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Spatial distribution of PAH concentrations and stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$) in mosses from three European areas – Characterization by multivariate analysis

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ABSTRACT

Polycyclic aromatic hydrocarbon (PAH) concentrations and N, C stable isotope signatures were determined in mosses *Hypnum cupressiforme* Hedw. from 61 sites of 3 European regions: Île-de-France (France); Navarra (Spain); the Swiss Plateau and Basel area (Switzerland). Total PAH concentrations of 100–700 ng g^{-1}, as well as $\delta^{13}C$ values of −32 to −29‰ and $\delta^{15}N$ values of −11 to −3‰ were measured. Pearson correlation tests revealed opposite trends between high molecular weight PAH (4–6 aromatic rings) content and $\delta^{13}C$ values. Partial Least Square regressions explained the very significant correlations ($r > 0.91$, $p < 0.001$) between high molecular weight PAH concentrations by local urban land use (<10 km) and environmental factors such as elevation and pluviometry. Finally, specific correlations between heavy metal and PAH concentrations were attributed to industrial emissions in Switzerland and road traffic emissions in Spain.

1. Introduction

Persistent organic pollutants (POPs) are defined as organic substances which are a concern for the environment and human health as they: possess toxic characteristics; are persistent; bioaccumulate; are prone to long-range transboundary atmospheric transport and deposition; are likely to cause significant adverse human health or environmental effects near to and distant from their source (UNECE, 1998). They are mainly of anthropogenic origin, show weak degradability and consequently are distributed in the environment worldwide, including in remote areas like Poles (Gustafsson et al., 2005). The combination of resistance to metabolism and lipophilicity means that POPs will accumulate in foodchains (Jones and de Voogt, 1999). The 1998 Aarhus Protocol on POPs (LRTAP Convention) and the 2001 Stockholm Convention on POPs – a global treaty under the United Nations Environment Programme (UNEP) – aim at eliminating and/or restricting the production and use of selected POPs.

Polycyclic aromatic hydrocarbons (PAHs) are a family of chemical compounds composed of carbon and hydrogen atoms which form at least two condensed aromatic rings. PAHs originate from fossil or non-fossil fuels by pyrolysis or pyrosynthesis. They are emitted into the atmosphere mainly from anthropogenic sources but they also originate from natural ones such as volcanic eruptions and forest fires (Simonich and Hites, 1995). The main sources of PAHs in the environment are aluminium production, coke production from coal, wood preservation and fossil fuel combustion (traffic, domestic heating, electricity production) (Wegener et al., 1992). PAHs are considered as POPs due to their low rates of degradation, toxicity and potential for both long-range transport and bioaccumulation in living organisms (Holoubek et al., 2007a). Regulation of PAH emissions and reliable monitoring of PAH concentration in ambient air is thus of paramount importance for public health.

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Despite recent effort to investigate PAH distribution and fate in air, water and soil, few studies have provided extensive information on spatial and temporal trends of PAH atmospheric deposition, due to high investment and running costs of adapted analytical devices. Biological monitoring is an alternative method consisting of an integrative technique able to assess the environment contamination based on studies of living organisms exposed to pollution. Because of their capacity to act as efficient interceptors and accumulators of chemicals, plants are widely used as passive biomonitors in urban and rural environments (Garrec and Van Haluwyn, 2002; Market et al., 2003). The most common biomonitor systems are conifer needles (Ratola et al., 2010), lichens (Blasco et al., 2011) and mosses (Harmens et al., 2013).

Bryophytes in particular have been employed over the past decades as biomonitors for the assessment of airborne deposition of heavy metals (Rühling and Tyler, 1969; Tyler, 1990; Harmens et al., 2012), radionuclides (Sumerling, 1984; Sawidis et al., 2009), POPs (Carlberg et al., 1983; Cipro et al., 2011), nitrogen (Solga et al., 2005; Harmens et al., 2011b) and stable isotopes of carbon, sulphur and nitrogen (Solga et al., 2005; Xiao et al., 2010; Cipro et al., 2011). Characteristics of mosses make them excellent subjects for biomonitoring. As they do not have any root system and barely no cuticle, mosses obtain most of their nutrients from the atmosphere (wet and dry deposition). Rhizoids can assure a part of mosses nitrogen nutrition by leading water from the soil to the plant by capillary external conduction (Schofield, 1981; Glime, 2007). However, Ayres et al. (2006) showed, for two different types of pleurocarpous mosses, that nitrogen assimilation from wet deposition is fairly more important than from the soil. Moreover, mosses high cationic exchange capacity and surface to volume ratio favour the accumulation of large amounts of pollutants (Gerdol et al., 2002a).

The present study takes part in a pilot project of the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP-Vegetation) established under the United Nation Economic Commission for Europe (UNECE) Convention on Long-Range Transboundary Air Pollution (LRTAP) to investigate the suitability of mosses as biomonitors of POPs at a large spatial scale (Harmens et al., 2011a). The participants of ICP-Vegetation have determined heavy metals and nitrogen contents in mosses sampled over Europe with a high spatial resolution (25 × 25 km) every 5 years (Rühling and Tyler, 1969; Tyler, 1990; Harmens et al., 2010, 2011b, 2012). The pilot study may lead to an extension of the programme to POPs, particularly to PAHs.

To further investigate the quantitative importance of different sources of deposition, the use of natural stable isotopes abundance levels in mosses provides a powerful approach for understanding environmental interactions. Isotope composition of elements, such as carbon and nitrogen, changes in predictable ways during their course through the biosphere, which makes them ideal tracers of the pathways and origins of these elements (Tcherkez, 2010). The ICP-Vegetation programme is therefore also focused on stable isotope signatures for studying atmospheric deposition (Harmens et al., 2011a).

This study presents the PAH concentrations and $\delta^{13}C$ and $\delta^{15}N$ measured in mosses *Hypnum cupressiforme* Hedw. from 61 sites of three European areas located in France, Spain and Switzerland. To identify common trends between pollutants and the parameters of influence on their content in mosses, data was submitted to multivariate analysis with site-specific and regional data.

2. Material and methods

2.1. Study area

The study was carried out over three European areas: Île-de-France, France (18 sites); Navarra, Spain (23 sites); Swiss Plateau and Basel region, Switzerland (20 sites). The sampling covered a total land surface of 40 000 km^2 (Fig. 1). The regions were chosen for their different geomorphologic, climatic and soil occupation characteristics. Île-de-France is located in the Paris Basin (43–173 m of elevation) and characterized by an oceanic climate (583–755 mm of rainfall) (MeteoFrance, 2011). The area is strongly urbanized by Paris metropolitan area (972 hab km^{-2}) but the land is also occupied by intensive cereal farming (INSEE, 2009). Located in the Western Pyrenees and close to the Biscay Bay, the sites of Navarra are mountainous (215–1220 m) and generally very humid (377–1900 mm) (GN, 2011). The area is covered by forests and grasslands dedicated to livestock farming. Urbanization is poor (97 hab km^{-2}) and population is concentrated in industrial areas such as Pamplona (Navarra's capital city) and the border with the Basque country in the Northwest (IEN, 2011). The Mitteland is formed by a vast plateau stretching across Switzerland, between the Alps and Jura mountain ranges (270–685 m). The climate is continental but relatively humid (826–1214 mm) due to the Alps which act as a weather front barrier (Meteotest, 2008). Population density is intermediate between the two other regions (450 hab km^{-2}) (OFS, 2011). The land is occupied by a mosaic of cities (Zürich, Geneva, Basel, Berne, Lausanne...) separated by intensive cereal farming areas. Basel area is characterized by very intense industrial activity.

