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Implication of vitality, seasonality and specific leaf area on PAH uptake in moss and lichen transplanted in bags

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ABSTRACT

In this work the moss *Hypnum cupressiforme* and the lichen *Pseudevernia furfuracea* were exposed in bags for six weeks alive and oven-devitalized during summer and winter; the content of 24 PAHs was quantified to evaluate the effect of vitality, seasonality and specific leaf area (SLA) on PAH uptake and profiling. Vitality was followed throughout the exposure by measuring PSII maximal photochemical efficiency (F_v/F_m). In summer, a limited PAH signal was detected, with no significant increase, or even loss, of these compounds. During winter, a significant increase of PAHs was measured in both biomonitors, especially in those devitalized, with a lower baseline PAH content compared to alive material. This result suggests that PAH uptake mostly relies on passive mechanisms. Accordingly, F_v/F_m demonstrated that moss and lichen exposed alive spent most of the exposure time in cryptobiosis. In both biomonitors 4-rings PAHs prevailed, followed by 2-3-rings in lichen and 5-6-rings in moss. Lichen performed better than moss, due to the ability to entrap PAHs in the body of thalli, preserving these compounds during the exposure. A formula was developed to express the accumulated PAHs in terms of flux, that resulted higher in lichen than in moss. Oven devitalized lichen exposed in winter provided the highest uptake, indicating that morphology, SLA and seasonality represent key parameters in PAH biomonitoring.

1. Introduction

Although all industrialized world and emerging countries recognize air pollution as a crucial problem, to be addressed by strategic environmental policies, air monitoring is still insufficiently considered due to the high costs and application restrictions. Further, the variability in air pollution patterns, underline the urgency of feasible approaches aimed to extensive screening of pollutants (De Nicola et al., 2016). Autochthonous and transplanted mosses and lichens have been exploited for several decades as biomonitors of atmospheric pollutants, taking advantage of their thallous structure, allowing the adsorption/absorption of wet and dry atmospheric depositions on the entire surface. For metals and metalloids, it is widely assessed that the uptake ability of mosses and lichens is related to their high surface to mass ratio, their capacity to intercept and retain particulate matter (Di Palma et al., 2017; Spagnuolo et al., 2013) and the specific characteristics of cell walls (González and Pokrovsky, 2014; González et al., 2016). Therefore, moss and lichen vitality does not affect metal uptake (Tretiach et al., 2007), since most of these elements are passively adsorbed to the thalli in form of PM.

Compared to metals, organic pollutants have been poorly investigated so far (e.g., Dolegowska and Migaszewski, 2011; Ötvös et al., 2004); among the organic pollutants, PAHs are widely distributed and are regarded as persistent organic contaminants in the environment (Ravindra et al., 2008). Of the total PAH emission into the urban environment, 40% is scavenged by vegetation via dry and wet deposition (Collins and Finnegan, 2010; Lehndorff and Schwark, 2004). Although a small fraction of PAHs can move from the soil to plant tissue, most of airborne PAHs enter vascular plants via stomata or via cuticle waxes; due to the large surface area, the foliar interface is considered as the main access for organic chemical accumulation (Desalme et al., 2013; Terzaghi et al., 2015). As for PAH detection by mosses and lichens, few, sometime contrasting data are available (Augusto et al., 2013, 2015; Capozzi et al., 2017; De Nicola et al., 2013; Keyte et al., 2009; Spagnuolo et al., 2017). In a one-year long experiment, Foan et al., (2015) demonstrated that PAH bioconcentration factor in mosses was significantly related to the water solubility (log K_{OW}) of these compounds. The absence of stomata and cuticle, in addition to the semi-volatile nature of PAHs (i.e., their partitioning between vapor and particle phases according to their molecular weight, temperature and

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humidity, and water solubility) make it difficult to predict the entrance routes of these pollutants by the moss and lichen thalli. Moreover, while the recent literature on the cell wall of mosses highlights the presence of several functional groups able to favor metal interception and adsorption (González and Pokrovsky, 2014; González et al., 2016), PAH interception and retention mechanisms by mosses and lichens could be different due to the prevalent hydrophobic nature of these compounds. Therefore, it appears unclear if mosses and lichens can be efficient biomonitors of atmospheric PAHs, especially when used as transplants.

In a recent study, Capozzi et al. (2017) tested devitalized *H. cupressiforme* exposed in bags in ten sites of southern Italy, set in proximity of random recurrent burnings; they evidenced that moss transplants could uptake high molecular weight (HMW) PAHs, but failed to accumulate low molecular weight (LMW) PAHs. This aspect was likely related to specific morphological features, as the leaves consisting of a single cell layer, and the absence of waxy cuticles, both traits impeding PAH uptake and their preservation in the cells. On the contrary, the ability to accumulate HMW PAHs relied on the well-known capacity of mosses to entrap and passively uptake particulate matter of various composition.

The use of devitalized moss and lichen was developed and applied in many experiments in the recent years (Adamo et al., 2011; Capozzi et al., 2016; De Nicola et al., 2013); this approach, sharing the advantage of bags ready to use, demonstrated highly reproducible and with lower internal variability of replicas, at least for metals and metalloids. But, when used to quantify organic molecules as PAHs, criticism could be raised to the approach with devitalized moss and lichen, which excludes all the uptake mechanisms linked to vitality.

