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Determination of polycyclic aromatic hydrocarbons, dibenzothiophene, and alkylated homologs in the lichen *Hypogymnia physodes* by gas chromatography using single quadrupole mass spectrometry and time-of-flight mass spectrometry

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ABSTRACT

Development of the Athabasca Oil Sands Region in northeastern Alberta, Canada has contributed polycyclic aromatic hydrocarbons (PAHs) and polycyclic aromatic compounds (PACs), which include alkyl PAHs and dibenzothiophenes, to the regional environment. A new analytical method was developed for quantification of PAHs and PACs in the epiphytic lichen bioindicator species *Hypogymnia physodes* for use in the development of receptor models for attribution of PAH and PAC concentrations to anthropogenic and natural emission sources. Milled lichens were extracted with cyclohexane, and extracts were cleaned on silica gel using automated solid phase extraction techniques. Quantitative analysis was performed by gas chromatography with selected ion monitoring (GC-SIM-MS) for PAHs, and by GC with time-of-flight mass spectrometry (GC-TOF-MS) for PACs. PACs were quantitated in groups using representative reference compounds as calibration standards. Analytical detection limits were $\leq$2.5 ng g^{-1} for all individual compounds. Precision as measured by laboratory duplicates was variable; for individual analytes above 5 ng g^{-1} the mean absolute difference between duplicates was typically <20%. Selection of single-analyte markers for source attribution should include consideration of data quality indicators. Use of TOF-MS to spectrally characterize PAC group constituents identified significant challenges for the accurate quantitation of PACs with more than two carbons in their side chain(s). Total PAH concentrations in lichen samples ranged from 12 to 482 ng g^{-1}. Total PACs in each sample varied from a fraction of total PAHs to more than four times total PAHs. Results of our analyses of *H. physodes* are compared with other studies using other species of lichens as PAH receptors and with passive monitoring data using polyurethane foam (PUF) samplers in the Athabasca Oil Sands Region (AOSR). This study presents the first analytical methodology developed for the determination of PACs in an epiphytic lichen bioindicator species.

1. Introduction

Bitumen extraction and upgrading activities in the Athabasca Oil Sands Region (AOSR) of northeastern Alberta, Canada are a source of polycyclic aromatic hydrocarbons (PAHs) and other petrogenic compounds in the region's environment [1–12]. Proper interpretation of environmental data for PAHs and other compounds requires an understanding of both their distribution within the environment, as well as an accounting for inputs. Sources of PAHs in the AOSR may include both natural air emissions (e.g., wildfires), water seepage from the exposed bitumen deposits, anthropogenic emissions related to bitumen extraction and upgrading operations, and non-operations sources such as on-road vehicular emissions.

Poor accessibility and lack of power infrastructure in the remote boreal forests surrounding the oil sands production facilities necessitated the use of bioindicators as ambient monitoring surrogates for elucidating the spatial distribution of atmospheric PAHs deposition in the AOSR. Lichens are well-known bioindicators of ecosystem health and accumulators of pollutants, including organ-

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ics [13,14]. Since the early 2000s, lichens have been studied as PAH bioindicators by a number of investigators, particularly in Portugal [15–21], Spain [22–26], Italy [27–36], India [37–41], as well as other countries [36,42–45]. An important component of the use of lichens as a biomonitoring tool is the demonstrated ability to relate lichen concentrations with standard measurements of airborne PAHs. Total PAHs measured in the lichen *Parmotrema hypoleucinum*, near a high-volume total suspended particulate (TSP) sampler, correlated with TSP ($r^2 = 0.71–0.85$, $p < 0.05$) [19]. A transplantation study using the lichen *Evernia prunastri* showed that uptake of PAHs by the lichens correlated well ($r^2 = 0.96$, $p < 0.05$) with uptake of PAHs by collocated polyurethane foam passive samplers over the same time period [32]. These and other studies provide evidence for the efficacy of lichens as biological monitors of ambient air PAH levels in remote regions like the AOSR.

Polycyclic aromatic compounds (PACs) is a term commonly used to denote PAHs with aliphatic side chains containing up to a total of four saturated carbons. The U.S. Environmental Protection Agency (EPA) has identified a list of "34 PAHs" that includes alkyl PAHs, and has described a method for their analysis [46]. PACs determined by the EPA method are reported in groups according to the parent PAH nucleus (and isomers) and the number of carbons in the side chain (e.g., C1-phenanthrenes includes methylphenanthrenes and methylanthracenes; C3 fluoranthenes includes trimethyl-, methylethyl-, and propylfluoranthenes and pyrenes). The term PACs also frequently includes dibenzothiophene and the alkyl dibenzothiophenes, and studies of PAH and PAC deposition in the AOSR have commonly included these in their studies [1–3,5–8,10–12]. PACs are also of environmental concern as their transformation into PAH-carboxylic acids may render them potentially more toxic [3,47].

Forensic data analysis tools called receptor models are used to extract information on the sources of air pollutants from measured concentrations at different receptor locations in the environment [48]. Since 2002, a number of investigators funded by the Wood Buffalo Environmental Association (www.wbea.org) have used the lichen species *Hypogymnia physodes* as a receptor organism for monitoring atmospheric deposition of inorganic species over an area of nearly 68,000 km^2 surrounding the AOSR surface mining and upgrading facilities near Fort McMurray, Alberta. In 2010 and 2014, pilot studies demonstrated the feasibility and suitability of using *H. physodes* as a PAH receptor [49,50]. In 2014, a larger study was initiated encompassing~130 sites across the AOSR to examine the use of *H. physodes* as a terrestrial receptor for PACs. Here we report the analytical methods employed in that study, including the use of gas chromatography with time-of-flight mass spectrometry (GC-TOF-MS) for determination of PACs, and present data and initial observations on this first use of a lichen species as a bioindicator of PACs.

2. Materials and methods

2.1. Lichen sample collection

Samples were collected from 127 sites within the AOSR at varying distances from the oil sands production and upgrading operations (~3–150 km). The sampling program for a similar study has been described previously [51]. Bulk composite samples of ~30 g of *H.physodes* were collected from at least 10 jack pine and/or black spruce host trees at approximately 1.5 m off the ground at each lichen sampling site. Field personnel wore vinyl gloves and used Teflon-coated tweezers for sample collection. Composited samples were placed into a pre-cleaned amber glass jar (I-Chem Class 200, IR 241–500, Thermo Fisher, Waltham, MA) labeled with tracking numbers representing the site and date of collection, then stored in a cooler for the duration of the day. Lichen samples were manually cleaned in the laboratory using clean handling techniques (by removing all foreign materials, bark and other debris) under a class 1000 clean hood. No drying of the samples was performed beyond that which occurred during cleaning (equilibration with the laboratory atmosphere) in order to minimize inadvertent losses of more volatile target analytes. Pilot studies by collaborators indicated that lichens prepared as described (n = 63) contained 3.27% ± 1.54% residual water [52]. Each cleaned sample was put into a new clean amber glass jar, and then stored frozen until shipment for analysis. Samples with chain of custody forms were shipped to RTI International (Research Triangle Park, NC, USA) and stored at −20 °C prior to further processing.

