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ISOTHECIUM MYOSUROIDES AND *THUIDIUM TAMARISCINUM* MOSSES AS BIOINDICATORS OF NITROGEN AND HEAVY METAL DEPOSITION IN ATLANTIC OAK WOODLANDS

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Abstract – Moss tissue chemistry is widely used as a bioindicator of atmospheric deposition. The objective of this study was to compare the tissue chemistry of two moss species in Irish Atlantic oak woodlands, *Isothecium myosuroides* [Im] and *Thuidium tamariscinum* [Tt], and to determine their relationship to indices of atmospheric deposition. Moss species were collected from twenty-two woodland sites during April 2013 and analysed for nitrogen, sulphur, and eleven heavy metals. Nitrogen content was significantly correlated between species ($r_S = 0.84$), but their mean values (Im = 1.23%, Tt = 1.34%) were significantly different. A simple linear regression suggested that nitrogen content was significantly related to atmospheric ammonia ($R^2 = 0.67$ [Im], $R^2 = 0.65$ [Tt]) and total nitrogen deposition ($R^2 = 0.57$ [Im], $R^2 = 0.54$ [Tt]). Many heavy metals had significant interspecies correlations (Al, V, Ni, Cu, Zn, As, Sb, Pb; $r_S = 0.46–0.77$). A few metals (As, Sb and Pb) were positively correlated with easting and northing for both species, which may suggest transboundary or national industrial emissions sources. The results suggest that both species could be used as bioindicators of deposition for nitrogen and some heavy metals, although further study of the relationship between tissue concentrations and atmospheric deposition is warranted. Furthermore, interspecies calibration is required to use both species in conjunction.

Keywords: bioindicator, nitrogen deposition, moss, Atlantic oak woodland, heavy metals

Introduction

Mosses are widely used as bioindicators and biomonitors of atmospheric pollution, including heavy metals (e.g., Zechmeister et al., 2003; Holy et al., 2009; Harmens et al., 2012; Vučković, 2013), nitrogen (N, e.g., Pitcairn et al., 1995; Bassingthwaight & Shaw, 2010; Harmens et al., 2011; Špirić et al., 2014) and persistent organic pollutants (POPs; summarized in Qimei et al., 2014), particularly in Europe where the International Co-operative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation, established under the UNECE Convention on Long-Range Transboundary Air Pollution) has co-ordinated European scale moss surveys since 2000 (URL: icpvegetation.ceh.ac.uk). Mosses generally source their nutrients from the atmosphere; as such, the concentration of trace elements is higher in moss tissue than in precipitation, facilitating analysis of a wide range of pollutants. Further, moss leaves have been reported to accumulate higher concentrations of trace elements than tree leaves (De Nicola et al., 2013) and lichens (Giordano et al., 2013). The analysis of moss at a large number of sampling sites can provide high spatial resolution observations on atmospheric deposition not practical (or cost effective) under traditional precipitation or air monitoring approaches.

Nonetheless, biomonitoring using mosses has limitations; for example, it is unlikely that a particular species of moss will be found at every sampling site, resulting in gaps in spatial

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coverage and associated mapping. This can be overcome by sampling a combination of species, but requires interspecies calibration owing to differences in uptake efficiency between moss species (Berg et al., 1995; Halleraker et al., 1998; Fabure et al., 2010). Further, the relationship between tissue chemistry and atmospheric deposition may be confounded by other environmental factors. Linear relationships have been identified between the percent (%) N content in moss tissue and both measured (Harmens et al., 2011) and modelled N deposition (Leith et al., 2008; Harmens et al., 2011; Stevens et al., 2011). Similarly, linear relationships have also been demonstrated for many trace metals (Berg et al., 1995), although deposition is not the predominant predictor of tissue concentration for all metals (e.g., Hg; Holy et al., 2009). Nonetheless, moss analysis compliments traditional deposition measurements and has been predominantly used to infer areas at risk from atmospheric pollutant deposition or map changes in deposition over time. Ireland's climate is dominated by relatively clean Atlantic air; however, total N deposition, dominated by emissions of reduced N from the agricultural sector, is relatively high (Hyde et al., 2003; Henry & Aherne, 2014). Further, transboundary air pollution from mainland Europe is associated with elevated deposition of sulphur, oxidized nitrogen, and heavy metals (Bowman & McGettigan, 1994; Burton et al., 2013), albeit decreasing in recent years (Burton & Aherne, 2011). Elevated deposition may impact ecosystem structure and function in semi-natural habitats, e.g., habitats listed in the European Union's Annex I (habitat types of conservation importance). One such habitat is acidophilous *Quercus*-dominated woodland, or Atlantic oak woodland. The objective of this study was to assess the potential of two moss species, *Isothecium myosuroides* and *Thuidium tamariscinum*, to be used as bioindicators of atmospheric deposition in Irish Atlantic oak woodlands. This study compared tissue chemistry between moss species for nitrogen, sulphur, and eleven heavy metals to determine if they could be used interchangeably for biomonitoring, and compared the tissue chemistry of each species against indices of atmospheric deposition to assess their suitability as bioindicators.

