 (8.52)	20.03 (13.72)	1.6	0.118	0.51
10	30.43 (5.37)	7.83 (1.42)	18.2	***	5.75	10	16.75 (9.06)	21.51 (14.35)	1.25	0.218	0.4
11	30.09 (5.94)	8.29 (0.94)	16.21	***	5.13	11	16.73 (8.12)	21.64 (14.41)	1.33	0.193	0.42
12	31.54 (6.05)	8.84 (1.86)	16.04	***	5.07	12	17.69 (8.52)	22.69 (15.02)	1.3	0.203	0.41
13	31.25 (5.55)	9.73 (1.96)	16.35	***	5.17	13	18.75 (7.59)	22.22 (14.64)	0.94	0.353	0.3
14	30.69 (6.27)	10.44 (2.60)	13.36	***	4.22	14	18.78 (6.42)	22.35 (14.63)	1	0.323	0.32
15	31.01 (6.03)	10.51 (2.34)	14.18	***	4.49	15	19.12 (6.75)	22.40 (14.56)	0.91	0.367	0.29
16	30.54 (5.66)	10.31 (2.49)	14.63	***	4.63	16	18.86 (7.12)	21.99 (14.07)	0.89	0.381	0.28
17	30.71 (5.75)	9.98 (2.14)	15.11	***	4.78	17	18.55 (6.95)	22.14 (14.45)	1	0.324	0.32
18	30.51 (5.63)	10.48 (2.09)	14.92	***	4.72	18	18.96 (6.75)	22.03 (14.02)	0.88	0.383	0.28
19	30.31 (5.33)	10.56 (2.61)	14.88	***	4.71	19	19.15 (6.38)	21.73 (14.01)	0.75	0.459	0.24
20	30.77 (5.47)	10.20 (2.20)	15.6	***	4.93	20	18.85 (6.89)	22.12 (14.30)	0.92	0.364	0.29

M – mean, SD – standard deviation, p – statistical significance, d – Cohen's d, *** – $p < 0.001$; I1, I2… – doses of water; E – *Pseudevernia furfuracea*; P – *Platismatia glauca.*

Appendix B. Results of Student's *t*-tests for independent samples – comparisons of interception between clean and polluted environment for the two lichen species.