2.2. Sample extraction, cleanup, and analysis

Lichens (0.5–1 g) were placed in polycarbonate vials and milled for 30 s in liquid nitrogen using a freezer mill (Model 6875, SPEX, Metuchen, NJ). After warming to room temperature, the finely divided solid (0.2 g) was weighed into a scintillation vial and 50 μL internal standard solution (100 ng mL^{-1} each of 16 deuterium labeled standards, see below) was added, followed by 2 mL cyclohexane (Fisher pesticide grade). Method blanks and controls were prepared without lichen. Method controls and lichen duplicate spikes received 50 μL of a PAH + PAC standard solution (100 ng mL^{-1} each component, see below). Samples were sonicated for 30 min (Model 8510, Bransonics, Danbury, CT), and decanted. The extraction was repeated two times, and the combined extracts were dried over anhydrous sodium sulfate (50 mg), centrifuged, and decanted. The extracts were concentrated to 0.2 mL using a TurboVap LV (Biotage, Charlotte, NC) with n-decane (Sigma-Aldrich, St. Louis, MO) as a keeper solvent. A typical batch included 13 unknowns, a method blank, and a method control. Near the end of the study, a subset of samples from early batches with higher concentrations of PAHs were selected for preparation of method duplicates or duplicate spikes.

Crude extracts were cleaned up using automated solid phase extraction (SPE) on a GX-271 ASPEC (Gilson, Middleton, WI). Glass SPE cartridges with 1 g silica (Macherey-Nagel, Bethlehem, PA) were pre-conditioned with 4 mL dichloromethane (Fisher GC Resolve®), followed by 4 mL hexane (Fisher GC Resolve ®). The extract was loaded on the column and washed with 2.9 mL hexane, then eluted with 4 mL 60% hexane/40% dichloromethane. The eluate was concentrated short of dryness on the TurboVap LV and the volume was adjusted to 0.2 mL with hexane. The extracts were transferred to autosampler vials with 250 μL glass inserts and stored at −20 °C prior to analysis.

2.3. Analytical standards

Target PAHs for calibration were obtained in a 24-component mix including 16 EPA PAHs, plus benzo(c)phenanthrene, benzo(j)fluoranthene, benzo(e)pyrene, and dibenzo(al/ai/ah)pyrenes at 500 μg mL^{-1} in dichloromethane/benzene (#H-QME-01, AccuStandard, New Haven, CT). A second-source PAH standard included the 16 EPA PAHs at 2000 μg mL^{-1} in dichloromethane (#31011, Restek, Bellefonte, PA). Deuterium-labeled PAHs for internal standards included the 16 EPA PAHs at 100 μg mL^{-1} in benzene-d6 (#ES-2528, Cambridge Isotope Laboratories, Andover, MA). Calibration standards also included ^{13}C-labeled standards for some analytes, but these were not used in this study.

The following PAC reference compounds were purchased from AccuStandard (New Haven, CT) as single-component solutions in toluene at 50 μg mL^{-1}: 7, 10-dimethylbenzo(a)pyrene, 1-methylbenz(a)anthracene, and 7-methylbenzo(a)pyrene. The

following targets or reference compounds were purchased from Chiron (Trondheim, Norway) as single-component solutions: 1-ethylpyrene, 200 μg mL^{-1} in toluene; and retene (500 μg mL^{-1}). The remaining PACs reported in this study were included in a 20-component mix from Chiron (S-4406-200-T) at 200 μg mL^{-1} in toluene.

All working solutions and calibration standards were prepared by dilution with hexane (pesticide residue grade, #H307, Thermo Fisher Scientific, Sunnvale, CA) in hexane-rinsed Class A volumetric glassware, using microliter syringes (Hamilton Co., Reno, NV). Internal standard spiking solution (D labels) was prepared at 100 ng mL^{-1}. PAH and PAC calibration standards were prepared at 7 levels from 0.5 to 50 ng mL^{-1}. All calibration standards contained internal standard at 25 ng mL^{-1}. All standards were stored at −20 °C when not in use.

2.4. GC-SIM-MS analysis

PAHs were determined initially using an Agilent 7890/5975 GC/MS with an Agilent DB-5MS column (60 m x 250 mm x 0.25 μm) with helium carrier at 1 mL min^{-1}. GC conditions were as follows: 1 μL injection; inlet, 250 °C splitless, carrier 1 mL min^{-1} He, oven program 70 °C for 3 min, ramp @ 7 °C min^{-1} to 325 °C, hold 20 min. The transfer line temperature was 290 °C. The detector was operated in electron ionization mode at 70 eV with selected ion monitoring (SIM). The source temperature was 230 °C and the quadrupole temperature was 150 °C. Monitored ions, SIM windows, and retention times and indices are presented in Table S1a. The instrument was calibrated using seven standards ranging from 0.5–50 ng mL^{-1}. Standards at low concentrations that did not give sufficient signal were dropped. For all PAHs, a quadratic fit with 1/x weighting was used, with correlations expected to yield $r^2 \geq 0.99$. Benzo(b)fluoranthene and benzo(j)fluoranthene were incompletely resolved and are reported together as benzo(bj)fluoranthene.

2.5. GC-TOF-MS analysis

PACs method development and analysis were performed on a LECO (St. Joseph, MI) Pegasus 4D gas chromatograph with time-of-flight mass spectral detection (GC-TOF-MS). TOF-MS provides complete unit mass spectra at each point in a chromatogram with only slightly less sensitivity than SIM methods, and as such is particularly suited to trace analysis of complex mixtures. GC conditions were slightly altered from the GC-SIM-MS analysis. The column was the same, with inlet, 280 °C splitless, carrier 1.5 mL min^{-1} He, oven program 50 °C for 5 min, ramp @ 10 °C min^{-1} to 325 °C, hold 18 min. The transfer line temperature was 280 °C. Mass spectrometer settings for the TOF-MS were as follows: acquisition delay, 600 s; mass range, 35–500 amu, acquisition rate, 4 s^{-1}; detector voltage, 350 V; source temperature, 275 °C; source voltage, 70 eV. The GC-TOF-MS was also calibrated with five to seven standards ranging from 0.5–50 ng mL^{-1}. In addition to the unique compounds 1-methylnaphthalene, 2-methylnaphthalene, dibenzothiophene, and the biomass combustion marker retene, a single compound was selected as representative of each PAC group for generation of a calibration curve. Quantitation ions, retention times and indices are summarized in Table S1b. Linear fit with 1/x weighting was used, with correlations expected to yield $r^2 \geq 0.99$.

