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Analytical progress and challenges for the detection of oxygenated polycyclic aromatic hydrocarbon transformation products in aqueous and soil environmental matrices: A review

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Abstract

Over the past 20 years, a growing body of research has raised concerns about the toxicity, fate, and transport of oxygenated transformation products of polycyclic aromatic hydrocarbons. Research targeting these diverse compounds in soil and water systems has been challenged by a lack of standard analytical techniques and suitable reference materials. However, recent efforts towards the consolidation of traditional analytical techniques as well as the development of novel approaches to improve sample preparation and hyphenated instrumental techniques show promise. This review discusses progress and challenges for both trends in analytical method development, and makes recommendations for supporting oxygenated PAH research.

Keywords: Polycyclic aromatic hydrocarbon metabolites, oxy-PAH, solid phase extraction, environmental analysis, soil analysis

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1. Introduction

Polycyclic aromatic hydrocarbons (PAH) represent some of the most widely reported persistent organic pollutants posing risk to soil and water systems at petroleum- and industrially-contaminated sites (Lawal, 2017, Alegbeleye et al. 2017, Abdel-Shafy and Mansour, 2016). Sixteen PAHs have been listed as priority pollutants by the United States Environmental Protection Agency (USEPA) for their known toxic and carcinogenic effects with similar lists prepared for environmental and food safety legislation in the EU (Lerda, 2011). Although this has been an important step for directing analytical developments, environmental research, and regulation of these pollutants, it has also limited the scope for considering additional risks associated with PAH-contaminated sites (Andersson and Achten, 2015). Many studies reporting the oxidative degradation of PAH in soils have also observed concurrent increases across a variety of toxicity and genotoxicity measures, indicating that parent PAH concentrations are not sufficient for the estimation of risk (Andersson and Achten, 2015). At the same time, it has long been understood that oxygenated PAH breakdown products including PAH functionalised with ketone, hydroxyl, and/or carboxyl groups may be more mobile, bioavailable, and more toxic than parent PAH (Arp et al., 2014; Boll et al., 2015; Chibwe et al., 2015; Knecht et al., 2013; Lundstedt et al., 2014). Indeed, workers have begun to call for the revision of the 16 EPA PAH regulatory list to include more compounds including oxygenated PAH metabolites (Andersson and Achten, 2015; Lundstedt et al., 2014).

It has been recognised for over 30 years that the inclusion of oxygenated PAH in environmental analyses could improve understanding of both site risks and in situ degradative processes. However, the lack of standardised analytical techniques has been a major challenge for the inclusion of oxygenated PAH alongside traditional hydrocarbons for monitoring and evaluating

site remediation (Lundstedt et al., 2014). In their recent paper 'Time to say goodbye to the 16EPA PAH?", Andersson and Achten (2015) suggest that three conditions should be met for the inclusion of additional polyaromatic compounds into regulatory lists: 1) sufficient evidence of toxicity, mutagenicity, or carcinogenicity; 2) evidence of their common occurrence in the environment; and 3) sufficient and practical analytical separations are possible. Of 10 carbonyl-modified PAH proposed as a starting point to form a list of oxygenated PAH for further monitoring in the environment, none were considered by the authors' estimation to yet have sufficiently validated analytical methods.

The difficulty in establishing standard techniques for oxygenated PAH analysis is primarily due to two key challenges: (1) the large diversity of these compounds and their wide-ranging chemical characteristics and (2) the complexity and variability of the matrices for which the analytical techniques must be validated. In addition, uncertainty in the selection and best use of surrogates, internal standards, and reference materials, as well as their limited commercial availability, remains a major issue for the establishment of robust methods (Walgraeve et al., 2010, Lawal, A.T., 2017; Lundstedt et al., 2014).

Yet despite these challenges, progress made in analytical techniques over the last two decades has provided new information regarding the distribution, persistence, and toxicity of various oxygenated PAH, and has begun to shed light on the role that oxygenated PAH metabolites may have in regulating PAH biodegradation pathways in the environment (Knecht et al., 2013; Lundstedt et al., 2007; Vaidya et al., 2017). This review provides a brief overview of oxygenated PAH and related mono-aromatic PAH transformation products in the environment and then discusses current analytical techniques available for their analysis in solid matrices including soil, sediment and sludge and aqueous freshwater matrices such as leachate, pore water, and

groundwater. Emphasis is placed on sample preparation, including more traditional solvent extraction and solid phase extraction (SPE) techniques, as well as novel sorptive extraction, miniaturization, and automation methods. Key insights into the use of hyphenated GC- and LC-instrumentation coupled to various detection apparatus are also discussed.

2. Oxygenated PAH and PAH metabolites in the environment

Oxygenated PAH are composed of a fused PAH architecture modified by the addition of oxygen-containing functional groups to one or more of the aromatic rings (See Figure 1 for selected examples, terminology, and abbreviations used in this text). These compounds may form alongside PAH during incomplete combustion processes (Obrist et al., 2015) along with other polyaromatic compounds including nitro-PAH and compounds containing N, S, or O heteroatoms (N,S, or O - PAC respectively, HPAC collectively) in the ring structure. However, oxygenated PAH may also be formed through subsequent transformation of PAH through natural or induced photo-, chemical-, or biological oxidation, which includes metabolic degradation by bacteria, algae, fungi, and higher order animals (Ghosal et al., 2016; Habe and Omori, 2003; Haritash and Kaushik, 2009; Meckenstock et al., 2016, for reviews). Some mono-aromatic compounds such as indanone, phthalate, catechol, and salicylate are formed at the later stages of PAH degradation and are considered collectively with oxygenated PAH species in the literature and in this review. In addition, fungi and higher-order animals also produce conjugated PAH metabolites through the substitution of one or more hydroxyl or carboxyl protons with glucoronide, glutathione, glycine, or sulfate groups; this facilitates excretion of the contaminant and increases environmental mobility (Boll et al., 2015; Schmidt et al., 2010a).

In 2007, the Swedish EPA reported that information regarding environmental concentrations of OPAH was lacking and subsequently in 2008 included these compounds in their national soil screening programme which investigated a variety of environmental matrices including soil, sludge, aerosol, breast milk, and some urban fish (perch) (Brorström-Lundén et al., 2010). Over the past 15 years, continued research has provided data for soils and aqueous systems obtained from both contaminated and uncontaminated sites across a number of countries, environments, and land use criteria (Table 1). For the majority of studies in soils and sediments, only OPAH have been evaluated, but a few studies have also investigated OHPAH and COOHPAH. Aqueous samples, which are more frequently monitored during experimental conditions, have also shown detectable to high levels of these compounds when obtained from environmental sources.

Direct inter-comparison of oxygenated PAH contents through available studies is difficult because different authors have included different compounds, matrix types, and sampling approaches. Indeed, one of the major improvements needed for developing this area of research is the establishment of a list of key target compounds, which could be extended where possible to determine additional transformation products via targeted or untargeted analysis. Nevertheless a few trends in the distribution of oxygenated PAH can be observed. Similar to PAH, highest concentrations of oxygenated PAH in soils are typically associated with contaminated industrial sites including former coking ovens, gasworks, and wood preservation sites, where oxygenated PAH may also be present in pore water, leachate, and groundwater (Arp et al., 2014; Enell et al., 2016; Ohlenbusch et al., 2002). Urban areas which receive atmospheric inputs from traffic or other combustion sources may also be substantially impacted, along with areas such as river deltas and harbours which receive large loads of terrestrial and atmospheric materials. Rural and natural areas also reveal the presence of these compounds, likely sourced from local use of wood stoves

(Avagyan et al., 2016) or agricultural equipment, natural or managed vegetation fires (Obrist et al., 2015; Wilcke et al., 2014a), or long range transport via atmospheric processes (Brorström-Lundén et al., 2010).

A substantial body of evidence now challenges the commonly-held assumption that because of their greater polarity, mobility, and bioavailability, oxygenated PAH are inherently more quickly degraded in the environment and are therefore less concerning than parent PAH (Lundstedt et al., 2007). Concentrations of OPAH that exceed parent PAH have been reported by several studies in both soil and water matrices (Bandowe et al., 2014; Brorström-Lundén et al., 2010; Kurihara et al., 2005; McKinney et al., 1999; Tidwell et al., 2017; Wilcke et al., 2014b). In several cases, studies have also demonstrated lower removal rates of some, though not all, of these compounds compared to the parent PAH (Hu et al., 2014), or have demonstrated similar stability to PAH under common environmental conditions (McKinney et al., 1999). Even the most polar constituents, conjugated metabolites, have been shown to be recalcitrant to mineralisation and to degrade at lower rates than parent PAH (Schmidt et al., 2010a). The longevity of these highly mobile PAH metabolites could bring concomitant downstream risks that are not currently recognised (Boll et al., 2015; Schmidt et al., 2010a). Moreover, even where further degradation of these compounds proceeds at reasonable rates, their production may still contribute to periods of elevated risk at contaminated sites, particularly in cases when these processes are accelerated by remediation initiatives (Chibwe et al., 2015; Lundstedt et al., 2006b).

3. Analyte chemical properties and matrix complexity - A double challenge

To date, an incredible variety of oxygenated PAH and PAH metabolites have been observed, though have not always been fully characterised (Letzel et al., 2001; Malmquist et al., 2013;

Walgraeve et al., 2010). These span a broad wide range of size, stability, volatility, solubility, sorption, polarity, acidity, and isomeric characteristics (Arp et al., 2014; Boll et al., 2015; Hanna et al., 2012; Letzel et al., 2001; Walgraeve et al., 2010). This breadth of physicochemical characteristics of oxygenated PAH and PAH metabolites can pose substantial challenges to the development of comprehensive analytical approaches. In general, researchers have addressed the challenge of analysing PAH metabolites by focusing on one class of compounds at a time (e.g. OPAH or OHPAH), most frequently the carbonyl-containing compounds in particulate matrices and the more soluble hydroxylated or conjugated forms in aqueous systems, though all compound groups may be found in either matrix type (see Table 1 and 2 for references). A few studies have addressed a wider range of compounds through the use of multi-component instrumental approaches (Ahmed et al., 2015) or multistep fractionation protocols (Bandowe and Wilcke, 2010; Meyer et al., 1999).

Fractionation can be particularly useful when target compounds require different processing (such as concentration and derivatization steps) or are better suited to different instrumental analyses (refer to Section 5 for further discussion). Subdivision by functional groups allows for some simplification in the analytical approach, as the grouped compounds tend to require similar treatments to render them detectable by GC- or LC-based techniques, but differences physicochemical characteristics within each group also remain substantial. For example, Walgraeve et al. (2010) provide a useful reference table providing estimated melting point, boiling point, vapor pressure, water solubility, and log octanol-water partition coefficient (K_{OW}) for 40 OPAH+OHPAH which shows estimated K_{OW} for four benzo[a]pyrene diones differ by over two orders of magnitude (log K_{OW} 3.05-5.24) and solubility for 2-3 ring OPAH by three orders of magnitude (1.48 – 4.10, mg/L) (Walgraeve et al. 2010), while two OHPAH isomers, 2-

hydroxyfluorene and 9-hydroxyfluorene, are estimated to have water solubility of 71 and 4900 mg/L, respectively at 25°C (Walgraeve et al., 2010).

In most cases, experimentally determined values for key chemical characteristics are not available, and estimated values can only be used as a starting point. Recent research has demonstrated that current tools for estimating the partitioning of oxygenated PAH between soil and water phases, using (K_{OW}) and/or total organic carbon (TOC) measures, can be misleading. Boll et al., (2015) observed that some sulfate-conjugated metabolites of pyrene and phenanthrene without a carboxylic acid group showed much lower sorption than estimates obtained through the Estimation Programs Interface (aqueous distribution coefficients up to 150 times higher than estimates), though those metabolites with the carboxylic acid group were more accurately predicted. They also reported that for the three soils investigated with TOC ranging between 2.8 and 64% and pH ranging between 8.4 and 9.1, soil organic carbon content was less important than analyte functional groups for understanding sorption, though the impact of TOC characteristics may be more notable over a broader range of soils and pH conditions (Boll et al., 2015). Arp et al., (2014) also demonstrate the value of predicting organic carbon distribution coefficients, $K_{TOC,}$ of PAH in heavily contaminated soils using Raoult's Law Coal Tar sorption model rather than K_{OW} alone, which tends to underestimate K_{TOC} for PAH. Substantially improved agreement between experimentally determined and predicted K_{TOC} for OPAH was also obtained using the new approach. Although these insights may have greater impact for risk assessment, they are also important to understand for analytical technique development, particularly the development of passive sampling devices.

In addition to the complexity of the target compounds, matrix interferences in environmental samples can be substantial (e.g. O'Connell et al., 2013; Pojana and Marcomini, 2007). Solid and

liquid environmental matrices contain additional macromolecules, humic substances, salts, and other interfering compounds. These may impact extraction efficiency (O'Connell et al., 2013, Sousa-Silva et al., 2015), preparative chromatography (Van de Wiele et al., 2004) and final analysis (Layshock et al. 2010). At the instrumental stage, interfering components may co-elute with target analytes or contribute to overall increased baseline noise, or specific signal suppressions or enhancements, which may be particularly problematic where samples or extracts have been concentrated to improve detection of low-concentration analytes (Walgraeve et al. 2010). At the same time, analytical techniques used for aerosol, atmospheric, and urine analysis which frequently inform or adapt techniques developed for soil, sediment, and environmental aqueous samples, may not be directly applicable to these matrices.

