Environ Sci Pollut Res
https://doi.org/10.1007/s11356-017-0415-2

REVIEW ARTICLE

Biological and analytical techniques used for detection of polyaromatic hydrocarbons

Sunil Kumar[1] · **Sangeeta Negi**[2] · **Pralay Maiti**[1]

Received: 8 July 2017 / Accepted: 3 October 2017

Abstract Polycyclic aromatic hydrocarbons contain two or more fused benzene rings that are considered as cosmo-pollutants ubiquitously found in the environment. The identification and monitoring of polycyclic aromatic hydrocarbons (PAHs) are of great interests for rapid and on-site detection. Therefore, many analytical and biological techniques have been proposed for the qualitative and quantitative assessments of PAHs. Non-biological analytical techniques such as infrared, Raman, and fluorescence spectroscopies are commonly exploited as non-destructive techniques while gas chromatography (GC) and high-performance liquid chromatography (HPLC) with multiple detectors are extensively employed for the separation and detection of an analyte. Even though spectroscopy and chromatography are more accurate, convenient, and feasible techniques, often, these methods are expensive and sophisticated which require high maintenance cost. On the other hand, biological approaches, i.e., immunoassay, PCR, and microarray, offer comprehensive high-throughput specificity and sensitivity for a similar analyte. Biosensor- and immunoassay-mediated detections of PAHs have opened up new avenues in terms of low cost, rapid determination, and higher sensitivity. In this review, we have discussed the strengths and limitations of biological and analytical techniques that were explored for precise evaluation and were trusted at both the legislation and research levels.

Responsible editor: Philippe Garrigues

✉ Sunil Kumar
sunilbt13@gmail.com

[1] School of Materials Science and Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi 221005, India

[2] Department of Biotechnology, Motilal Nehru National Institute of Technology, Teliyarganj, Allahabad 221004, India

Keywords PAHs · Chromatography · Spectroscopy · Biosensor · Nanoparticles

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are categorized under the group of organic pollutants that persist ubiquitously in the environment. The relative production rates of PAHs have been increased due to the establishment of the industrial revolution. PAHs are one of the regular pollutants produced during the incomplete combustion of fossil fuel consisting of two or more condensed benzene rings in their molecular structure. The calculated molecular diagnostic ratios (MDRs) for anthracene/(anthracene + phenanthrene) were ranged from 0.037 to 0.078 indicating that the sources of PAHs were primarily petrogenic because the ratios were less than 0.1 (Zhao et al. 2015). However, the soot emitted from vehicles, coal-fired power plants, coking, and other industries comprises a range of PAHs altering the physicochemical characteristics of biotic and abiotic systems (Kim et al. 2009; Thomas et al. 2016; Rein et al. 2016). The diameter of soot particles is reported in nano dimension which are inhaled during respiration causing a reduction in the vital lung capacity of industrial workers. The superfluous presence of soot particles in the atmosphere impairs the factual composition of the milieu. Heavy-particle sediment on aquatic and terrestrial ecosystems participates in biomagnifications and threatens the life of biotic systems (Forbes 2010). The characteristics and standard limit of PAH contaminant in the soil are reported in Tables 1 and 2 (Piskonen and Itävaara 2004; Malawska and Wiołkomirski 2001).

The rating organizations, the US Environmental Protection Agency (US-EPA) and the European Food Safety Authority, legitimately declared a list of 16 PAH pollutants. Among

Published online: 14 October 2017

Table 1 Characteristics of the PAH-contaminated soil

Parameter	Characteristic
Type	Sandy loam
pH	7.7
Ash content (%)	9
WHC (ml g^{-1})	0.85
Total PAHs (μg kg^{-1})	222,000
Naphthalene	2410
Acenaphthalene	14,300
Acenaphthene	2030
Fluorene	5150
Phenanthrene	20,200
Anthracene	15,200
Fluoranthene	27,000
Pyrene	30,700
Benz(a)anthracene	18,700
Chrysene	17,600
Benzo(b)flouranthene	10,200
Benzo(k)flouranthene	16,000
BaP	21,200
Indeno(1,2,3-c,d)pyrene	10,800
Dibenz(a,h)anthracene	1590
Benzo(g,h,i)perylene	9100

Source: Piskonen and Itävaara (2004)

them, eight PAHs are considered to be hazardous pollutants due to their toxicity, mutagenicity, and carcinogenicity causing a significant health risk. For instance, benzo[a]pyrene (BaP) is a kind of PAH which is a concerning endocrine disruptor that affects the cognitive development of children (Perera et al. 2006; European Commission 2002). The European Scientific Committee on Food (SCF) distinguished benzo[a]pyrene as a marker for carcinogens and recommended the maximum residue level (MRL) for benzo[a]pyrene at 2 μg kg^{-1} (Wang and Guo 2010). The International Agency for Research on Cancer has classified benzo(a)pyrene to the group 1 of carcinogens, i.e., compounds that have proven carcinogenic effect on humans (Zachara et al. 2017). The estimated data reported for the maximum intake of BaP from food is about 6–8 ng kg^{-1} body weight day^{-1} (Danyi et al. 2009) while the European Union (EU) and the World Health Organization (WHO) endorsed that the maximum admissible concentration for BaP is of 10 ng L^{-1} in water intended for human consumption (Bortolato et al. 2008). In another report, the levels of BaP content in chocolate samples containing various cocoa are ranged from 0.07 to 0.63 μg kg^{-1} in the German market and 1.62 μg kg^{-1} of chocolate in the Indian market (Fromberg et al. 2007). The occurrences of PAHs in foods during food processing such as fermenting, drying, roasting, frying, and smoking are the primary source in human exposure. Therefore, the "Code of Practice for the Reduction of Contamination of Food with PAHs from Smoking and Direct Drying Processes" has guided people on how to avoid PAH contamination in cocoa beans and its derivatives (Raters and Matissek 2014). To implement the above guideline, a series of validation data such as calibration range, recovery rate (50–120%), inter- and intraday precisions, limit of detection (LOD ≤ 0.30 μg kg^{-1}) and limit of quantitation (LOQ ≤ 0.90 μg kg^{-1}), and $HORRAT_r < 2$ (Horwitz ratio: acceptability of methods of analysis) were evaluated by regulation (EU No. 836/2011) for the determination of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene in food. The comparative study of LOD, LOQ, and percentage recovery of the different methods for detection of benzo(a)pyrene which were obtained during experimental measurements is shown in Table 3.