Lichens and mosses have been studied in deep, concerning mechanisms related to desiccation tolerance (Proctor, 2000; Kranner et al., 2008). Many bryophytes and lichens can withstand drying to water contents of 5–10% of their dry weight. As soon as water is available, these organisms can recover quickly, within a few minutes or hours, and switch to normal metabolism (Oliver et al., 1998). Chlorophyll fluorescence provides a rapid and non-invasive means of exploring aspects of the behavior of the photosynthetic system during and following environmental stress (Lichtenthaler and Rinderle, 1988; Maxwell and Johnson, 2000). To understand if devitalized moss and lichen are good biomonitors of air pollution also in the case of PAHs or if, losing vitality, these organisms drop part of their ability to accumulate PAHs, we set up an experiment aimed to: i) test the effect of vitality on the uptake and profiling of PAHs in transplanted moss and lichen; ii) compare PAH uptake in both biomonitors during summer and winter; iii) follow how biomonitor vitality was affected during the exposure duration, using photosynthetic parameters as proxy. Moreover, for comparison with data from physical chemical devices, a formula expressing the accumulated PAHs in terms of flux was implemented.

2. Materials and methods

2.1. Experimental design, bags preparation and exposure

The species used for this study were the moss *Hypnum cupressiforme* Hedw. and the lichen *Pseudevernia furfuracea* (L.) Zopf. Moss carpets for summer exposure were collected from tree trunks (*Fagus sylvatica* L.) at Montevergine, (40°59′38.69″N; 14°41′4.53″E – 850–950 m a.s.l.); moss carpets for winter exposure were collected at Taburno-Camposauro Regional Park on barks of *F. sylvatica* (1000 m a.s.l. – 41°6′18.34″N; 14°35′36.81″E). Lichen thalli were collected from trunks of *Abies alba* Mill. at Monte Matese (41°27′49.14″N; 14°23′5.71″E – 1650 m a.s.l.). The collection sites are proximal natural sites, far, at least 5 km, from known sources of pollution; the collections were completed in a single day.

Moss and lichens were brought to laboratory and manually cleaned; only green and brown-green moss shoots (distal 4–5 cm) were used for bag preparation. Distal lobes of lichen (3–5 cm) were selected and treated as for moss. Moss and lichen were extensively washed and homogenized in distilled water according to Tretiach et al. (2007) and Ares et al. (2012), to obtain clean moss and lichen material. At this stage, the two biomonitors were partitioned in two aliquots of approximately 100 g each; one moss and lichen aliquot was devitalized in a dry oven al 100 °C for 24 h to obtain dead moss and lichen (OM and OL, respectively); another aliquot was left alive to obtain (WM and WL, respectively).

Eight samples of dead (oven devitalized, O) and alive (washed, W) moss and lichen materials (OM, OL, WM, WL), were analyzed prior to exposure to assess the baseline PAH contents, and to determine the after-exposure enrichment of moss and lichen with reference to the respective pre-exposure biomaterial.

According to the guidelines for standardization of bag preparation and exposure (Capozzi et al. 2016), subspherical bags were prepared with 2.5 g of each bio-material ensuring a density inside the bags of 15 mg cm^{-1}. Eight bags of each biomaterial were suspended on a latticework by nylon strings and exposed for 6 weeks in open air, without any artificial shading, on the roof of the Biology Department building in the urban area of Naples city. One additional bag filled with WM and WL was also exposed and used for the evaluation of biomonitor vitality during the exposure, by photochemical measurements (see paragraph below). The exposure started in June 2017 and was repeated for comparison in January 2018, to test how PAH uptake ability was affected by seasonality. Details on meteorological parameters recorded during the two exposure periods in the study area, are given in Table 1.

In the second exposure (winter), based on the results obtained in summer, the experimental design was simplified: lichen was exposed both alive and after oven treatment (WL and OL), while moss was exposed only devitalized (OM). The lichen used for this exposure was a sub-sample of the spring 2017 collection, stored in cold greenhouse and its vitality was checked as explained below (see paragraph 2.5).

After exposure, moss and lichen materials were removed from bags, dried at 20 °C, manually milled and homogenized with a ceramic mortar and pestle using liquid nitrogen and stored in the dark at 4 °C before the analyses.

2.2. PAH analyses

According to the protocols EPA 3550 C 2007 and EPA 8270 D 2014 for PAH analyses, two grams of each biomaterial were sonicated twice by a Falc Sonicator, in 25 mL of dichloromethane for 20 min each. The extracts, purified through activated silica gel, were dried to a volume of 200 mL under a gentle nitrogen stream. Afterwards, the samples were analyzed by GCeMSD (Agilent 5975C with a VF-17MS column) with helium as gas carrier at 1.3 mL min^{-1}. The oven temperature program started at 50 °C, increased with ramp rate 30 °C min^{-1}, to 350 °C and held for 9 min. All analyses were performed in selected ion monitoring (SIM). The analyses were performed by the certified Mérieux NutriSciences Company (Resana, Italy).

Table 1
Summary of meteorological parameters during the exposure periods.