4. Extraction, fractionation, and storage

4.1 Preliminary sample processing

A variety of methods have been used for the preparation of soil and water samples prior to instrumental analysis (Figure 2). Extraction and clean-up methods are generally emphasised in the literature, but the impact of sample handling during pre-treatment and intermediate steps can also be substantial. Sample pre-treatment includes the separation of particulate and aqueous fractions, sieving and drying of sediments and the filtration of aqueous samples, as well as other steps including enzymatic de-conjugation or pH adjustment, which are discussed in later sections. For soils and similar matrices, the presence of water can adversely impact extraction, preparative chromatography, and instrumental detection. Excess water is therefore usually removed before extraction, and further drying steps may be included before final analysis. Very wet samples may

be centrifuged (Hu et al., 2014), but typical preparations involve moderate air drying followed by chemical drying by sodium sulfate, which may also be performed after extraction or during sample clean-up (see Section 4.5). The sample is also ground and sieved to remove bulk materials. The <2mm fraction is usually selected for extraction, though the finer fractions tend to have the highest levels of PAH and OPAH and therefore may be used. Since PAH and OPAH can also sorb strongly to organic materials such as wood fibres, where these bulk materials are a substantial portion of the matrix, for example during composting remediation tests, it should be remembered that sorption may be an apparent source of loss from the final analysed soil matrix.

Filtration is sometimes performed prior to extraction of aqueous samples and may be applied to organic extracts prior to analysis. However, it has been generally recognised that hydrophobic organic compounds including PAH and many metabolites may adsorb on to filter membrane surfaces, leading to underestimates of analyte content (Enell et al., 2016). Despite this, filtration of samples or organic extracts is commonly overlooked as a source of loss during sample preparation. Workers report use of glass fibre (Boll et al., 2015; Niederer, 1998), cellulose acetate (Santos et al., 2017), mixed cellulose ester (Ohlenbusch et al., 2002), nylon (Avagyan et al., 2015; Hu et al., 2014), PTFE (Lankova et al., 2016; Malmquist et al., 2013), polyamide (Lundstedt et al., 2006b), PVDF (Hanna et al., 2012), or more ambiguously, 'organic filter membrane' (Liao et al., 2014), 'filter paper' and 'centrifugal filter' (Toriba et al., 2016), or just 'filtered' samples (Siemers et al., 2015), but most of these studies have not evaluated the potential impact of the filtration step. A few studies indicate that nylon may be appropriate for OHPAH prepared in methanol or methanol/DCM (1:1) (Avagyan et al., 2015; Hu et al., 2014) and PVDF may be appropriate specifically for filtering aqueous samples containing naphthoic acids (Boll et al., 2015). In general, however, centrifugation rather than filtration is the recommended approach for separating very wet

soil samples or for removing particulate materials from aqueous samples (Enell et al., 2016). Where an aqueous sample has large colloid load, e.g. a simulated leachate (shaken aqueous extracts of soils), analysis of centrifuged samples may lead to overestimation of the analytes; sampling protocols may be adapted to avoid this error, for example, through the use of flow-through or recycled leachate collection systems (Enell et al., 2016).

4.2 Extraction from solid matrices

Soxhlet, ultrasonic-assisted extraction and pressurised liquid extraction (PLE) are the most frequently used techniques for the extraction of oxygenated PAH in soils and sediments. Although less common, microwave assisted extraction (Cai et al., 2017; McKinney et al., 1999; Sun et al., 2017) and supercritical fluid extraction (SFE) (Han et al., 2015) have also recently been used for analyses of OPAH and NPAC. With the exception of SFE which uses CO_2 as an extraction solvent (Han et al., 2015), typical solvent systems include dichloromethane (DCM) or hexane mixed with DCM or acetone if PAH or OPAH are of interest, while acetonitrile, methanol, ethyl acetate, acids, or occasionally water may be used to improve the extraction of more polar OHPAH and COOHPAH metabolites (Meyer et al. 1999, Bandowe and Wilcke 2010 Wang et al 2012, Blum 1997). For phenolic compounds specifically, citrate buffer may be added and is recommended over ethylenediaminetetracetic acid (EDTA) or water alone (Blum, 1997)Although sodium hydroxide (NaOH) is sometimes used for more exhaustive extraction at elevated pH, humic acids also become soluble and may cause matrix interference in subsequent chromatography (Vinken et al., 2005). Additional materials such as sand, diatomaceous earth, activated copper powder, sodium sulfate,

or sorptive materials are sometimes added to the sample or extraction vessel in order to improve extractions, remove interferents, or facilitate fractionation.

In recent years PLE has become a dominant technique for the extraction of PAH, OPAH and to a lesser extent OHPAH in sediments. Many of these PLE methodologies for OPAH extraction have evolved from the work of Lundstedt et al. (2000) which initially focused on the recovery of PAH and was then extended to extract OPAH (Andersson et al., 2003; Lundstedt et al., 2003, 2006a). More recently, it has been used as the basis for a laboratory inter-comparison study for the analysis of PAH, OPAH, and NPAC in spiked soils and unspiked reference soils (Lundstedt et al., 2014). The original work optimised PAH extraction and found best results were obtained using small sample mass (1 g), two short static extraction cycles and a large rinse volume (11 mL). Temperature was optimised at 150^0C to limit wear and tear on the instrument, while extraction pressures ranging from 6.9-19 MPa had limited influence on PAH recovery. Several solvents provided acceptable results but use of 1:1 hexane:acetone was preferred for minimising the use of chlorinated solvents and for its better suitability for subsequent silica clean-up. In order to capture OPAH in a subsequent study (Lundstedt et al., 2003), a second extraction cycle using 99:1 methanol:acetic acid was introduced as well as a solvent exchange step before silica clean-up, but PLE conditions were otherwise not re-optimised. Other authors have built on this core method with some modifications to improve clean-up and detection of OHPAH (see Table 3). A further development involved the addition of silica to the cell below the sample to perform extraction and fractionation/clean-up in an automated 2-cycle operation. This method provided comparable results to Soxhlet extraction followed by silica clean-up (Lundstedt et al., 2006a). During the inter-comparison study, 6 out of 8 laboratories used PLE methodologies without the integrated silica fractionation, though with some variations in instrument and solvent parameters; one other

laboratory followed the integrated PLE-fractionation method; and the final laboratory used ultrasonication instead of PLE, with comparable results. Although none of the seven laboratories showed consistently higher or lower results, substantial inter-laboratory RSD was observed (21-97%), suggesting the need for further understanding of PLE parameters which specifically impact OPAH recovery (Lundstedt et al., 2014). Of particular concern for PLE extractions is the possible conversion of some PAH to PAH quinones, for example anthracene to anthraquinone (Lundstedt et al., 2014) and the rearrangement of PAH quinones and some ketones (Walgraeve, 2010). Bandowe and Wilcke (2010) reported low, highly variable, or unrealistic recoveries (e.g. 159+/-44%) for 9,10-phenanthrenequinone, 1,2-acenaphthenequinone, and 1,4-naphthoquinone, respectively which they attributed to the lower stability of quinones during GC-MS analysis, but also indicated that the elevated temperatures used in the PLE process may also be involved in the observed conversions. Rearrangement has also been observed by workers studying these compounds in aerosols, when PLE or elevated-temperature ultrasonic extraction was used (Kishikawa and Kuroda, 2014; Lintelmann et al., 2005). Although widely reported as a concern, best practices to address this issue have not yet been established.

Attempts to use PLE to investigate OHPAH and COOHPAH have yielded mixed results. Bandowe and Wilcke (2010) used a solvent mixture comprised of ethyl acetate/DCM/TFA (250:125:1 v/v/v), to extend their DCM-based PAH and OPAH extraction to include these compounds. Several OPAH and mono-hydroxylated OHPAH showed enhanced recovery, but the majority of OHPAH and COOHPAH had recoveries that were moderate to poor (0-7% for fourteen compounds). For the most polar compounds, only 1-3% improvement in recoveries were observed with the inclusion of this acidified system compared to DCM extraction alone, suggesting that insufficient acidity of the solvent was not the primary source of loss. At the same time, the extent

to which the subsequent silica fractionation protocol contributed to the low recovery was not independently evaluated. Although further studies to extend PAH/OPAH PLE protocols should be undertaken, greater successes have generally been obtained in studies where OHPAH have been investigated as a sole target class (Avagyan et al., 2015; Wang et al., 2012). By using methanol extractions performed at higher temperatures (i.e. 200°C) than usually used for OPAH, workers have obtained OHPAH recoveries of 70-102% for extracts of filters, with recoveries from soil deviating from these values up to 6% after clean-up (Avagyan et al., 2015). In a more unusual approach, Wang et al. (2012) explored a water/acetonitrile system for PLE extraction of 8 OHPAH from wetland sediments followed by dispersive liquid-liquid micro-extraction (DLLME) concentration procedure and obtained recoveries of 57-91% after both procedures.

4.3 Extraction from aqueous samples

Table 2 summarises the advantages and disadvantages of various techniques for the preparation of aqueous samples and references their use in primarily environmental contexts, but also in urine and culture media where much of the method development for the detection of mono-hydroxylated PAH metabolites in aqueous samples has been undertaken (see Table 2 for references). In some cases, if the matrix is sufficiently free of interferents and metabolite concentrations are sufficiently high, extraction may be forgone entirely, and the sample applied to an LC-based detection method. More commonly, however, solvent or sorbent based extraction is performed first. Liquid-liquid extraction (LLE), and reverse phase solid phase extraction (SPE) are the most common approaches for the extraction of aqueous samples. Miniaturization techniques such as DLLME and single drop micro-extraction (SDME), and other sorption techniques, including dispersive solid phase

extraction (dSPE), solid phase micro-extraction (SPME), stir bar sorption, and passive sampling devices have also been utilised. These represent a growing field of sample preparation which aims to reduce sample preparation time, solvent use, and costs, and to facilitate the analysis of smaller sample volumes, or to investigate specific characteristics such as environmental partitioning or bioavailability. Although they have not yet widely been adopted for the analysis of oxygenated PAH more general reviews are available for factors affecting these techniques and their use in environmental analysis (Bizkarguenaga et al., 2013; U. Ghosh et al., 2014; Jain and Verma, 2011; Piri-Moghadam et al., 2016; Souza-Silva et al., 2015).

Both LLE and reverse phase SPE as well as other aqueous sorptive extraction techniques take advantage of the relatively elevated K_{OW}s of target analytes and depend on facilitating the efficient fractionation of these compounds into a comparatively nonpolar liquid or solid phase. Ethyl acetate and DCM are most frequently used for the organic phase during LLE, though toluene and trichloroethylene (TCE) are also used, particularly in DLLME and SDME approaches; C18 and polymeric (polystyrene-divinyl benzene) sorbents are most frequently used for solid phase extraction, while polydimethylsiloxane (PDMS) and polyacrylate have been investigated for SPME and stir-bar sorptive techniques respectively. Passive sampling devices, which also depend on this fractionation but utilize a longer sample exposure period, have been developed using low density polyethylene (LDPE), polyoxymethylene (POM), and silicone, as well as Tenax and HLB resins. When DLLME is used, an additional organic modifier which is miscible with water such as acetone, acetonitrile, or ethanol is used as a disperser solvent in order to increase the interaction between the aqueous sample and the extraction solvent (Gupta et al., 2015; Wang et al., 2012). A similar strategy may be used during the conditioning of non-polar sorbents for aqueous samples.

During extractions, modification of the aqueous phase may also enhance transfer of more polar analytes, particularly those which dissociate in water. Acidic PAH metabolites and small phenols which may be ionised in aqueous solution must be converted into the unionised form in order to facilitate interaction with the organic phase. This can be achieved by reducing sample pH below the pKa of target analytes, typically to pH <2. Similarly, basic compounds such as NPAC co-analytes are better transferred at higher pH (Siemers et al., 2015). Salts may also be added to promote the aqueous exclusion of organic materials and facilitate analyte transfer to the organic phase (Letzel et al., 2001). The addition of HCl and NaCl to SDME protocols has improved recovery of OHPAH (Wang et al., 2017) and yielded no significant effect for OPAH recovery (Santos et al., 2017).