Table 2 Standard limit of PAH content (μg kg^{-1}) in the soil surface layer

Total PAH content	Pollution class	Soil assessment
< 200	0	Unpolluted (natural content)
200–600	I	Unpolluted (increased content)
600–1000	II	Slightly polluted
1000–5000	III	Polluted
5000–10,000	IV	Heavily polluted
> 10,000	V	Very heavily polluted

Source: Malawska and Wiołkomirski (2001)

Our aim is to explore the analytical, molecular, and biological techniques to address the environmental burden of PAHs and their consequences on health issues taken during food consumption. That is why we emphasize for the detection of pollutant, mainly PAHs, by various techniques. Analytical techniques reported for the determination and identification of PAHs are FTIR, Raman spectroscopy, fluorescence spectroscopy, mass spectrometry (MS), gas chromatography (GC), and high-performance liquid chromatography (HPLC) (Yang and Watts 2005). All these techniques are promising analytical tools which provide information about the structure and function of hydrocarbons. However, each technique has its own advantages and limitation. Vibrational spectroscopy, ATR-FTIR, Raman spectroscopy, and surface-enhanced Raman spectroscopy (SERS) have been applied extensively in order to scrutinize the PAH profile at the laboratory level (Chen et al. 2015; Pejcic et al. 2013) while gas chromatography with mass detector (GC-MS) offers unambiguous identification of volatile components which are used to examine the molecular-level characterization of analogous PAHs (Dijkmans et al. 2014; Sugumaran et al. 2015; Kumar and Negi 2015; Kumar et al. 2012). Furthermore, qualitative and quantitative assessments of PAHs have been estimated by HPLC–photodiode array detection (PDA) (García-Delgado et al. 2013). Today, fluorescent materials are being extensively used in biological fields for the rapid detection of organic pollutants. The

Table 3 The comparative study of LOD, LOQ, and percentage recovery of the different methods for detection of benzo(a)pyrene which were obtained during experimental measurements

S. no.	Methods	LOD	LOQ	Recovery (%)	References
1	Foods specified in Regulation (EC) No. 1881/2006	≤ 0.30 μg kg^{-1} for each of the four substances	≤ 0.90 μg kg^{-1} for each of the four substances	50–120	European Commission Regulation No. 836/2011
2	HPLC-FD	0.07 μg kg^{-1}	0.17 μg kg^{-1}	90–100	Raters et al. (2014)
3	HPLC/UV-FLD	0.1 μg kg^{-1}	0.3 μg kg^{-1}	88–91–97	Danyi et al. (2009)
4	GC-MS/MS	0.002 μg kg^{-1}	0.006 μg kg^{-1}	92	Rozentale et al. (2017)
5	GC-HRMS	0.006 μg kg^{-1}	0.021 μg kg^{-1}	108	
6	Ultra-HPLC	0.08 μg kg^{-1}	0.25 μg kg^{-1}	89.77	da Silva et al. (2017)
7	HPLC-FLD	0.18 μg kg^{-1}	0.25 μg kg^{-1}	108.65	Zachara et al. (2017)
8	GC-MS/MS	0.011 μg kg^{-1}	0.037 μg kg^{-1}	57	Veyrand et al. (2007)
9	GC-MS	0.1 μg kg^{-1}	0.5 μg kg^{-1}	88–100	Simon et al. (2006)
10	Isotope dilution GC/MS	0.02 μg kg^{-1}	0.03 μg kg^{-1}		Kacmaz et al. (2016)
11	Excitation–emission spectroscopy coupled to U-PLS/RBL	0.29 ng L^{-1}		72	Vásquez et al. (2013)
12	Excitation–emission spectroscopy coupled to U-PLS/RBL	5.5 ng L^{-1}			Bortolato et al. (2008)
13	MSPE-GC-MS method	0.37 ng g^{-1}	1.2 ng g^{-1}	108.8 ± 3.4	Zhao et al. (2011)
14	HPLC	0.3 ng g^{-1}	0.9 ng g^{-1}		Kumari et al. (2012)
15	Redox-labeled electrochemical immunoassay	2.4 ng mL^{-1}			Wei et al. (2009)
16	Monoclonal antibody-based ELISA	24 ng L^{-1}		IC_{50} 65 ng L^{-1}	Matschulat et al. (2005)
17	Protein microarrays using DNA/dye conjugate	24.5 μg L^{-1}		IC_{50} 37.2 μg L^{-1}	Fan et al. (2012)

spectral characteristics of flow cytometry have discriminated the planktonic cells that are with and without PAHs (Cerezo et al. 2017). The intensity generated as a result of interaction between an aromatic pollutant and macromolecules (nucleic acids/protein/lipids) is recorded by extending the molecular techniques such as polymerase chain reaction (PCR), probe designing, and microarray. These techniques furnish precise and rapid recognition of aromatic hydrocarbon with a lower limit of detection. The real-time fluorescence quantitative immuno-PCR (RTFQIPCR) is applied to detect trace amounts of anthracene in the environment using a molecular beacon as a probe (Ye et al. 2009). Further, engineering and designing of highly specific primers for PCR and microarray are targeted for the selective screening of genomic sequences. The identified potent genes for the degradation of PAHs are enumerated as *nahA3*, *nahAc*, *nagAc*, *ndoB*, *ndoC2*, *pahA3*, *pahAc*, *phnAc*, *phnA1*, *bphA1*, *bphAc*, *dntAc*, and *arhA1* in Gram-negative bacteria and *narAa*, *nidA/pdoA1*, *phdA/pdoA2*, and *nidA3/fadA1* in Gram-positive bacteria (Chen et al. 2015). Moreover, the specificity of primers restricts the range of PAH degraders and permits to isolate ideal PAH bacterial taxa. 16S ribosomal RNA (rRNA) gene sequencing based on PCR amplification has been exploited to examine the microbial community. Therefore, the biodegradation potential of a PAH-degrading consortium has been evaluated widely through real-time PCR in contaminated environments (Cébron et al. 2008). PCR-based detection of the PAH degrader, *Mycobacterium*, has been studied to correlate the rate of mineralization with the mycobacterial cell concentration in the soil slurry (Wang et al. 1995). Rhee et al. have developed comprehensive 50-mer oligonucleotide microarrays using 1657 probes from all 2402 known genes that are involved in biodegradation and metal resistance (Rhee et al. 2004). Similarly, antibodies against PAHs might become a promising tool for PAH screening. Antibody-based detection of signature molecules, which represent biomarkers, has been carried out via immunochemical assay showing excellent reproducibility at the parts-per-billion level (Alarie et al. 1990). Recently, multiple immunoassay-dependent PAH detection tools are invented and are distinct such as enzyme-linked immunosorbent assay (ELISA), fluorescence, quartz crystal microbalance (QCM), electrochemical immunoassay (ECIA), and radioimmunoassay (RIA) (Wei et al. 2009). The US EPA has executed an immunoassay test kit (PAH RaPID assay) for the determination of total PAHs in soil (Spier et al. 2011). In this review, a comprehensive description of analytical and biological techniques for the detection of PAHs has been discussed. But we cannot compare the data obtained from chromatographic and biological assays because of the fundamental differences in methods. Chromatography-based assays are dependent on

chemical or physicochemical properties of the molecule, while biological assays are based on the affinity of antibody–antigen interaction. Despite that, GC and HPLC are widely accepted and validated methods by legislation while spectroscopy and biological techniques are still exploited for research purpose that is further endorsed by GC and HPLC. Therefore, the data generated from GC and HPLC are trusted for both legislation and research levels.