I [%]	Clean E	Polluted E				*I* [%]	Clean P	Polluted P			
	M (SD)	M (SD)	t	p	d		M (SD)	M (SD)	t	p	d
1	7.24 (1.13)	0.42 (0.38)	18.07	***	8.08	1	12.59 (1.76)	1.13 (0.20)	20.41	***	9.13
2	10.34 (1.21)	1.25 (0.23)	23.43	***	10.48	2	15.39 (0.51)	2.15 (0.45)	61.27	***	27.4
3	13.24 (1.22)	2.82 (0.38)	25.74	***	11.51	3	20.28 (0.92)	2.51 (0.33)	57.67	***	25.79
4	14.32 (1.15)	3.25 (0.29)	29.46	***	13.18	4	21.52 (1.37)	3.82 (0.47)	38.67	***	17.29
5	16.35 (1.28)	5.04 (0.38)	26.81	***	11.99	5	25.00 (2.79)	5.16 (0.50)	22.13	***	9.9
6	17.45 (1.41)	5.19 (0.27)	27	***	12.07	6	27.04 (1.54)	6.15 (0.69)	39.14	***	17.5
7	18.55 (1.24)	5.70 (0.77)	27.72	***	12.4	7	29.39 (2.56)	6.35 (0.54)	27.88	***	12.47
8	20.16 (2.38)	5.62 (0.63)	18.63	***	8.33	8	32.38 (2.82)	6.68 (0.34)	28.62	***	12.8
9	22.43 (2.21)	6.10 (0.49)	22.8	***	10.19	9	33.32 (2.22)	6.75 (0.41)	37.24	***	16.66
10	25.44 (1.22)	8.06 (1.98)	23.64	***	10.57	10	35.42 (2.03)	7.59 (0.47)	42.34	***	18.93
11	24.55 (1.77)	8.92 (0.59)	26.42	***	11.82	11	35.62 (1.80)	7.65 (0.79)	45.01	***	20.13
12	25.86 (0.91)	9.51 (1.99)	23.59	***	10.55	12	37.22 (2.17)	8.16 (1.52)	34.66	***	15.5
13	26.07 (1.41)	11.43 (0.74)	29.04	***	12.99	13	36.42 (1.88)	8.02 (1.03)	41.85	***	18.72
14	24.86 (1.60)	12.69 (1.44)	17.83	***	7.98	14	36.52 (2.19)	8.18 (0.93)	37.63	***	16.83
15	25.58 (1.55)	12.66 (0.96)	22.42	***	10.03	15	36.44 (2.97)	8.36 (0.59)	29.29	***	13.1
16	25.47 (2.37)	12.25 (2.05)	13.35	***	5.97	16	35.61 (2.23)	8.37 (0.74)	36.73	***	16.43
17	25.28 (0.78)	11.83 (0.91)	35.57	***	15.91	17	36.15 (1.88)	8.13 (1.11)	40.58	***	18.15
18	25.49 (1.10)	12.43 (0.46)	34.66	***	15.5	18	35.52 (3.12)	8.54 (0.79)	26.52	***	11.86
19	25.30 (1.07)	13.00 (0.90)	27.78	***	12.42	19	35.32 (1.74)	8.13 (0.58)	46.92	***	20.99
20	25.52 (0.95)	12.19 (0.71)	35.49	***	15.87	20	36.02 (0.97)	8.21 (0.96)	64.43	***	28.81

M – mean, SD – standard deviation, p – statistical significance, d – Cohen's d, *** – $p < 0.001$; I1, I2… – doses of water; E – *Pseudevernia furfuracea*; P – *Platismatia glauca.*

Appendix C. Results of Student's t-tests for independent samples – comparisons of interception of the two species in clean and polluted environment.

I [%]	Clean E	Clean P				*I* [%]	Polluted E	Polluted P			
	M (SD)	M (SD)	t	p	d		M (SD)	M (SD)	t	p	d
1	7.24 (1.13)	12.59 (1.76)	8.08	***	3.61	1	0.42 (0.38)	1.13 (0.20)	5.15	***	2.3
2	10.34 (1.21)	15.39 (0.51)	12.16	***	5.44	2	1.25 (0.23)	2.15 (0.45)	5.68	***	2.54
3	13.24 (1.22)	20.28 (0.92)	14.57	***	6.52	3	2.82 (0.38)	2.51 (0.33)	1.95	0.067	0.87
4	14.32 (1.15)	21.52 (1.37)	12.73	***	5.69	4	3.25 (0.29)	3.82 (0.47)	3.29	0.004**	1.47
5	16.35 (1.28)	25.00 (2.79)	8.91	***	3.99	5	5.04 (0.38)	5.16 (0.50)	0.6	0.553	0.27
6	17.45 (1.41)	27.04 (1.54)	14.52	***	6.49	6	5.19 (0.27)	6.15 (0.69)	4.08	0.001**	1.83
7	18.55 (1.24)	29.39 (2.56)	12.06	***	5.39	7	5.70 (0.77)	6.35 (0.54)	2.17	0.044*	0.97
8	20.16 (2.38)	32.38 (2.82)	10.47	***	4.68	8	5.62 (0.63)	6.68 (0.34)	4.66	***	2.08
9	22.43 (2.21)	33.32 (2.22)	11	***	4.92	9	6.10 (0.49)	6.75 (0.41)	3.24	0.005**	1.45
10	25.44 (1.22)	35.42 (2.03)	13.35	***	5.97	10	8.06 (1.98)	7.59 (0.47)	0.73	0.474	0.33
11	24.55 (1.77)	35.62 (1.80)	13.85	***	6.19	11	8.92 (0.59)	7.65 (0.79)	4.07	0.001**	1.82
12	25.86 (0.91)	37.22 (2.17)	15.25	***	6.82	12	9.51 (1.99)	8.16 (1.52)	1.71	0.104	0.77
13	26.07 (1.41)	36.42 (1.88)	13.92	***	6.22	13	11.43 (0.74)	8.02 (1.03)	8.5	***	3.8
14	24.86 (1.60)	36.52 (2.19)	13.57	***	6.07	14	12.69 (1.44)	8.18 (0.93)	8.29	***	3.71
15	25.58 (1.55)	36.44 (2.97)	10.24	***	4.58	15	12.66 (0.96)	8.36 (0.59)	12.06	***	5.39
16	25.47 (2.37)	35.61 (2.23)	9.85	***	4.41	16	12.25 (2.05)	8.37 (0.74)	5.65	***	2.53
17	25.28 (0.78)	36.15 (1.88)	16.91	***	7.56	17	11.83 (0.91)	8.13 (1.11)	8.16	***	3.65
18	25.49 (1.10)	35.52 (3.12)	9.59	***	4.29	18	12.43 (0.46)	8.54 (0.79)	13.39	***	5.99
19	25.30 (1.07)	35.32 (1.74)	15.52	***	6.94	19	13.00 (0.90)	8.13 (0.58)	14.39	***	6.44
20	25.52 (0.95)	36.02 (0.97)	24.4	***	10.91	20	12.19 (0.71)	8.21 (0.96)	10.56	***	4.72