MATERIALS AND METHODS

Sampling and laboratory analysis

Moss species were collected during April 2013 from thirty-nine woodland sites across the south-eastern region of Ireland. All study sites were surveyed for vegetation under the Irish National Survey of Native Woodlands (Perrin et al., 2008) and were classed as *Quercus petraea-Luzula sylvatica* woodlands (Atlantic oak woodlands). Two pleurocarpus moss species, *Isothecium myosuroides* (Im) and *Thuidium tamariscinum* (Tt) were present at a sub-set of twenty-two sites (Figure 1); these sites were retained for subsequent analysis. Clippings of each species were taken from 5−7 different locations within a 20 m radius from the centre of the study site and composited by species to reduce potential differences between individual plants, such as changes in tissue content due to plant age (Hill et al., 2002). Unwashed samples were stored in paper bags and oven-dried at 60°C for 70 hrs; care was taken to remove any soil, bark, or other contaminants. Dried samples were pulverized in a mixing mill (SPEX Mixer Mill 6000D; three minutes per sample). Samples were analysed for nitrogen (N) and sulphur (S) content (%) using an Elementar vario Marco CNS analyzer. Heavy metal concentrations for eleven metals (aluminum (Al), vanadium (V), chromium (Cr), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), antimony (Sb), and lead (Pb)) were determined using a Triple-Quad ICP-MS analyzer following acid digestion (Mars 6 microwave digester, EPA method 3052).

Regional datasets and statistical analysis

Long-term (1991−2010) total N deposition (wet NH_4^+ and NO_3^- plus dry NH_3 and NO_X for forests) data were obtained from Henry & Aherne (2014) on a 5 km × 5 km grid and atmospheric ammonia (NH_3) concentration (1999−2000) on the same grid spacing was obtained from de Kluizenaar and Farrell (2000). Non-marine sulphate ($nmSO_4^{2-}$) deposition maps were obtained from Aherne et al. (2014). Total N deposition and atmospheric NH_3 concentrations were considered indices of N deposition and $nmSO_4^{2-}$ deposition was considered an index of sulphur deposition for comparison with moss tissue % N and % S. Site data were extracted from the regional datasets using Quantum GIS (QGIS, 2014).

The % N and % S tissue content, and heavy metal concentrations, were compared between species using Spearman's rank correlation test. Tissue chemistry was compared between moss species using a two-tailed paired Student's t-test. Interspecies ratios (Im:Tt) were calculated for N, S and heavy metals. Spearman's rank correlation tests were used to determine whether there were significant correlations between moss tissue chemistry and atmospheric deposition indices (total N deposition, atmospheric NH_3 concentrations, $nmSO_4^{2-}$ deposition, and easting and northing, which were assumed to be surrogates for long-range atmospheric transport). Simple linear regression