Non-biological techniques for the detection of PAHs

Spectroscopic identification

The qualitative aspects of spectroscopy are based on the distribution of energy possessed by a molecule at any given moment. PAHs constitute a class of conjugated π electrons which is a fundamental prospect for the molecular-level characterization of compounds which have similar molecular weights but different chemical structures.

IR spectroscopy

The qualitative aspect of infrared spectroscopy is to determine the functional groups of molecules in the sample. Structure–spectral relationships of IR radiation between 4000 and 400 cm^{-1} signify unique functional groups of an associated molecule at characteristic frequencies. Fundamental principles of FTIR are based on the molecular vibrations possessed by a molecule at any given moment. It could easily differentiate saturated (C–C), unsaturated (C=C), and aromatic hydrocarbons (C–C=C) along with asymmetric (2924 cm^{-1}) and symmetric (2853 cm^{-1}) stretch vibrations of the CH_2 group. Most of the aromatic hydrocarbon consists of C–H stretching absorption which represents overlapping band in the the 3100 to 2700 cm^{-1} region. In order to observe absorption peak, molecules must be active in the IR region and one of the vibrational motions must alter the net dipole moment of the molecule (Hsu 1997; Cann and Spikes 2005). The sensitivity of IR spectroscopy for various PAHs is based on empirical correlations and out-of-plane C–H bending vibrations. The intensity of IR signals could be improved by simulation of the IR spectra with density functional theory (DFT) calculations that are highly dependent on the delocalization of π-conjugated C–H and polarization of vibrational displacements (Tommasini et al. 2016). Thus, infrared spectroscopy is one of the most widely used spectroscopic techniques for PAH analysis. The molecular structure might be predicted by examining the changes in vibrational and rotational spectra of PAHs. Spectra of solid PAHs consistently display νC–H stretching overtone features near ~ 880, ~ 1145, and ~ 1687 nm which are shifted slightly for some heterocycles and different side groups (Izawa et al. 2014). In the recent years, the mass spectrometric techniques have been employed to determine the hydrocarbon classes and their derivatives during petroleum fractions (Hsu 2003). Fourier transform infrared spectroscopy/mass spectrometry (FTIR/MS) and Fourier transform ion cyclotron resonance (FT-ICR) spectrometer based on mass analyzer have played a vital role in the identification and characterization of polyaromatic and heteroaromatic hydrocarbons (Pakarinen et al. 2007; Negi and Kumar 2012). A high-resolution mass spectrometry (HR-MS) technique has been employed to characterize the petroleum fractions into 33 hydrocarbon classes (HC33), comprising 5 classes of saturates, 13 classes of aromatics, and 15 classes of sulfur aromatics ranging from diesel to vacuum gas oil (VGO). Further, the correlation of sulfur-containing compounds with HC33 has been validated through wavelength-dispersive X-ray fluorescence (WDXRF) (Sugumaran et al. 2015). The sensing-dependent spectroscopy has been used in molecular selectivity and deployed in the field of geochemical mapping to monitor the concentration profile of a number of hydrocarbons. Therefore, sensor-based attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) has been exploited for determining the concentration of volatile organic compounds (VOCs) and PAHs in water. Sensors accurately measure the concentration of benzene, toluene, ethylbenzene, xylenes, and naphthalene (BTEXN) in an oil–water mixture. A miniaturized prototype of an ATR-IR sensor system could easily discriminate meta- and para-xylene (Fig. 1a) showing advantages over conventional analytical techniques (Pejcic et al. 2013). The efficacy of mid-infrared spectroscopy as powerful tool has been examined in discriminating structurally related aromatic hydrocarbons, which is further quantified at the parts-per-billion level by thermal desorption (TD)-FTIR hollow waveguide (HWG). This technique is valid compared to GC-FID and could be employed in real field monitoring of environmental air samples (Young et al. 2011).

Raman spectroscopy

The characteristics of Raman spectroscopy corresponding to the vibrational mode are computed by density function theory. The use of Raman spectroscopy has opened the possibility for the identification and quantitative examination of molecules without the need for chromatographic separation. It is limited by its low sensitivity as compared to fluorescence techniques. Therefore, recent developments and enhancement factors such as SERS are recognized as important analytical surface techniques for the characterization of adsorbates and make it possible to observe the Raman spectrum of even a single molecule (Costa et al. 2006). SERS offered us an effective technique for the detection of PAHs showing five and seven distinctive bands for anthracene and pyrene (Fig. 1b). The detection limits of measured SERS spectra were of 8 and 40 nM for

anthracene and pyrene, respectively. Trace detection of PAHs by SERS on a metal sandwich substrate was bridged by 1,10-decanedithiolis (Gu et al. 2013). Chen et al. (2015) have rapidly identified seven PAH compounds using Raman and SERS assays and compared them to the experimentally acquired spectra of DFT. Thus, the Raman spectra of selected PAHs help in understanding the electronic and vibrational structures of the π conjugate that describes π-π* electronic excitations. SERS nanosensors were developed by using silver nanoparticles (Ag NPs) functionalized with viologen dications (VGDs) which can be used for the specific detection of PAHs. The sensing ability of VGD-NP systems depends on VGDs to form intermolecular cavities at interparticle junctions which leads to a massive intensification of the Raman emission of the target molecule (Guerrini et al. 2009).