M – mean, SD – standard deviation, p – statistical significance, d – Cohen's *d*, *** – $p < 0.001$; I1, I2… – doses of water; E – *Pseudevernia furfuracea*; P – *Platismatia glauca.*

Appendix D. Results of Student's *t*-tests for independent samples – comparisons of PAHs content and maximum water storage capacity of the two species in two environments.

	Clean	Polluted			
	M (SD)	M (SD)	t	p	d
SUM PAH	881.02 (224.96)	1909.94 (455.16)	9.06	***	2.87
S_{max} [%]	30.64 (5.39)	10.34 (2.14)	15.65	***	4.95
	E	P			
	M (SD)	M (SD)	t	p	d
SUM PAH	1230.30 (434.14)	1560.65 (754.36)	1.7	0.098	0.54
S_{max} [%]	18.92 (6.72)	22.07 (14.15)	0.9	0.374	0.28
	Clean E	Polluted E			
	M (SD)	M (SD)	t	p	d
SUM PAH	898.49 (273.85)	1562.12 (279.71)	5.36	***	2.4
S_{max} [%]	25.44 (0.75)	12.39 (0.45)	47.15	***	21.09
	Clean P	Polluted P			
	M (SD)	M (SD)	t	p	d
SUM PAH	863.55 (176.53)	2257.75 (300.47)	12.65	***	5.66
S_{max} [%]	35.84 (0.80)	8.29 (0.39)	98.15	***	43.89
	Clean E	Clean P			
	M (SD)	M (SD)	t	p	d
SUM PAH	898.49 (273.85)	863.55 (176.53)	0.34	0.738	0.15
S_{max} [%]	25.44 (0.75)	35.84 (0.80)	29.97	***	13.4
	Polluted E	Polluted P			
	M (SD)	M (SD)	t	p	d
SUM PAH	1562.12 (279.71)	2257.75 (300.47)	5.36	***	2.4
S_{max} [%]	12.39 (0.45)	8.29 (0.39)	21.96	***	9.82

M – mean, SD – standard deviation, p – statistical significance, d – Cohen's *d*, *** – $p < 0.001$; SUM_PAH – total concentration of polycyclic aromatic compounds; S_{max} – maximum water storage capacity; P – *Platismatia glauca*; E – *Pseudevernia furfuracea*