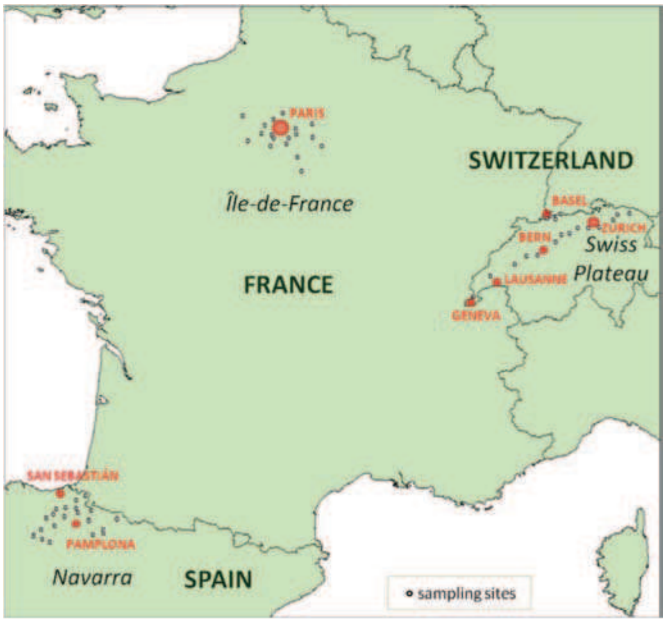

Fig. 1. Situation of the 61 sampling sites in three areas of Europe.

2.2. Study design and sample collection

The study was designed and the samples were collected following the recommendations of the ICP-Vegetation manual elaborated for moss surveys (ICP-Vegetation, 2010). Sampling was carried out with a minimal resolution of 20 × 20 km. All sites were located in forests, at least at 300 m from main roads (highways), villages and industries, and at least at 100 m from smaller roads and houses. One composite sample, consisting of five to ten subsamples, was collected from each sampling point within an area of 50 × 50 m. In order to determine the overall variability associated with the entire procedure (sampling and analysis), triplicate moss samples were collected from 10 sites of Île-de-France.

Sampling was conducted between August and October 2010: Aug 18–Oct 18 in Switzerland, Sept 19–Oct 16 in Spain and Oct 4–Oct 10 in France. Approximately 0.04 m^2 of the species *Hypnum cupressiforme* Hedw. was collected per sample from horizontal parts of non decomposed deadwood (bark of branches or tree stumps) with disposable powderless nitrile gloves. Samples were taken exclusively from wood to reduce contamination by soil and run-off water. The canopy was mainly constituted by deciduous broad-leaved trees in France and Spain and by mixed forests of deciduous broad-leaved trees and conifers in Switzerland. The sub-samples were laid side by side in 1 L polyethylene bags, and then placed in iceboxes for transportation to local laboratories, where they were frozen at −20 °C: *Museum National d'Histoire Naturelle* (MNHN) in France, *Laboratorio Integrado de Calidad Ambiental* (LICA) in Spain and *Forschungsstelle für Umweltbeobachtung* (FUB) in Switzerland. The samples were kept frozen during transportation to the *Laboratoire de Chimie Agro-industrielle* (LCA) in Toulouse (France) where sample preparation was conducted.

2.3. Sample preparation

Extraneous material attached to the moss samples was removed with stainless steel tweezers. The green and green–brown shoots from the last three years growth

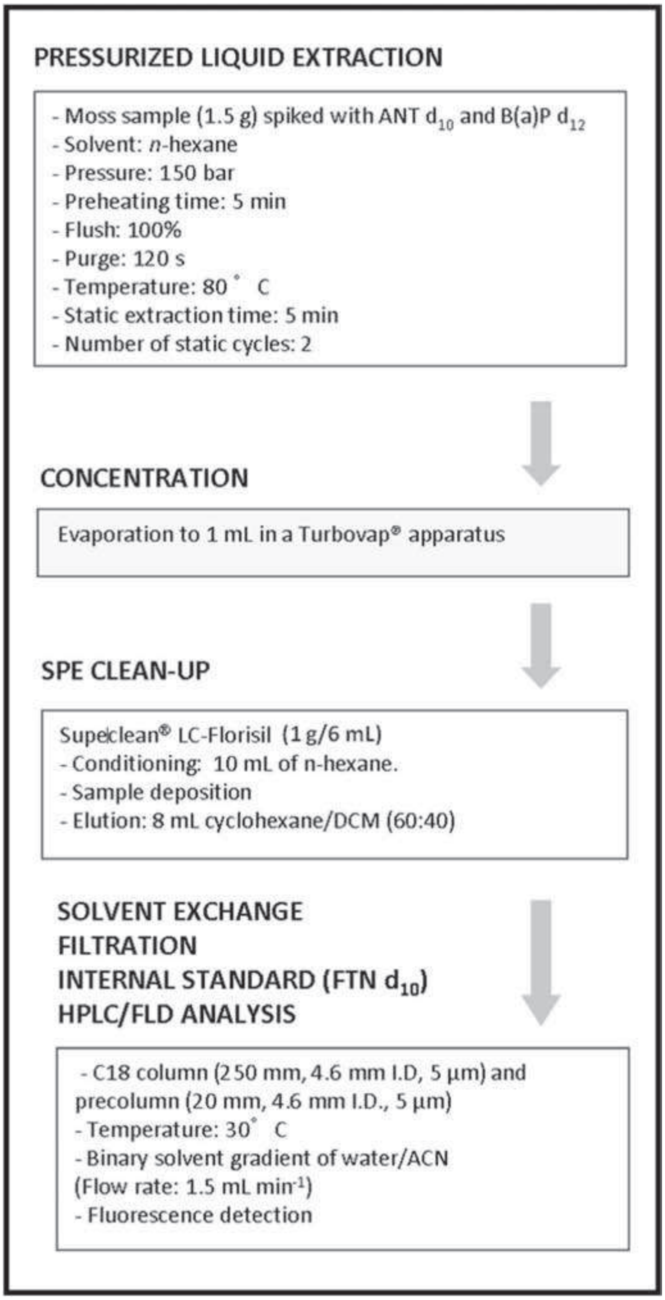

Fig. 2. Diagram illustrating all operations of the analytical procedure to determine PAH concentrations in moss samples by solvent extraction.

were withdrawn using stainless steel tweezers and scissors. The unwashed samples were freeze-dried with an Alpha 2–4 LD apparatus for 24 h (Martin Christ, Osterode am Harz, Germany), then ground to a fine powder in a stainless steel mill (particle size < 0.5 mm).