Fluorescence spectroscopy

Fluorescence is a radiative phenomenon which generates characteristic spectra for each PAH at a particular wavelength in which excitation wavelengths are ranged from 245 to 400 nm and emission wavelengths from 280 to 550 nm (Christensen et al. 2005). Moreover, fluorescence emissions strongly depend on the concentration of fluorophore and quencher along with the physical characteristics of the compounds (Steffens et al. 2011). The efficiency of the fluorescence process is measured in terms of quantum yield, quenching rate constants, energy transfer, lasing ability, and radiative and non-radiative rate constants, from which the whole photophysical behavior can be deduced (Fery-Forgues and Lavabre 1999). Therefore, wavelengths of maximum absorbance were set as the excitation wavelengths (Rivera-Figueroa et al. 2004). The emission of light obtained during fluorescence corresponds to the relaxation of the molecule from the excited state to the ground state under the same spin orientation. Thus, the excitation–emission spectra provide both qualitative and quantitative information during analysis. Fluorescence spectroscopy is exploited owing to its better optical detection, high sensitivity, and proficient diagnostic potential. The fluorescence spectra of PAH mixture having a concentration range from 0 to 8 μg L^{-1} has been determined using fluorescent quencher as a humic acid by means of four-way parallel factor analysis (PARAFAC). The excitation–emission matrices of phenanthrene, pyrene, anthracene, and fluorine at varying concentrations of fluorescent quencher are shown in Fig. 1c (Yang et al. 2016). Photoluminescent graphene quantum dots (PL-GQDs) of pyrene, benzo[a]pyrene, or naphtho[2,3–1]pyrene precursor are reported based on the carbonization with strong acid followed by hydrothermal reduction with hydrazine hydrate. The fluorescence of GQDs showed a sensitive and selective quenching effect to Fe^{3+} with a detection limit of 5×10^{-9} M. The tunable and low cytotoxicity effect of GQDs could be used as fluorescent probes for cellular imaging (Zhou et al. 2013). CdTe quantum dots (QDs) prepared on TiO_2 nanotube sensor (TiO_2 NTs) were applied for the quick detection of PAHs based on fluorescence resonance energy transfer (FRET) in which CdTe QDs act as donors and PAHs as receptors. FRET has enhanced the sensitivity of BaP by about two orders with respect to the direct fluorescent spectrometry with a detection limit of 15 pM, set by the US Environment Protection Agency (Yang et al. 2010). Fluorescence synchronous acquisition based on the 0–0 transition energy has recorded multiple signals that are dependent on the number of rings of the PAHs. Emission signals of fluorimetry validated through the comparison with the data obtained by gas chromatography (GC) led to the conclusion that fluorimetric analysis can be a new and cost-effective approach for the analysis of PAHs (Tarpani et al. 2011). The high concentrations of PAHs, as a result of the hurricane Sandy (October 29, 2012), have been detected in raw fish oil (*Brevoortia tyrannus*) using excitation–emission matrix (EEM) spectroscopy (Bentivegna et al. 2016). The measurement of excitation–emission fluorescence matrices of the analytes enhances the sensitivity (limits of detection below part-per-billion levels) and selectivity and reduces the determination time by about 4 min per sample (Carabajal et al. 2017). The simultaneous determination of benzo[a]pyrene and dibenz[a,h]anthracene by chemometric-assisted excitation–emission fluorescence spectroscopy on nylon membranes has been developed to detect at the parts-per-trillion levels. The value of LODs and sensitivity for BaP were quantified to be 5.5 and 0.3 ng L^{-1} by applying PARAFAC and unfolded partial least squares coupled to residual bilinearization (U-PLS/RBL). The excitation–emission fluorescence spectroscopy enhances the power of sensitivity and selectivity of BaP by about six times faster than HPLC (Bortolato et al. 2008). The U-PLS/RBL-predicted concentrations were compared; furthermore, the LODs obtained using EEFMs on nylon membranes coupled with U-PLS/RBL are comparable to the values obtained using HPLC-FLD. The limit of detection was ranged from 0.29 to 1.0 μg kg^{-1} with recoveries between 64 and 78%. The predicted U-PLS/RBL could be a suitable alternative to the chromatographic method (Vásquez et al. 2013). Thus, the excitation–emission fluorescence using PARAFAC analysis has been employed to develop a fingerprinting approach for the classification and matching of oil samples. This method was complementary to GC-FID for the initial screening of oil samples (Christensen et al. 2005). The detection of hydroxy-polycyclic aromatic hydrocarbons using *N*-doped carbon dots as a new matrix by negative-ion matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) exhibited higher sensitivity and excellent reproducibility owing to its abundant π-conjugated system.