		Summer	Winter
T (°C)	Min	20.9	6.4
	Mean ± SD	27.7 ± 1.8	10.5 ± 2.3
	Max	30.1	14.3
Rain	Days	4	25
	mm/day	< 10	> 10
Sky	Days	96% Sunny	86% Cloudy
RH (%)	Mean ± SD	58.4 ± 10.2	71.3 ± 9.6
WIND (km/h)	Mean ± SD	9.6 ± 3.2	9.1 ± 4.9
	Max	20.2	19.8
	Main direction	SSW	ENE

The concentration of the following 24 PAHs were quantified by multi-point calibration curves and labelled internal standards; (2-rings): naphthalene (Naph); 2-methylnaphtalene (2M-Naph); (3-rings): acenaphthene (Ace), acenaphthylene (Acy), fluorene (Flu), phenanthrene (Phen), anthracene (Ant); (4-rings): fluoranthene (Flt), pyrene (Pyr), perylene (Prl), benz[a]anthracene (B[a]A); chrysene (Chrys), (5-rings): benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[j] fluoranthene (B[j]F), benzo[a]pyrene (B[a]P), benzo[e]pyrene (B[e]P), dibenzo[a,h]anthracene (DB[a,h]A), (6-rings): benzo[g,h,i]perylene (B[g,h,i]P), indeno[1,2,3-c,d]pyrene (IP), dibenzo[a,i]pyrene (DB[a,i]P), dibenzo[a,h]pyrene (DB[a,h]P), dibenzo[a,e]pyrene (DB[a,e]P), dibenzo[a,l]pyrene (DB[a,l]P).

Labelled PAHs (naphthalene D8, acenaphthene D10, phenanthrene D10, chrysene D12, perylene D12) were used as surrogates for the quality control of the procedure and their recovery percentages (from 82% to 120%) were included to correct the concentration of each compound. The minimum detectable PAH concentration was 1 ng g^{-1} d.w. for each compound.

2.3. Calculation of specific leaf area and deposition flux for moss and lichen

Twenty individual dry moss shoots were randomly collected and weighted; then, for each shoot the average number of leaves was counted by the aid of a stereomicroscope Leica M8. Afterword, 20 leaves per shoot were randomly selected, photographed, and pictures acquired by a Leica LMD 6500 dissecting light microscope. The photographs were then processed using the free source ImageJ software to calculate the leaf area and the specific leaf area according to the formula:

SLA = Leaf area (m^2)/dry weight (g)

For the lichen, 20 dried thalli were selected, individually weighed, and photographed by the aid of a scanner. The acquired pictures were similarly processed by the ImageJ software to calculate SLA.

Here we propose a formula to calculate the pollutant deposition flux, or daily uptake, starting from the data obtained by the biomonitoring (i.e. a mass to mass ratio, usually ppm or ppb, that represents the concentration of a certain pollutant per unit mass of biological tissue) in order to facilitate the comparison with other non-biological monitoring devices.

The flux was calculated using the following formula:

$$\mathbf{\Theta_{DF}} = \mathbf{M_{acc}}/(\mathbf{S} * \mathbf{d})$$

where $\mathbf{\Theta_{DF}}$ is the deposition flux [$ng*m^{-2}*d^{-1}$]; $\mathbf{M_{acc}}$ is the amount of each pollutant (expressed as *ng*) accumulated during the exposure period, obtained by subtracting the pollutant amount before exposure M_0 from that measured after the exposure M_f; **S** is the exposed surface of the organism (i.e., leaf area), expressed as m^2; **d** is the duration of exposure, expressed as days.

Since **S** is directly linked to the **SLA** (see the above paragraph), the same deposition flux can be quickly obtained by dividing the pollutant concentration $\mathbf{[M]_{acc}}$ (expressed as $ng\,g^{-1}$) by the 2*SLA ($cm^2\,g^{-1}$) multiplied for the day of exposure (**d**): $\Theta_{DF} = \mathbf{[M]_{acc}}*(2*\mathbf{SLA}*\mathbf{d})^{-1}$

2.4. Assessment of vitality in moss and lichen

The vitality assessment was studied through two experiments carried out during both summer and winter exposures. In the first experiment we built vitality response curves for control (pre-exposed) and for mosses and lichens exposed in bags in open air (WL and WM). Aliquots of control samples, coming from pristine sites, were dried in an oven at 38 ± 2 °C for almost one hour until constant weight and then subjected to rehydration dynamics in controlled laboratory conditions in order to assess the intrinsic capability of recovery. The rehydration of samples was obtained in relative humidity (RH%) saturated atmosphere including the lichen lobes and the moss shoots in a closed box filled with wet paper, sprayed with distilled water. The vitality of moss and lichen was measured on fully dried samples (time t = 0) and at different time intervals (2, 5, 12, 20, 30, 40 and 90 min) up to 90 min after rehydration, monitoring the PSII maximal photochemical efficiency (F_v/F_m), together with the fresh weight of samples. To measure F_v/F_m on the same spot at each interval of time, the samples were marked with a narrow adhesive ribbon.

To assess the rehydration degree, the samples were weighed at each time, and chlorophyll fluorescence emission measurements were performed. These measurements were considered as a reference for the kinetic of recovery for the samples exposed to open air in moss and lichen bags.

Moss and lichen samples exposed in bags in open air (WM and WL) were periodically collected (after 1, 2, 3, 4 and 5 weeks from exposure) carried in laboratory, immediately weighed and subjected to fluorescence emission measurements (t = 0). After 15, 40, 60, 70, 90 min after rehydration in vapor saturated atmosphere, the samples of both species were again weighed and F_v/F_m measured to assess the vitality. As no difference was found in the kinetics of recovery of F_v/F_m and fresh weight in samples after each week of exposure, a single graph was built considering the behavior of both species during the time of recovery from desiccation.