2.4. *PAH determination*

PAH content in mosses was determined by pressurized liquid extraction (PLE) and solid-phase extraction (SPE) cleanup, in association with analysis by high performance liquid chromatography coupled with fluorescence detection (HPLC–FLD). Main parameters are summarized in Fig. 2. Details on the analytical method, its optimization, quality assurance and quality control (QA/QC) are described in a previous study (Foan and Simon, 2012). The linearity range for the PAH analysis extends from 0 to 150 ng mL^{-1} with regression coefficients of 0.9993–0.9999. The repeatability of the analysis, determined with a 2 ng mL^{-1} standard solution ($n = 10$), showed uncertainty lower than 10%. Quantification limits in mosses range between 0.1 and 2.5 ng g^{-1} (dry weight). The reproducibility of the method, tested by repeating the entire extraction–purification–analysis procedure ($n = 6$), showed standard deviations for each PAH between 1 and 22%. The accuracy was also verified with the reference material IAEA-140-OC (ANALAB, Bischheim, France). All PAHs measured were included in the reference concentration ranges (95% confidence intervals) except for B(a)A, overestimated because of interferences with other reference molecules (PCBs or pesticides).

A standard mix containing acenaphthene (ACE), fluorene (FLR), phenanthrene (PHE), anthracene (ANT), fluoranthene (FTN), pyrene (PYR), benz(a)anthracene (B(a)A), chrysene (CHR), benzo(b)fluoranthene (B(b)F), benzo(k)fluoranthene (B(k)F), benzo(a)pyrene (B(a)P), dibenz(a,h)anthracene (D(ah)A), and benzo(ghi)perylene (B(ghi)P) at 10 µg mL^{-1} of acetonitrile, was used for calibration (Mix 16 HAP, LGC Standards, Teddington, UK). Deuterated PAHs were used as surrogate standards (anthracene d$_{10}$ and benzo(a)pyrene d$_{12}$ at 10 µg mL^{-1} in acetonitrile) and as internal standard (fluoranthene d$_{10}$ at 100 µg mL^{-1} in acetonitrile) (LGC Standards, Teddington, UK). All solvents were HPLC grade: acetonitrile (ACN), cyclohexane, dichloromethane (DCM) and *n*-hexane were provided by Scharlau (Sentmenat, Spain) and Milli-Q water by Millipore (Molsheim, France).

2.5. *Elemental analysis and isotopic measurements*

Moss samples were also analysed at the *Laboratoire de biogéochimie et écologie des milieux continentaux* (Paris, France) for their carbon and nitrogen content and their respective stable isotope signatures (δ^{13}C and δ^{15}N). Tissue C and N contents (%, dry weight) were determined by an elemental analyzer (EA1108, Eurovector, Milan, Italy) with an analytical precision of 0.1%. Calibration was performed using tyrosine (ThermoQuest Italia, Radano, Italy).

Moss samples were thermally decomposed to CO_2 and N_2 and isotope ratios of total carbon and nitrogen were determined by an elemental analyser coupled to a continuous flow isotope ratio mass spectrometer (EA-CF-IRMS) (Elementar-Isoprime, Manchester, UK). Based on the measurements of %C and %N, samples were weighed and put in tin capsules (250 µg for C and 150 µg for N). Carbon and nitrogen isotope data are expressed in the usual delta notation (δ^{13}C and δ^{15}N), defined as:

$$\delta_{\text{sample}}(‰) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000$$

where R is the isotope ratio ^{13}C/^{12}C or ^{15}N/^{14}N. The international standard for C is Pee Dee Belemnite (PDB) for which the ratio ^{13}C/^{12}C is 0.01112372. The international standard for N is atmospheric nitrogen (Mariotti, 1983, 1984), for which the ratio ^{15}N/^{14}N is 0.0036765.

Different reference materials were used for carbon and nitrogen: tyrosine ($\delta^{13}C = -23.2‰$, $\delta^{15}N = 9.98‰$, laboratory standard), IAEA-N$_1$ ($\delta^{15}N = 0.3‰$), IAEA-N$_2$ ($\delta^{15}N = 20.3‰$) and IAEA-N$_3$ ($\delta^{15}N = 4.5‰$) and VPDB ($\delta^{13}C = -23.2‰$). Analytical precision is about $\pm 0.1‰$ for δ^{13}C and $\pm 0.2‰$ for δ^{15}N.

2.6. *Statistical analysis*

One-way analysis of variance (ANOVA) was performed to validate the sampling procedure and to identify significant differences between moss data obtained in the three European areas. To study correlations between the variables, Pearson's correlation coefficients were calculated. Partial least square (PLS) regression was performed to determine the influence of site-specific and regional parameters on pollutant content in mosses. The statistical analysis was conducted using XLSTAT 2008 (Addinsoft, Paris, France) software.

3. Results

3.1. *Validation of the sampling procedure*

At a 95% confidence level, individual PAH concentrations were determined with a maximal variability between the triplicate samples ranging between 16% and 25%, for benzo(k)fluoranthene and pyrene respectively. Total PAH concentrations (for 13 compounds) were measured with a maximal variability of 20%. Elemental analysis revealed up to 4% and 11% variability for carbon and nitrogen respectively, as δ^{13}C and δ^{15}N values were measured with respective maximal uncertainties of 2% and 15% (95% confidence level). ANOVA showed significantly higher differences between PAH concentrations, C and N content and stable isotope signatures measured in the mosses from the 10 French sites than the variability between triplicates collected at the sites ($p < 0.0001$). Therefore, composite sampling with 5–10 subsamples appears to integrate well spatial variability on the sampling site.

3.2. *PAH concentrations*

Individual and total PAH concentrations of 13 compounds measured in mosses from the three areas of Europe are presented in Table 1. Total concentrations ranged from 98.1 to 697.8 ng g^{-1} (dry weight). Average content was significantly higher in France and Switzerland, with respective values of 264.0 and 241.5 ng g^{-1}, against 182.1 ng g^{-1} in Spain (ANOVA, $p < 0.05$). The mosses from Switzerland showed the widest range of concentrations (98.1–697.8 ng g^{-1}), those from France the lowest (148.5–359.9 ng g^{-1}). However, the minimal total content was significantly higher in French samples (ANOVA, $p < 0.05$). In the three regions, the major compounds were, by increasing order of importance, phenanthrene, fluoranthene, fluorene and pyrene. Their content in mosses generally exceeds 10 ng g^{-1} and can reach values over 100 ng g^{-1}. On the contrary, acenaphtene, anthracene and dibenzo(a,h) anthracene presented concentrations close to quantification limits ($\sim$ng g^{-1}). Several compounds of high molecular weight (B(a)A, CHR, B(b)F, B(k)F, B(a)P, B(ghi)P) presented low concentrations in mosses from Spain (0.8–13.4 ng g^{-1}), but were relatively more important in samples from France (4.9–33.9 ng g^{-1}) and Switzerland (2.6–70.6 ng g^{-1}). For example, benzo(a)pyrene, considered as the most toxic PAH (IARC, 2010), showed respective average concentrations of 11.2 and 9.9 ng g^{-1} in France and Switzerland, against 3.5 ng g^{-1} in Spain.