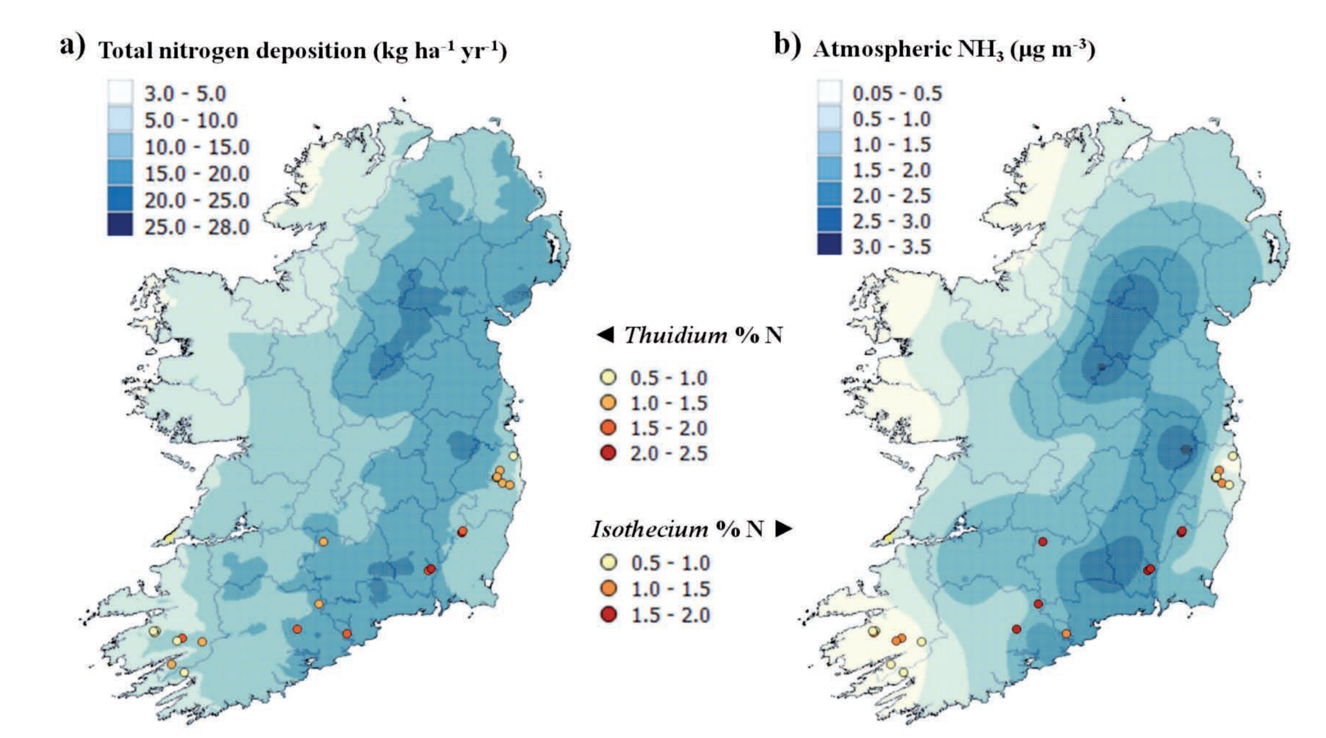

Figure 1. Map of Ireland showing (a) mapped total nitrogen deposition for deciduous forests and (b) atmospheric ammonia concentrations. Study site locations (filled circles) are shaded to represent % N tissue content of (a) *Isothecium myosuroides*, and (b) *Thuidium tamariscinum*. Map lines indicate county borders.

was used to determine the relationship between tissue N content (%) and total N deposition (kg N ha^{-1} yr^{-1}), and atmospheric NH_3 concentrations (µg m^{-3}). All statistical analysis was conducted in R 3.0.3 (2014). Obvious outliers were removed prior to analysis (seven values, Im: Al = 1, Cr = 1, Ni = 1, Pb = 1; and Tt: Al = 1, Ni = 1, Zn =1).

Results

The % N tissue content in Im ranged from 0.65–1.93% (mean = 1.23%, median = 1.06%, standard deviation (s.d.) = 0.39), compared with 0.83–2.28% (mean = 1.34%, median = 1.39%, s.d. = 0.42) in Tt (Table 1). Nitrogen tissue content (%) was significantly correlated between species (r_s = 0.84, p < 0.001), but the mean value between species was significantly different (t = –2.1, p < 0.05). Tissue % S content ranged from 0.07–0.20% (mean = 0.10%, median = 0.9%, s.d. = 0.03) in Im and 0.07–0.18% (mean = 0.10%, median = 0.10%, s.d. = 0.02) in Tt. No interspecies correlation was found for % S, and the mean was not significantly different between species.

Table 1. Statistical summaries of tissue content analysis for heavy metals (Al, V, Cr, Fe, Ni, Cu, Zn, As, Cd, Sb, Pb: mg kg^{-1}), and nitrogen and sulphur (%). *p*-value = significance of Student's t-test between species; r_s = results of Spearman's rank correlation test, (** = p < 0.001, * = p < 0.05).