In the second experiment, the kinetic of dehydration was evaluated; to this aim the rehydrated moss and lichen after each acquisition of F_v/F_m and fresh weight, were put again in their bags and exposed in open air to monitor the time needed for their complete desiccation. The kinetics of dehydration was followed by F_v/F_m and fresh weight measurements until the complete drying of the samples.

The chlorophyll *a* fluorescence emission was measured by means of a Pulse-Amplitude-Modulated fluorometer (Junior-PAM, Walz, Germany) supplied with a monitoring Leaf-Clip JUNIOR-B (Walz, Germany), on 30′ dark-adapted samples. The background fluorescence signal, F_o, was induced on 30 min dark adapted thalli, by a blue LED internal light of about 2–3 µmol photons $m^{-2}\,s^{-1}$, at a frequency of 0.5 kHz. The maximal fluorescence level in the dark-adapted state (F_m) was induced by 1 s saturating light pulse (8000 µmol photons $m^{-2}\,s^{-1}$) at a frequency of 10 kHz; the maximal PSII photochemical efficiency (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_o)/F_m$ (Maxwell and Johnson, 2000).

2.5. Data analysis

Microsoft Excel and STATISTICA ver. 8.0 (StatSoft, Inc. 2008) software were used to process the data. Shapiro Wilk's test and Levene's test were used to assess the normality and homogeneity of the variances, respectively. Analysis of Variance (ANOVA) was used to assess the differences among the different theses; in case of rejection of the null hypothesis the Tukey's significance post-hoc test was applied ($p < 0.05$).

3. Results

3.1. PAH contents in lichen and moss in summer and winter exposures

Average PAH contents in moss and lichen before and after summer and winter exposures are reported in Table 2 and 1s (supplementary table), whereas PAH accumulation in both seasons is shown in Fig. 1. Based on the contents, a different PAH profile was observed in the lichen depending on the pre-exposure treatment: WL showed contents of total and 4-rings PAHs significantly higher than OL; oven-devitalization induced a loss of 2-3 and 4-rings PAHs, also affecting the total PAH content (see Table 2, 234 ng g^{-1} in OL vs. 372 in WL). In summer, a general, but not significant decline of PAHs was observed in WL after the exposure (see Table 2, 372 ng g^{-1} vs. 326 ng g^{-1} and Fig. 1), especially concerning 2-3 rings PAHs. By contrast, a moderate not significant increment was observed in OL (see Table 2, 234 ng g^{-1} vs.

Table 2
Average PAH contents (ng g^{-1} DW) in moss and lichen in summer and winter exposure. Each value represents the mean ± SD, n = 8. Different letters indicate significant differences among the treatments according to Tukey's post-hoc test.

	Σ2-3 rings	Σ4 rings	Σ5-6 rings	ΣPAH
	Summer exposure			
WL_pre	184.5 ± 12.6b	178.0 ± 20.6c	10.8 ± 1.5d	372.3 ± 22.1c
OL_pre	131.5 ± 14.3bc	91.5 ± 8.5ef	12.1 ± 2.1cd	234.3 ± 10.6de
WL_post	142.2 ± 16.4bc	170.8 ± 17.7c	13.1 ± 2.0cd	326.4 ± 35.2c
OL_post	127.3 ± 15.5bc	116.8 ± 16.4de	16.2 ± 1.6cd	260.2 ± 26.1d
WM_pre	120.5 ± 15.8bc	58.8 ± 7.6f	34.1 ± 4.6ab	213.3 ± 7.2de
OM_pre	127.1 ± 18.2bc	68.3 ± 9.8f	39.5 ± 8.6a	234.8 ± 30.2de
WM_post	105.8 ± 16.3c	59.0 ± 11.6f	28.5 ± 5.3ab	193.2 ± 27.3e
OM_post	99.0 ± 16.4c	60.1 ± 9.0f	35.3 ± 3.7a	194.3 ± 25.3e
	Winter exposure			
WL_pre	184.5 ± 12.6b	178.0 ± 20.6c	10.8 ± 1.5d	372.3 ± 22.1c
OL_pre	131.5 ± 14.3bc	91.5 ± 8.5ef	12.1 ± 2.1cd	234.3 ± 10.6de
WL_post	271.6 ± 67.4a	365.3 ± 11.9b	29.2 ± 4.3ab	665.9 ± 39.3b
OL_post	258.2 ± 86.4a	451.9 ± 29.4a	30.4 ± 5.2ab	740.5 ± 69.2a
OM_pre	77.4 ± 35.4c	77.35 ± 35.7f	22.9 ± 3.4bc	177.6 ± 33.9e
OM_post	80.1 ± 22.5c	144.8 ± 23.5 cd	37.1 ± 3.6a	262.0 ± 20.1d

260 ng g^{-1} and Fig. 1) depending on the increase of 4-6 rings PAHs.

Both PAH profiles and contents were at all comparable in pre-exposure WM and OM (see Table 2 total PAH content 213 and 235 ng g^{-1} respectively), in which 2–3 rings PAHs represented the main fraction, followed by 4-rings and 5–6 rings. In summer, a limited, not significant loss of PAHs was observed after the exposure in both materials (see Table 2, 193 and 194 ng g^{-1} and Fig. 1), especially concerning 2-3 rings PAHs.