3.3. *Elemental and isotopic measurements*

Carbon and nitrogen content as well as δ^{13}C and δ^{15}N measured in mosses from the three areas of Europe are summarized in Table 1. Elemental analysis revealed carbon content ranging between 38.4 and 47.3% (dry weight) and nitrogen content ranging between 0.9 and 2.1% (dry weight). No significant differences were observed between the three regions studied. Stable isotope signatures ranged from −32.3‰ to −28.7‰ for δ^{13}C and from −10.3‰ to −3.0‰ for δ^{15}N. δ^{13}C values were significantly higher in Spain than in the two other areas (ANOVA, $p < 0.05$), with an average value of −29.5 ± 0.6‰ ($n = 23$), against −31.0 ± 0.7‰ ($n = 18$) and −30.8 ± 0.7‰ ($n = 20$) in France and Switzerland respectively. δ^{15}N values showed similar ranges in France (−8.0‰ to −3.3‰) and Spain (−7.9‰ to −3.0‰), and close average values: −6.0‰ in France and −5.6‰ in Spain. The mosses from Switzerland presented a wider range for δ^{15}N, with values from −10.8‰ to −3.3‰ and a significantly lower average value of −6.9% (ANOVA, $p < 0.05$).

3.4. *Correlation between variables*

A Pearson correlation test was carried out on the data obtained from the 61 sites (Table 2). All individual PAH concentrations are linearly correlated with the total concentrations. Particularly, PHE and FTN concentrations are very significantly correlated with the total concentrations, with $r = 0.903$ and 0.975 respectively ($p < 0.0001$). Several groups of individual PAH concentrations show significant linear correlations:

- light PAHs (2-ring compounds): ACE and FLR ($r = 0.625$, $p < 0.0001$),
- intermediate PAHs (3 and 4-ring compounds): PHE, ANT, FTN and PYR (r ϵ [0.500; 0.886], $p < 0.0001$),
- heavy PAHs (4, 5 and 6-ring compounds): B(a)A, CHR, B(b)F, B(k)F, B(a)P, B(ghi)P, D(ah)A (r ϵ [0.914; 0.997], $p < 0.0001$).

There are also significant correlations between FLR and PHE ($r = 0.532$, $p < 0.0001$), as well as between PHE, FTN and all heavy PAHs (r ϵ [0.506; 0.759], $p < 0.0001$).

Concerning elemental and isotopic analysis, δ^{15}N values showed a low positive linear correlation with N content ($r = 0.305$; $p < 0.05$) and a low negative linear correlation with C content ($r = -0.290$, $p < 0.05$). N content showed low linear correlations with heavy PAHs such as B(a)A, CHR, B(b)F and B(k)F (r ϵ [0.253; 0.283], $p < 0.05$). δ^{13}C values showed low negative linear correlations with FTN, all heavy PAHs and total PAH concentration (r ϵ [−0.431; −0.302], $p < 0.05$).

3.5. *Correlation with site-specific and regional characteristics*

Partial least square (PLS) regression was carried out between the variables measured in mosses (quantitative variables) and site-specific and regional characteristics (explicative variables): altitude, annual precipitation and soil occupation, obtained from the CORINE Land Cover database. Regression coefficients obtained by PLS are given in Table 3. Altitude of sampling sites appeared as

Table 1

Mean, range and standard deviation (SD) of the values measured in mosses *Hypnum cupressiforme* Hedw. from three European countries: individual and total PAH concentrations (ng g^{-1}, dry weight), carbon and nitrogen content (%) and isotope ratios (‰). In italic, the total concentration of the 13 PAHs is given and is annotated *$\Sigma_{13}PAH$*.

	France ($n = 18$)			Spain ($n = 23$)			Switzerland ($n = 20$)		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
ACE	2.5	1.5–4.0	0.7	3.8	1.5–9.9	2.2	3.7	2.0–5.9	1.1
FLR	39.1	26.9–55.0	9.0	46.1	22.1–99.2	24.1	38.5	21.2–63.4	10.6
PHE	29.2	19.8–42.0	6.4	27.5	16.0–46.3	12.4	30.8	16.7–81.4	13.9
ANT	1.3	0.7–2.1	0.4	1.5	0.6–3.5	0.8	1.4	0.5–3.9	0.8
FTN	38.7	19.2–60.2	10.6	23.6	8.1–55.4	19.5	35.6	12.7–116.8	23.8
PYR	66.1	33.2–115.9	23.3	52.9	11.7–135.7	56.2	60.6	17.6–151.3	34.1
B(a)A	13.7	6.1–20.3	3.7	2.9	0.8–7.0	1.7	9.3	1.8–59.7	14.4
CHR	20.0	11.2–30.6	5.5	7.1	3.5–13.4	2.8	14.5	5.2–61.1	13.3
B(b)F	17.2	8.9–25.7	4.8	5.0	1.8–9.7	2.2	14.6	4.8–70.6	18.0
B(k)F	9.1	4.9–13.0	2.3	2.9	1.4–5.0	0.9	7.8	2.6–36.4	8.9
B(a)P	11.2	5.9–17.2	3.3	3.5	1.8–5.5	1.0	9.9	3.3–47.0	11.5
D(ah)A	2.6	1.3–3.8	0.7	1.1	0.5–1.9	0.4	2.4	0.9–10.9	2.8
B(ghi)P	13.3	5.3–33.9	6.6	4.3	1.9–8.0	1.6	12.4	4.1–55.1	14.1
$\Sigma_{13}PAH$	*264.0*	*148.5–359.9*	*61.0*	*182.1*	*99.7–355.7*	*105.3*	*241.5*	*98.1–697.8*	*139.8*
%C	42.7	38.4–44.6	1.8	44.2	41.1–47.3	1.4	45.0	42.8–46.9	1.2
%N	1.6	1.2–2.1	0.2	1.3	0.9–1.9	0.3	1.4	1.1–1.9	0.2
δ^{13}C	−31.0	[−32.3; −29.9]	0.6	−29.5	[−30.7; −28.7]	0.7	−30.8	[−31.9; −29.4]	0.7
δ^{15}N	−6.0	[−8.0; −3.3]	1.3	−5.6	[−7.9; −3.0]	1.3	−6.9	[−10.8; −3.3]	2.0

Table 2
Pearson correlation coefficients between individual and total PAH concentrations (ng g^{-1}, dry weight), C, N content (%), $\delta^{13}C$ and $\delta^{15}N$ (‰) measured in the mosses from the 61 European sites. The coefficients in bold are significant.