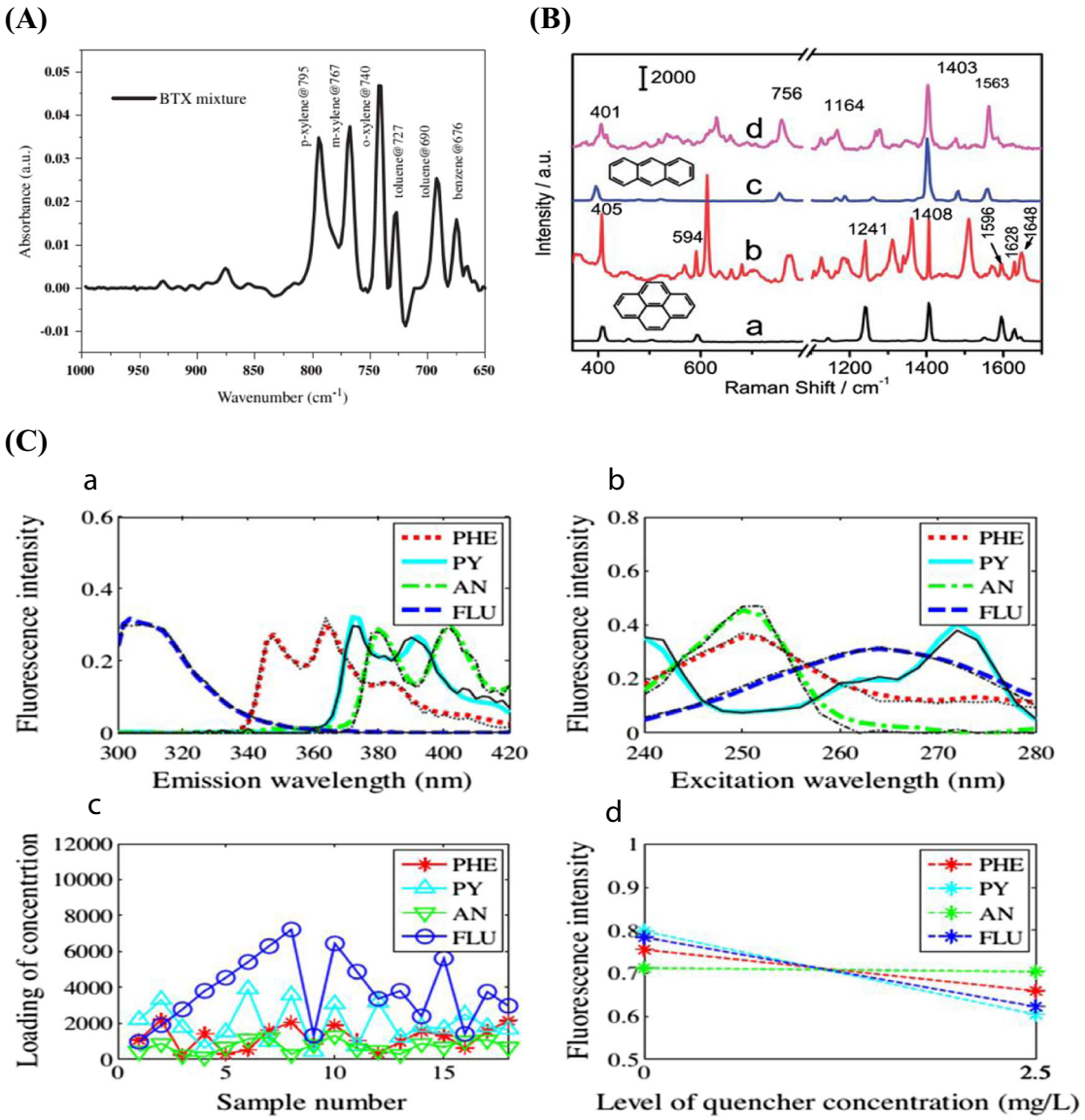

Fig. 1 **a** IR absorption signal obtained at planar silver halide waveguide sensor of a BTX sample mixture in aqueous solution after enrichment with a thin layer of ethylene–propylene copolymer (E/P-co) coat achieving higher sensitivity compared to ATR-IR sensor consisting of a zinc selenide (ZnSe) waveguide with the surface modified by a thin poly(isobutylene) (PIB) coating (Pejcic et al. 2013). **b** Normal Raman spectra of pyrene (a) and anthracene (c) and surface-enhanced Raman scattering (SERS) spectra of 0.1 mM pyrene (b) and anthracene (d) residing in the sandwich structure (Gu et al. 2013). **c** The four profiles of four-way PARAFAC decomposition: emission profile (a), excitation profile (b), sample profile (c), and fluorescence quantum yield (d). All the black lines represent the reference spectra and colored lines the resolved spectra. A visual comparison between the estimated and their corresponding reference spectra in the emission and excitation modes is well-matched (Yang et al. 2016)

The mass concentrations of Σ-hydroxy-pyrene, Σ-dihydroxy-anthraquinone, and Σ-dihydroxy-benzo(a)pyrene were ranged from 0.125 to 0.136, 0.039 to 0.052, and 0.053 to 0.072 ng m^{-3}, respectively (Lu et al. 2016).

Chromatographic identification

Recent advances in chromatography and mass spectrometry have given invaluable compositional and quantification information. Various chromatographic techniques such as HPLC, GC, and LC-MS coupled with multiple detectors such as electron impact ionization (EI), field ionization (FI), field desorption (FD), flame ionization detector (FID), thermal conductivity detector (TCD), electron capture detector (ECD), mass selective detector (MSD), and flame photometric detector (FPD) offer very low detection limits for specific compounds (Cheng et al. 2012). Chromatography techniques have been widely exploited by researchers in order to detect and quantify the parts-per-million or sub-parts-per-million level of aromatic compounds.

Liquid chromatography

The separations of non-volatile and trace polar compounds are typically achieved by liquid chromatography. In this procedure, separation takes place only up to a certain extent because of the limited plate length. The separation of an analyte is highly affected by the humidity and temperature. Moreover, liquid chromatography (LC) does not provide complete information about each PAH and alkyl-substituted PAH as GC and HPLC. LC extended with fluorescence or MS detector furnishes an efficient method for the determination of individual PAHs in complex mixtures. The uptake of pyrene, benzo[a]pyrene, and benzo[a]anthracene from cigarette smoke corresponding to 1-hydroxypyrene (1-OHP), 3-hydroxybenzo[a]pyrene (3-OHBaP), and 3-hydroxybenz[a]anthracene (3-OHBaA) in human urine, respectively, was detected by exploring liquid chromatographic tandem mass spectrometry method (LC-MS/MS) depicting good precision, accuracy, and notably high sensitivity. Thus, the detection of three monohydroxylated polycyclic aromatic hydrocarbons, even in low concentration, in human urine has become possible by the use of liquid chromatographic tandem mass spectrometry (Zhang et al. 2015). The validation of online LC with two different detector fluorescence (FL) and UV–diode array (DAD) has been employed for the quantification and identification of PAHs with detection limits in the range of 0.1–1 mg kg^{-1} of dried sludge. Fluorescence detector and UV-DAD has been exploited for the quantification and confirmation of PAHs, respectively, generating characteristic and distinguished spectra for each PAH (Miège et al. 1998).

Gas chromatography

Gas chromatography is a quantitative technique usually employed for the identification of volatile and non-polar compounds in which gases are used as mobile phase instead of liquid. The uses of a gas as mobile phase bring rapid equilibrium with stationary phases offering fast analysis in a short time with high precision. Therefore, during injection, samples should be thermally stable and should not be degraded during thermal conductivity. Thus, we can conclude that GC analysis is restricted to only volatile samples that must be thermally stable. Multi-dimensional gas chromatography mass spectrometry (GCxGC-qMS) in combination with solid-phase microextraction (SPME) was applied to isolate and to distinguish the isomers of the same molecular mass compounds (Lamani et al. 2015). The ultrasound-assisted emulsification microextraction (USAEME) method was applied for the extraction of PAHs before and after spiking in seawater followed by gas chromatography (GC-FID) for their determination (Saleh et al. 2009). The two-dimensional GC coupled to FID and sulfur chemiluminescence detector (SCD) has been employed to quantify both polycyclic aromatic hydrocarbons and sulfur-containing polycyclic aromatic hydrocarbons in three commercial gas oils being separated according to the class of carbon number ($C_nH_xS_y$) (Dijkmans et al. 2014). The suitability of microwave-assisted extraction methods attached with headspace solid-phase microextraction (MAE-HS-SPME) followed by GC-MS was validated for biomonitoring assessment and quantification of 16 PAHs in bark and pine needles of two different species (*Pinus pinaster* Ait. and *Pinus pinea* L.). This study proves that plant species are the natural monitors of contaminants (Ratola et al. 2012). The headspace solid-phase microextraction (HSSPME) procedure coupled to GC/MS has shown impressive detection limit ranging from 1.1 to 3.3 ng for the determination of PAHs (Ré et al. 2015). The high-resolution capillary gas chromatography equipped with mass selective detector (HRGC-MS) and LC with time-programmed FD was explored in order to evaluate the quantitation aspects of PAHs. HRGC-MS is preferred compared to LC-FD in the separation and detection of PAHs because of the higher linear range of MS compared to the fluorescence detector and because of practical considerations (Berset et al. 1999). The isotopic dilution-mediated GC-MS has been taken into account for the identification and quantification of the eight PAHs in olive pomace. The limits of detection was under (0.1–0.4 μg kg^{-1}) the acceptable maximum permitted limit of 2 μg kg^{-1}, and the average recovery rates were ranged from 69.0 to 97.5% (Diletti et al. 2005). Furthermore, the occurrence of PAHs in air quantified by GC–MS/MS based on isotope dilution was estimated between 279 and 1243 ng m^{-3}, with an average concentration of 514 ng m^{-3} (Zhao et al. 2015). However, low-resolution GC-MS using ^{13}C-labeled PAHs as internal standards has been employed for formal validation in an inter-laboratory collaborative study (Rose et al. 2007). The value of relative standard deviation (20%), limits of quantification (0.5 ng g^{-1}), and recoveries (81–96%) for fortified corn oil was quantified by GC-MS using three deuterated PAH surrogate standards (Wang and Guo 2010). Thus, GC-MS with isotope dilution might be exploited for tracing and compensating analyte losses during the particular stages of the analytical procedure, which makes the results more accurate, precise, and selective.