In winter, both WL and OL significantly accumulated PAHs during the exposure, particularly the oven-devitalized material, showing a total PAH content of 740 vs. 666 ng g^{-1} found in WL. Oven-devitalized lichen showed contents two folds (for 2-3 rings) and four folds (for 4-rings) higher than pre-exposure contents, while the 6-rings PAHs were substantially unchanged after the exposure (Fig. 1). In OM a significant increase was observed after the exposure (177 vs. 262 ng g^{-1} and Fig. 1), specially concerning 4-rings PAHs which doubled their concentration.

3.2. Lichen vs. moss

The comparison lichen vs. moss was based on the winter exposure, because during the summer, PAH loss prevailed on the accumulation. All the biomaterials showed higher accumulation performances in winter ($p < 0.05$); in case of loss of individual PAHs, no significant difference occurred between the pre- and post-exposed contents ($p > 0.05$; see Table 1s).

In general, based on PAH content, lichen performed better than moss ($p < 0.05$, see Fig. 1; Tables 2 and 1s); the oven-devitalization in lichen, lowered PAH baseline content, (significant for Flu, Phen, Ant, and Flt, $p < 0.05$), allowing this biomaterial to measure the highest significant increment during the 6 weeks exposure. Accordingly, based on the average accumulation of the single PAHs (Post-exposure minus pre-exposure contents; Table 3), OM showed the highest accumulation only for two PAHs, WL for 6 and OL for 10 PAHs; therefore, OL had overall the best accumulation performance. Considering the relative percentages of PAH – grouped by rings – accumulated in moss and lichen during the winter (Fig. 2), it is evident that both biomonitors mainly accumulated 4-rings PAHs, 71 and 80% respectively in lichen and moss; conversely, lichen accumulated LMW PAHs (25%), while moss HMW PAHs (17%).

3.3. SLA and deposition flux

The calculation of the SLA for the moss *H. cupressiforme* and the

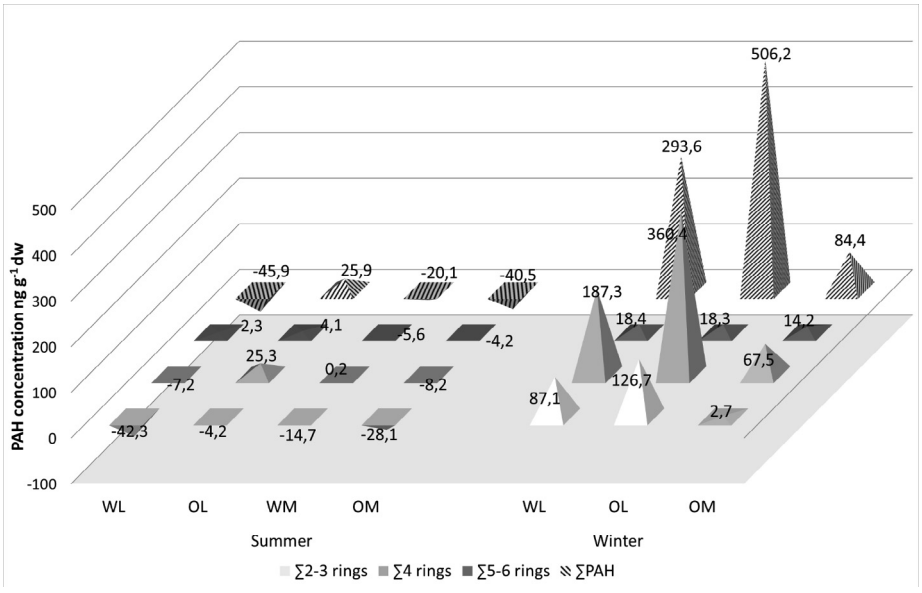

Fig. 1. PAH accumulation (post- minus pre-exposure values) in lichen and moss alive (WL, WM) and devitalized (OL, OM) exposed during summer and winter.

Table 3
PAH accumulation (post- minus pre- exposure contents, ng g^{-1} dw) in OM, WL and OL exposed in winter; maximum values are marked in grey.

	OM	WL	OL
Naph	-8.9	51.5	77.75
2-M-Naph	-1	35	18.7
Acy	-1.7	-1.5	12
Ace	1	-0.2	2.5
Flu	-2.9	-2.1	-1.3
Phen	16	5.3	15
Ant	0.3	-1.5	1.8
Flt	25.6	48.9	127.2
Pyr	29.9	117	205.4
B[a]A	3.7	5.7	6.9
Chrys	11.7	20.7	20.8
B[b]F	2.8	5.9	4.3
B[k]F	1.1	1.8	1.6
B[j]F	1.7	3.8	2.7
B[e]P	4.65	-2	-2
B[a]P	0.75		
IP	1.1	2.5	2.3
B[g,h,i]P	1.7	7.5	8.8

lichen *P. furfuracea* is summarized in Table 4; the deposition flux based on the accumulation of PAH during the exposure (post- minus pre-exposure contents; Table 5) was calculated only on oven devitalized materials exposed in winter, because for these materials a significant accumulation was measured. Deposition flux can be obtained by PAH accumulation for all plant materials and SLA measured in moss and lichen according to the formula reported in the paragraph 2.3; however, to provide a summary picture of PAH flux in the two biomonitors, examples of this calculation for single, illustrative PAHs per each n-ring number type (2-3- 4-5- rings), in addition to the summation of each PAH class (2-3 rings, 4 and 5-6 rings) and total PAHs, are given in Table 5.