Variables	ACE	FLR	PHE	ANT	FTN	PYR	B(a)A	CHR	B(b)F	B(k)F	B(a)P	D(ah)A	B(ghi)P	Σ_{13}HAP	%C	%N	$\delta^{13}C$	$\delta^{15}N$
ACE	**1**																	
FLR	**0.625******	**1**																
PHE	**0.389**	**0.532******	**1**															
ANT	0.130	0.172	**0.723******	**1**														
FTN	**0.292***	**0.341****	**0.886******	**0.650******	**1**													
PYR	**0.401****	**0.463*****	**0.730******	**0.500******	**0.819******	**1**												
B(a)A	−0.038	0.039	**0.579******	**0.459*****	**0.699******	0.204	**1**											
CHR	−0.041	0.037	**0.609******	**0.465*****	**0.759******	**0.311***	**0.962******	**1**										
B(b)F	−0.062	−0.048	**0.506******	**0.439*****	**0.626******	0.104	**0.968******	**0.932******	**1**									
B(k)F	−0.051	−0.021	**0.531******	**0.435*****	**0.644******	0.122	**0.973******	**0.938******	**0.997******	**1**								
B(a)P	−0.039	−0.035	**0.528******	**0.461*****	**0.647******	0.123	**0.971******	**0.932******	**0.996******	**0.995******	**1**							
D(ah)A	−0.004	−0.023	**0.518******	**0.435*****	**0.622******	0.109	**0.955******	**0.914******	**0.984******	**0.984******	**0.984******	**1**						
B(ghi)P	−0.004	−0.028	**0.511******	**0.479******	**0.621******	0.146	**0.914******	**0.929******	**0.933******	**0.927******	**0.927******	**0.921******	**1**					
Σ_{13}PAH	**0.336****	**0.449*****	**0.903******	**0.644******	**0.975******	**0.761******	**0.759******	**0.805******	**0.691******	**0.708******	**0.706******	**0.691******	**0.695******	**1**				
%C	0.135	−0.069	−0.131	−0.013	−0.212	−0.190	−0.228	−0.210	−0.120	−0.141	−0.115	−0.065	−0.102	−0.208	**1**			
%N	**−0.259***	−0.036	−0.046	−0.064	0.119	−0.003	**0.264***	**0.283***	**0.262***	**0.253***	0.237	0.219	0.221	0.134	−0.226	**1**		
$\delta^{13}C$	0.150	0.094	−0.194	−0.078	**−0.352****	−0.168	**−0.388****	**−0.431****	**−0.371****	**−0.372****	**−0.390****	**−0.302***	**−0.341****	**−0.322***	0.122	−0.126	**1**	
$\delta^{15}N$	0.027	0.056	−0.031	0.084	−0.020	0.014	0.009	−0.063	−0.078	−0.087	−0.064	−0.075	−0.098	−0.024	**−0.290***	**0.305***	0.087	**1**

p-Values obtained by Student's *t* test and their degree of "statistical significance": $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Table 3
Partial Least Square (PLS) regression coefficients between variables measured in mosses and site-specific and regional characteristics. The coefficients in bold are significant.

Predictor	ACE	FLR	PHE	ANT	FTN	PYR	B(a)A	CHR	B(b)F	B(k)F	B(a)P	D(ah)A	B(ghi)P	Σ_{13}HAP	%C	%N	$\delta^{13}C$	$\delta^{15}N$
Altitude	**0.251**	**0.319**	0.025	0.029	−0.236	0.031	**−0.488**	**−0.527**	**−0.468**	**−0.461**	**−0.458**	**−0.413**	**−0.416**	−0.213	**0.304**	−0.191	**0.510**	0.014
Annual precipitation	0.070	0.098	−0.156	0.170	**−0.324**	−0.215	**−0.316**	**−0.400**	**−0.287**	**−0.297**	**−0.284**	**−0.284**	−0.245	**−0.291**	**0.278**	0.020	**0.284**	0.149
Agricultural land use (10 km radius)	0.029	−0.208	−0.224	**−0.295**	−0.081	−0.082	−0.110	−0.053	−0.060	−0.054	−0.048	−0.047	−0.012	−0.130	0.151	0.036	−0.162	**−0.339**
Agricultural land use (25 km radius)	0.040	**−0.282**	−0.172	**−0.250**	0.054	0.022	0.006	0.081	0.036	0.044	0.043	0.048	0.069	−0.021	0.116	0.102	**−0.293**	**−0.288**
Agricultural land use (50 km radius)	−0.137	**−0.275**	−0.102	−0.196	0.202	0.062	**0.279**	**0.376**	**0.285**	**0.288**	**0.279**	**0.247**	**0.297**	0.156	−0.015	**0.301**	**−0.491**	−0.239
Forestal land use (10 km radius)	0.032	0.044	0.016	0.199	−0.148	−0.022	−0.207	**−0.261**	−0.190	−0.197	−0.209	−0.173	−0.166	−0.128	0.056	−0.032	**0.338**	0.071
Forestal land use (25 km radius)	0.172	0.165	0.136	**0.304**	−0.112	−0.007	−0.204	**−0.275**	−0.166	−0.174	−0.173	−0.144	−0.115	−0.072	0.144	−0.167	**0.382**	0.088
Forestal land use (50 km radius)	**0.290**	0.153	0.144	0.239	−0.097	−0.023	−0.228	**−0.295**	−0.159	−0.163	−0.149	−0.127	−0.103	−0.074	**0.358**	**−0.318**	**0.307**	0.036
Urban land use (10 km radius)	−0.215	−0.070	0.163	0.033	**0.343**	0.074	**0.512**	**0.553**	**0.489**	**0.495**	**0.502**	**0.428**	**0.393**	**0.340**	−0.172	0.232	**−0.563**	0.087
Urban land use (25 km radius)	**−0.265**	−0.086	0.088	−0.028	**0.275**	0.062	**0.450**	**0.502**	**0.429**	**0.430**	**0.434**	**0.373**	**0.337**	**0.282**	−0.201	0.241	**−0.531**	0.017
Urban land use (50 km radius)	**−0.309**	−0.146	0.060	−0.057	**0.290**	0.070	**0.485**	**0.527**	**0.456**	**0.460**	**0.452**	**0.390**	**0.342**	**0.286**	**−0.322**	**0.345**	**−0.563**	−0.008
Urban land use (75 km radius)	**−0.326**	−0.192	0.038	−0.080	**0.300**	0.074	**0.510**	**0.557**	**0.479**	**0.483**	**0.467**	**0.409**	**0.380**	**0.293**	**−0.366**	**0.425**	**−0.578**	−0.020
Urban land use (100 km radius)	**−0.321**	−0.206	0.028	−0.084	**0.298**	0.084	**0.495**	**0.553**	**0.458**	**0.463**	**0.447**	**0.387**	**0.390**	**0.287**	**−0.369**	**0.427**	**−0.593**	−0.035

Sources : – Annual precipitation : http://france.meteofrance.com; http://meteo.navarra.es; http ://www.meteotest.ch. – Soil occupation data: http://www.eea.europa;eu/publications/COR0-landcover.