Quantitation of PAC groups first required the identification of retention time ranges. Retention indices (RI) for single targets and reference compounds [53] were calculated, substituting benzo(ghi)perylene for picene as the reference value RI = 500 [54]. Archived chromatograms from GC-TOF-MS analysis of lichen sample extracts with high concentrations of PAHs were examined to generate RI windows for groups. For a given group, the chromatogram of the molecular ion of a particular PAC group was displayed. Using published RI data for C1 and C2 PACs [54,55], approximate retention time windows for the groups were calculated. The mass spectral display feature of the TOF-MS instrument software was used to examine individual peaks within each window and refine the retention window parameters. Groups were integrated either by expanding the baseline of the reference compound or by adding a peak and expanding the baseline to fit the window. Groups were quantitated in the software if the reference compound was used, or outside the instrument software system using response factors (calculated as the ratio of the group area to the internal standard peak area) and the calibration regression parameters for the reference compound (e.g., 2-methyl phenanthrene for the methylphenanthrenes).

Because all of the lichen samples had measurable PAHs, the method detection limits (MDLs) were estimated using seven replicate matrix-free spikes carried through the entire method at the equivalent of the lowest calibration level, equivalent to 0.5 ng g^{-1}. MDLs were calculated as the one-tailed Student's t value at p = 0.01 multiplied by the standard deviation of the seven measurements.

In all analytical runs, up to 10 samples (including blanks and controls) were bracketed by solvent (hexane) blanks and continuing calibration verification (or calibration check) standards at one or more concentrations (usually 12.5 ng mL^{-1}). Solvent blanks were expected to be at or near noise level and to show no evidence of carryover. Check standards were expected to be with 15% of the nominal value. In addition, each batch included (i) one second source calibration check standard at 25 ng mL^{-1} for each analyte (EPA 16 PAHs) that was expected to be within 15% of the nominal value, and (ii) one duplicate injection to be used in assessing instrument precision. All results were blank-subtracted using the corresponding batch method blank. Post-processing, corrections were made to PAH data to compensate for isobaric interferences from co-eluting ^{13}C-labeled standards in the calibration curves (Table S2).

3. Results and discussion

Initial analytical development efforts were based on the method of Guidotti et al. [28] who extracted the lichen *Pseudevernia furfuracea* with cyclohexane and ultrasound. Cyclohexane was preferred due to its compatibility with the analytes of interest, and its propensity to minimize the amount of more polar or lipid-like materials extracted from the host biological matrix. To increase the extraction efficacy, we sought to maximize the surface area of the lichen matrix. Manual grinding was ineffective due to the tough texture of *H. physodes*. Ultimately we found that milling the lichen in liquid nitrogen for 30 s was sufficient to yield a uniform, fine powder for further processing.

Due to the logistical challenges of collecting samples from multiple, remote locations in the AOSR, and our need to reserve a majority of the sample for other analyses, we sought to limit our sample size for extraction to 0.2 g. This small a sample still contained substantial chromatographic interferences after drying, centrifuging, and concentrating. The automated SPE procedure that was used for cleanup of these extracts was a scaled-down version of the Guidotti et al. [28] procedure. All-glass SPE cartridges, including glass frits, were used to avoid contact with plastic; custom racks were provided by Gilson to accommodate the glass cartridges. Similarly, all components of the automated SPE instrument that came into direct contact with sample or solvents were glass, fluoropolymer, or stainless steel.

During pilot studies [49], samples were spiked with deuterium-labeled surrogate standards (naphthalene-d^8, acenaphthylene-d^8, phenanthrene-d^{10}, fluoranthene-d^{10}, pyrene-d^{10}, benzo(a)pyrene-

Table 1
Method detection limits for PAHs and PAC reference compounds by GC-SIM-MS and/or GC-TOF-MS (ng g^{-1}).

Analyte	SIM-MS	TOF-MS
Naphthalene	1.75	3.4[b]
Acenaphthylene	0.40	0.30
Acenaphthene	0.29	0.29
Fluorene	0.33	0.31
Phenanthrene	0.46	0.28
Anthracene	0.45	0.52
Fluoranthene	0.33	0.69
Pyrene	0.20	0.12
Benzo(c)phenanthrene	0.33	0.34
Benz(a)anthracene	0.14	0.26
Chrysene	0.26	0.44
Benzo(bj)fluoranthene	0.58	0.66
Benzo(k)fluoranthene	0.45	2.5[a]
Benzo(e)pyrene	0.95	0.55
Benzo(a)pyrene	1.09	2.5[a]
Indeno(1,2,3-c,d)pyrene	0.23	2.5[a]
Dibenz(a,h)anthracene	0.30	2.5[a]
Benzo(g,h,i)perylene	0.25	2.5[a]
Dibenzo(ai)pyrene	0.35	2.5[a]
Dibenzo(al)pyrene	0.21	2.5[a]
Dibenzo(ah)pyrene	1.44	2.5[a]
1-Methylnaphthalene	Not determined	0.81
2-Methylnaphthalene	Not determined	0.51
1-Methyl-9H-Fluorene	Not determined	0.57
Dibenzothiophene	Not determined	0.17
2-Methyldibenzothiophene	Not determined	0.23
2-Methylphenanthrene	Not determined	0.98
Retene	Not determined	1.02
1-Methylfluoranthene	Not determined	0.59
1-Methylchrysene	Not determined	0.59
7-Methylbenzo(a)pyrene	Not determined	2.5[a]
1,6-Dimethylnaphthalene	Not determined	0.45
2,4-Dimethylphenanthrene	Not determined	2.5[a]
1-Ethylpyrene	Not determined	2.5[a]
6-Ethylchrysene	Not determined	2.5[a]
7,10-Dimethylbenzo(a)pyrene	Not determined	5.0[a]

[a] Instrument quantitation limit.
[b] Estimated from method blanks.

d^{12}, and benzo(g,h,i)perylene-d^{12}) prior to extraction, with internal standards added after cleanup. Calculated concentrations of all analytes were adjusted in groups according to the recovery of the appropriate surrogate standard. We consistently recovered 80% or more of surrogates, and, therefore, discontinued their use for concentration correction. Subsequently, we began calibrating each analyte to its labeled analog or a very closely related PAH, and added a mixture of all 16 deuterium-labeled EPA standards at the start of the procedure, completely eliminating the need for *a posteriori* surrogate corrections.