High-performance liquid chromatography

HPLC is widely accustomed for both qualitative and quantitative analyses of many substances. Usually, it is based on the principle of refractive index (RI) detector: greater RI difference between the sample and the mobile phase offers higher sensitivity. Sometimes, analytes in a complex mixture overlap their refractive index value to the mobile phase which causes them to be invisible to the detector. High-performance liquid chromatography is commonly employed for the rapid separation and detection of biological molecules, pharmaceuticals compounds, and environmental pollutants. It is suitable for the separation of non-volatile species and enantiomers of

racemic drugs with one or more chiral centers by choosing the proper stationary and mobile phases. Polycyclic aromatic hydrocarbons extracted from spent mushroom compost have been quantified using high-performance liquid chromatography–photodiode array detection (García-Delgado et al. 2013). The concentrations of 15 PAHs possessing two to six rings were determined by using HPLC with florescence detector while the concentrations of two nitrated PAHs, 1-nitropyrene (1-NP) and 6-nitrocrysene (6-NC), were quantified using HPLC coupled with chemiluminescence detector (Nassar et al. 2011). An ultrafast liquid chromatography coupled with fluorescence detector has been optimized for the determination of 15 polycyclic aromatic hydrocarbons using a recent Kinetex-C18 column (250 mm × 4.6 mm) (Goulas et al. 2015). The liquid–liquid extraction (LLE) procedure yields better result than HPLC to a low extent in PAH-containing water samples as compared to solid-phase extraction (SPE) procedure. Upon comparing the performance of the two chromatographic techniques, HPLC-FLU-UV and GC-MS are used to obtain the resolution of all PAHs. The LLE-HPLC-FLD-UV technique is somewhat more sensitive than the GC-MS technique for the determination of PAHs which gives slightly higher recoveries and requires shorter analysis times although both techniques are competent and worthy (Díaz-Moroles et al. 2007). In Dionex Corporation Application note 95 (1994), it was mentioned that the HPLC methods for the determination of PAHs from contaminated soil were analyzed by using UV and fluorescence detector. The naphthalene is retraced through a UV detector whereas benzo(b)fluoranthene, benzo(k)fluoranthene, and benzo(a)pyrene are virtually absent in the UV chromatogram but are easily quantifiable with fluorescence detector. The standard mixture of 16 PAHs was distinguished by using both UV and fluorescent detector. The peaks 1–4 are prominent with UV detector while the remaining 12 PAHs have been observed using fluorescence detector (Fig. 2a). Thereafter, unknown polyaromatic hydrocarbons from contaminated soil are extracted and identified by comparing with standard mixtures (Fig. 2b). Furthermore, all detection limits are matched to those specified in the US EPA Methods 550, 810, and 8310 (Dionex Corporation Application note 95 1994). The maximum limits for occurrence of carcinogenic marker PAHs4 (benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), and BaP) in foodstuffs (cocoa beans and their derived products) have been determined and subsequently validated by an HPLC-FD method. It was observed that none of the cocoa beans and derived products exceeded the maximum value fixed by Regulation (EU) No. 835/2011 of 5 μg kg^{-1} fat for BaP and 35 μg kg^{-1} fat for PAHs4 (Raters and Matissek 2014). The four PAHs in smoked rice were further quantified by HPLC in terms of detection limit (ranged 0.05–0.12 ng mL^{-1}), recoveries (87 to 98%), and repeatability with a relative standard deviation between 3.68 to 7.47% (Mahmoudpour et al. 2017). Moreover, the online coupling of donor acceptor complex chromatography and reversed-phase HPLC with fluorescence detector has been accounted for the determination of PAHs in edible oils. The donor acceptor complex chromatography is based on the principal of π-π interactions in which the stationary phase (DACC column) acts as an electron acceptor that retains the PAHs (electron donors). Thereafter, PAHs are transferred online to the analytical reversed-phase column and identified according to retention time using external calibration (Rose et al. 2007).

Biological methods used for detection of PAHs

Biosensor-mediated detection

Progress in biological science and technology has encouraged on-site and in situ detections of aromatic compounds. Recombinant microbial biosensors are known to be very simple, cheap, and efficient monitoring tools for the detection of various environmental pollutants. The recombinant microbial biosensor was to be engineered by the fusion of nahR (encoding the NahR regulatory protein for naphthalene degradation)::lac Z genes for the monitoring of PAHs. The system containing the nahR::Psal::lacZ fusion genes has shown sensitivity to salicylate and naphthalene in both gas and aqueous phases through color development. β-Galactosidase expressed in the presence of salicylate or naphthalene hydrolyzes chlorophenol red-ß-D-galactopyranoside (CPRG) developing a red color that could be observed with the naked eye and measured at 570 nm (Cho et al. 2014). Similarly, a bacterial whole cell was used as biosensor tool for on-line and in situ detections of aromatic compounds. Promoter-sensing aromatic genes were fused with reporter genes monitoring electrochemical changes at real-time and online as early warning system for environmental hazards (Paitan et al. 2004). The electrochemical biosensors were designed using four different types of DNA (calf thymus single-stranded DNA (ssDNA), calf thymus double-stranded DNA (dsDNA), salmon testis ssDNA, and salmon testis dsDNA) on graphite screen-printed electrodes for benzo(a)anthracene and phenanthrene as model analytes. The increase in DNA guanine oxidation peak during interaction between PAHs and immobilized DNA was demonstrated as positive detection of PAHs**.** The difference detected by the analyte interaction was calculated in terms of the percentage of response variation (*R*%), as the ratio of the guanine peak area after the interaction with the sample and in buffer solution (Fig. 3a). 3.5- and 2.7-fold increases in the *R*% values were measured at 20 and 50 ng mL^{-1}, respectively, using UV-activated BaP (Del Carlo et al. 2008). An antibody-based sensor has been explored for the detection of PAHs having concentrations as low as 0.3 μg L^{-1}, within 10 min of sample acquisition, which was compared with a laboratory-based gas chromatography–mass spectrometry method revealing great promise as a field instrument for the rapid monitoring of PAH pollutant (Spier et al.