significantly correlated with light PAH concentrations: ACE ($r = 0.251$) and FLR ($r = 0.319$), carbon content ($r = 0.304$) and δ^{13}C values ($r = 0.510$), as well as significantly anti-correlated with heavy PAHs: B(a)A, CHR, B(b)F, B(k)F, D(ah)A and B(ghi)P ($r = [-0.527; -0.413]$). Annual precipitation was significantly anti-correlated with several individual PAHs: FTN, B(a)A, CHR, B(b)F, B(k)F, B(a)P, D(ah)A ($r = [-0.400; -0.284]$) and total PAH concentrations ($r = -0.291$). Soil occupation by agriculture showed significant positive correlations with heavy PAH concentrations ($r_{50\ \mathrm{km}} = [0.247; 0.376]$) and nitrogen content ($r_{50\ \mathrm{km}} = 0.301$), as well as negative correlations with FLR ($r_{25\ \mathrm{km}} = -0.282$) and ANT ($r_{10\ \mathrm{km}} = -0.295$) concentrations, δ^{13}C values ($r_{50\ \mathrm{km}} = -0.491$) and δ^{15}N values ($r_{10\ \mathrm{km}} = -0.339$). Forest land use was significantly correlated with ACE ($r_{50\ \mathrm{km}} = 0.290$) and ANT ($r_{25\ \mathrm{km}} = 0.304$) concentrations, carbon content ($r_{50\ \mathrm{km}} = 0.358$) and δ^{13}C values ($r_{25\ \mathrm{km}} = 0.382$), and significantly anti-correlated with CHR concentrations ($r_{50\ \mathrm{km}} = -0.295$) and nitrogen content ($r_{50\ \mathrm{km}} = -0.318$). However, the most important correlations appeared between the variables and urban land use, with maximal positive values for urban occupation within a 10 km radius and FTN ($r = 0.343$), heavy PAHs ($r = [0.393; 0.553]$) and total PAH ($r = 0.340$) concentrations, as well as for urban occupation within 100 km radius and nitrogen content ($r = 0.427$). Maximal negative correlations appeared between urban land use within a 100 km radius and ACE concentrations ($r = -0.321$), carbon content ($r = -0.369$) and δ^{13}C values ($r = -0.593$).

The PLS regressions obtained to explain PAH content in mosses with soil occupation data are represented in Fig. 3. The correlations circle shows a t1 axis negatively correlated with urban land use at 10–100 km and positively correlated with forestry land use at 10–50 km, as well as a t2 axis positively correlated with agricultural land use at 10–50 km. Most of the PAH concentrations are explained by the t1 axis: mosses sampled close to urban areas are highly contaminated by high molecular weight PAHs, as those from rural areas are characterized by lighter weight PAHs. The individuals from the different regions are clearly differenciated by the PLS regressions: Île-de-France sites (France) are negatively correlated with t1, due to high urbanization of this area; Navarra sites (Spain) are positively correlated with t1, due to the high occupation of the land by forests and low urbanization; Switzerland shows an intermediate position. The individuals from the three areas are dispersed along the t2 axis, due to highly variable agricultural activity.

3.6. Correlations with additional moss data

Heavy metal concentrations were determined in *Hypnum cupressiforme* samples collected synchronously in Switzerland and Spain following the method described in Gonzalez-Miqueo (2009). A Pearson correlation test revealed for the mosses from Switzerland significant linear correlations ($p < 0.01$) between heavy PAHs (B(a)A, CHR, B(b)F, B(k)F, B(a)P, D(ah)A and B(ghi)P) and several heavy metals: silver ($r > 0.677$), bismuth ($r > 0.827$), cadmium ($r > 0.605$), cobalt ($r > 0.789$), lead ($r > 0.823$) and zinc ($r > 0.935$) (Table 4). As a result, total PAH concentrations were significantly correlated with all heavy metal concentrations measured ($r > 0.544$, $p < 0.01$), which is particularly interesting as the different elements presented various levels: 0.01–0.07 μg g^{-1} for Bi, 0.1–0.7 μg g^{-1} for Co, 2.0–6.5 μg g^{-1} for Pb and 17–170 μg g^{-1} for Zn. In Navarra, significant linear correlations ($p < 0.01$) were also measured between concentrations of several heavy metals and heavy weight PAHs (Table 4):

- Cu (2.3–7.7 μg g^{-1}) with B(b)F and B(k)F ($r > 0.501$),
- Hg (0.02–0.07 μg g^{-1}) with B(b)F, B(k)F, B(a)P and B(ghi)P ($r > 0.568$),
- Pb (1.0–10.3 μg g^{-1}) with B(b)F, B(k)F, B(a)P and B(ghi)P ($r > 0.494$),
- Sb (0.04–0.12 μg g^{-1}) with B(b)F, B(a)P and B(ghi)P ($r > 0.518$),
- Zn (13–38 μg g^{-1}) with B(b)F, B(k)F, B(a)P and B(ghi)P ($r > 0.539$).

Nitrogen content showed significant linear correlations with the heavy metals concentrations ($r > 0.54$; $p < 0.01$). Common trends were also observed between metals and δ^{15}N values, with a maximum correlation for Zn ($r = 0.54$; $p < 0.01$).

4. Discussion

Several European studies revealed the same range of total PAH concentrations in mosses *Hypnum cupressiforme* Hedw. Indeed, values of 107–640 ng g^{-1} were measured in Wienerwald Nature

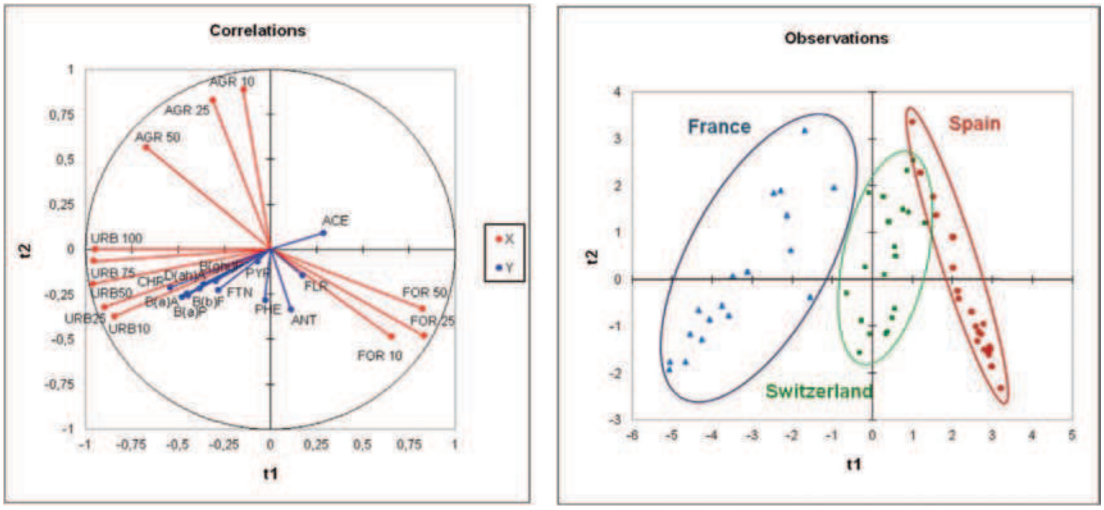

Fig. 3. Graphical representation of the PLS regressions of PAH content in mosses in function of soil occupation data for 61 European sampling sites. The individuals are the European sites presented in the "Observations" graphic. On the correlations circle, the quantitative variables (*Y*) are individual PAH concentrations and explicative variables (*X*) are soil occupation data (URB: urban areas; AGR: farmland; FOR: forests; in a radius of 10–100 km).