Although C18 and polymeric sorbents are most often cited for SPE work with oxygenated PAH, several studies have reported limitations of these sorbents (Table 2) and have applied mixed-mode or tandem devices. Newer sorbent materials and devices reviewed recently by Płotka-Wasylka et al., (2016) have yet to be applied to oxygenated PAH and may offer an area of continued analytical development. However, standardisation of SPE techniques is generally lacking (Andrade-Eiroa et al., 2016) and consolidation of existing techniques for oxygenated PAH may be more important for regularising the analysis of these compounds. This is particularly important because sub-optimal use of SPE may lead to poor precision, lead to sample loss, or provide incomplete method comparisons. For example, Wang et al., (2012) prepared subcritical water extracts (SWE) of soils with 20% ACN and concluded that clean-up by dispersive liquid-liquid micro-extraction (DLLME) had three times higher recovery than clean-up by C18 SPE; however the SPE method used had originally been developed for water samples without ACN, and its presence may have adversely impacted chromatography and contributed to premature elution of the target OHPAH.

Van de Wiele et al., (2004) also reported loss of both low molecular and high molecular weight OHPAH in their C18 SPE methodology and attributed it to loss during a wash step, possibly enhanced in part by competition of matrix components for nonpolar interaction sites.

4.4 Conjugated metabolites

Although conjugated metabolites may be formed in soils and sediments, they are most often studied in aqueous matrices. For their analysis, enzymatic deconjugation is frequently, though not always, performed prior to extraction. Deconjugation improves interaction with the organic LLE solvent (e.g. (Cerniglia et al., 1982) or nonpolar sorbent system (Lankova et al., 2016) and may also be required in order to make use of established GC derivatization and analysis techniques. As the availability of conjugated reference standards is limited (Ayala et al., 2015) deconjugation also facilitates detection through comparison to more readily available unconjugated standards.

The deconjugation process involves the application of phosphate or acetic acid buffer as well as hydrolysing enzymes including arylsulfatase and beta-glucoronidase followed by incubation at 37 °C, typically overnight. Working with urine samples, Jacob et al., (2007) found that glucuronidase and arylsulfatase isolated from *E. coli* and *Aerobacter aerogenes* respectively gave cleaner extracts and better recovery than commonly-utilised enzyme mixtures obtained from *Helix pomatia*. The sample is then extracted and cleaned by the desired protocol, although hydrolysed samples may have altered consistency compared to original samples, which can lead to clogging of SPE tubes (Lankova et al., 2016).

Since deconjugation steps inherently render conjugated and unconjugated metabolites indistinguishable, sample preparation must be planned to differentiate the components when consideration of the two groups separately is important. In one approach, analysis can be

completed with and without the deconjugation step (Jacob et al., 2007). Alternatively, stepwise extraction may be used to fractionate these metabolite groups: for example, fungal naphthalene degradation microcosms were first extracted with ethyl acetate to obtain unconjugated metabolites, then deconjugation enzymes were added to the remaining aqueous phase, followed by a second extraction with ethyl acetate to obtain the newly deconjugated products (Cerniglia et al., 1982). More recently, LC-MS (and LC-FLD-MS) methods have been used to identify both conjugated and unconjugated metabolites directly in a variety of aqueous matrices and extracts (Ayala et al., 2015; Boll et al., 2015; Malmquist et al., 2013; Schmidt et al., 2010a; Tang et al., 2016). Because of the consistent structure of the conjugate unit, its presence may also help with MS identification of metabolites.

4.5 Clean-up of organic extracts

Due to the wide range of compounds present in environmental matrices, the use of broad specificity solvent systems, and the concentration steps required to improve detection of trace metabolites, crude extracts tend to require additional clean-up or fractionation prior to analysis. The majority of these methods have been developed in the context of extracts from solid matrices, but some have also been applied to LLE extracts of aqueous samples. Most often, this involves preparative chromatography using open column (larger sorbent volumes), SPE (smaller sorbent volumes), or PLE-based methods (sorbent packed extraction cells) which allow for simultaneous extraction and fractionation (Lundstedt et al., 2006a). Table 3 presents a summary of extraction, clean-up, and fractionation methods that make use of preparative column chromatographic techniques. In some cases, gel permeation chromatography or size exclusion chromatography (SEC) has been

recommended to be used prior to SPE, and the latter has been reported to substantially improve baseline noise although total recovery may be reduced with the inclusion of this step (Bandowe and Wilcke, 2010; Layshock et al., 2010). Activated copper powder and sodium sulfate are sometimes added to SPE protocols in order to remove sulphur and residual water respectively. As recovery values presented in Table 3 include different stages of the methodology, these values are not directly comparable in all cases, but give an indication of method performance

Similar issues regarding standardization and optimal use of SPE protocols described above for aqueous applications are also of concern here. Selecting appropriate sorbent and eluents is a key step, but determining appropriate conditioning steps, sample analyte load, load solvent type and volume, and flow rates are also essential. Insufficient separation may either lead to direct analyte loss (e.g. nonpolar compounds lost during wash step), highly complex chromatograms (Letzel et al., 2001), the necessity of recombining separated fractions (Chibwe et al., 2015; Layshock et al., 2010) or non-ideal for further processing (e.g. OHPAH being missed in an underivatized OPAH fraction). In general, fractionation need only be sufficient to the question at hand, and while some workers (Chibwe et al., 2015) have been able to demonstrate some interesting trends in toxicity associated with smaller fractionation bands, the inclusion of 14 elution cycles recombined into 6 composite fractions, is overly complex and undesired for most investigations. Pre- or post- SPE concentration, dilution, or derivatization steps, must also be carefully considered, as improvements to these steps could help reduce analyte loss observed in some protocols.

A variety of sorbents have been used for the clean-up and separation of PAH and OPAH in soil organic extracts. Silica is the most widely used, with partial deactivation through addition of 2-10% water to the silica often recommended. Since deactivation reduces analyte sorption, it can reduce the solvent intensity required for subsequent elution steps and limit the release of unwanted

compounds (Lundstedt et al., 2006a). Considering additional common sorbents for a PLE-based methodology, Lundstedt et al., (2006a) included Florisil and alumina (each deactivated 1.2 %) as a potential contenders for PAH/OPAH separation and clean-up. They found somewhat similar results between Florisil and silica during screening tests (see also Witter and Nguyen, 2016), but preferred the silica due to its reduced retention of PAH which led to greater PAH/OPAH separation efficiency. Alumina is not preferred for the fractionation of PAH and OPAH (Lundstedt et al., 2014) but has been used separately or in tandem alumina/silica sorbent systems for the removal of humic substances, macromolecules, and polar interferences in soil, water, and especially aerosol extracts. In these applications silica may also be used for fractionation of aliphatic and aromatic compounds (Albinet et al., 2014, 2006; Cai et al., 2017; Qiao et al., 2013; Sun et al., 2017; Van Gestel et al., 2003; Walgraeve et al., 2010). The specific advantages of base-modified silica sorbents (KOH-impregnated silica gel, and amonopropyl silica) for PAH/OPAH analysis has not been discussed in detail in soil the literature, though these likely improve the removal of acidic interferents, and may reduce RSDs for OPAH quantitation compared to deactivated silica (Lundstedt et al., 2014).

Less commonly, silica has been used to determine OHPAH and COOHPAH, with mixed results. Strong recoveries have been obtained for OHPAH when they were the sole class investigated and methanol was used as both the extraction and elution solvent. Using these conditions, workers attributed only 5-10% losses in recovery of standards to the SPE methodology itself (Avagyan et al., 2015). However, when other workers have extended exisiting PAH/OPAH fractionation techniques for acidified DCM/acetone extracts to include OHPAH and COOHPAH, recovery of these compounds was either not effected, or was low to moderate (Bandowe and Wilcke, 2010). In this case, the use of methanol and acidified methanol did not improve and recovery in part

because eluates exhibited turbidity, and were unworkable for subsequent derivatization (Bandowe and Wilcke, 2010). It is possible that the use of acidified methanol could also lead to the unintentional methylation of carboxylic acid groups of COOHPAH analytes (Antolovich et al., 2004).

The challenge of obtaining OH- and COOHPAH has been best addressed through the use of base-modified sorbents using the methodology of Meyer et al. (1999). This work used successive extraction on silica/strong base and strong acid sorbents to specifically isolate four classes of PAH-related compounds: (1) PAH and S- and O-PACs; (2) neutral metabolites and neutral NPACs; (3) acidic metabolites and (4) basic NPACs. An abbreviated version of their method allows for the separation of PAH, neutral metabolites, and acidic metabolites, without consideration of HPACs. This technique has been further validated in composting microcosm studies of spiked soils (Meyer and Steinhart, 2001), where 30 metabolites of 2-4 ring PAH and 4 additional metabolites of NPACs were identified, including metabolites with carbonyl, hydroxyl, diol, carboxyl, dicarboxyl anhydride, and mixed functionalities. Although this method provides more extensive separation capacity for a wide variety of target analytes, it involves large solvent volumes and a lengthy Soxhlet extraction, and with the second column may be more involved than required for the analysis of PAH metabolites alone. Nevertheless, it is one of the few methods that has been evaluated for the range of PAH, OPAH, OHPAH, and COOHPAH which should be of interest in metabolite detection studies.

4.6 Undesired volatilization, sorption, and leaching

Many workers report low recoveries and high variability for volatile and semi-volatile target analytes including naphthalene, indanone, and phenolic compounds, attributed to evaporative losses during sample preparation. Volatilization may be minimised by avoiding harsh drying conditions including lyophilisation, excessively low vacuum, heating, or high drying gas flow rates and complete dry down of samples and extracts. Where possible, solvents should be selected to minimise the need for subsequent solvent exchange through dry down and reconstitution, a step sometimes implemented e.g. to allow for effective derivatization or appropriate HPLC chromatography. The addition of keeper solvents, which are less volatile than the primary sample solvent, such as heptane (Meyer et al. 1999), dodecane (Fan et al., 2012; Li et al., 2014; Woudneh et al., 2016), and toluene (Bandowe and Wilcke, 2010; Siemers et al., 2015) may be helpful in preventing losses due to excessive drying, but these have not been specifically evaluated, and could lead to reduced solubilisation of more polar analytes. In many cases, keeper solvent use during PAH analysis has not substantially improved recovery of the smallest targets (Dabrowski, 2016). In some cases, non-extractive LC-MS or headspace techniques may be preferred for the recovery of the most volatile constituents.

Losses due to sorption on extraction equipment have also been reported and may impact recovery of both LMW and HMW components. Substantial losses of alklylated phenols have been attributed to free silanols in glassware, and recoveries of these compounds have been improved by >100% when rotary evaporation components were silanised prior to sample concentration (Berkner et al., 2004), a step that is also recommended for OHPAH analysis in atmospheric samples (Woudneh et al., 2016) and PAH in soil pore water (8272 - USEPA, 2007). Greater losses observed when PLE was used rather than ultrasonication have been attributed to losses in the PLE tubing (Berkner et

al., 2004), and other workers have implemented a flush back mechanism during PLE extraction (Walgraeve et al., 2010). Discussed above (Section 4.1), use of any filters should be carefully considered. PTFE vial caps and other implements are recommended. The use of plastics is not recommended, especially as phthalate contamination is common and can lead to interferences not only for the analysis of phthalate-related metabolites, but other analytes as well (3630C USEPA, 1996). Since not all losses are possible to control, depletion tests can be conducted to estimate losses due to sorption (Boll et al., 2015). Method calibration and the use of surrogate standards is also recommended though the latter approach may not be feasible (See section 6).

4.7 Storage and stability

PAH and metabolites may be structurally altered or otherwise lost during extraction and storage. As discussed, quinones have been shown to be susceptible to rearrangement when heat is applied during extraction methods such as PLE or sonication and during GC-analysis (Bandowe et al., 2010; Lundstedt et al., 2014; O'Connell et al., 2013). The impact of elevated heat on other metabolites is less documented but studies suggest that stability under common storage conditions is less dependent on temperature and more dependent on solvent type and sample preparation. Loss of OHPAH occurred in water samples stored at temperatures from -80ºC to +20ºC for 14 days (recovery ranging between 27 and 64%), and temperature itself did not majorly impact degradation of standards; however samples stored in toluene were well preserved for the 14 days within the same tested temperature range (Woudneh et al., 2016). OPAH stored in ethyl acetate at 4ºC were stable for 111 days (O'Connell et al., 2013).

Deconjugation tends to reduce while derivatisation tends to increase storage stability. When deconjugation was applied to urine samples prior to 14 days sample storage in clear vials at

ambient temperatures, analyte loss was dramatic with less than 20% recovery of PAH metabolites; without deconjugation, recovery was 55-75% under the same conditions (Woudneh et al., 2016). Similar losses from deconjugated urine were observed (Motorykin et al., 2015), while undeconjugated urine samples containing OHPAH have also shown mean variation of only 15% of the original analytical results after 1 year of storage at -20 ºC (Jacob et al., 2007). Derivatization has been shown to improve subsequent recovery of several OHPAH compared to underivatized aqueous preparations. OHPAH derivatized with N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) to form trimethylsilyl (TMS) derivatives, and prepared in a solution of 2-ME, acetonitrile, and toluene were stable for 55 days at -80ºC when stored in glass inserts (Woudneh et al., 2016). TMS derivatives in N,O-bis(trimethylsilyl)acetamide (BSA)-derivatized extracts prepared in hexane showed good stability for 24 hours at room temperature and -20ºC, but lost over 10% for some analytes after 1 week storage at room temperature (Toriba et al., 2016). Formation of TMS derivatives using N, O-Bistrifluoroacetamide (BSTFA) may also improve OHPAH stability (Schummer et al., 2009). *Tert*-butyldimethylsilyl (TBDMS) derivatives prepared using N-Methyl-N-tert-butyldimethylsilyltrifluoroacetamide (MTBSTFA) in acetonitrile showed less than 10% loss when stored up to one week at -20 with the exception of 1,3 dihydroxynaphthalene and 9-hydroxyfluorene, and with some smaller quinone species also being lost by over 10% after two weeks (Motorykin et al., 2015). Pentafluorobenzyl ether derivatives showed good stability at room temperature for at least 24 hours, and at -20ºC for at least 5 days. Dansylated derivatives were also stable within 10% over 4-weeks storage at -20ºC.