(A) **Peaks:**

1.	Naphthalene	10 mg/L	9.	Benzo(a)anthracene	1
2.	Acenaphthylene	20	10.	Chrysene	1
3.	Acenaphthene	10	11.	Benzo(b)fluoranthene	2
4.	Fluorene	2	12.	Benzo(k)fluoranthene	1
5.	Phenanthrene	1	13.	Benzo(a)pyrene	1
6.	Anthracene	1	14.	Dibenzo(a,h)anthracene	2
7.	Fluoranthene	2	15.	Benzo(g,h,i)perylene	2
8.	Pyrene	1	16.	Indeno(1,2,3-cd)pyrene	1

(B) **Peaks:**

1.	Naphthalene
2.	Fluoranthene
3.	Pyrene
4.	Benzo(a)anthracene
5.	Benzo(b)fluoranthene
6.	Benzo(k)fluoranthene
7.	Benzo(a)pyrene

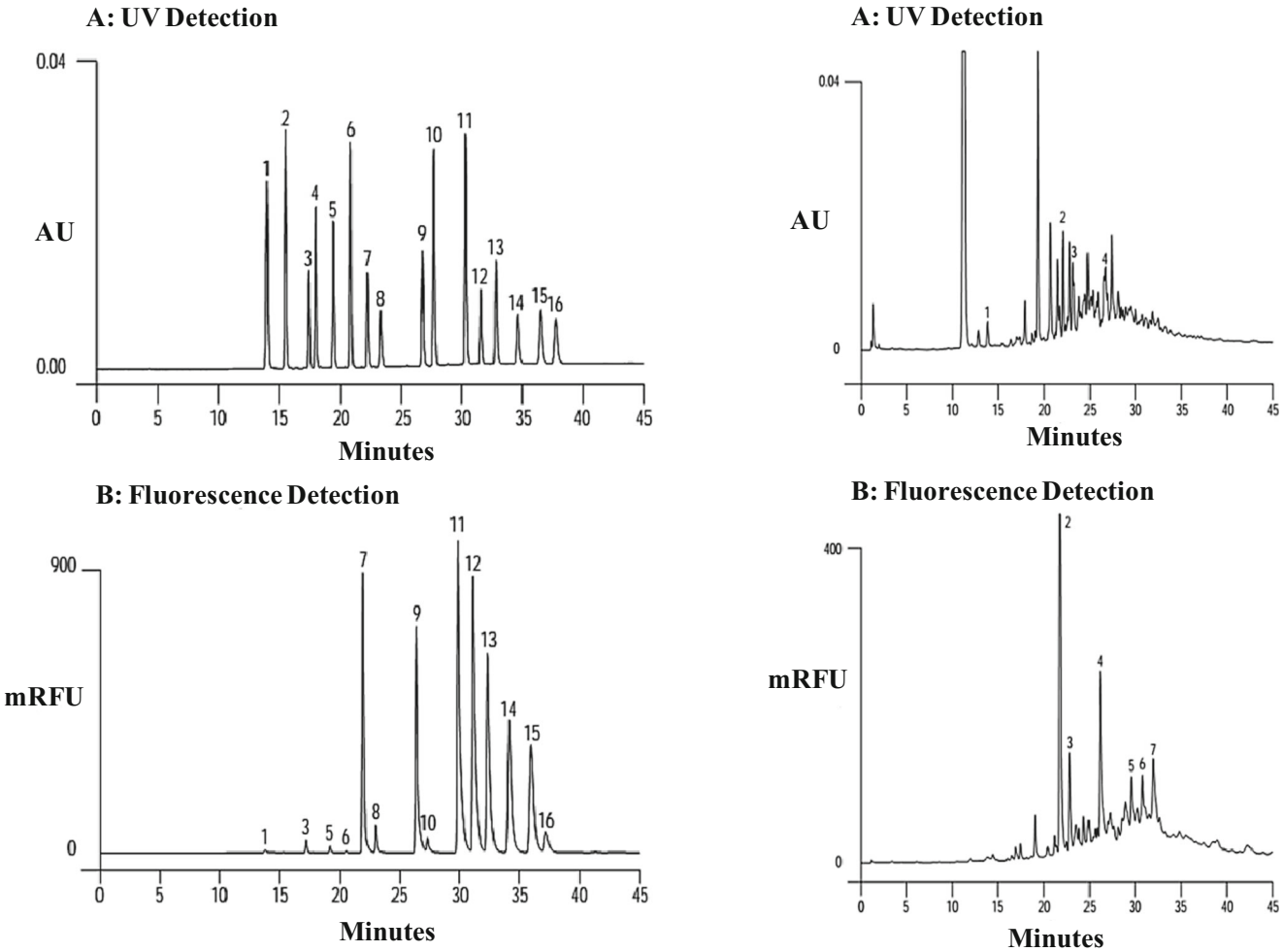

Fig. 2 Gradient separation of **a** standard mixture of 16 PAHs and **b** extract of PAHs-contaminated soil analyzed by reversed-phase HPLC using SUPELCOSIL™ LC-PAHs column (250 mm × 4.6 mm × 5 μm) under both UV (254 nm) and fluorescence (excitation 280 nm) detections (Dionex Corporation Application note 95 1994)

2011). The US EPA has adopted an immunoassay test kit for PAHs which is based on monoclonal anti-PAH antibodies that selectively specify the occurrence of PAHs in soil and water ecosystems. A schematic fundamental representation of the immunoassay is shown in Fig. 3b (Lin et al. 2007). Meimaridou et al. (2010) developed a flow cytometry-based immunoassay (FCIA) using a color-encoded microbead technology to detect BaP using a monoclonal antibody (Mab22F12) against BaP. A highly sensitive assay measured IC_{50} of 0.3 μg L^{-1} using a monoclonal antibody (Mab22F12) against BaP similar to ELISA. The result of FCIA was eight times more sensitive compared to a surface plasmon resonance (SPR)-based

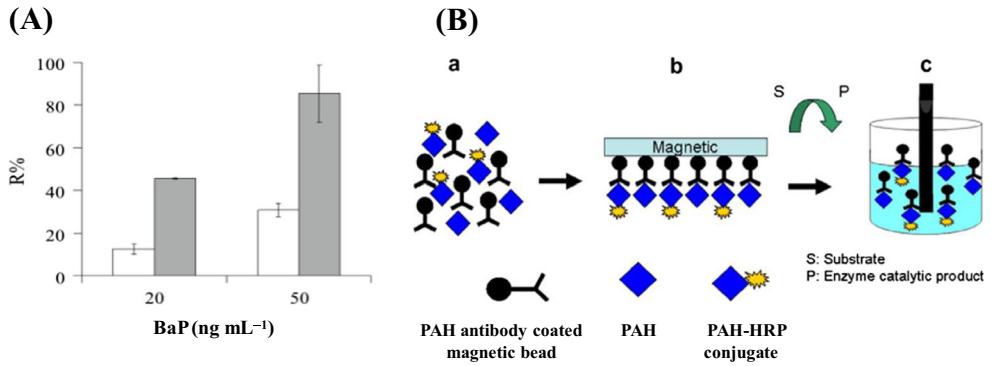

Fig. 3 **a** Effect of the exposure of the electrochemical biosensors to UV-irradiated BaP solutions with respect to non-irradiated solutions. A significant increase of the guanine oxidation peak was observed at both tested concentration levels comparing the *R*% values for UV-exposed BaP (gray bars) and non-exposed BaP (white bars) (Del Carlo et al. 2008). **b** Bioelectrochemical magnetic immunoassay. Liquid-phase competitive immunoreaction among PAH antibody-coated MBs, PAH analyte, and HRP-labeled PAHs (a). Magnetic separation and washing of immunoreaction complex with a magnetic processing system (b). Electrochemical detection (Lin et al. 2007) (c)