The SLA mean value of moss was about five folds that of lichen; thus, when considering the deposition flux, all values are far higher (in some cases more than one order of magnitude) for lichen compared to moss.

3.4. Assessment of vitality

Table 6 lists F_v/F_m and fresh weight values measured in fully rehydrated pre-exposed moss and lichen. The monitoring was followed for 90 min. The reference values of F_v/F_m measured before exposure in moss and lichen collected fresh from the field in a pristine area in moist conditions, were respectively 0.729 ± 0.041 and 0.557 ± 0.023, for the same samples the weights in grams were 28.34 ± 2.02 and 11.10 ± 0.92.

Table 4
Estimated SLA*2 (expressed as $m^2\ g^{-1}$) for the two biomonitors; n = 20.

Species	Min	Mean ± SD	Max
Hypnum cupressiforme	0.094	0.135 ± 0.061	0.280
Pseudevernia furfuracea	0.021	0.026 ± 0.004	0.034

The kinetics of rehydration were different in moss and lichen. More specifically, the maximal PSII photochemical efficiency (F_v/F_m) was reached after 90 min from moisturizing in the moss (0,714) and just after 20 min in the lichen (0,554). As regards the fresh weight, the original weights were recovered after 30 min in the lichen (9,99 g), and at 90 min in the moss (27,60 g) (Table 6).

Fig. 3 shows the values of fresh weight and F_v/F_m measured in dried moss and lichen exposed in bags (WM and WL) in open air soon after sampling and at different times from remoistening. The fresh weight in both species reached the steady state after 40′ from rehydration; in fact, no further increase of fresh weight was observed up to 90′ (Fig. 3, bars). On the contrary, moss and lichen showed different kinetics in F_v/F_m: more specifically, in the lichen this parameter recovered after 40′ from rehydration, whereas in the moss it was not restored, even after 90′ (Fig. 3, lines). It is noteworthy that after 24 h from rehydration, both species showed a full recovery of F_v/F_m compared to pre-exposed condition (data not shown).

The dehydration experiments showed very rapid drop (within 15′) of the fresh weight in the lichen compared to the moss (Fig. 3); in parallel also a decline of F_v/F_m was observed. Conversely, the moss remained moistened, with a minimal photochemical activity up to 80′, confirming the resistance of this species to desiccation. As for the experiment carried out during the winter exposure, no significant difference was observed in both transplanted species in the kinetics of fresh weight and F_v/F_m (data not shown).

4. Discussion

4.1. PAH accumulation and profile in the two biomonitors

After emission, PAHs can accumulate in plants through absorption and adsorption mechanisms depending on their availability in the environment, physical chemical properties (e.g., the partitioning between vapor and particle phases, the K_{OW} coefficient), environmental conditions (e.g., temperature, humidity, radiation) and plant traits (Harmens et al., 2013; Loppi et al., 2015). These parameters influence the levels of PAHs and their profile in a variable manner in a given species, even collected in relatively close background areas (Gerdol et al., 2002).

The total PAH pre-exposure contents in our samples were in line with the contents reported by Harmens et al. (2013) for mosses from background areas, in which the average concentration for up to 17 PAHs ranged from ca. 100 to 600 ng g^{-1} dry wt. In addition, according to Gerdol et al. (2002), in pre-exposure material collected in pristine

Fig. 2. Lichen and moss PAH uptake profile calculated on devitalized materials (OL and OM) exposed in winter. Light grey: 2-3 rings; medium grey: 4- rings; deep grey: 5-6 rings PAHs.

Table 5
Deposition flux [Θ_{DF} – $ng*m^{-2}*d^{-1}$] for moss and lichen calculated for some illustrative PAHs, PAHs grouped by rings and total PAHs.

	Naph	Phen	Chrys	B(b)F	Σ2-3 rings	Σ4 rings	Σ5-6 rings	ΣPAH
OM_W	0.00	2.82	2.06	0.49	0.53	11.99	2.50	14.81
OL_W	71.20	13.74	19.05	3.94	116.30	250.92	16.76	463.37

Table 6
Moss and lichen fresh weight and maximal PSII photochemical efficiency (F_v/F_m) measured in laboratory soon after desiccation in oven at 38 ± 2 °C (t = 0) and at different time intervals from rehydration in control healthy individuals. Each value is the mean ± SE (n = 3).

	Moss		Lichen	
Time (min)	Fresh weight (g)	F_v/F_m	Fresh weight (g)	F_v/F_m
0	8.16 ± 1.23	0.042 ± 0.014	3.26 ± 0.65	0.011 ± 0.012
2	13.26 ± 1.34	0.475 ± 0.031	7.27 ± 1.02	0.459 ± 0.031
5	13.89 ± 1.13	0.489 ± 0.201	8.01 ± 0.99	0.461 ± 0.041
12	15.88 ± 2.00	0.603 ± 0.021	8.68 ± 1.00	0.498 ± 0.021
20	16.89 ± 1.46	0.614 ± 0.031	9.10 ± 0.89	0.554 ± 0.040
30	18.00 ± 1.87	0.625 ± 0.031	9.99 ± 1.29	0.501 ± 0.031
40	19.02 ± 2.20	0.644 ± 0.024	10.75 ± 2.12	0.545 ± 0.019
90	27.60 ± 2.45	0.714 ± 0.031	10.81 ± 1.92	0.546 ± 0.023

sites, the greater fraction was represented by LMW PAHs. The different content of PAHs in pre-exposed mosses, noticeably higher in those collected in spring at Montevergine (213 ng g^{-1}) than in those collected in autumn at Taburno mountain (177 ng g^{-1}), could depend on the different deposition rate in the two sites, but also on the different season. The higher PAH content in moss collected in spring could reflect PAHs accumulated during the preceding winter, whereas the lower PAH content in mosses collected in autumn could be affected by PAH degradation and loss occurred in the preceding summer (Harmens et al., 2013). In line with the total PAH content measured in the present work in native lichen, Augusto et al. (2013) found concentrations of 16 EPA-PAHs in lichens between 58 and 556 ng g^{-1}, with the highest values found during the coldest winter months.