In general, conditions that facilitate oxidation should be avoided during all sample handling and storage. Photodegradation is a well-documented issue for aromatic compounds and samples should be kept away from light by using amber or foil-wrapped extraction and storage vessels and

darkened work conditions (Woudneh et al., 2016), with some workers recommending using UV filters (Ahmed et al., 2015), or only red light (Lin et al., 2015; Ma et al., 2016). In addition, storage in toluene can minimise photodegradation compared to water (Woudneh et al., 2016). Processing samples under N_2 and with the addition of antioxidants has improved recovery of OHPAH in water and urine samples. In one study, 2-mercaptoethanol (2-ME) was preferred over butylated hydroxytoluene (BHT) for its solubility in both organic and aqueous mixtures and its improved characteristics for the subsequent HPLC methodology employed (Woudneh et al., 2016). However, the impact of antioxidants was of short duration and their addition did not improve analyte recovery over 14 days of storage (Woudneh et al., 2016). Antioxidants R-tocopherol, BHA, quercetin, gallic acid have also improved OHPAH recoveries during urine sample preparation (Jacob et al., 2007). For studies of anaerobic samples, even further care is required, as hydroxylation of the aromatic ring may also occur during sampling when reduced acceptors are present (Meckenstock et al., 2016). Microbial oxidation may be a concern for some samples. Filter-sterilised solutions containing phenolic acids and citrate showed little degradation of the acids at 9ºC over a 6 day period (Blum, 1997), but filter sterilisation should be avoided if the filtration step has not been validated. Methanol and acetonitrile (5-20%) are sometimes added to aqueous solutions to inhibit microbes, and may have the benefit of maintaining target analytes in solution when the sample is stored at reduced temperatures (Boll et al., 2015; Jaitz et al., 2011; Ohlenbusch et al., 2002).

5. Hyphenated instrumental techniques

Both gas chromatography and (ultra) high performance liquid chromatography (UPLC/HPLC, inclusively, LC) have been used for the analysis of PAH, OPAH, OHPAH, COOHPAH, and conjugated PAH metabolites in environmental samples. Mass spectral analysis (MS) is preferred

for metabolite work as it can offer full or partial identification of unknown compounds and help improve detection or quantitation of co-eluting compounds often present in complex environmental samples. Recent work has focused on developing LC-MS protocols and simplifying existing GC-MS techniques for the analysis of these compounds. Detection is ultimately performed using ion trap or quadrupole MS, MS/MS, or high resolution MS (HRMS) including quadrupole-time of flight (QTOF) or TOF detectors, operating in scan, single ion monitoring (SIM), and, increasingly, multiple reaction monitoring (MRM) modes. Scan mode provides more information for compound identification and is best suited to untargeted analysis. For the investigation of unknown compounds, Chibwe et al. (2015) suggested a hierarchical approach to compound identification of oxygenated PAH using LC-MS and GC-MS spectra, as follows: "(1) authentic standard (experimental mass spectral match and retention time match with an authentic standard), (2) isomer (experimental mass spectral match but retention time mismatch with an authentic standard), (3) library or database (mass spectral match with library, database or literature), (4) group (evidence for possible structures but insufficient for one exact structure allowing the definition of structural class or presence of certain functional groups), and (5) unknown (molecular formula or exact mass could only be assigned to structure, or poor library matching)." (Chibwe et al., 2015)

Using this approach, they found that most of the compounds which increased by at least 1.5x post bioremediation, and which therefore were of interest for potentially contributing to the increased toxicity observed after this treatment, were only able to be classified as level 5 compounds, though 11 compounds met criteria for levels 2-4, with most containing at least two phenyl rings and oxygen. At the same time, they observed that of 40 compounds identified by LC-MS, and 48 identified by GC/GC-MS showed little overlap. This highlights the importance of viewing LC-MS

and GC-MS as complimentary approaches, and also indicates the need for further development of compound libraries and increased diversity of authentic standards. SIM and MRM are preferred for targeted analysis as they offer reduced matrix interferences, lower detection limits, and especially in the case of MRM, greater confidence in compound identification. In order to take advantage of these different attributes, newer instruments which support the fast cycling of multiple modalities have also been used, though tradeoffs in sensitivity should be expected (Cochran et al., 2012)

Other hyphenated techniques, especially LC-fluorescence (FLD) detection and LC-diode array detection (DAD) offer specific advantages for some analyses and are used separately or in tandem with either LC-MS or GC-MS. LC-GC-MS has also recently been used to analyze OPAH in aerosols in order to take advantage of the different separation capacities of the two chromatographic systems (Ahmed et al., 2015). Atmospheric-pressure solid-analysis probe mass spectrometry (ASAP-MS), which involves minimal sample preparation and does not use chromatographic separation, has also recently been investigated as a semi-quantitative screening tool for OPAH (Carrizo et al., 2015).

Despite their growing use, information on instrumental method development for oxygenated PAH for environmental soil and water analysis is still scarce. Instead, techniques have often been adopted from studies of aerosols, urine, or pure substances, or adapted from PAH methodologies (Hayakawa et al., 2017; Lundstedt et al., 2014; Walgraeve et al., 2010). These studies have provided useful insights into the factors impacting instrumental analysis, but greater attention within the community may be needed to understand particular matrix effects associated with soil and environmental aqueous samples, or to address particular research questions: e.g. the detection of bacterial or fungal metabolites in heavily contaminated samples.

5.1 Liquid chromatography and optical detection techniques

Liquid chromatographic techniques take advantage of the same differences in size, polarity, and acidity that can make addressing the wide array of PAH transformation products difficult. Typically, samples are separated by reverse phase chromatography with C18 used most often as the stationary phase. Although C18 may provide inadequate resolution of the more polar PAH metabolites in complex samples, it has been successfully applied for the separation of diverse oxygenated PAH with different functionalities during the same run, for example, both conjugated and unconjugated metabolites (Malmquist et al., 2013; Tang et al., 2016). Phenyl-modified silica has been preferred by some workers since the enhanced polarizability of the stationary phase can facilitate interaction with aromatic nuclei and improve separation of closely-eluting compounds; methods are available which address up to 81 PAH and oxygenated PAH of varying and mixed functionalities including low molecular weight phenolic acids in the same run (Letzel et al., 2001). Nevertheless, sample complexity and matrix interferences may present significant challenges to achieving adequate separation or identification of analytes, and most workers focus only on a subclass of these compounds after prior fractionation utilizing SPE, online SPE (Olmos-Espejel et al., 2012) or a more selective extraction procedure (Wang et al., 2012).

UV absorbance detection, DAD, and FLD take advantage of highly conjugated π bond systems which absorb UV light. Compared to UV and FLD, DAD is more often used for the investigation of unknown compounds as not all oxygenated PAH fluoresce, and spectra may be used to support compound identification (Meyer and Steinhart, 2000; Pramauro et al., 1998; Wischmann and Steinhart, 1997). More selective and sensitive, FLD is widely used for monitoring PAH in water (Lerda, 2011), OPAH in aerosols (Hayakawa et al., 2017), OHPAH in urine (Fan et al., 2012; Onyemauwa et al., 2009) as well as metabolite production under pure culture conditions (Olmos-

Espejel et al., 2012). Despite improved selectivity, interferences in complex matrices are common, and some OHPAH do not offer good sensitivity with this technique (Fan et al., 2012). Similarly, many quinones require derivatization to enable fluorescence detection (Kishikawa and Kuroda, 2014). Rather than being used as singular technique, more often recently FLD, DAD, or DAD+FLD is used in-line with LC-MS to provide complementary evidence for identifying different PAH and oxygenated PAH in environmental samples (Boll et al., 2015; Hollosi and Wenzl, 2011; Letzel et al., 2001; Van de Wiele et al., 2004).

5.2 Liquid Chromatography - Mass Spectrometry

The use of LC-MS is growing in popularity for investigations of oxygenated PAH in both medical and environmental samples. LC-MS shows some specific advantages over GC-MS including the potential for direct analysis of aqueous samples, the capacity to analyze a greater number of compounds with carboxyl and hydroxyl groups without derivatization, and lower operating temperatures which may limit the rearrangement and loss of quinones.

Ionization of target analytes has been a key concern in the literature, particularly as small and nonpolar compounds may resist ionization in common LC-MS sources. In general, LC-MS techniques are best applied to conjugated or acidic oxygenated PAH since these are either already ionized or may be easily ionized in the sample matrix. Grosse and Letzel, (2007) compared the ionization of 30 non-conjugated PAH metabolites using electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI), using both negative and positive ionization modes. Nearly all tested metabolites yielded higher signals using APCI and APPI than ESI, which also failed to detect several compounds. Most compounds were preferentially ionized in the negative mode, except for lactones and ketones which were better

ionized in the positive mode. Other workers have reported higher sensitivity of negative ESI for mono-hydroxylated PAH metabolites, but larger issues with ESI matrix interferences have also led these workers to prefer APCI for their specific applications (Jacob et al., 2007; Sakuma et al., 2011). ESI has been used in the quantification of more polar components including COOHPAH in contaminated groundwater (Ohlenbusch et al., 2002), OHPAH in wastewater (Pojana and Marcomini, 2007), and OHPAH and conjugated metabolites in supernatants of fungal PAH degradation incubations as well as urine (Schmidt et al., 2010a; Tang et al., 2016). At the same time, the use of APCI is growing for these more polar compounds and is generally preferred for OPAH analysis, particularly in atmospheric samples where the technique has been more widely adopted (Boll et al., 2015; Cochran et al., 2016; Ghosh et al., 2014; Kishikawa and Kuroda, 2014; Nyiri et al., 2016; O'Connell et al., 2013; Walgraeve et al., 2010). APPI may be preferred for the analysis of parent PAH (Hollosi and Wenzl, 2011) and has been used more recently for analysis of OHPAH in soils (Avagyan et al., 2015)

Matrix effects can be substantial for all LC-MS applications, in particular as other constituents including salts, buffers, or the solvent itself can cause residue build-up in instruments, compete with analytes, change charge-transfer characteristics, or lead to additional interactions such as ion-pairing. In addition to ensuring sufficient cleanup during sample preparation, the addition of dopants post-column can also improve ionization of resistant PAH and breakdown products through matrix modification. Hollosi and Wenzl (2011) compared dopants acetone, toluene, anisole, and xylene, separately and in combinations to improved detection of PAH by APPI and found that pure anisole yielded highest signal intensities for these compounds. Other researchers have found that a combination of methanol/toluene and toluene/anisole also support the ionization of OPAH and OHPAH respectively by APPI (Avagyan et al., 2015; Ghislain et al., 2012), and that

acetone improves signals of most classes of oxygenated PAH over methanol/water eluents alone (Grosse and Letzel, 2007). Another study using APCI demonstrated higher signals for individual OPAH when chloroform or hexane was used as a solvent compared to methanol (Ghislain et al., 2012). O'Connell et al., (2013) found that the addition of DCM improved detection of OPAH by LC-APCI-MS up to five-fold while the addition of ammonium formate and formic acid did not improve OPAH detection. Nevertheless, detection of these more resistant compounds may be best assisted by the use of inline DAD or FLD devices as discussed above, or further application of the sample to GC-MS either offline (O'Connell et al., 2013), or online using LC-GC-MS (Ahmed et al., 2015).

5.3 Gas Chromatography - Mass Spectrometry

GC-MS is the most widely used instrumentation for the analysis of PAH transformation products in environmental soil and water samples. GC-MS demonstrates advantages over LC for oxygenated PAH analysis in its higher resolution chromatography (Toriba et al., 2016), enhanced separation of isomers (Grova et al., 2013), lower instrumental detection limits (O'Connell et al., 2013), more normative ionization parameters, and more established compound reference libraries. Method optimisation for oxygenated PAH has been conducted primarily for aerosol samples, and is reviewed elsewhere (Hayakawa et al., 2017; Kishikawa and Kuroda, 2014; Walgraeve et al., 2010), but several points warrant particular consideration or have not yet been reviewed.