Table 4
Significant Pearson correlations between heavy metal concentrations measured in mosses from Switzerland ($n = 20$) and Spain ($n = 23$) and individual PAH, total PAH (Σ_{13}HAP) concentrations, nitrogen content and δ^{15}N values.

	r ($p < 0.01$)	B(a)A	CHR	B(b)F	B(k)F	B(a)P	D(ah)A	B(ghi)P	Σ_{13}HAP	%N	δ^{15}N [‰]
Switzerland	Ag	0.727	0.741	0.737	0.737	0.706	0.677	0.692	0.627	n.s.	n.s.
	Bi	0.877	0.827	0.896	0.884	0.876	0.852	0.879	0.644	n.s.	n.s.
	Cd	0.666	0.667	0.662	0.660	0.639	0.605	0.632	0.544	n.s.	n.s.
	Co	0.827	0.794	0.836	0.824	0.820	0.789	0.815	0.650	n.s.	n.s.
	Pb	0.854	0.843	0.863	0.857	0.842	0.823	0.838	0.691	n.s.	n.s.
	Zn	0.958	0.935	0.980	0.977	0.969	0.962	0.965	0.721	n.s.	n.s.
Spain	Cu	n.s.	n.s.	0.501	0.569	0.461	n.s.	0.475	n.s.	0.870	0.498
	Hg	n.s.	n.s.	0.681	0.684	0.568	n.s.	0.679	n.s.	0.801	0.278
	Pb	n.s.	n.s.	0.592	0.494	0.551	n.s.	0.568	n.s.	0.686	0.402
	Sb	n.s.	n.s.	0.518	0.473	0.575	n.s.	0.570	n.s.	0.537	n.s.
	Zn	n.s.	n.s.	0.665	0.598	0.595	n.s.	0.539	n.s.	0.641	0.542

n.s.: non-significant.

Reserve (Austria) for the 13 studied PAHs (Krommer et al., 2007) and mosses from Kosetice observatory (Czech Republic) showed concentrations ranging from 47 to 112 ng g^{-1} for the 16 PAHs classified as priority pollutants by USEPA (Holoubek et al., 2007b). Moreover, total concentrations measured in Navarra (Spain) are of the same order as previous studies carried out in that area, which revealed values of approximately 130 ng g^{-1} (Foan et al., 2010; Foan and Simon, 2012).

Previous studies also showed the same major compounds: FLR, PHE, FTN, PYR. Their concentrations were significantly correlated ($r > 0.50$, $p < 0.0001$), due most importantly to their emission from petrol and diesel vehicles in urban and traffic areas (Ho et al., 2002; Omar et al., 2002; Orlinski, 2002; Ravindra et al., 2006). No common trends between the major compounds and soil occupation was identified, as rural sites can be occupied by high traffic density. It is notably the case of Navarra region, which is crossed by main roads connecting Spain to France and is only occupied by 1% of urban land (Fig. 4). Correlations between PAHs and heavy metals Cu, Hg, Pb, Sb and Zn in this region ($r > 0.46$; $p < 0.01$) are related to their common emission by road traffic (Pearson et al., 2000; Zechmeister et al., 2006).

The high molecular weight PAHs (B(a)A, CHR, B(b)F, B(k)F, B(a)P, B(ghi)P) concentrations were very significantly correlated ($r > 0.91$, $p < 0.0001$) and were significantly correlated with the percentage of urban land use at 10 km around the sampling sites ($r = [0.40; 0.55]$, $p < 0.05$). Local soil occupation (within a 10 km radius) shows the highest correlations with PAH data, probably due to the fact that PAH concentrations in mosses follow an exponential decrease in the vicinity of contamination sources (Ares et al., 2009). Synchronous emission of these compounds by industries located in urban areas may explain these trends (Ares et al., 2009, 2011). This is probable as strong correlations with the heavy metals Ag, Bi, Cd, Co, Pb and Zn emitted by industrial activity were observed in Switzerland ($r > 0.66$, $p < 0.01$). Heavy PAH concentrations were therefore relatively higher in Île-de-France and Switzerland, due to the high percentage of industrialized urban areas in these two regions (Fig. 4). Mosses from high altitudes were depleted in heavy PAHs, certainly due to fractionation of the compounds by particle sedimentation and PAH condensation under lower vapour pressures (Liu et al., 2005). Heavy PAH concentrations also tended to decrease with high precipitation levels. This fact may be related with particle leaching by wet deposition (Kinnersley et al., 1996; Couto et al., 2004).

Elemental and isotopic analysis also revealed similar values to previous studies. Indeed, in mosses *Hypnum cupressiforme* Hedw. from Navarra (Spain), carbon content ranged from 30.9% to 43.9%, nitrogen content from 0.8% to 1.5%, δ^{13}C values from −30.5‰ to −28.5‰ and δ^{15}N from −6.0‰ to −4.0‰ (Foan et al., 2010). Mosses *Haplocladium microphyllum* (Hedw.) Broth. sampled between 2005 and 2007 in China showed carbon content ranging from 37.8% to 45.7% in a mountainous and urban area respectively, and δ^{13}C values ranged from −30.2‰ in the urban area to −26.5‰ in the mountains (Liu et al., 2008, 2010). Analysis of mosses *Pleurozium scheberi* (Brid.) Mitt. and *Scleropodium purum* (Hedw.) from 8 rural sites in Germany showed nitrogen content of 0.7–2.3% and δ^{15}N values from −7.9‰ to −2.9‰ (Solga et al., 2005). Active biomonitoring in Italy with *Tortula muralis* Hedw. revealed values for δ^{15}N of −6‰ to 2‰ at urban areas (industrial, residential and city centers), against −7‰ to −2‰ at rural sites located at more than 10 km from stationary emission sources and more than 1 km from

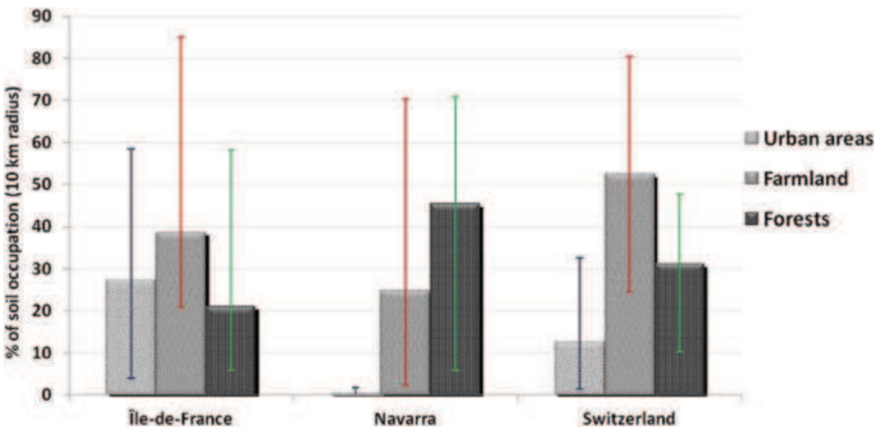

Fig. 4. Average percentages of soil occupation at a 10 km radius around the sites of the three studied regions. Error bars represent the values ranges (minimum – maximum).

roads (Gerdol et al., 2002b). A study carried out in Chinese cities with mosses *Haplocladium microphyllum* showed values of −8 to 4‰ (Xiao et al., 2010).