The devitalization effect in moss and lichen (OM and OL) produce biomaterials with a lower baseline content of PAHs, compared to the living counterparts (WM and WL), and, thus, higher sensitivity to detect PAH inputs during the exposure period.

Moss and lichen showed different profiles of accumulated PAHs: in both species, the prevalent accumulated fraction is represented by 4-rings PAHs; for the remaining fractions, moss mainly accumulate 5-6 rings while lichen mostly 2-3 rings PAHs. These results agree with previous reports; specifically, for lichens it is known that PAH profile is mostly dominated by 2-3-, and 4-rings PAHs (Augusto et al., 2013). Differences in thallus morphology between the two biomonitors could explain this result; mosses having typically single-cell-layered leaves, do not accumulate 2-3 rings PAHs mainly in vapor phase, but they have the capacity to retain PAHs in particle phase due to their high surface to mass ratio and cell wall chemical properties (González and Pokrovsky, 2014; Tretiach et al., 2007). In lichens, instead, the structure of cortex and medulla allows for the entrapment and staying of PAHs in vapor phase. This observation well agrees with a previous study based on laboratory experiments, showing that in *Xanthoria parietina* gas phase PAHs easily cross lichen surface and accumulate in the thallus (Augusto et al., 2015). Although the mechanisms of interception of PAHs by plant surface are not completely exploited, a feasible hypothesis could be the adsorption on thallus surface by weak interactions as for example, p-interactions between benzene rings of PAHs and polar molecules of the cell wall, like phenolic compounds and acid polysaccharides (Capozzi et al. 2018).

The different PAH profile found in moss and lichen transplanted in bags in urban context seems to conflict with PAH content and profiles reported for species from natural background areas, where both moss and lichen prevalently contain 2-3 rings PAHs. It is assessed that PAHs in the gaseous phase are generally transported to pristine areas, far from pollution sources, while HMW PAHs are generally deposited in larger proportions near emission sources, like urban and industrial areas (Thomas, 1986). The discrepancy observed between PAH profiles found in native and transplanted biomonitors could depend on the different traits of natural and anthropogenic environments, the first characterized by climatic conditions preserving the permanence of PAHs in vapor phase, the latter promoting their decomposition due to higher temperature and radiation. This last aspect also affects the different levels of PAHs found in the two exposures, higher in winter compared to summer. Meteorological parameters recorded during the exposure periods support the seasonal variations observed; e.g., mean

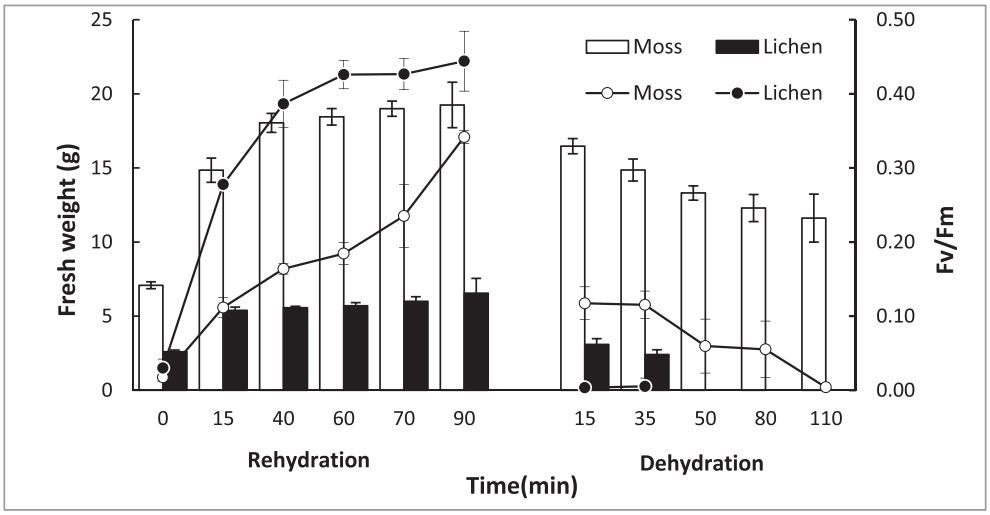

Fig. 3. Evaluation of vitality of moss and lichen exposed in bags during rehydration and dehydration cycles, expressed as F_v/F_m (lines) and fresh weight (bars). See text for further details.

temperature was more than twice in summer compared to winter, and sunny days were 96% in summer exposure and only 14% in winter exposure (Table 1).

