Biomonitoring of PFAS Using Animals and Lichens – A Short Overview of Approaches, Findings, and Perspectives

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Abstract

Per – and polyfluoroalkyl substances (PFAS) are emerging contaminants, detected globally. Due to their persistent nature, concerns about their presence in the environment has been raised. Biomonitoring provides valuable insight into PFAS exposure, distribution and bioaccumulation. Wild boars, honey bees or lichens serve as effective bioindicators, reflecting local contamination sources and atmospheric pathways. However, most analysis monitor only a limited number of PFAS species. Advances in analytical techniques, such as sum parameters and element specific detectors allow broader detection of PFAS. Integrating biomonitoring with advanced analytical techniques enhances our knowledge and understanding on PFAS distribution, transformation and exposure, especially in total areas to detect background contamination as well as supporting improved environmental and health management.

Background

Large-scale production and application of per – and poly-fluoroalkyl substances (PFAS) have become a globally present contaminant. Their exceptional physical and chemical stability originating from the high C-F bond energy and diverse functional groups makes them persistent in the environment (1, 2). To date, the International Stockholm Convention has regulated several PFAS such as perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), and long chain (C_9-C_{14}) perfluorocarboxylic acids are now persistent organic pollutant candidates. Moreover PFBS and GenX are considered as substances of very high concern. (3-8). Despite these measures, PFAS remain widespread due to historical use, resistance to degradation and precursor transformation (9). PFAS biomagnification through the food web (10-12), underlines the need for continuous monitoring. Biomonitoring provides an excellent strategy to assess the emission of legacy (perfluorocarboxylic – and sulfonic acids, PFCA and PFSA) as well as emerging PFAS (13).

Biomonitoring

Biomonitors are living organisms (plants, animals, etc.), or biological samples (blood, organs etc.) that are used to assess and quantify pollution levels and ecosystem health (14). A practical biomonitor should be widespread, ecologically relevant, well characterised biologically and regularly exposed to environmental media of interest (14). For terrestrial biomonitoring the commonly used matrices include mammals, invertebrates or lichens.

Wild boar (*Sus scrofa*) liver is a valuable bioindicator due to their omnivorous diet, their widespread distribution, and their high place in the trophic level (15), placing wild boars near the top of the food chain. As polar PFAS, which are mostly analysed, bind to protein (16), rather than lipids, livers are highly suitable matrix for biomonitoring, allowing insight into PFAS originating from biota and soil (17-22). Indeed, studies found that PFAS pattern in wild boars are site-specific and related to the local contamination source (20-22). PFOS, which is usually the most dominant PFAS in biological samples, was found to make up 86 % of $\Sigma c(PFAS_{Target})$ determined in wild boars from a contaminated area close to the Rhine-Ruhr area in Germany (Felder et al., 2022) and 66 % to $\Sigma c(PFAS_{Target})$ in wild boars from an area with background contamination (Rupp et al., 2023). In contrast, in boar livers from Bohemian Forest National Park, PFOS contributed only 30 % to $\Sigma c(PFAS_{Target})$. Moreover, PFOA and PFNA concentrations (figure 1) were an order of magnitude higher compared to other samples from areas with background PFAS contamination (21). These suggest the influence of a nearby contamination source. Indeed, a nearby fluoropolymer manufacturing company Dyneon GmbH (23) reported PFAS contamination through air and wastewater between 1968 and 2008 with contamination being present even after the production ceased. The elevated PFAS observed in the wild boar can be also linked to this, making it unsafe for human consumption (24).

Honey bees (*Apis mellifera*) are emerging biomonitors, because they collect pollutants such as PFAS through air, water, soil dust, and plants during foraging. Although individual bees are vulnerable to pollution, colonies are resilient and accumulate or respond to stressors without collapsing (25). Furthermore, they provide different non-invasive sampling matrices (bees, honey, pollen or propolis) that reflect on environmental contamination patterns. Similarly to the wild boars, a site specific PFAS profile was observed in honey bees, influenced by not only local contamination source, but seasonal variations, including bloom timing and rainfall as well (figure 2) (26), thus making data interpretation challenging. Due to their small foraging area (2-3 km) and short lifetime (4-6 weeks), honey bees are well-suited for pointing towards local PFAS

point sources, allowing high time and spatial resolution and monitoring acute, short term and seasonal changes in contaminants, which reflects on more recent exposure as opposed to years of accumulation.