All of the PAH calibrations required a quadratic fit on the Agilent 5975 GC–MS. We have observed this consistently for a variety of analytes on single quadrupole instruments, from volatile organics to organochlorine pesticides and PCBs. In contrast, a linear fit with $r^2 \geq 0.99$ for all PAHs and PACs was obtained on the TOF-MS. During TOF-MS method development, we found that TOF-MS instrument linearity extends to at least 2500 ng mL^{-1}. Linearity is a desirable method characteristic in that it is unbiased by the relative recovery of analytes and internal standards. Table 1 compares MDLs for PAHs, determined by SIM-MS and by TOF-MS, and for PACs, determined by TOF-MS. For most analytes, MDLs were <1 ng g^{-1}. Although comparison of chromatograms of the lowest level calibration standard suggested SIM-MS would have better sensitivity, variability in analyte recovery proved to be more important in many cases so that TOF-MS yielded a comparable MDL to SIM-MS. In some cases, instrument sensitivity did not extend to the lowest calibration level, and MDLs were estimated from instrument response.

Components of precision were assessed using data from instrument (injection) duplicates (n = 12) and laboratory duplicates of milled lichen samples (n = 8). For comparison, we also include in Table 2 precision data from seven spiked replicates at the concentration of the lowest calibration standard, used in calculating the MDLs. At this spike level, precision may be strongly influenced by analytical background (e.g., higher for naphthalene) and sensitivity (e.g., higher mass PAHs).

Caution is needed in determining within-sample precision when dealing with a large number of analytical targets with widely varying concentrations in complex mixtures. For some analytes, within-sample precision at lower concentrations may be adversely affected, for example, by analytical background, while other analytes may be at concentrations that do not represent the population. For this reason, we took the approach of duplicate extractions, across batches, that best represents laboratory variability (extraction, cleanup, and analysis) across a range of concentrations for each analyte. Table 2 summarizes the results of injection and laboratory duplicates, as measured by the mean absolute percent difference between duplicates. The analytical components of precision presented in Table 2 varied by analyte, in part due to the low frequency of detection for some of them, and due to low concentrations for others. Variability in the method blank values for a particular analyte also affected precision in some cases. Both injection and laboratory duplicates tended to be slightly more variable on the TOF. Precision for individual analytes occurring above 5 ng g^{-1} was typically in the range of 10% to 20%. For TOF PAC groups we observed higher imprecision (sometimes >30%) that was associated with greater chromatogram noise in the region of interest, and with challenges of consistently integrating very complicated peak groups (sometimes with quite different presentation in the gas chromatogram). We note that detailed data quality indicators are not consistently provided in the published literature for determining PACs in environmental samples from the AOSR. Our observations suggest that analytical precision data in particular are needed to enable comparison of monitoring data produced by different groups, and for identifying specific compounds and/or groups for use in source attribution.

Method control and sample duplicate spike recovery data are summarized in Table 3. Target recoveries were 70–130%; most analytes fell in the 80–110% range. Outliers among PAHs tended to fall among analytes that did not have deuterated analogs as internal standards. The duplicate spike data were not indicative of mass spectral signal suppression.

Implementation of a method for PAC determination by GC-SIM-MS requires *a priori* knowledge of the retention time window for each PAC group. Although published retention index data were available as a guide, we needed to allow for differences in PAC profiles among region-specific PAC sources. The use of TOF-MS reduced the level of effort involved in MS method development compared to SIM (SIM requires identifying retention windows for monitored ions, which becomes complicated when analyte groups overlap), and eliminated the need to re-determine windows in the event of a shift in retention times (which is commonly observed after preventive instrument maintenance in the course of GC studies involving large numbers of samples). The preservation of complete mass spectral data across the chromatogram by TOF-MS provides the advantage of being able to probe the composition of each retention window in detail.

During the identification of the retention windows for PACs using a reference sample, the importance of the complete mass spectral data became clear. The chromatogram for sample JQ-9410, a lichen sample collected from a location less than 10 km from two oil sand mines, was used for identifying PAC group windows, and included 8 peaks with mass 198 eluting within 16 s of the reference compound 2-methyldibenzothiophene (2-MeDBT), all with

Table 2
Instrument, method, and 0.5 ppb[a] precision.