Generally, separation of oxygenated PAH is conducted using DB-5, DB-5MS or HP5-MS-coated capillary columns, but these are not considered the most appropriate stationary phases for these analytes which may exhibit tailing or poor resolution of isomers. A comparison of DB17MS and DB-XLB columns for the analysis of OPAH demonstrated that the former provided improved

separation, improved quantitation, and higher signal areas for most compounds. The study also presented concerns of the likelihood of overestimation of some compounds, particularly benzanthrone, in reference materials, when columns with similar selectivity as DB-XLB including DB-5MS are used (Nocun and Schantz, 2013). In another study, it was shown that RTX®-2330 provided the best separation of OHPAH and specifically methyl-naphthols when compared with column phases RTX®-440 and RTX®-50 and ZB-5MS but also led to peak broadening of late eluting compounds and required extended run times compared to the other columns; the authors therefore recommended using the ZB-5MS for applications where providing a summary area of all methyl-naphthol isomers was appropriate (Li et al., 2014). Use of GC×GC methods may improve resolution, but difficulties in integration may also contribute to deviating results (Lundstedt et al., 2014).

Electron impact ionization (EI) at 70eV is the most common ionization technique used for oxygenated PAH. Negative ion chemical ionization (NICI) utilizing methane as the ionization gas is used more widely in the study of OPAH in atmospheric materials and is preferred when nitro-PAH are included as target analytes (Cochran et al., 2012; Hayakawa et al., 2017). It may also offer improved stability of OPAH compared to EI (Albinet et al., 2006). However, higher LODs (0.5-51x) using methane-NICI compared to EI have also been reported for OPAH (Cochran et al., 2012), and this method has only occasionally been used for soil, where its utility remains unknown (Niederer, 1998).

Due to their greater polarity, most COOHPAH and OHPAH are insufficiently volatile and require derivatization prior to GC analysis. Derivatization of OHPAH and COOHPAH involves the replacement of the –H, or -OH of hydroxyl or carboxyl groups with silyl, or less frequently, alkyl or acetyl groups (Itoh et al., 2005; Orata, 2012). Schummer et al., (2009) compared two of the

most commonly applied derivatization agents for the analysis of phenolic compounds and OHPAH: BSTFA and MTBSTFA. Both reagents were successfully utilized, but the use of MTBSTFA was recommended based on the increased signal strength, more consistent fragmentation patterns, and improved chromatographic resolution of OHPAH using an ULTRA-2 column. Further advantages of using MTBSTFA may also include improved the stability of OHPAH derivatives when trace amounts of residual water are present (Motorykin et al., 2015). Nevertheless, it was also observed that for compounds such as 9-hydroxyfluorene which exhibit steric hindrance, MTBSTFA is less suitable; in these cases, the smaller BSTFA agent more readily accesses the hindered proton, and yields clearer signals (Schummer et al., 2009). In some GC-MS setups, it is possible to perform derivatization online in the auto sampling system or directly in the injection port. This can simplify sample preparation, reduce standard error measures, and reduce the loss of target analytes (Bizkarguenaga et al., 2013). MTBSTFA has been applied in in-port derivatization techniques for acidic and polar organic pollutants (Bizkarguenaga et al., 2013), while BSTFA has been used specifically for automated derivatisation of OHPAH for in-port (Gupta et al., 2015) and SPME on-fiber applications (Luan et al., 2007).

PAH and OPAH do not require derivatisation and are frequently analysed directly in the same fraction. However, higher operational temperatures may make GC-MS unsuitable for analysis of thermally unstable OPAH, which are frequently reported to exhibit rearrangement, nonlinear or non-quantitative characteristics when this instrumentation is used (Cochran et al., 2012; Nocun and Schantz, 2013; O'Connell et al., 2013). Reducing initial oven temperatures (60 °C compared to 70 °C) has been shown to improve recovery of early eluting quinones such as 1,4 bezoquinone by ~400 fold (O'Connell et al., 2013), while the use of pulsed injection has been demonstrated to improve signal areas (Cochran et al., 2012). Signals may increase with higher injection port

temperatures, but more moderate injection port temperatures (e.g. 140 °C) may also be preferred (Albinet et al., 2014). Quadratic fitting of calibration curves has been used to extend the calibration range of some compounds which would otherwise be considered non-quantitative or with calibration ranges limited to one order of magnitude (50-750ng/mL) (O'Connell et al., 2013). Increased variability and reduced signal areas including non-detection of 1,2-naphthoquinone has been observed when glass wool was used as packing in the injection port liner compared to the use of CarboFrit™ filter liners or dimpled, unpacked glass liners (O'Connell et al., 2013). Another approach to improving the detection of quinones by GC-MS is to perform derivatisation after reduction of the ketone functional groups. The use of zinc and acetic anhydride has improved signals for 1,4 naphthoquinone by ~100 times (Kishikawa and Kuroda, 2014). A combination of zinc or dithiothreitol (DTT) with BSTFA effected the conversion of several PAH quinones to double TMS derivatives, while the use of the three reagents together allowed for the identification of all 37 PAH quinones studied as their TMS derivatives and doubled the signal intensities for orthoquinones compared to the use of zinc and BSTFA alone (Toriba et al., 2016). Further work demonstrated additional increases when a mix of BSA+ trimethylchlorosilane (TMCS)+ trimethylsilylimidazole (TMSI) (3:2:3) was used as the silylation reagent (Toriba et al., 2016).

6. Assessing method performance

6.1 Internal and surrogate standards

Techniques for conducting quantitation and assessing recovery using surrogates or internal standards vary substantially (Table 3). Due to the range of compounds considered and the number of steps involved in sample preparation, multiple isotopically labelled standards are needed to fully

describe and/or correct for method recovery and variation in instrument sensitivity. Deuterated or ^{13}C labelled standards of oxygenated PAH have limited commercial availability, and this has been identified as a cause for the limited number of studies of oxygenated PAH in environmental samples (Nocun and Schantz, 2013). Since the use of large numbers of labelled reference compounds entails high expense or requires in-house production, researchers have often omitted their use or have relied on compounds with alternate chemistries to the target analytes, e.g. deuterated PAH (Obrist et al., 2015) or nitro-PAH (Niederer, 1998). Some labelled compounds are becoming more available (Walgraeve et al., 2010) and deuterated anthraquinones and fluorenones are increasingly used for OPAH recovery-correction in soil analyses (Enell et al., 2016; Layshock et al., 2010; Lundstedt et al., 2014; O'Connell et al., 2013). Nevertheless uncertainty in the selection of appropriate representative analogues remains. Bandowe and Wilcke, (2010) found that benzophenone-2,3,4,5,6-d5 was suitable for representing OPAH, but 1-hydroxynaphthalene-d7, transcinnamic acid-d6, 1,4-naphthoquinone-d6 exhibited substantial losses or unpredictable behavior which made them unsuitable to represent broader OHPAH, COOHPAH, and OPAH classes. Where labeled OPAH may also exhibit rearrangement during analysis, this might compensate for rearrangements of the native unlabeled compounds, but could also lead to complications in interpreting the amount of deuterated standard and any labeled rearrangement products, as well as any additional unlabeled analytes they are intended to represent (Lundstedt et al., 2014).

6.2 Certified reference materials

Spiked test samples do not fully reflect real-world matrices in part because analyte-matrix interactions can change with time. Enhanced sorption through soil ageing tends to 'lock away' polyaromatic components, and spiked soils may be more easily extracted than heavily aged soils

(Arp et al., 2014). For some matrices and instrumentation, it may also be very difficult to compensate matrix effects through the use of spiked surrogates (Lankova et al., 2016). More generally, spiking soils or water samples during individual laboratory studies does not provide an opportunity for ongoing detailed method comparisons, since variations in matrix may confound comparison of analytical techniques.

In order to address these gaps, suitable Certified Reference Materials (CRMs) are needed. Currently no CRMs are available which provide reference analytical values for oxygenated PAH in soil or environmental water matrices (Lundstedt et al., 2014). This has presented challenges for the validation and inter-comparison of analytical methods used in research and will continue to be a barrier to the adoption of a regulatory framework for these compounds (Lundstedt et al., 2014).

In the meantime, the use of CRMs for PAH analysis and/or alternate matrices provides a starting point for methodological comparisons in the literature. Workers have reported concentrations of OPAH obtained for diesel particulate matter and extracts (NIST SRMs 1650, 1650b, 2975, 1975), urban dust and fine particulate material (NIST SRMs 1648a, 1648b, 2786, 1649a), and mussel tissue (NIST SRM 2977) with comparative information to support instrumental method development provided by several researchers (Albinet et al., 2006; Fushimi et al., 2012; Nocun and Schantz, 2013; O'Connell et al., 2013; Toriba et al., 2016). CRMs for select OHPAH are available for medical (urine NIST SRMs 3672 and 3673) (See Li et al., 2014) and marine studies applications (fish bile BCR-720 and BCR-721), and these compounds are occasionally included in studies of the aerosol materials indicated above (Albinet et al., 2006). In soils specifically, limited data are available for OPAH superfund site soil 103-100 and NIST SRM 1941 (Lundstedt et al., 2006a; Obrist et al., 2015). Initial analysis of NIST SRM 1944 NJ river sediment has also been undertaken and includes comparison of GC-MS and LC-APCI-MS analyses of these sample

extracts, and further demonstrates that in some cases, particular matrix interferences may also lead to substantially different results when internal standards are used for quantitation compared to standard addition (Layshock et al., 2010; O'Connell et al., 2013). More extended data sets are also now available for reference soils ERM CC013a and BCR-524 (Lundstedt et al., 2014).

7. Conclusions

Significant gaps remain in understanding how PAH breakdown occurs in situ, what aromatic co-factors may modulate bioremediation strategies, and how to monitor and assess the extent to which PAH breakdown products contribute to risk at both contaminated and remediated sites, as well as for downstream receptors. These questions must continue to be supported by the development of robust analytical techniques that capture a sufficient range of PAH transformation products. Establishing methods for the extraction, identification, and quantification of oxygenated PAH from environmental matrices has excited increasing interest in the last 15 years. At the same time, the tandem concepts of cost (time) saving and green chemistry is driving a movement towards simplified analysis through reduced sample preparation, miniaturization, simultaneous or online derivatization, and the use of newer sorptive devices for extraction and passive sampling. This is an exciting area of development, but more work is still needed for method consolidation of oxygenated PAH analyses in soil and environmental water matrices. In soils, OPAH analyses may be the first to be standardized, but a concerted effort to formulate best practices to address OPAH rearrangement during extraction and analysis is still needed, as well as other parameters that may improve OPAH inter-laboratory data comparability and further push forward the certification of suitable CRMs. At the same time, more polar compounds which may more readily enter water

systems should not be ignored, and continued extension of techniques should be undertaken. For oxygenated PAH in water, where these compounds may be most bioavailable, efforts to define initial target compound lists with acceptable limits of detection validated through toxicological assays would help establish analytical benchmark criteria and improve inter-study comparability. At the same time, it is expected that currently unidentified oxygenated PAH compounds will continue to emerge as relevant factors impacting site risk and management, and continued collaboration which supports sharing of compound mass spectral libraries and reference compounds will be needed.

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Table 1: Concentrations of oxygenated PAH in various soil and aqueous environmental matrices

Location	Compounds	Land Use/ Matrix type	Concentration[1]	Reference
Soils, Sediments, Sludge			***ng/g***	
Sweden	Σ10 OPAH	background sediments	~195-410	Brorström-Lundén et al., 2010
		background soils	~110	
		urban sediments	~50-310	
		urban soils	~50-460	
		sewage treatment plant sludge	~800-2200	
		storm water treatment sludge	~250-700	
Germany, Mainz	Σ7 OPAH;	garden soil	6.6; *16.4*	Bandowe and Wilcke, 2010
Germany, Berlin	*Σ11OHPAH+COOHPAH*	former gasworks site soil	15,681; *518*	
Brazil, Manaus		forest soil	170.2; *36.3*	
Uzbekistan, Angren	Σ7 OPAH; *Σ5 OHPAH*	rural-industry gradient soils	29-1848; *7-63*	Bandowe et al., 2010
Slovakia, Bratislava	Σ9 OPAH; *Σ5 OHPAH*	urban soils, variable land use histories	33.8-2640; *nd-50.5*	Bandowe et al., 2011
Argentina	Σ15 OPAH	rural soils along a climatic gradient	0.1–124	Wilcke et al., 2014
Thailand, Bangkok	Σ15 OPAH	urban soils	6-234	Bandowe et al., 2014
Slovakia	Σ14 OPAH	forest soils near aluminium smelter	30-2900	Bandowe et al., 2018
Sweden, Karlstad	Σ11 OPAH	gasworks plant	6240-23570	Arp et al., 2014
Sweden, Riksten		tar factory	110-108080	
Sweden, Holmsund		wood impregnation site	219500-243710	
Belgium		gasworks plant	32820	
France		coking plant	10950-203900	
		coking and metallurgy site	9550-106070	
		gas factory	26860	
USA	Σ9 OPAH	remote forest sites	6-39	Obrist et al., 2015
China, Yangtze River	Σ4 OPAH	river delta topsoils	2.1-834.1	Cai et al., 2017
China	Σ4 OPAH	agricultural soils	1–42	Sun et al., 2017
Hong Kong	Σ5 OPAH *Σ8 OHPAH*	mangrove sediments	47.9-397; *36.0-180*	Wang et al., 2015
Aqueous Samples			***µg/L***	
Former gasworks site	Σ7 COOHPAH [2]	groundwater - former gasworks site	2-98	Ohlenbusch et al.. 2002
Tokyo, Japan	Σ9 OPAH + O-OHPAH [3]	seawater (Tokyo and Suruga Bays)	0.0107-0.2116	Kurihara et al., 2005
Wastewater treatment plant	Σ3 OHPAH	municipal wastewater influent	0.110	Pojana and Marcomini, 2007
		industrial wastewater influent	1.417	
		wastewater treatment plant effluent	0.02	
Sweden, Belgium, France	Σ11 OPAH	porewater - industrial soils	0.00194-168	Arp et al., 2014
Germany	Σ17 OHPAH +OPAH[4]	river water (Elbe and Weser)	0.0128-0.0558	Siemers et al., 2015
North Sea		sea water	0.0027-0.0092	
Sweden, France	Σ11 OPAH	leachate - industrial soils	0.2518-160.64	Enell et al., 2016
Argentina	Σ3 OPAH	lake water	3.05	Guiñez et al., 2018
		drinking water	0.82	
Brazil	Σ2 OPAH	sea water	1605	Santos et al., 2017
		river water	1868	
		groundwater	nd	
China	Σ4 OPAH	river water,	0.13	Qiao et al., 2014
		untreated wastewater	0.17	
		treated wastewater	0.12	
USA	Σ22 OPAH	river water (Portland Superfund site)	0.279	Tidwell et al., 2017