4.2. Deposition flux

In this work we suggested a formula to calculate a deposition flux (i.e. a daily uptake normalized to SLA), to provide a conversion of the uptake expressed as mass to mass, into a flux expressed as mass to surface; this step is necessary in view of an intercalibration between bioaccumulators and conventional deposimeters. The values calculated for SLA in *H. cupressiforme* and *P. furfuracea* well agree with those previously reported for the same species (Adamo et al., 2007). The higher values of deposition flux found in the lichen compared to moss depend on the higher thallus thickness (inversely related to SLA), allowing for the accumulation of PAHs (especially LMW) in the body of thalli. Specific leaf area was considered as an ecological trait proportionally linked to uptake of PAHs in vascular plants (Terzaghi et al., 2015). Under this respect, lichen thalli behave as leaves of higher plants; by contrast, moss is loser since its leaves are only one cell thick; this feature can explain the loss of LMW PAHs in moss during the exposure and the consequent null flux for these compounds (e.g. Naph). Of course, fluxes reflect PAH accumulation, with 4-rings PAHs representing the most abundant fraction for both biomonitors. When considering HMW PAHs, their accumulation is strongly dependent on biomonitor surface, significantly higher in moss than in lichen (Adamo et al., 2007), since these compounds are mostly in particle phase.

4.3. PAH accumulation and vitality

One of the aims of the present work was to understand if and how the vitality of the two transplanted biomonitors was linked to PAH uptake and accumulation using photochemical efficiency as an indicator of vitality. The fluorescence index maximal PSII photochemical efficiency (Fv/Fm) can be considered a good proxy of the impairment of photosynthetic function following desiccation, and recovery subsequent the rehydration (Seel et al., 1992; Tuba et al., 1996; Csintalan et al 1999; Tretiach et al., 2007). In parallel, rehydration can be easily evaluated through the measurement of the fresh weight. Surprisingly, these two parameters not only provided different response in moss and lichen, but their kinetics were not synchronized. Our results indicate indeed, that the completeness and subsequent course of the recovery varied from one species to another. During the rehydration, the moss has a tardive photochemical recovery compared to lichen even if the tissues restore the moisturizing in about 40′. The lichen appears more resilient than moss from a photochemical point of view, showing a substantial restoration of the vitality after 15′ rehydration. Notwithstanding, the two species, when exposed in bags, loose their vitality (within few minutes the lichen, less rapidly the moss), entering a cryptobiotic state. In this situation, the uptake and accumulation of PAHs can be considered as a mix of adsorption and absorption, both occurring by passive mechanisms, in which vitality is only marginally involved.

The vitality of these biomonitors is limited to a very narrow time frame, in which humidity conditions due to rain or fog are compatible with photosynthesis; in other words, for most or all exposure time alive cryptogams behave as dead biomonitors, thus not differently from the devitalized material. In agreement with our results, in a previous study Tretiach et al. (2007) found a decline of photosynthetic pigments, chlorophyll fluorescence and CO_2 gas exchange in the lichen *Pseudevernia furfuracea* exposed in bags and concluded that transplanted biomonitors are comparable to a “still life”. The papers focused on biomonitoring of air quality by “alive” moss and lichen in which bags are periodically sprayed with water (e.g., Sorbo et al., 2008) should be reconsidered in the light of these results, indicating that transplanted moss and lichen spent most or all exposure time in a cryptobiotic steady state even when sprayed.

The decline of vitality parameters in transplanted biomonitors during dehydration is an expected result, considering that even in natural condition many bryophytes and lichens can preserve their vitality by cryptobiosis when their water content is drastically reduced due to drought (Oliver et al., 1998). Surprisingly instead, oven devitalization, which irreversibly stops any active uptake mechanism, does not influence PAH accumulation within the lichen thalli, which results comparable in WL and OL for LMW and HMW PAHs (see Fig. 2 and Table 2); this supports the idea that the entrapment and retention of these compounds is principally grounded on mechanisms independent from vitality.

5. Conclusion

The above described experiments provide for the first time an overview on PAH accumulation in moss and lichen bags in relation to vitality and morphological traits; the implications of the exposure season and SLA were evaluated as well. The exposure modified PAH profile and determined a loss of PAHs in summer, due to high temperature and radiation; whereas a significant accumulation of PAHs occurred in winter, indicating that cold season is the most appropriate for PAH biomonitoring by transplants, and any comparison between surveys carried out in different seasons should be avoided. Lichens performed better than mosses in terms of accumulation and flux, due to their ability to entrap PAHs in the body of thalli, preserving the most labile fraction (2-3 rings) of PAHs during the exposure. According to morphological traits, lichen PAH profile mainly covers 2-4 rings PAHs (vapor and particle phase), whereas moss prevalently accumulates 4-6 rings PAHs (mostly particle phase). Oven devitalization does not reduce the uptake ability but drops the baseline PAH content enhancing the sensitivity of the biomaterial, allowing PAH detection even at low concentration in the environment. Specific leaf area must be considered as an important feature in the analysis of PAH uptake, since it considers both surface and thickness of thalli, two key traits to clarify accumulation mechanisms of these pollutants. Under this respect, vitality experiments highlighted that PAH accumulation takes place mainly by passive mechanisms, since biomonitors spend most of the exposure time in cryptobiosis, even when exposed alive. Due to the PAH profile found in lichen and moss transplants, partly overlapping, their combined use could be more appropriate for PAH biomonitoring in anthropized environments lacking natural species. Finally, the calculation of the daily deposition flux should be compared to passive samplers to test the real possibility to use transplants to assess PAH deposition and related human health risk.

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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.2019.105727.

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