	Isothecium myosuroides		***Thuidium tamariscinum***			
	Mean	**Standard deviation**	**Mean**	**Standard deviation**	***p*-value**	r_s
%N	1.23	0.39	1.34	0.42	< 0.05	0.84**
%S	0.10	0.03	0.10	0.02	0.31	0.25
Al	68.60	39.57	39.34	20.23	< 0.005	0.48*
V	0.76	0.21	0.68	0.21	0.08	0.57*
Cr	1.04	0.37	1.28	0.45	< 0.05	0.20
Fe	262.26	107.33	242.09	103.86	0.38	0.35
Ni	1.17	0.28	0.99	0.27	< 0.005	0.66**
Cu	2.51	0.82	3.17	0.97	< 0.05	0.46*
Zn	26.43	14.18	20.64	5.88	< 0.05	0.56*
As	0.12	0.08	0.09	0.07	< 0.001	0.72**
Cd	0.16	0.09	0.07	0.03	< 0.001	0.41
Sb	0.06	0.03	0.03	0.02	< 0.001	0.59**
Pb	2.00	0.99	1.08	0.63	< 0.001	0.77**

The trace metal with the highest mean concentration was Fe, followed by Al > Zn > Cu > Pb > Ni > Cr > V > Cd > As > Sb (Table 1). Interspecies correlation was observed for Al, V, Ni, Cu, Zn, As, Sb and Pb. In contrast, no interspecies correlation was observed for Cr, Fe or Cd (Table 1). Mean concentrations were significantly different for all metals except V and Fe (Pairwise two-tailed t-test, $p < 0.05$). The Im:Tt interspecies ratios varied between metals (Figure 2), with ratios falling above and below 1.0 (Im = Tt) for all elements except Pb. However, with the exceptions of Cu and Cr, the median for each trace metal was above 1.0, suggesting that the metal concentrations in Im were generally higher than in Tt.

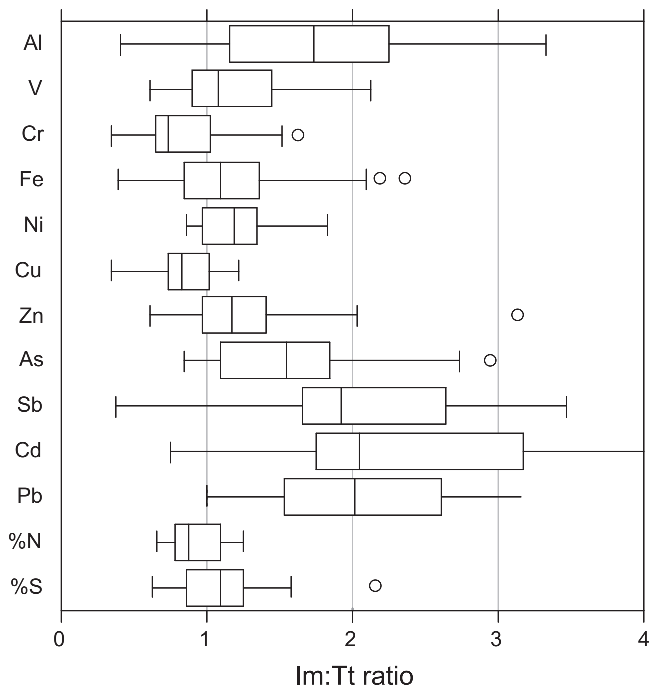

Figure 2. Boxplots of interspecies Im:Tt ratios (*Isothecium myosuroides:Thuidium tamariscinum*) of tissue concentrations for eleven trace metals, nitrogen and sulphur. The boxplot midline represents the median, edges of the box represent first and third quartiles, whiskers extent 1.5 × the width of the box, and outliers are indicated by open circles.