Entries in italics indicate a second set of target analytes; *nd* - not detected (<LOD);

1 mean concentrations, mean concentration ranges, or in some cases approximate concentrations.;

2 naphthalene derivatives;

3 anthracene derivatives;

4 primarily phenolics

Table 2: Methods for the extraction of oxygenated PAH from aqueous samples

Advantages	Disadvantages and Challenges	References and applications			
		Additional factors	Reference	Compounds identified	Matrix
1.Direct Analysis by LC-MS or LC-DAD - Best for relatively clean, concentrated samples, and the analysis of conjugated metabolites					
• Minimal sample preparation • Polar compounds preserved in sample; are not lost during extraction • Matrix may better reflect real world sample matrix • Conjugated metabolites may be analysed directly • Conjugated and deconjugated metabolites can be considered alongside each other • Demonstrated utility in samples with expected higher concentrations (incubation studies)	• Limitations in the detectability of some analytes • Matrix interferences not removed prior to analysis, increased baseline noise or other instrumental problems; • Without fractionation, chromatograms may be overly complex • Pramauro et al. (1998) identified more analytes with LLE • Instrument LOD must be very low or samples must be concentrated prior to injection • Identification of compounds may require more analyst experience • Filtering prior to direct injection can lead to analyte loss • Aqueous samples may have reduced storage stability	*detector/ion source* FLD-ESI-QTOF FLD- ESI-QTOF FLD-ESI-QTOF ESI- MS/MS and ESI-Q-TOF UV	 Boll et al., 2015 Schmidt et al., 2010 Malmquist et al., 2013 Tang et al., 2016 Pramauro et al., 1998	 sulfate-conjugated and COOHPAH sulfate-conjugated and COOHPAH conj. and unconj.metabolites conj. and unconj.metabolites OHPAH, OPAH	 fungal mycelial microcosms fungal mycelial microcosms seawater/sediment microcosms urine irradiated soil wash solutions
2. LLE techniques - Best for simplified method development, in reasonably concentrated samples, less polar compounds more easily extracted, or when a fractionation technique developed for organic extracts is desired					
• Utilises simple laboratory equipment • As for soil extracts, further clean-up, fractionation, concentration, and derivatization techniques may be applied • Sample volume range 10mL -1L • Opportunities for scaling the technique • May be performed sequentially to target different analytes • May provide better recovery of large nonpolar compounds compared to direct SPE (Siemers et al., 2015)	• Solvent volumes are frequently larger than for direct SPE • Extraction of large volumes of aqueous sample is not tenable • pH adjustment and/or ionic adjustments required for recovering phenolic, acidic, and basic compounds • Transfer of polar analytes may be incomplete after pH adjustment • Conjugated metabolites require deconjugation before extraction • Recovery for nonpolar compounds may be reduced compared to some SPE methods (Siemers et al., 2015)	*solvent/disperser solvent* *pH and salts/subsequent sorbent* *shaking, temperature, number of extraction cycles*			
A. LLE + application to analytical instrument with or without derivatization					
• Minimises further sample preparation and potential sources of analytes loss • Reduces method development time • Does not require chromatographic apparatus or consumables	• Matrix effects of raw extract may be substantial, particularly if extract must be concentrated prior to analysis • No opportunity for further preparative fractionation and application of different techniques to different compound classes	DCM/pH <2 hexane+toluene ethyl acetate/pH <2 pentane+toluene	Pramauro et al., 1998 Siemers et al., 2015 Cajthaml et al., 2001 Li et al., 2014	PAH, OPAH, OHPAH, COOHPAH PAH, HPAC, OHPAH, OPAH OPAH, OHPAH, COOHPAH OHPAH	irradiated TiO2-treated soil washing solutions river water, sea water fungal mycelial microcosms urine (deconjugated)
B. LLE + Normal phase SPE					
• Allows for sample clean-up and removal of interferents • Allows for fractionation • Provided load solvent and volume is the same, fractionation technique developed for other organic extracts such as soils may be applied to LLE extract • Depending on the treatment used, different fractions may be analysed by varying techniques	• Direct SPE may be preferred to reduce sample processing steps • More time- and materials than direct analysis of LLE extracts • Scaling method may be time consuming. • Some solvents used for LLE must be exchanged prior to load step • Additional steps over direct LLE may result in analyte loss	DCM/silica DCM/silica DCM/pH 5-8, 10-11 /KOH-silica toluene/NaCl/ silica	Liao et al., 2014 Lundstedt et al., 2006b Enell et al., 2016 Letzel et al., 2010	PAH, OPAH PAH, OPAH PAH, OPAH, NPAC phenols, OPAH OHPAH	aq. phase soil slurry aq. phase soil slurry leachate - contaminated soils lignite pyrolysis wastewater
C. LLE + Dispersive SPE					
• Simple purification protocol with simple lab equipment obtained better results than direct SPE (C18) for urine (C18) (Lankova et al., 2016)	• Methods typically intended for purification but not fractionation • Underdeveloped for aqueous environmental samples	ethyl Acetate/Z-Sep; also tested Z-Sep+, C18, PSA and ENVI-Carb	Lankova et al., 2016	OHPAH	deconjugated urine
D. Dispersive Liquid Liquid Microextraction (miniaturisation technique)					
• Very low solvent volumes (<500µL); high enrichment factors		toluene/ACN	Wang et al., 2012	OHPAH	sediment extracts H_2O:ACN

• Low cost, high speed option • Utilises simple laboratory equipment • Yielded strong recoveries for 4:1 H_2O:ACN sediment extracts (Wang et al., 2012)	• Although method optimization has been reported for urine, further optimization • Applicability to highly contaminated samples is unknown • Back –extraction may be necessary (Guiñez et al., 2018) • Limited number of OPAH have been tested	toluene/ACN	Wang et al., 2015	OHPAH	sediment extracts H_2O:ACN
		TCE/ EtOH/ pH6, NaCl	Gupta et al., 2015	OHPAH	Deconjugated, spiked urine
		dodecanol/MeOH/ ACN	Guiñez et al., 2018	OPAH, PAH , Nitro-PAH	lake water, drinking water
E. Single Drop Microextraction (miniaturisation technique)					
• Extremely small solvent volumes; high enrichment factors • Derivatization may be completed during extraction step, with good efficiency models for OHPAH (Wang et al. 2017) • Can be automated • Better recovery and LODs than C18 SPE or SPME have been obtained (Wang et al.; 2017 Santos et al., 2017)	• Manual extraction may be challenging, specific automation apparatus recommended for some approaches • Matrix effects may be substantial and greatly increase LODs • Applicability to contaminated samples is unknown • When derivatization is completed simultaneously analytes recalcitrant to derivatization may not be fully quantifiable	*toluene:cyclohexane* /HCl+NaCl	Wang et al., 2017	OHPAH	estuarine waters
		toluene; cyclohexane, isooctane/ HCl+NaCl also tested	Santos et al., 2017	OPAH, PAH , Nitro-PAH	river water, sea water, groundwater
3. Direct SPE Techniques - Preferred for removal of matrix interferents and processing larger sample volumes					
• Requires less solvent than many LLE protocols • Larger sample volumes may be processed e.g up to 1L • Concentrates target analytes • Provides clean-up and fractionation opportunities • May increase recovery of small polar analytes over LLE • Storage on column possible for some applications	• May require increased method development time • Few methods cover a broad range of oxygenated PAH • Methods cannot be adapted from methods for organic extracts • Fractionation is largely untested for oxygenated PAH • Typically requires vacuum manifold and pump • Storage of oxygenated PAH on SPE devices not yet investigated	*specific sorbent* *maximum load* *sample pH* *eluent(s)* *compatibility with further processing*			
A. C18- based sorbents					
• Well known sorbents in environmental literature • Adequately retain OHPAH (Olmos-Espejel et al. 2012; Luan et al., 2007) • Well known in medical literature • Methods available for PAH and EPA phenols • May be preferred for OPAH analysis, as more polar compounds are less well retained (Qiao et al., 2014)	• Premature elution of polar compounds • pH stability lower than polymeric sorbents; may be particular issue with acidified or base-treated samples for analytes • Strongly hydrophobic constituents may be retained on the column • Insufficient selectivity including poor removal of humic acids (Ferrer and Barceló, 1999) and urinary interferents • Cleaner chromatograms obtained using stir bar sorption, LLE+ dispersive SPE for urine (Zhao et al., 2013; Lankova et al., 2016)	Strata-E ;DSC-18 Envi-18 Envi-Chrom P	Pojana and Marcomini, 2007	OHPAH	treated and untreated wastewater
		Sep-Pak	Luan et al., 2007	OHPAH	water
		Chromabond C18-PAH	Olmos-Espejel et al., 2012	PAH, OPAH	aqueous phase algal degradation study
		PrepSep C18	Van de Wiele et al., 2004	OHPAH	simulated human gastrointestinal matrix
		C18	Qiao et al., 2013, 2017	OPAH	river water, wastewater
B. Polymeric Sorbents (Polystyrene Divinyl Benzene)					
• Relatively well known sorbents in environmental literature • Higher retention of polar compounds than C18 • May capture a broad range of compounds • Recovery of conjugated metabolites (Malmquist et al., 2013) • Environmental brands demonstrate utility in medical literature • Methods available for PAH and EPA phenols	• Generally non-specific sorbents • May show reduced recovery of PAH compared to some LLE applications (Siemers et al., 2015), or more variable response to OPAH (Qiao et al., 2013) • Recovery of PAH may be improved by the inclusion of C18 column in the protocol (Motorykin et al., 2015)	Lichrolut EN	Siemers et al., 2015	Phenols PAH, few O/OHPAH, NPAC	river water, sea water
		Oasis HLB	Malmquist et al., 2013	conjugated pyrene metabolites	seawater- spiked sea sediment microcosms
		Focus, Isolute 101, Bond Elut Plexa	Motorykin et al., 2015	OHPAH, PAH	deconjugated urine
C. Additional nonpolar sorbents : Cyclohexyl, Phenyl, C8					
• Strong recovery has been reported for naphthols and phenols with cyclohexyl and phenyl phases • Moderate to good recovery of PAH using these phases	• Few follow up studies using cyclohexyl phase • Phenyl phase also led to overestimation of compounds • C8 not recommended as tested	cyclohexyl, phenyl,C8, C18	Rostad et al., 1984	Phenols, Naphthols, PAH, HPACs	groundwater from contaminated site
D. Mixed mode sorbents (Polymeric-Weak Anion Exchange) (P-WAX)					
• May facilitate the retention of more than one class of analyte • P-WAX may facilitate the capture of acidic, and charged, and conjugated oxygenated PAH metabolites (Boll et al., 2015)	• Limited studies for oxygenated PAH in environmental samples	P-WAX	Boll et al., 2015	acidic and sulfate-conjugated metabolites	aqueous fungal microcosms
		Oasis Max	Kakimoto et al., 2008	OHPAH conjugates	urine
E. Tandem SPE					