	Instrument					Laboratory					0.5 ppb (7 replicates)	
		SIM		TOF			SIM		TOF		SIM	TOF
	TOF range (ng/g)	# Pairs > MDL	Mean absolute% difference	# Pairs > MDL	Mean absolute% difference	TOF range (ng/g)	# Pairs > MDL	Mean absolute% difference	# Pairs > MDL	Mean absolute% difference	Mean absolute% difference	Mean absolute% difference
Naphthalene	4.4–19.1	13	4.1%	10	7.3%	6.3–7.9	7	31.0%	6	28.0%	108.7%	<MDL
Acenaphthylene	0.4–0.7	4	16.4%	2	7.3%	0.4–0.4	0	N/A	1	15.2%	21.7%	19.3%
Acenaphthene	0.4–2.5	12	21.8%	7	24.5%	0.7–1.2	5	20.4%	4	37.0%	17.6%	18.5%
Fluorene	1–8.2	13	6.6%	13	6.3%	1.1–4.2	8	14.0%	8	18.7%	20.5%	19.9%
Phenanthrene	7.2–84.9	13	1.1%	13	2.7%	11–30.9	8	6.2%	8	14.3%	29.3%	17.7%
Anthracene	0.8–16.3	8	14.2%	7	17.1%	1.1–3.8	4	23.8%	5	33.4%	28.8%	33.2%
Fluoranthene	3–15.8	13	7.2%	13	4.3%	4.3–8.3	8	19.6%	8	15.7%	20.2%	44.0%
Pyrene	1.3–38.7	13	4.9%	13	7.9%	1.5–11	8	16.9%	8	25.0%	11.2%	7.9%
Benzo(c)phenanthrene	1.2–4	2	9.0%	2	11.2%	0–0	2	11.3%	0	N/A	20.7%	21.9%
Benz(a)anthracene	0.7–43.9	13	5.8%	11	8.0%	0.5–9.3	8	11.9%	8	42.1%	8.4%	16.3%
Chrysene	1.2–85	13	5.4%	12	11.5%	1.7–19.6	8	12.0%	8	20.1%	16.1%	27.7%
Benzo(bj)fluoranthene	2.5–27.6	13	19.7%	11	14.2%	3.1–9.7	8	68.9%	7	64.1%	35.3%	43.0%
Benzo(k)fluoranthene	4.8–4.8	0	N/A	1	6.4%	0–0	0	N/A	0	N/A	25.7%	<MDL
Benzo(e)pyrene	2.2–43.4	6	5.7%	8	37.4%	1.3–50.3	4	19.2%	6	46.2%	61.0%	35.3%
Benzo(a)pyrene	3–48.5	12	7.4%	5	34.6%	5.6–45	4	30.1%	3	66.7%	65.3%	<MDL
Indeno(1,2,3-c,d)pyrene	11.4–11.4	12	25.0%	1	18.3%	0–0	3	76.9%	0	N/A	13.5%	<MDL
Dibenz(a,h)anthracene	15.5–15.5	2	4.8%	1	3.5%	0–0	2	38.3%	0	N/A	18.3%	<MDL
Benzo(g,h,i)perylene	3.1–29.5	13	4.3%	3	1.9%	5.2–5.2	8	16.2%	1	21.3%	14.5%	<MDL
Dibenzo(ai)pyrene	8.7–8.7	0	N/A	1	3.2%	0–0	3	4.0%	0	N/A	21.0%	<MDL
Dibenzo(al)pyrene	0–0	0	N/A	0	N/A	0–0	0	N/A	0	N/A	13.1%	<MDL
Dibenzo(ah)pyrene	0–0	0	N/A	0	N/A	0–0	0	N/A	0	N/A	<MDL	<MDL
1-Methylnaphthalene	2.6–41.3	N/A	N/A	12	3.7%	5.7–13.3	N/A	N/A	8	15.4%	N/A	51.9%
2-Methylnaphthalene	1.3–17	N/A	N/A	12	6.3%	2.7–7.4	N/A	N/A	8	18.3%	N/A	32.3%
Methylfluorenes	1.8–30	N/A	N/A	12	20.4%	2.9–9.4	N/A	N/A	8	60.9%	N/A	36.5%
Dibenzothiophene	1–46	N/A	N/A	13	7.8%	1.1–8.8	N/A	N/A	8	17.6%	N/A	10.7%
4-Methyldibenzothiophene	1.6–93.3	N/A	N/A	13	9.2%	2–25.5	N/A	N/A	8	21.7%	N/A	N/A
2/3-Methyldibenzothiophene	1–65.7	N/A	N/A	13	14.6%	1.2–16.2	N/A	N/A	8	23.0%	N/A	14.7%
1-Methyldibenzothiophene	0.6–39.9	N/A	N/A	13	14.7%	0.6–11.6	N/A	N/A	6	30.6%	N/A	N/A
Methylphenanthrenes	10.8–242	N/A	N/A	13	6.3%	17.8–83.1	N/A	N/A	8	22.9%	N/A	N/A
Retene	3.2–59.2	N/A	N/A	13	4.2%	6.7–25.6	N/A	N/A	8	15.9%	N/A	65.2%
Methylfluoranthenes	5.3–117	N/A	N/A	13	16.9%	7–33.4	N/A	N/A	8	31.8%	N/A	N/A
Methylchrysenes	3.4–252	N/A	N/A	12	12.8%	5.1–54.8	N/A	N/A	8	23.5%	N/A	N/A
Methylbenzopyrenes/methylbenzofluoranthenes	63.6–207	N/A	N/A	2	21.4%	40.4–40.4	N/A	N/A	1	14.1%	N/A	N/A
C2-Naphthalenes	1.6–79.2	N/A	N/A	13	9.7%	12.9–33.7	N/A	N/A	8	16.9%	N/A	N/A
C2-Dibenzothiophenes	5.8–272	N/A	N/A	13	14.7%	2–86.6	N/A	N/A	8	40.0%	N/A	N/A
C2-Phenanthrenes	19.4–312	N/A	N/A	13	11.2%	22.6–121	N/A	N/A	8	27.9%	N/A	N/A
C2-Pyrenes	8.4–241	N/A	N/A	13	17.7%	12–66.7	N/A	N/A	7	52.3%	N/A	N/A
C2-Chrysenes	8.6–312	N/A	N/A	9	19.4%	20.6–55.5	N/A	N/A	5	53.3%	N/A	N/A

[a] Spike equivalent to 0.5 ng/g in lichens. N/A = insufficient or no data; <MDL = spike level was below MDL.

Table 3
Recovery of PAHs and reference PAC compounds from method controls and duplicate spikes, 25 ng g^{-1}.

		Method controls (n = 12)		Duplicate spikes	
	Instrument method	Mean recovery	RSD	30B16	JP312
Naphthalene	SIM	108%	4%	110%	101%
Acenaphthylene	SIM	124%	4%	127%	112%
Acenaphthene	SIM	105%	2%	111%	104%
Fluorene	TOF	140%	6%	90%	100%
Phenanthrene	SIM	97%	4%	101%	97%
Anthracene	SIM	102%	6%	105%	84%
Fluoranthene	SIM	102%	6%	100%	94%
Pyrene	SIM	98%	2%	97%	89%
Benzo(c)phenanthrene (50 ng/g)	SIM	89%	11%	74%	84%
Benz(a)anthracene	SIM	91%	6%	95%	85%
Chrysene	SIM	98%	6%	107%	91%
Benzo(bj)fluoranthene (75 ng/g)	SIM	85%	18%	106%	93%
Benzo(k)fluoranthene	SIM	97%	7%	101%	92%
Benzo(e)pyrene (50 ng/g)	SIM	95%	19%	63%	68%
Benzo(a)pyrene	SIM	92%	8%	89%	80%
Indeno(1,2,3-c,d)pyrene	SIM	93%	5%	106%	79%
Dibenz(a,h)anthracene	SIM	90%	5%	101%	91%
Benzo(g,h,i)perylene	SIM	91%	6%	100%	86%
Dibenzo(ai)pyrene	SIM	77%	23%	150%	114%
Dibenzo(al)pyrene	SIM	61%	38%	173%	107%
Dibenzo(ah)pyrene	SIM	47%	49%	226%	108%
1-Methylnaphthalene	TOF	110%	6%	123%	100%
2-Methylnaphthalene	TOF	110%	6%	122%	105%
1-Methyl-9H-Fluorene	TOF	99%	5%	105%	71%
Dibenzothiophene	TOF	72%	9%	98%	88%
2-Methyldibenzothiophene	TOF	60%	16%	94%	79%
2-Methylphenanthrene	TOF	106%	7%	98%	89%
Retene	TOF	106%	2%	102%	93%
1-Methylfluoranthene	TOF	105%	3%	112%	98%
1-Methylchrysene	TOF	101%	4%	180%	165%
7-Methylbenzo(a)pyrene	TOF	103%	5%	124%	107%
1,6-Dimethylnaphthalene	TOF	100%	9%	98%	68%
2,8-Dimethyldibenzothiophene	TOF	43%	25%	113%	82%
2,4-Dimethylphenanthrene	TOF	109%	9%	54%	121%
1-Ethylpyrene	TOF	96%	29%	72%	106%
6-Ethylchrysene	TOF	100%	4%	111%	99%