Easting (E) and northing (N) were significantly correlated with As (Spearman's rank correlation $r_s = 0.60$ [E], 0.52–0.54 [N]), Sb (r_s = 0.63–0.70 [E], 0.54–0.71 [N]), and Pb (r_s = 0.73–0.81 [E], 0.67–0.72 [N]) in both moss species (Im–Tt), and easting was correlated with Cd (r_s = 0.48–0.53) in both mosses, with V ($r_s = 0.43$) in Im, and with Cu ($r_s = 0.52$) in Tt. Non-marine SO_4^{2-} deposition was correlated only with V ($r_s = 0.43$) and Ni ($r_s = 0.53$) in Im mosses. A significant linear relationship was observed between % N tissue content and atmospheric NH_3 concentrations (Figure 3a; Im: $y = 0.80 + 0.43x$, $R^2 = 0.67$; Tt: $y = 0.88 + 0.47x$, $R^2 = 0.65$), as well as total N deposition (Figure 3b; Im: $y = -0.05 + 0.10x$, $R^2 = 0.57$; Tt: $y = -0.03 + 0.11x$, $R^2 = 0.54$). In contrast, S tissue content was not related to nmSO_4^{2-} deposition.

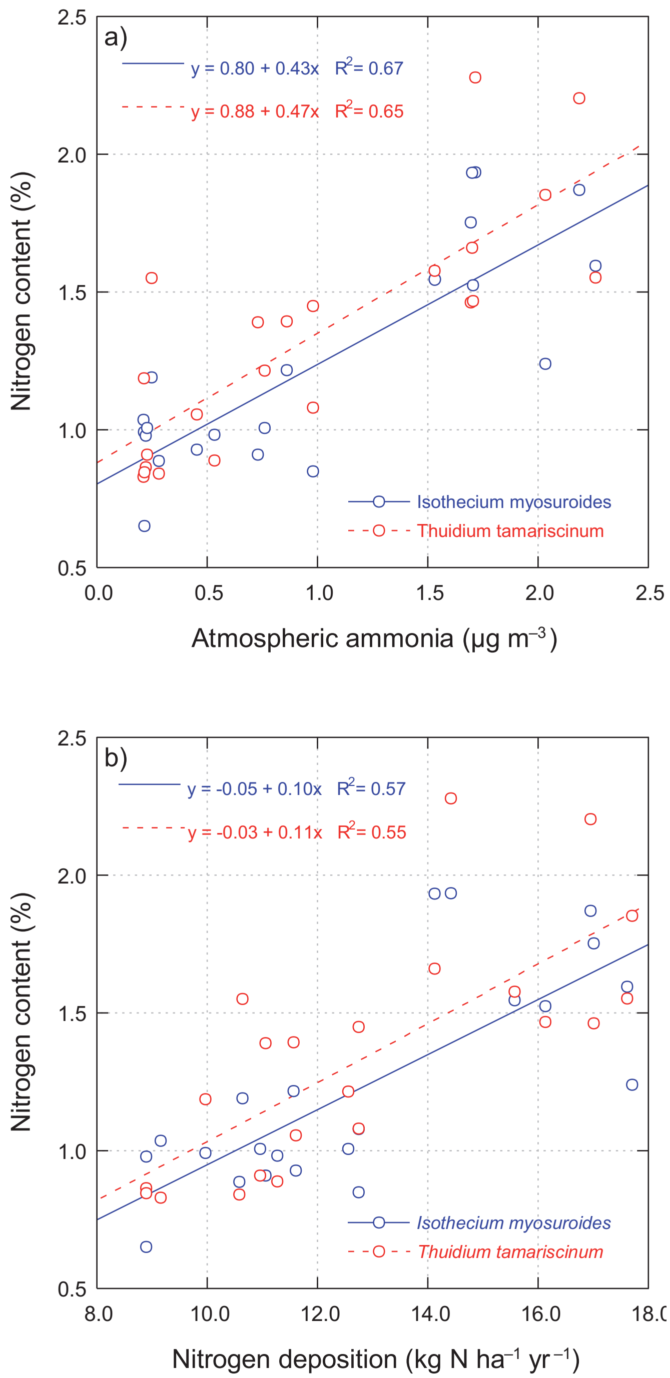

Figure 3. Simple linear regression between moss tissue nitrogen content (%) and (a) atmospheric ammonia concentrations (µg m^{-3}) and (b) total nitrogen deposition (kg N ha^{-1} yr^{-1}) for *Isothecium myosuroides* and *Thuidium tamariscinum*.

Discussion

Relationships to environmental indices

The ICP Vegetation recommend *Pleurozium schreberi* and *Hylocomium splendens* as bioindicators, followed by *Hypnum cupressiforme* and *Pseudoscleropodium purum* (Moss Monitoring Manual: Harmens, 2009).