Liu et al. (2010) observed over an urban to rural transect that moss carbon content was significantly correlated with nitrogen content and significantly anti-correlated with $\delta^{13}C$ values. Urbanized sites were characterized by higher C and N contents and low $\delta^{13}C$ values (strongly negative). This study did not reveal any significant linear correlation between C, N contents and $\delta^{13}C$ values, probably because of the wide geographical extent and the various emission sources. However, $\delta^{13}C$ values were significantly anti-correlated with the percentage of urban land use ($r = [-0.531; -0.593]$, $p < 0.01$), showing a clear depletion of ^{13}C in mosses from urbanized areas. The significantly higher $\delta^{13}C$ values measured in Navarra (Spain) may therefore be explained by the low urbanization of the area (Fig. 4). The inversed trends between $\delta^{13}C$ and PAHs are probably related with the emission of both CO_2 and PAHs during combustion of fossil fuels and biomass. The fact that mosses collected at high altitude sites were enriched in ^{13}C is difficult to interpret, as isotope fractionation is influenced by the composition of source CO_2 as well as physiological factors (Körner et al., 1991).

Solga et al. (2005) showed a significant positive linear correlation between nitrogen concentrations in mosses and in bulk deposition, and a significant negative linear correlation between $\delta^{15}N$ values in mosses and $NH_4^+ - N/NO_3^- - N$ concentration ratios in bulk deposition. Xiao et al. (2010) also observed a significant negative linear correlation between $\delta^{15}N$ and NH_4^+ concentrations in bulk deposition. It emerges that urbanized areas, characterized by high emissions of NO_x originating from road traffic, show high $\delta^{15}N$ levels in mosses (close to 0), whereas rural areas, characterized by NH_3 emissions from agriculture, show low $\delta^{15}N$ values (strongly negative). In this study, the $\delta^{15}N$ levels measured are characteristic of rural sites, probably due to their remoteness from emission sources. N content and $\delta^{15}N$ values are barely correlated, probably because N content is function of the total atmospheric nitrogen deposition, as $\delta^{15}N$ is influenced by various emission sources. Significant linear correlations were observed in Navarra between N content, $\delta^{15}N$ values and several heavy metals, probably due to the intensive road traffic emissions in that area. $\delta^{15}N$ was negatively correlated with the percentage of agricultural land use at a 10 km radius ($r = -0.339$, $p < 0.01$). Indeed, farm and agricultural activities induce the liberation of ammonia (gas), which is poorly enriched in ^{15}N. The mosses collected in areas close to farmland may have absorbed NH_3 and are therefore depleted in ^{15}N. Lower $\delta^{15}N$ values in Switzerland than the other areas may therefore be explained by the intensive agricultural activity over the Swiss Plateau. Indeed, at a 10 km radius around the sampling sites, farmland occupies 52% of the soil (Fig. 4). Liu et al. (2010) showed that high carbon content in urban areas can be related with the fertilizing effect of N deposition. This relation may explain the low anti-correlation between C content and $\delta^{15}N$ values in mosses ($r = -0.290$, $p < 0.01$). Finally, the common trends between N content and heavy PAH concentrations may be explained by their important deposition in urban areas (industry, road and domestic emissions…). Nevertheless, it should be noted that the forest canopy may also have contributed to the N content and $\delta^{15}N$ values in the European mosses, by enriching throughfall deposition in organic nitrogen (Cape et al., 2010; Drápelová, 2012).

5. Conclusions

Total PAH concentrations measured in mosses *Hypnum cupressiforme* Hedw. from three European regions ranged from 100 to 700 ng g^{-1}. Samples collected in Navarra, a rural area of Spain, were significantly less contaminated by PAHs than those from Île-de-France (Paris metropolitan area) and the Swiss sampling points, especially those of the Basel region. The major compounds FLR, PHE, FTN and PYR were significantly correlated ($r = [0.34; 0.89]$, $p < 0.01$). Their distribution was not explained by soil occupation data (percentage of farmland, forests and urban areas), as they are emitted mainly by road traffic. In further studies, predictions may be improved by introducing traffic density data in the PLS regressions. Heavy PAH concentrations (B(a)A, CHR, B(b)F, B(k)F, B(a)P, B(ghi)P) were very significantly correlated ($r > 0.91$; $p < 0.0001$). Their distribution was partially explained by local urbanization in a 10 km radius ($r = [0.40; 0.55]$, $p < 0.05$). Spanish samples were therefore characterized by relatively low percentages of heavy PAHs due to low urbanization in this area (1%). Environmental parameters such as elevation and precipitation appeared as anti-correlated with heavy PAH content (respectively $r = [-0.53; -0.43]$ and $r = [-0.40; -0.28]$, $p < 0.05$). The trends observed in Navarra may therefore also be attributed to the relief and heavy rainfall of the area. Specific correlations with heavy metal concentrations confirmed that the main PAH emission sources were industrial activity in Switzerland and road traffic in Navarra.

$\delta^{13}C$ values, which ranged from −32‰ to −29‰, were significantly anti-correlated with local urbanization in a 10 km radius ($r = -0.53$; $p < 0.05$). This may be attributed to ^{13}C depletion in anthropogenic emissions. $\delta^{13}C$ values were also correlated with altitude ($r = 0.51$; $p < 0.05$). Significantly higher $\delta^{13}C$ values measured in mosses from Navarra (−29‰ against −31‰ in the two other areas) can therefore be explained by the low urbanization and relief of this area. Heavy PAHs and $\delta^{13}C$ followed opposite trends ($r = [-0.30; -0.43]$; $p < 0.05$). $\delta^{15}N$ values, which ranged from −11‰ to −3‰, were anti-correlated with land occupation by farming in a 10 km radius ($r = -0.34$, $p < 0.01$), due to N emission under the reduced form depleted in ^{15}N. The very low minimum value measured in mosses from the Swiss Plateau (−11‰) can therefore be attributed to the high percentage of farmland in this area (52%). However, no clear trends were observed in the three regions as $\delta^{15}N$ is also influenced by NO_x emissions in urban and traffic areas, as well as canopy leaching on the sampling sites. Interpretation of isotope signatures in mosses is difficult. Future studies will have to include the analysis of source N and C composition for further exploitation of bioindication results. Moreover, for complementary information to identify contamination sources, sulphur isotope signatures ($^{34}S/^{32}S$) should be measured. Several participants of ICP-Vegetation have recently started measuring them in mosses across Europe.

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