• Found to be advantageous for some medical applications • Facilitates removal of specific interferents or target recovery of more than one class of compounds • Improved recovery of larger PAH compared to polymeric column alone (Motorykin et al., 2015)	• More materials intensive than single-phase SPE • Insufficiently tested for environmental matrices	Bond Elut Plexa/ C18	Motorykin et al,. 2015	OHPAH, PAH	deconjugated urine
		C18/silica; aminop-propyl silica, cyano- and diol	Chetiyanikornkul et al., 2006	OHPAH	deconjugated urine
		Immuno-sol gel / C18	Letzel et al., 2001	OPAH	deconjugated urine
F. Solid Phase Disk Extraction					
• Larger surface area for sample and solvent interaction • Longer dwell times for ample transfer on to stationary phase. • May be used in passive sampling applications • Vacuum manifold or other device not required	• Limited studies for oxygenated PAH in environmental samples • Directional fractionation not possible	Empore C18 and Empore SDB-XC	Kurihara et al., 2005	OPAH and OHPAH	Seawater Tokyo Bay
		Envi C-18 Disk	Lundstedt et al., 2003	OPAH	aqueous phase of Fenton agent slurry
4. Other sorptive extractions - Key advantages are application specific - may be preferred for low solvent use, high concentration factors, and field sampling options					
• Minimal or no solvents used (0-5mL) • Reusable materials- reduces waste and cost • More selective sorbents available –reduces interfering compounds, baseline noise • Potential for developing field-extraction protocol • Options for thermal or solvent desorption • Automated or in-situ derivatization protocols may be applied • On-site applications may be feasible	• Fractionation not used • Only individual classes of oxygenated PAH have been studied • More selective membranes may also exclude compounds of interest • On-site methods are underdeveloped, • Calibration may be a challenge • Storage stability of analytes on devices not known	*sorbent material and format, thickness, sampling time, pH, ionic strength, matrix effects mixing desorption conditions*			
A. Solid Phase Micro Extraction (SPME)					
• Minimal or no solvents; significant concentration factors • Sample preparation time can be reduced • Aqueous sampling and headspace applications possible • OHPAH have been successfully analysed • Substantially lower LODs achieved compared to C18 SPE (Luan et al., 2007) • Established methods for PAH, phenolics in aqueous media. • Adaptation for GC-MS or HPLC possible, though latter not yet studied for oxygenated PAH • Range of sorbents available • Automation is possible and recommended	• Underdeveloped for environmental samples • Small sorptive surface area; better for cleaner samples • Competition for binding sites may be issue for dirty samples • Extraction efficiency matrix dependent • Calibration can be challenging • Lower upper calibration limits and poorer precision than traditional C18 SPE (Luan et al., 2007) • Manual injection finicky, automation recommended • OHPAH and COOHPAH require on-fibre derivatization • Initial investment in devices can be expensive • Thin fibres are expensive and can easily break	*Material*			
		polyacrylate	Luan et al., 2007	OHPAH	water, culture media, algal degradation experiments
		polyacrylate	Luan et al., 2006	OHPAH	aqueous phase of PAH degradation experiments
		polyacrylate	Smith et al., 2002	OHPAH	deconjugated urine
		polyacrylate	Gmeiner et al., 2002	OHPAH	deconjugated urine
B. Stir Bar Sorptive Extraction					
• Larger surface area and more robust apparatus than SPME • More easily applied to large volumes than SPME or SPE • Minimal solvent use compared to LLE or SPE (200µL-5mL) • Improved clean-up over C18 SPE for OHPAH in urine, with strong recoveries (Zhao et al., 2013). • Can apply traditional solvent-based derivatization	• Recovery of OHPAH was somewhat low when acetylation-derivatization applied prior to sample extraction; • Recovery of higher molecular weight aromatics was reduced when compared to a direct-SPE method using Oasis HLB sorbent; (Poulain et al , 2016) • Automation is not possible	PDMS	Itoh et al., 2005	OHPAH, naphthoquinone	seawater, puddlie water
		PDMS	Zhao et al., 2013	OHPAH	deconjugated urine
5. Passive sampling devices -Special applications, field deployment, estimation of mobile or bioavailable fractions					
• Can be used to estimate mobile or bioavailable fraction in combined water/sediment systems • May be deployed in field	• Current techniques have not addressed OHPAH or COOHPAH • Applicability and QC required for field applications not established for oxygenated PAH • May require long periods to equilibrate - e.g. 7-28 days	*material and device format, conditioning, sampling duration, extraction, calibration*			
A. Tenax and HLB in dialysis tubing					
• Used to investigate distribution of genotoxic elements	• Equilibration period of one week	Tenax/HLB	Hu et al., 2014	PAH, OPAH	phosphate-buffered

• Use of dialysis tubing to suspend sorbent simplifies sample preparation compared to direct application of Tenax beads without adverse impacts on recovery	• Though it reflects the most mobile constituents, the bioavailable fraction of genotoxic elements in the whole slurry was not fully represented through this technique, • Larger solvent volumes, and longer extraction period (overnight) used than for other sorptive techniques				suspension of contaminated soil bioreactor slurry
B. Polyoxymethylene strips (POM)					
• OPAH, PAH, and NPAC can be analyzed at the same time • Extracts can be further processed as other organic extracts • Used to estimate porewater/KTOC parameters for OPAH	• Considered complimentary but not replacement technique for leachate sampling (Enell et al., 2016) • Long equilibration period (28 days for studies referenced here) • Larger solvent volumes used than for other sorptive techniques	POM	Enell et al., 2016	PAH, OPAH, NPAC	pore water/leachate from contaminated soils
		POM	Arp et al., 2014	PAH, OPAH, NPAC	simulated pore water from contaminated soils
		POM	Josefsson et al., 2015	PAH, OPAH	water
C. Silicone and Low Density Polyethylene (LDPE) passive sampling devices					
• Have been used to quantify OPAH in river water • Silicone provides greater sensitivity for individual OPAH than LDPE passive sampler, which may be preferred for PAH analysis	• Notable differences were observed for some OPAH between using standard addition and internal standard quantitation methods • Silicone sampling device contributed to matrix effects, extensive pre-cleaning required; selection of sorbent format important	Silicone, LDPE	O'Connell et al., 2013, 2014	OPAH	river water
		LDPE	Tidwell et al., 2017	OPAH	river water

Table 3: Preparative chromatographic methods for the cleanup and fractionation of soil extracts for the analysis of oxygenated PAHs

Reference and sample type	Sample Preparation and Extraction[1]	Column[2]	Procedure[3]	Instrumental Analysis	Analytes (Recovery %[4])	*Comments*; further applications and references
Lundstedt et al., 2003 solid phase of bioslurry treatment of former gasworks soil	**PLE** 1g soil sample mixed with 5g Na_2SO_4 Extract 1: HEX:ACE (1:1) Extract 2: MeOH:acetic acid (99:1) Combine extracts Evap. aliquot of 10% Reconstitute in 1mL HEX	**Open Column** 15mm 1g anhydrous Na_2SO_4 5g silica gel deact. 10%	Condition: HEX 20mL Load: Sample Wash: HEX 5mL Elute PAH, OPAC, SPAC: HEX:DCM (3:1) 15mL Elute OPAH, NPAC: DCM 30mL Evap. to dryness Reconstitute in toluene	GC-MS	69 PAH (64-102%*fa* for 9 tested) 23 OPAHs + isomers 26 NPAC + isomers	*PLE conditions optimised for PAH analysis* Andersson et al., 2003- Soil compost mixtures, sample pretreatment included acidification step, and only first extraction cycle was used. OHPAH also identified after BSTFA derivatisation. Lundstedt et al., 2006b and Liao et al., 2014 - Fractionation method applied to LLE extracts (DCM) of slurry liquid phase Lundstedt et al., 2006a - Fractionation method applied to soxhlet extracts and compared to PLE-fractionation Lundstedt et al., 2014
Lundstedt et al., 2006a spiked uncontaminated and contaminated industrial soils	**PLE/fractionation** 1-5g soil homogenised with 20g Na_2SO_4	**PLE Cell Column** 11mL Isolute sorbent cellulose filter soil homogenate (0.5-1g) cellulose filter 4g silica gel deact. 2%	Extract /elute PAH : CycloHEX/DCM (9:1) Extract/elute OPAH: CycloHEX/DCM (1:3)	GC-MS	33 PAH (55-91%*ma* for 33 tested) 13 OPAH (47-129%*ma* for 8 tested)	*Additional sorbent tested: florisil 1.2% deact., and alumina 1.2% deact., silica 5% deact. and activated* *Solvent selection and extraction temperature discussed* *Comparison to soxhlet and open column chromatography technique of Lundstedt et al 2003 with comparable results* Lundstedt et al., 2006b - Treatment slurry solids Lundstedt et al., 2014 Liao et al., 2014 - treatment slurry solids, extraction solvent modification, Na_2SO_4 in place of the diatomaceous earth
Avagyan et al., 2015 soil samples from industrial area wood smoke particulates	**PLE** 1.5g soil or 40mg wood smoke particulates Extract: MeOH Evap to 0.5mL	**SPE** 100mg silica Biotage Isolute	Condition: 3mL HEX Load: 0.5mL prepared sample Wash: 0.5mL HEX Elute OHPAH: 3mL MeOH Concentrate to 0.5mL Filter 0.2µm nylon	HPLC/ APPI-MS	12 OHPAH and mixed O-OHPAH species (70-102%*ea)* (95-100%*fa* standard solutions) (92-104%*fa* spiked soil extracts)	*PLE optimization explored, with temperature and pressure significantly and positively related to extraction recovery; static time and number of cycles were less important for recovery* *losses of 5-10% reported for evaporation step* *Matrix effects ranged from -15% to +20% without clean-up but were substantially reduced by SPE method*
Obrist et al. 2015 forest soils	**PLE** 1-5g soil Extract 1: DCM Extract 2: ACE Extracts dried by Na_2SO_4	**SPE-** silica Supelclean LC-Si	Elute PAH and OPAH: HEX/benzene (1:1)	Ion trap GC-MS	26 PAH and alkyl PAH 9 OPAH	

Bandowe and Wilcke, 2010 soils OPAH spiked and unspiked	**PLE** 20g soil mixed with diatomaceous earth Extract 1: DCM Extract 2: ACE/DCM /TFA (250:125:1 v/v/v). Combine extracts Dry with Na_2SO_4 Evap. and solvent exchange to 1 mL HEX	**Open Column** 8mL borosilicate glass glass wool 5g silica gel deact. 10% glass wool	Condition: 10mL HEX Load: 1mL sample preparation Elute PAHs and al.kylPAH (F1): 9mL HEX/DCM (5:1) Add toluene and evap. to 0.5mL Elute OPAH, OHPAH, and COOHPAH (F2): 5mL ACE Evap. to 1mL, add 10mL ACE Evap. to 0.5mL Derivatise F2 (BSTFA/TMCS)	GC-MS	7 deuterated PAH 36-84%*ma* 8 OPAHs (34-96%*mc*, with most 78-96%*ma*) 7 OHPAH and COOHPAH (2-70%*ma*), 8 OHPAH and COOHPAH targets not recovered, most with two or more OH or COOH groups.	*Further elution with MeOH and acidified MeOH did not improve recovery of OHPAH and COOHPAH* Bandowe et al., 2010 Bandowe et al., 2011 Bandowe et al., 2014 (OPAH and azarenes) Wilke et al., 2014a (OPAH and azarenes) Wilke et al., 2014b Bandowe et al., 2018 (OPAH and Azarenes)
Wischman et al., 1996 soil/compost obtained from PAH-spiked microcosms	**Ultrasonication** 20g soil mixed with 1mL 4N HCl dried with 20g Na_2SO_4 Extract: 2x DCM (40mL) Evap. each extract to 5mL Combine extacts Evap. to 5mL	**Open Column** 8mL borosilicate glass PTFE frit 2g silica gel deact. 6% PTFE frit	Condition: 8mL HEX Elute PAH: 12mL HEX:DCM(9:1) Elute OPAH: 6mL DCM Elute OHPAH and COOHPAH:5mL 1% acetic acid in MeOH Evap. polar fractions Solvent exchange to 1mL MeOH 100µL aliquot dried Derivatise (BSTFA/TMCS)	GC-MS	6 OPAH (40-88%*ma*) 3 OHPAH and COOHPAH (49-89%*ma*)	*Higher recoveries obtained for most compounds when acidification was not used, but substantially lower recovery for COOHPAH and naphthalic anhydride*
Qiao et al,. 2013 river sediments river water suspended particulates	**PLE** Extract: 2x ACE: DCM(1:1) Solvent exchange Concentrate	**Open Column** 10mm alumina (6 cm) deact. 3% silica (12cm) deact. 3% Na_2SO_4 (1cm**)**	Condition: sorbents stored in HEX prior to use Elute: F1: 15mL HEX), F2: 75mL DCM:HEX (3:7), F3: 60mL DCM:HEX (1:1), F4: 60mL DCM:HEX (7:3) F5: 60mL DCM Evap. to 0.5mL	GC-MS	23 PAH 4 OPAH(101-148%*ma*) 4 nitro-PAH	*Target compounds recovered in F2, F3, and F4* Qiao et al., 2014 Qiao et al., 2017 wastewater particulates
Witter et al., 2016 urban stream sediments	**PLE fractionation** 10g lyophilised sediment	**PLE Cell Column + SPE** 1. PLE cell column 34mL diatomaceous earth florisil copper granules cellulose filter 2. SPE Silica Biotage	1. Extract sample with DCM Evap. to 1mL 2. SPE Condition: 20mL DCM:HEX (1:4) Load: 1mL prepared sample Elute PAH, OPAH, NPAC: 2x20mL DCM:HEX (1:4)	GC-MS	10 OPAH (85-110%*ma*) 4 HPAC (81-111%*ma*)	