similar mass spectra. Only four isomers of MeDBT are possible; the remainder of the peaks are methylnaphthothiophenes (MeNTs) [55]. Using a "baseline" approach, integrating all peaks within a fixed window, would result in over-reporting the DBTs as a group. The over-reported amount would depend on the relative amount of MeNTs in the group, as well as on the width of retention time window. Methods reported in other studies are mostly ambiguous with respect to the size of that window. Although quantification of DBTs was not part of a methodological debate regarding the accuracy of alkyl PAH methods [54,56,57], the same issues with respect to intended use of the data apply here. If there is a demonstrable relationship between a group quantitation and a unique property of that group, analogous to GC-FID determination of total petroleum hydrocarbons, then there may be defensible value to that type of measurement. Chemically, however, it may be more accurate to refer to that group less specifically (e.g., as a C1-DBT region group, or C1-polycyclic sulfur heterocyles (PASHs)).

C1 groups were defined cleanly, as spectra of methyl PAHs are characterized primarily by a M − 1 peak from tropylium ion formation, and only a small number of isomers are possible for each methyl-PAH. Beginning with C2 groups, however, spectral characteristics become more complicated, with molecular ions becoming less dominant in the spectra with important consequences for relative response factors across isomers within a group [54]. Detailed examination of the C3-phenanthrene and C3-fluoranthene groups, in sample JQ9410, illustrates some of the issues.

The chromatogram of the molecular ion (m/e 220) of the C3-phenanthrene region of JQ9410 is shown in Fig. 1a with selected mass spectra in Fig. 1b–e. A reference mass spectrum, for 1,2,8-trimethylphenanthrene, is presented in Fig. 1b; that is also peak D in Fig. 1a. The deconvoluted spectrum of Peak A in Fig. 1c includes the key features represented in the reference spectrum, but there are strong signals at unit masses 252 and 237. It may be argued that these are co-eluting peaks and the interfering peak does not contribute to the peak abundance at m/e 220, but analyte validation would be needed to confirm that hypothesis. Similarly, the spectrum of de-convoluted peak B (Fig. 1d) looks very much like a monomethyl PAH, even though it elutes at the same time as another C3 analyte, 1,2,6-trimethylphenanthrene. Peak C is retene, a C4 phenanthrene. In samples recently exposed to wildfire smoke, retene may dominate the C3 phenanthrene group and result in significant over-reporting unless it is screened out.

Similarly, the chromatogram of the molecular ion (m/e 244) of the C3-fluoranthene region of JQ9410, with mass spectra, is shown in Fig. 2. In the chromatogram, the C3 region is in the range 1840–1900 s. The trend for groups to appear as unresolved bands of peaks in the form of humps, as mass and possible substitution patterns increase, is apparent here. Our selection of 1-*n*-propylpyrene as a reference compound illustrates some of the challenges in pursuing the more alkylated peaks; the M–15 peak frequently observed with dimethyl or ethyl PAHs is completely absent. Selecting the most intense peak for mass spectral display, at 1862seconds, we see the 244 and 229 masses, but no 215 mass as seen in the reference compound. It is apparent that the reference compound used might not be appropriate for calibration purposes. The challenge of identifying an appropriate reference compound has been pointed out by other investigators [54,58].

Current efforts to improve the characterization of alkyl PAH groups have taken two approaches. One is to continue to expand the number of individual compounds that are well characterized