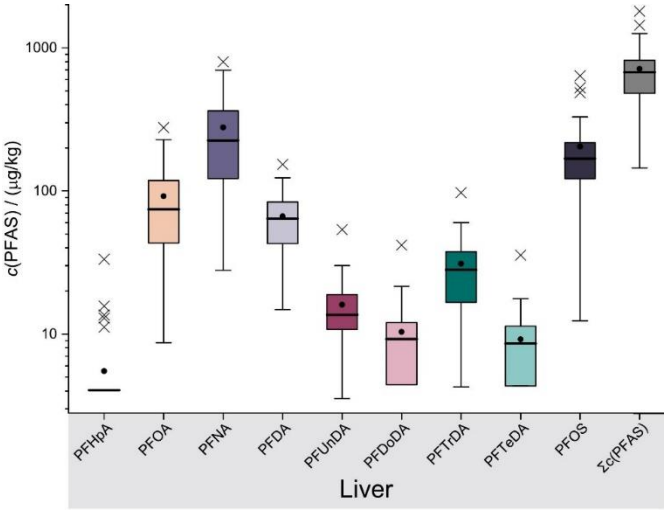

Figure 1: PFAS profile in wild boar livers from Bohemian Forest National Park. Source: (22)

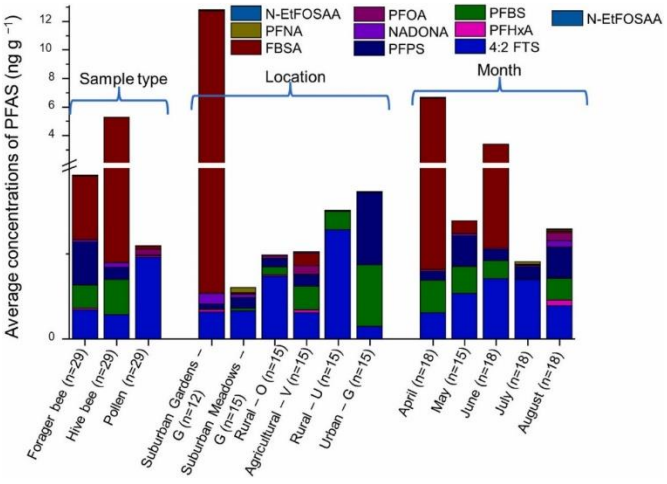

Figure 2: Average PFAS concentrations found in the samples. The graphs are grouped by sample type, location and month. Source: (26)

Lichens are symbiotic associations between fungi and algae or bacteria (27, 28). As lichens absorb components from air directly, thus reflecting the direct input from the atmosphere, they are established air pollution biomonitors. Lichens from the Arctic contained PFAS with the PFAS profile dominated by the odd carbon chain lengths ($C_8 < C_9$ and $C_{12} < C_{13}$, $C_{10} < C_{11}$), representing the direct input from the atmosphere (12), while those from the Antarctica showed no detectable PFAS, possibly due to reduced particulate transport and ionic nature of PFAS (29). Preliminary analysis on *Umbilicaria spp*. and *genus Stereocaulon arenarium* lichen from East Greenland (Ammassalik Island) by us, showed the presence of only PFOA, PFOS and PFHxS, and no odd chain PFCA, at higher concentrations than what was reported before (12) (figure 3), confirming atmospheric PFAS deposition even in remote regions.

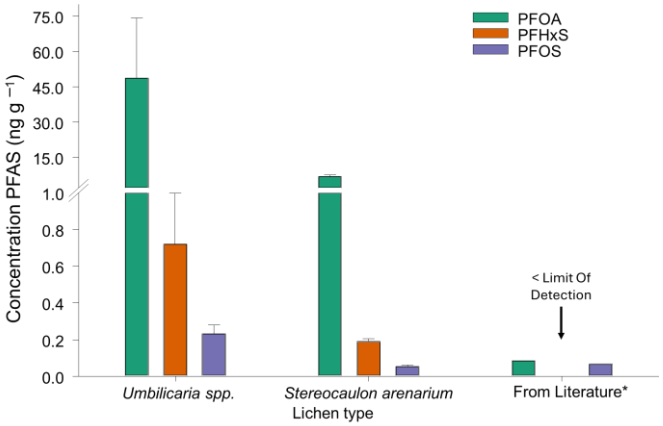

Figure 3: Preliminary data on PFAS concentration found in two different lichen type from the Arctic, and the observed PFAS concentrations compared to the literature values. *Ref. 12.

Emerging techniques for PFAS monitoring

PFAS measurement is analytically challenging due to their lack of chromophores, water solubility, low volatility and excessive presence in consumables, chemicals and instruments (30). Currently, liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) is the standard analytical technique, suitable for a wide range of matrices and offering very low detection limits (ppt range). However, with this approach, only those PFAS that are easily ionisable and have standards available for can be analysed, which often excludes the numerous precursor PFAS.

Sum parameter methods

Sum parameters, such as total oxidizable precursor (TOP) assay, adsorbable organofluorine (AOF) or extractable organofluorine (EOF), allows bulk quantification of PFAS without requiring (new) individual standards. TOP assay oxidises precursor PFAS into perfluoroalkyl acids (PFAA), which can be easily targeted with standard LC–MS/MS approach, estimating the amount of oxidisable PFAS content present in the samples (9). EOF quantifies all organofluorines which can be extracted with a given organic solvent [usually methanol, although others can be used as well (31)] after combustion at high temperature, while AOF is suitable for organofluorines that can be adsorbed onto activated carbon (also hydrophobic ones) (32). Combined together with LC–MS/MS, these approaches reveal hidden PFAS burden. For example, in wild boar livers from the Bohemian Forest, targeted analysis accounted for a max. 33% of the EOF. Even after TOP assay, during which a wide range of PFAA formed (21, 22), unidentified organoflourine still remained (figure 4). This highlights the need to use different instrumentation to successfully close the fluorine mass balance.