Arp et al., 2014 contaminated and uncontaminated soils	**PLE** 1g soil mixed with solvent-washed sand to fill cell Extract: HEX/ACE(1:1) Evap half of extract	**Open Column** 16mm ID glass column 5g KOH-impregnated silica gel	Elute PAH, OPAH and NPAC: 30mL DCM Evap and solvent exchange to 1mL toluene for soils and 0.5mL toluene for worm tissue	GC-MS	16 PAH, 11 OPAH, 4 NPACs	*Soil/water partitioning and bioaccumulation considered* *Worm tissue also analyzed* Enell et al., 2016 - applied fractionation method to LLE extracts (DCM, KOH) of leachate obtained from contaminated soils
Meyer et al., 1999 spiked soil/compost mixtures and creosote and wood-impregnation site contaminated soils	**Soxhlet** 20g soil mixed and ground with 1mL 1M HCl and 20g Na_2SO_4 Soxhlet extraction: DCM (210 mL) + HEP (10mL) Rotary evap. to 5mL	**Open Column + SPE** 1: Open Column PTFE frit 0.7g Chromabond SB PTFE frit 2.0g of silica gel (10% deact.) PTFE frit 2: SPE Chromabond SA cartridge	1: Open Column Condition: 12mL HEX Load: 5mL extract Elute PAH, SPAC, OPAC (F1): 3mL HEX, 12mL HEX/ DCM (85:15; v/v), 2mL DCM Elute OPAH, OHPAH, and NPAC (F2): 1mL DCM, 6mL MeOH, 3mL 0.05N HCl in MeOH Elute COOHPAH (F3): 6mL 0.05N HCl in MeOH 2. SPE (F2 only) Condition: 5mL MeOH Load and elute neutrals (F2a): F2 eluate + 5mL MeOH Elute Basics (F2b): 5mL 1N ammonia in MeOH Dilute fractions	F1,F2a : GC-MS F2a, F2b, F3, HPLC-DAD	14 PAH (31-98%*ma*) 6 OPAH (32-96%*ma*) 2 OHPAH (87-94%*ma*) 6 COOHPAH (93-100%*ma*) NPAC (60-101%*ma*) OPAC and SPAC (29-102%*ma*)	*Abbreviated method can be used without further separation of F2* Meyer and Steinhart, 2000 (PAH, OPAC, SPAC, NPAC) Meyer and Steinhart, 2001 (30 PAH metabolites identified. F2 used without further fractionation)
Layshock et al., 2010 reference materials: sediment mussel tissue urban dust diesel particulate matter	**PLE** Solid samples mixed with approximately 30x Na_2SO_4 PLE extraction: DCM Some treated with SEC all samples: evap and solvent exchange to 1mL HEX prior to SPE	**SPE** Discovery 1000mg aminopropylsilica and/or Discovery 1000mg bonded silica (used separately or in tandem)	Elute nonpolar compounds: 3x2mL HEX:DCM 9:1 Elute polar compounds: 3x2mL 4:1 HEX:DCM Evap. Recombine fractions	GC-MS	*ma* 9 OPAH (34-97%*ma* for standards)	*Diesel extract also tested, applied directly to SPE* *Fractionation during preparative chroamtography may not have been adequate since eluates were recombined prior to analysis* *Losses of OPAH during fractionation were reported to be not attributable to the use of silica or aminopropyl silica* *Authors report SEC improved chromatographic resolution of OPAH from sediment extracts compared their tandem SPE method, but also led to lower recovery for some OPAH compounds*

[1]HEX, ACE, MeOH, DCM, and HEP denote hexane, acetone, methanol, dichloromethane, and n-heptane respectively

[2]measurements in mm refer to column internal diameter; column materials are listed top to bottom, following the vertical setup; deact. denotes deactivation with water

[3]F# denotes a fraction number for further reference;

[4]Reported recoveries reflect different stages of the process and are indicated in the brackets in italics: *m*=full method, spiked compounds added before extraction and all subsequent clean-up and evaporation steps included; *e*=extraction only- recovery evaluated before cleanup/fractionation *f*= fractionation, spiked compounds added after extraction and before cleanup/fractionation; *a*=absolute recoveries – i.e. not corrected for surrogate spike recovery; *c*= corrected for surrogate spike recovery.

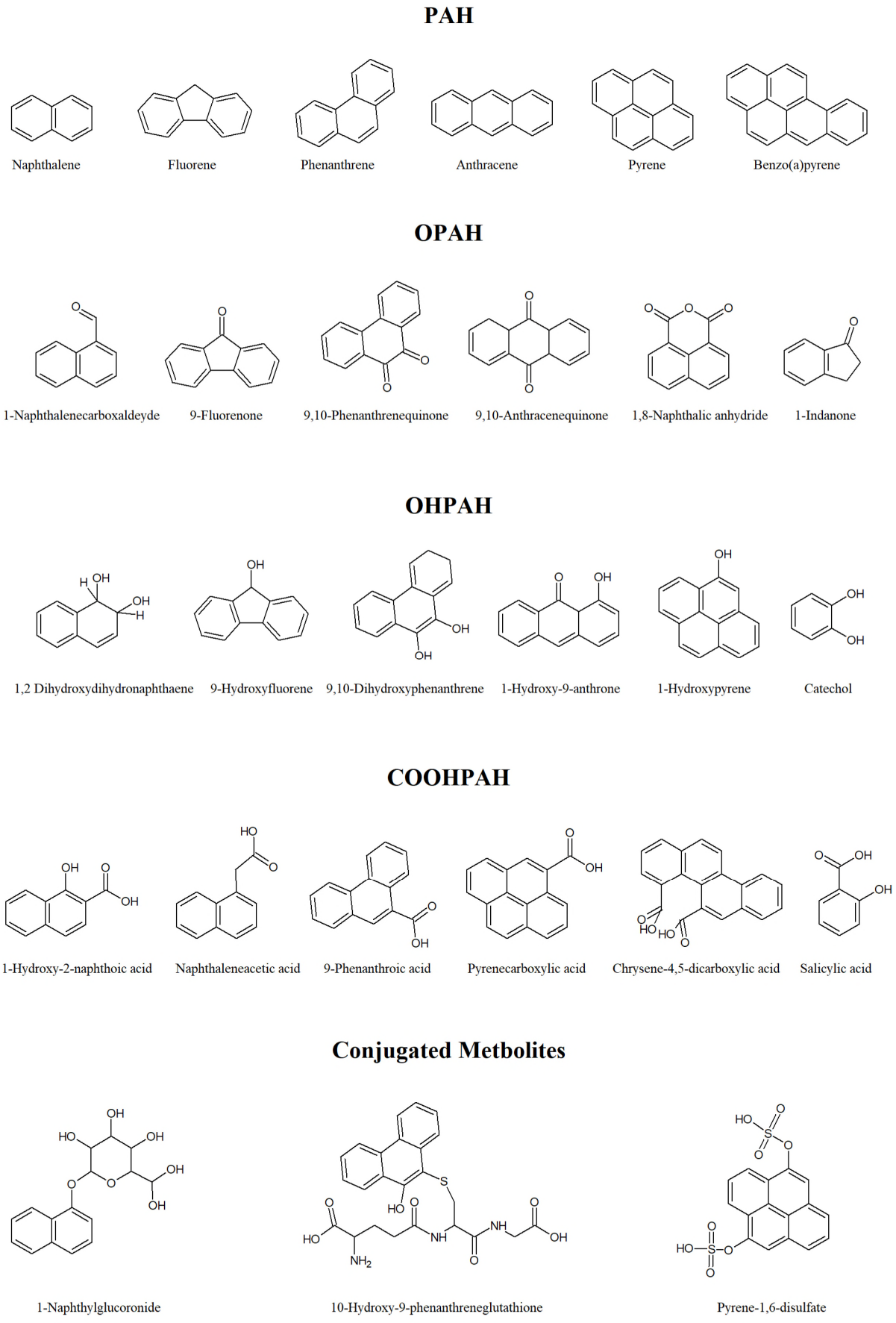
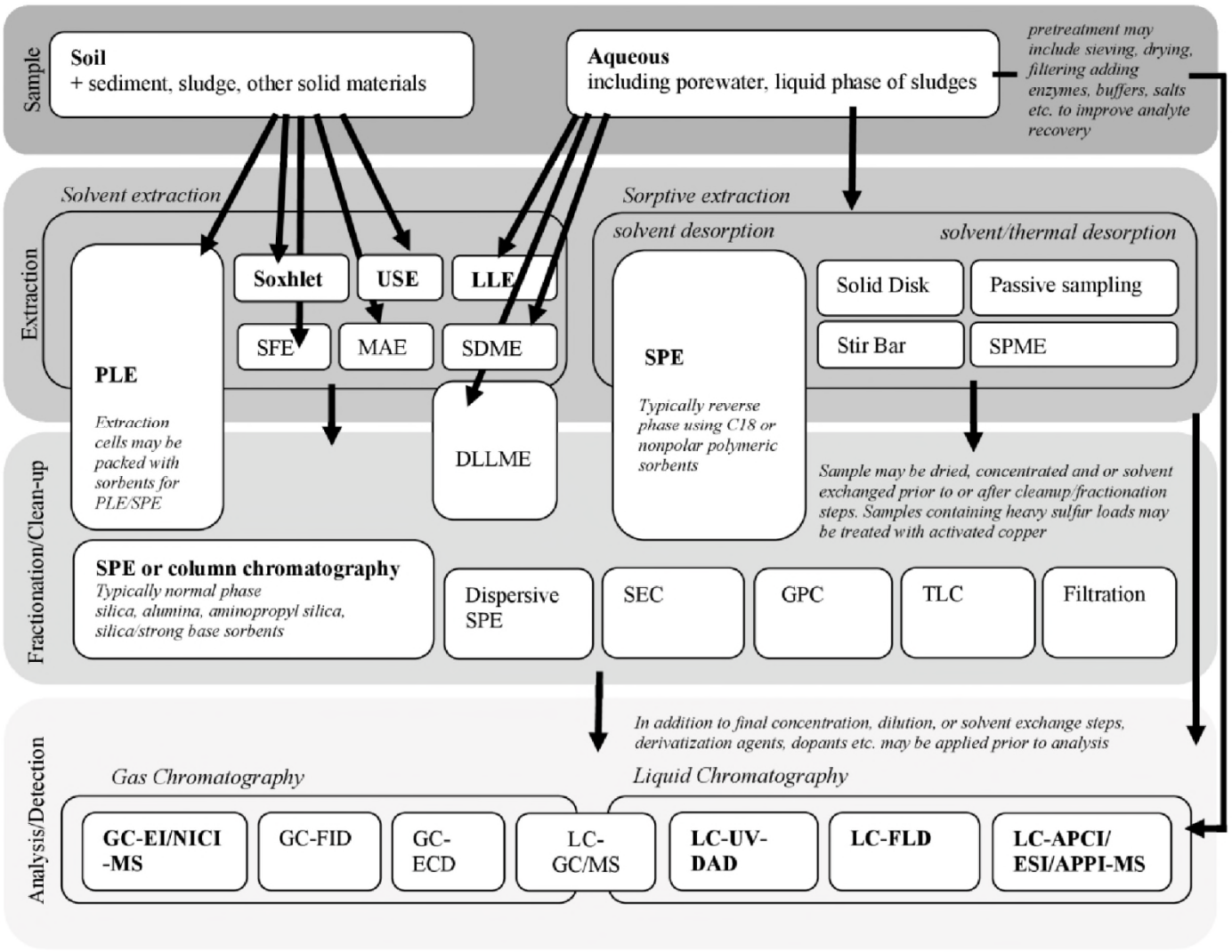


Figure 1: Selected PAH and oxygenated PAH breakdown products grouped by most polar functional group: OPAH, OHPAH and COOHPAH modified by carbonyl, hydroxyl, and carboxyl groups, respectively. Note that monoaromatic breakdown products are included.



PLE pressurized liquid extraction
USE ultrasonic-assisted extraction
LLE liquid liquid extraction
SFE supercritical fluid extraction
MAE microwave assisted extraction
SDME single drop micro extraction
SPE solid phase extraction
SPME solid phase microextraction
SEC size exclusion chromatography
GPC gel permeation chromatography
TLC thin layer chromatography

LC liquid chromatography
EI electron impact ionization
NICI negative ion chemical ionization
FID flame ionization detection
ECD electrochemical detection
UV ultraviolet
DAD diode array detection
FLD fluorescence detection
APCI atmospheric pressure chemical ionization
ESI electrospray ionization
APPI atmospheric pressure photoionization

Figure 2: Analytical techniques for the detection of PAH degradation products

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Analytical progress and challenges for the detection of oxygenated polycyclic aromatic hydrocarbon transformation products in aqueous and soil environmental matrices: A review

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