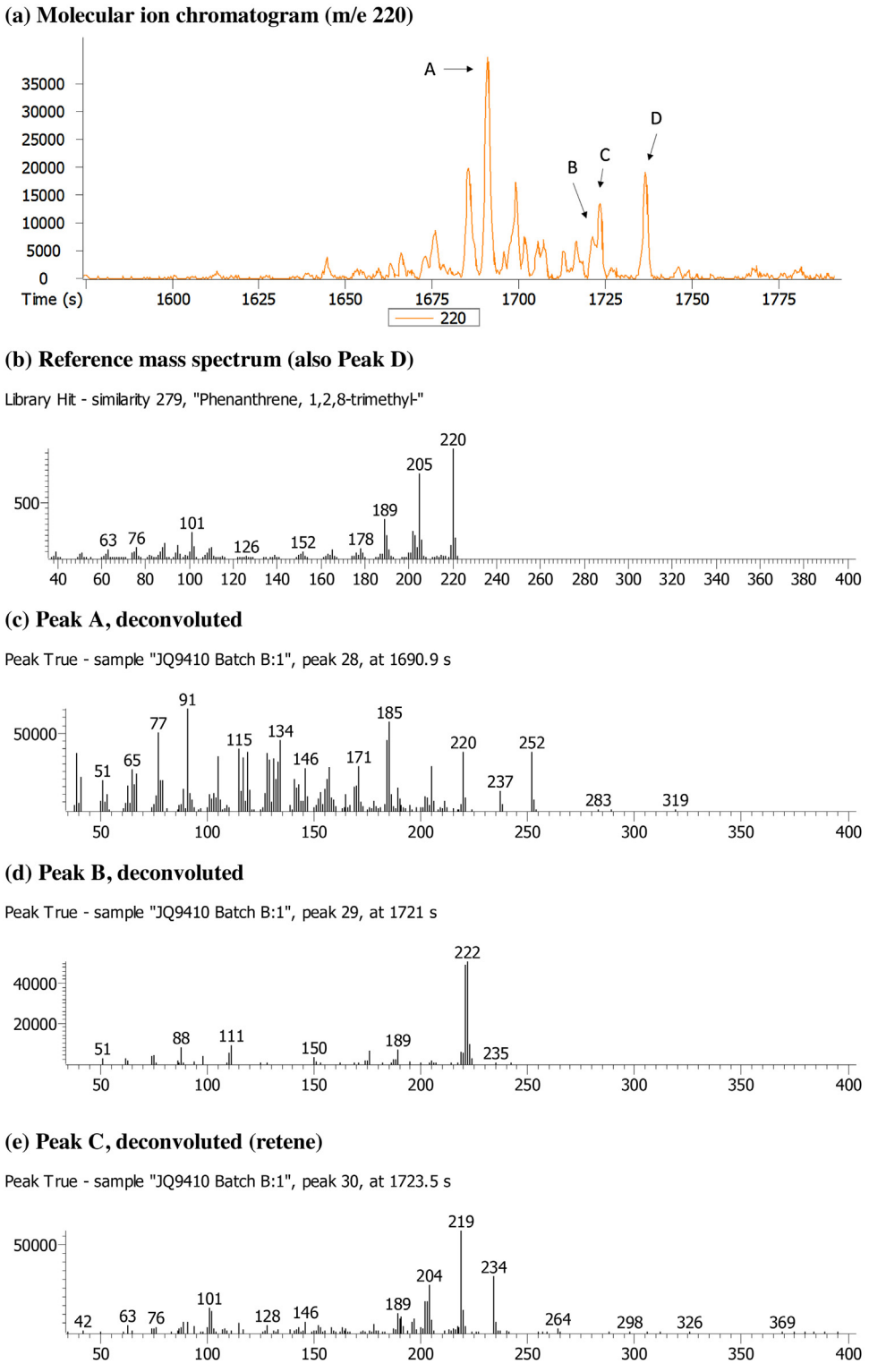

Fig. 1. TOF-MS evaluation of C3-phenanthrene region.

physically, spectroscopically, chromatographically, and construct libraries that can inform method development [58,59]. Alternatively, de-convolution, 2-dimensional GCMS, and GC x GC–MS may be applied to samples to improve resolution of individual components of a sample [58,60–63]. However, given the current state of the science, and the results from our samples, we restricted our group characterization to the C1 and C2 groups where we obtained clean peaks or clusters with acceptable spectral quality, and were supported by published retention index data.

Summary statistics for the analysis of 134 samples (including field duplicates) of *H. physodes* collected from the AOSR during August and September, 2014, are presented in Table S3. The data provide insight into the relative abundance of PAHs and PACs in these environmental samples. Both PAHs and PACs of up to four, and in some cases five rings, are found in over half the samples, at con-

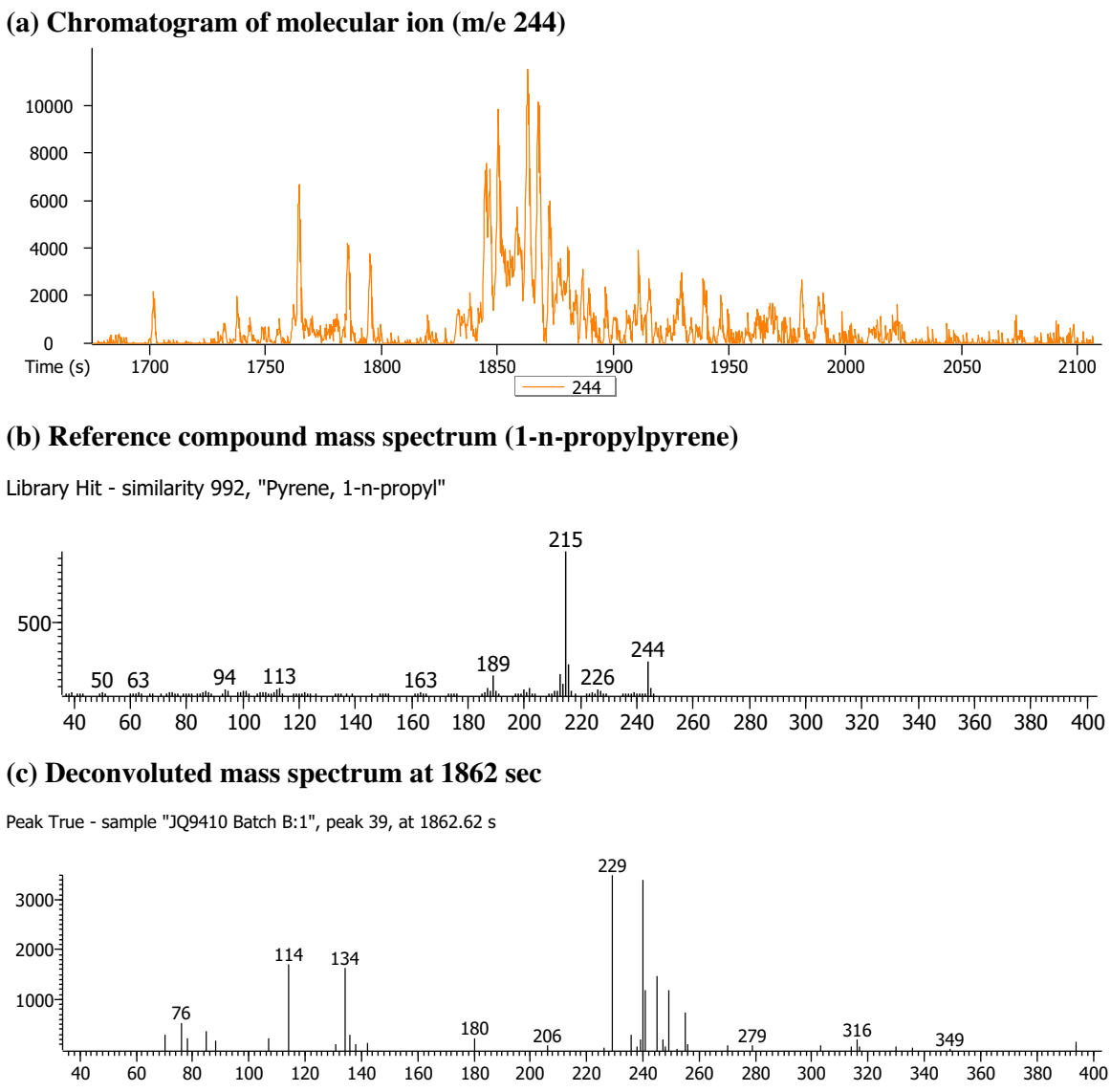

Fig. 2. TOF-MS evaluation of C3-fluoranthene/pyrene region.

centrations that are inversely related to distance from the oil sands operations (Figs. S1 and S2). Individual compounds were found at concentrations <100 ng g^{-1} lichen, and most were <50 ng g^{-1}, whereas grouped analytes (e.g., C1-phenanthrenes/anthracenes) were are found at concentrations up to 300 ng g^{-1}. The data illustrate the value of this methodology in that the PACs, especially dibenzothiophene and its alkylated homologs, are more specific markers of petrogenic materials. PAHs are indicative of both petrogenic and pyrogenic sources, but the PACs improved differentiation of petrogenic source contributions to sample measurements.