However, none of these species were frequent at the oak woodland plots in the current study; as such, the most commonly observed pleurocarpus mosses were evaluated for their potential as alternative bioindicators. The % N tissue content of Im and Tt moss species was correlated with atmospheric NH_3 concentrations and total N deposition, suggesting that these species may be a valuable tool for assessing regions or habitats that are potentially threatened by atmospheric N deposition in Ireland. Previous studies have also demonstrated similar significant linear relationships (Pitcairn et al., 1995; Leith et al., 2008; Harmens et al., 2011). Bassingthwaight & Shaw (2010) were unsuccessful at relating % N tissue content in *Isothecium myosuroides* to measured N deposition at four sites in British Columbia, Canada; however, they did find a relationship between tissue N and modelled N deposition in a survey of fifty-seven sites. The lack of correlation between % N tissue content and site coordinates in this study suggests local rather than transboundary emissions sources of N, which is consistent with studies that suggest NH_3 emissions are mostly deposited close to their source (Pitcairn et al., 1998; Sutton et al., 1998; Dore et al., 2008; Vogt et al., 2013). Dry deposition of NH_3 from agricultural sources constitutes the majority of total N deposition in Ireland (Hyde et al., 2003; Henry & Aherne, 2014), emphasizing the need for monitoring programs with high spatial resolution.

Few trace metals were correlated with deposition indices used in this study. However, As, Sb, and Pb showed a significant increase with increasing easting and northing, and V, Cu, and Cd with easting, suggesting that their sources were potentially anthropogenic, as the majority of Ireland's industry is located in northern and eastern regions, or possibly from transboundary sources in mainland Europe. This is in agreement with Bowman & McGettigan (1994), who reported higher oxidized nitrogen and non-marine sulphur deposition in Ireland associated with easterly winds.

Interspecies correlations

The range of N tissue content found in moss tissue in this study (0.65−2.28%) was within the range reported across Europe (for a variety of moss species) by Harmens et al. (2011; 0.34−3.82%); further, the N content range (0.44−2.45%) reported for sites in the United Kingdom (n = 170), was very similar to the current study. In concert with other studies comparing tissue chemistry between moss species (Halleraker et al., 1998), our results show interspecies correlations for most of the elements. The correlations found between species for % N tissue content, and Al, V, Ni, Cu, Zn, As, Sb, and Pb metal concentrations, suggest that Im and Tt could be used in combination as bioindicators for these elements in Atlantic oak woodlands. However, significant difference in means for all correlated elements except V indicates that calibration factors are needed prior to mapping the combined results (e.g., Halleraker et al., 1998). In contrast, no significant interspecies correlations were found for %S, Cr, Fe, and Cd, suggesting that Im and Tt cannot be used interchangeably as bioindicators for these elements. Most trace metals (Al, Ni, Zn, As, Cd, Sb, and Pb) had higher concentrations in Im than Tt; the opposite was true for Cr and Cu. Berg & Steinnes (1997) and Halleraker et al. (1998), compared *Hylocomium splendens* and *Pleurozium schreberi* mosses and also reported interspecies ratios both greater and less than 1.0.

Conclusions

Both *Isothecium myosuroides* and *Thuidium tamariscinum* are common species in Atlantic oak woodlands (Atherton et al., 2010) and have been utilized in similar studies comparing tissue content to indices of deposition (Im: Mitchell et al., 2004; Leith et al., 2008; Bassingthwaight & Shaw, 2010; Tt: Leith et al., 2008). However, neither species is recommended by the ICP Vegetation (Moss Monitoring Manual: Harmens, 2009). The results of the current study suggested that tissue chemistry of *Isothecium myosuroides* and *Thuidium tamariscinum* could be used for assessing and monitoring atmospheric deposition in Irish Atlantic oak woodlands (Annex 1 habitat: 91A0), particularly for atmospheric NH_3 and some trace elements (Cu, As, Sb, and Pb). However, the variation between species, with higher concentrations in Im for most metals, suggests that interspecies calibration is required, and should include an assessment of site variability. As well, it should be noted that Im and Tt are both epiphytic species, and tissue content may be influenced by stemflow and throughfall (Leith et al., 2008). Further, this study focused on the east and south east of Ireland, increasing the spatial coverage of study sites would help identify regions in Ireland potentially threatened by atmospheric deposition.

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