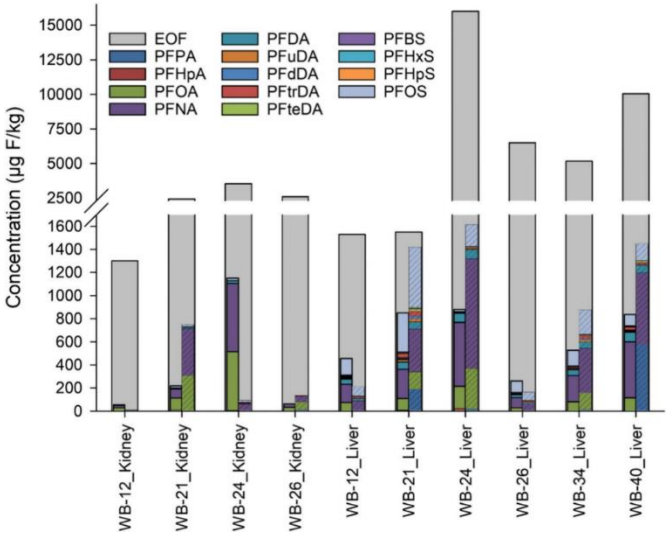

Figure 4: Concentration of target PFAS species in selected livers and kidneys before dTOPA (solid colours on the left) and after dTOPA (striped on the right), as well as the EOF (grey at the background) content. All concentration is expressed as µg F/kg. Source: Ref. 22.

Alternative ionisation and chromatography

To detect PFAS that are non-ionisable with normal electrospray ionisation, alternative sources, such as atmospheric pressure chemical ionisation and photoionisation (APCI and APPI) (33) and microplasma sources, such as atmospheric pressure glow discharge [APGD (34)] can be used. These methods cover less polar e.g. telomeralcohols, partially fluorinated or aromatic organofluorine species, side chain fluoropolymers and fluoroalkanes. Gas chromatography (GC)-MS can further identify volatile and hydrophobic species. It works similarly to LC methods and still relies on structural information and available standards and databases. The analytical problem is that although more than 12 000 PFAS exist and only about 100 can reliably be determined with current analytical techniques based on molecular mass spectrometry (35).

Element specific detection

Element specific detectors such as atomic emission (AED), inductively coupled plasma (ICP) coupled to mass analysers or ^{19}F nuclear magnetic resonance (NMR) can detect fluorine, and help closing the mass balance. AED monitors fluorine emission at 690 nm wavelength (36), while ICP–MS/MS measures fluorine indirectly as $[^{138}Ba^{19}F]^+$ at m/z 157 (37). Recently, as interest in PFAS content increased, the need to develop new instruments and methodologies arose. Raab et al. (38) revisited negative ion ICPMS using a modern commercial ICP-MS with few modifications to test whether fluorine detection with reasonable sensitivity would be possible. These techniques can be coupled to chromatographic separation techniques, allow not only the detection of total organic fluorine, but also speciation, although for identification, standards and molecular information are still needed. The simultaneous use of ICP-MS and LC high resolution MS provides a reliable, species independent quantification (39, 40) as the ICP-MS enables the fluorine quantification, while LC-MS delivers complementary molecular identification and accurate mass. ^{19}F NMR allows the identification of total organofluorine in the samples as well as offers structural information through the chemical shifts, leading to the identification and quantification of both known and unknown organofluorines (41). A significant drawback to all of these techniques is the high detection limit, which remain a major challenge due to high background contamination, the often-low level of PFAS in the samples and low instrumental sensitivity to fluorine. Therefore, the continuous refinement of these element specific detection techniques is crucial for real world environmental application.

Conclusion

Biomonitoring using several types of organisms is indispensable for getting a more comprehensive picture of PFAS exposure and tracing contamination pathways. However, harmonised sampling and analytical protocols, as well as international collaborations are needed to translate biomonitoring results into effective environmental management and public-health protection. Conventional LC-MS/MS analysis targets only a subset of the total organofluorine burden. Incorporating advanced techniques like sum parameters or element specific detection is essential to capture the unknown organofluorine fraction and close the mass balance. The combination of biomonitoring and advanced analytical approaches strengthens PFAS monitoring and improves risk assessment. Continued international collaborations and method development are key to mitigate PFAS pollution and protect environmental and public health.

Acknowledgement

The authors would like to express sincere gratitude to Till Schröder, Marc Preihs, Jan Borovička, Raquel Gonzalez de Vega, Andrew Kindness, Eileen Prieler, Robert Brodschneider, and Andrea Raab, whose previous publications have significantly contributed to the development of this review. Their previously published works have been thoroughly cited and referenced throughout this manuscript, and their contributions have been invaluable in shaping the insights presented here.

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