It is useful from a methodological standpoint to compare results from the AOSR, using *H. physodes*, with those from studies using other lichen species in other locales, to evaluate if our data falls within published ranges. In Table 4 we present current results, as range of total PAHs, with data from other recent studies [49,64]. Such comparisons must be interpreted with a significant amount of caution, because the studies may calculate their "total PAH" using a different suite of individual PAH species. Some studies focus on regions that are industrialized or have significant on-road vehicular activity, with less data from areas with lower levels of PAH deposition from those sources. Additionally, different lichen species are used, and in most cases neither relative uptake between species, nor analyte selectivity between species, have been studied. Still, our data from AOSR samples are in the range of what other investigators have observed, with the lowest values (<50 ng g^{-1} total PAH) occurring at locations well removed (>50 km) from the extraction and upgrading facilities, and reflecting the remote nature of much of the AOSR.

Because PACs in lichens have not been reported previously, the same comparisons cannot be made for those compounds. However, PAHs and PACs have been reported in other environmental matrices from the AOSR. Table 5 compares relative amounts of total 3-ring and 4-ring PAHs to total C1 and C2 3-ring and 4-ring PACs and total dibenzothiophenes from our data, with data reported from a study using polyurethane foam (PUF) passive samplers in the AOSR [65]. PUF samples in that study were collected at air monitoring stations (AMS) located at distances of 0–70 km from a fixed central point (AMS 1); boreal forest sites ranged from 6 to 106 km away from that point and were more reflective of the environments from which our lichen samples were collected. Naphthalene and alkylnaphthalenes tend to dominate total PAH and total PAC measurements and obscure trends in the other PAHs in the Harner et al. [65] data, as a result, they were excluded from the totals. Data for the alkylbenzopyrenes and alkylbenzofluoranthenes were not

Table 4
Comparison of current study data with selected studies using lichens as PAH bioindicators.

Citation	Species	Study location	# Samples	Sample type	∑PAH range (ng/g lichen)
Guidotti et al. [28]	*Pseudevernia furfuracea*	Rieti, Italy	41	Transplanted	36–375
Blasco et al. [26]	6 spp.	Pyrenees, Spain	156	Endogenous	238–6240
Satya et al. [39]	*Rinodina sophodes*	Kanpur City, India	18	Endogenous	189–494
Augusto et al. [15], Augusto et al. [16]	*Parmotrema hypoleucinum; Xanthoria parietina*	Sines, Portugal	34	Endogenous	127–599
Fernandez et al. [42]	*Pyxine coralligera*	Caracas, Venezuela	11	Endogenous	240–9080
Augusto et al. [18]	*Parmotrema hypoleucinum*	Sines, Portugal	13	Endogenous	58–556
Loppi et al. [32]	*Evernia prunastri*	Molise, Italy	7	Transplanted	19–682
Studabaker et al. (current study)	*Hypogymnia physodes*	Alberta, Canada	127	Endogenous	13–484 (GC-SIMMS) 11–510 (GC-TOFMS)

Table 5
ΣPAC/ΣPAH ratios in lichens and passive PUF samplers for 3-ring and 4-ring PACs and PAHs in the AOSR.

	n	Min	Median	Max
PUF AMS [59]				
ΣC1 PAC/ΣPAH	8	0.65	1.23	2.78
ΣC2 PAC/ΣPAH	8	0.23	0.57	1.32
ΣDBTs/ΣPAH	8	0.34	1.10	1.52
PUF boreal [59]				
ΣC1 PAC/ΣPAH	10	0.81	1.49	4.49
ΣC2 PAC/ΣPAH	10	0.07	0.73	3.26
ΣDBTs/ΣPAH	10	0.39	1.48	4.32
Lichens (GC-TOF-MS data)				
ΣC1 PAC/ΣPAH	127	0.34	1.15	1.71
ΣC2 PAC/ΣPAH	127	0.16	1.58	4.46
ΣDBTs/ΣPAH	127	0.11	0.72	1.83

reported by Harner et al. [65], and those groups were also excluded from calculated results in Table 5. The ranges of relative amounts of alkyl PAHs to PAHs tended to be slightly lower in our study for C1 PACs and DBTs, but higher for C2 PACs. This could be due to methodological differences, such as response factors of reference compounds used for calibration and the size of the integration window for each group, differences in uptake and retention of PAHs and PACs between PUF samplers and lichens, or lichen-specific matrix interferences. More standardized analytical methodology for identification, quantification, and reporting of PAC groups will help narrow sources of variability between data from different studies. Standardized approaches should, at minimum, include selection of quantitation standards with MS response comparable to the actual composition of PAC groups, and reporting of retention indices for all windows, with associated metadata.

4. Conclusions

Given the level of interest in PAHs and PACs in the environment of the AOSR, more uniformity in extraction/analytical methodologies and reporting is necessary to establish data comparability benchmarks. This is the first report of PACs in lichens, enabled by the acquisition of detailed TOF-MS spectral data at trace concentrations, from <1 ng g^{-1} to >100 ng g^{-1}. Both PAHs and PACs were extracted from lichens using freezer milling and cyclohexane extraction with sonication, and cleaned for gas chromatography on silica gel. Analysis of QC samples by GC-SIM-MS for PAHs and GC-TOF-MS for PAC reference compounds demonstrates acceptable recovery and precision. Use of TOF-MS to guide selection of chromatographic windows for PAC groups showed that for C3- and C4-PACs in lichen extracts, even deconvoluted peaks within a window could have poor fit with reference compound mass spectra, and could even be misclassified. The method was used to determine PAHs, C1 PACs, and C2 PACs in 127 lichen samples collected from the AOSR. The range of PAH concentrations is consistent with published concentrations using other analytical approaches. Use of TOF-MS was a significant step forward for quantitation of trace concentrations of PACs in samples with limited mass by demonstrating the utility of lichens as PAC bioindicators. TOF-MS will be an important tool going forward for improved characterization of the composition of PAC groups in ambient air quality bioindicator species and for the identification of appropriate analytical reference compounds. Our results demonstrate the added value of instrument methods that yield higher data content for characterizing complex mixtures of environmentally important materials. Continued improvement, such as characterization and quantification of C3 and C4 PACs in bioindicators, may rely on more advanced instrumental methods with greater compound resolution (GC x GC) and mass resolution (HRMS).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2017.02.051.

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