biosensor immunoassay (BIA). The FCIAs based on Mab22F12 measured 800 and 96 μg L^{-1} of BaP equivalents in the smoked carp extract and contaminated wheat flour extract, respectively. The selectivity of the FCIAs was also tested with other PAHs, apart from BaP, such as indenol[1,2,3-cd]pyrene (IP), BaA, and CHR. Therefore, the European Food Safety Authority has approved FCIA for the detection of PAH contaminants in food (Meimaridou et al. 2010). The direct competitive electrochemical immunoassay for PAHs has been assembled by using anti-PAH monoclonal antibody immobilized on (3-glycidoxypropyl)-trimethoxysilane (GPTMS)-modified indium tin oxide (ITO) electrodes. Signals were recorded as electro-catalytic current during the interaction between redox-labeled tracer and anti-PAH monoclonal antibody. The detection limits of benzo[a]pyrene (2.4 ng mL^{-1}) and pyrenebutyric acid (10 ng mL^{-1}) were quantified even at the nano level (Wei et al. 2009). Disposable amperometric immuno-sensor based on monoclonal anti-phenanthrene antibodies for the detection of phenanthrene was developed using screen-printed electrodes. The indirect co-exposure competition assay for phenanthrene has reduced the assay time and showed greater sensitivity with a LOD of 0.8 ng mL^{-1} while indirect displacement assay showed a LOD of 2 ng mL^{-1} for phenanthrene, similar to the co-exposure competition assay (Fähnrich et al. 2003). The oxidation of highly reduced aromatic hydrocarbons into a series of hydroxylation derivatives using oxygenase was well-characterized through enzyme-based biosensor. The activity of oxygenase was determined in vivo using BD Oxygen Biosensor™ plates to measure oxygenase-mediated oxygen depletion in the presence of specific aromatic analytes (Tizzard and Lloyd-Jones 2007). A highly sensitive monoclonal antibody-based ELISA has been developed for the detection of benzo[a]pyrene in potable water. Fourteen monoclonal antibodies were generated for immunoassay in mice using novel BaP derivatives. The immunoassay had shown the least interference and the best sensitivity with the purified antibody (clone 22F12) with average IC_{50} of 65 ng L^{-1} for BaP and corresponding detection limit of 24 ngL^{-1} (Matschulat et al. 2005).

Microarray-based estimation

Microarray analysis is a high-throughput screening method simultaneously measuring the expression levels of a large number of genes. The study of genomics via microarray is a powerful technology for observing the expression of thousands of genes simultaneously in a single experiment (Tian et al. 2013). Protein microarray analysis is one of the fastest growing analytical tools which offer rapid screenings of numerous contaminants in environmental matrices. Indirect competitive immunoassays for protein microarrays were developed to achieve higher sensitivity and simultaneous detection of 17β-estradiol (E2), BaP, and 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) in the sample. The IC_{50} and limit of detection (LOD) were calculated 0.32 and 0.022 μg L^{-1} for E2, 37.2 and 24.5 μg L^{-1} for BaP, and 31.6 mg L^{-1} and 2.8 μg L^{-1} for BDE-47, respectively. In this array, a DNA/SYTOX Orange conjugate with antibody labeling was employed to increase the fluorescence signal and sensitivity of the immunoassays. There was no cross-reaction being detected during the immunoassay of these three environmental chemicals. Thus, microarray-based immunoassays could be useful tools for the precise evaluation of numerous environmental pollutants (Fan et al. 2012). The rat livers exposed to PAHs [benzo[a]anthracene (BA), benzo[a]pyrene (BP), phenanthrene (PA) and naphthalene (NT)] were investigated via transcriptome analysis and genome-wide expression profiles. The characteristic of signature molecular for PAHs existing in liver hepatocytes distinguishes different PAHs at an early exposure time. Therefore, gene expression of signature molecules can be used to predict surrogate markers for the detection of biological responses of PAHs (Jung et al. 2013).

The low-density oligonucleotide microarray with 465 probes assessed the impact of benzo[a]pyrene on marine mussels. The potential of the new array was tested on two target tissues, the gills and the digestive gland, of *Mytilus galloprovincialis* on exposing BaP at 5, 50, and 100 μg L^{-1} for 3 days. Transcriptional data were further confirmed by qRT-PCR (Banni et al. 2017). However, Ma et al. (2014) have developed a simple and rapid method to detect the nucleic acid-based PAH probes in which complementary and mismatched sequences of DNA were detected using amplified resonance light-scattering signals. Multiple spectra were generated during groove binding between PAHs and double-stranded DNA. A highly sensitive and robust real-time fluorescence quantitative immuno-PCR (RTFQ-IPCR) using molecular beacon (MB) probe was employed to detect trace anthracene in the environment. Based on its sensitivity and reproducibility, MB-based RTFQ-IPCR was found to be feasible for on-site field tests (Ye et al. 2009). A comprehensive 50-mer-based oligonucleotide microarray has been used for detection of genes from soil microcosms. Genomic DNA extracted from a pure culture of *Thauera aromatica* K172 randomly labeled with Cy5 was used for the detection sensitivity of the 50-mer FGA-based hybridization. Detection sensitivity evaluation in the presence of heterogeneous non-target DNAs involved the mixing of genomic DNA (10 to 1000 ng) from *T. aromatica* K172 and 1-μg MR-1 DNA of *Shewanella oneidensis* showed hybridization signals with 50 ng of *T. aromatica* K172 DNA for the target 2-oxoglutarate ferredoxin-oxidoreductase beta subunit gene (gi19571178) while hybridization signals were barely detectable using 25 ng of genomic DNA (Fig. 4a). Furthermore, *Pseudomonas putida* PpG7 cells were grown using naphthalene as a sole carbon source and RNA detection sensitivity was also examined. The RNA hybridization signal intensity of the gene (gi17863959) labeled with Cy5 using reverse transcriptase was significantly higher than the background signal when the cell number was 1.3×10^7 indicating gene-dependent detection sensitivity (Fig. 4b) (Rhee et al. 2004). The changes in crude oil composition and microbial functional gene responses during ozonation and bioremediation treatments were also examined through microarray-based functional gene analysis (Liang et al. 2009a).

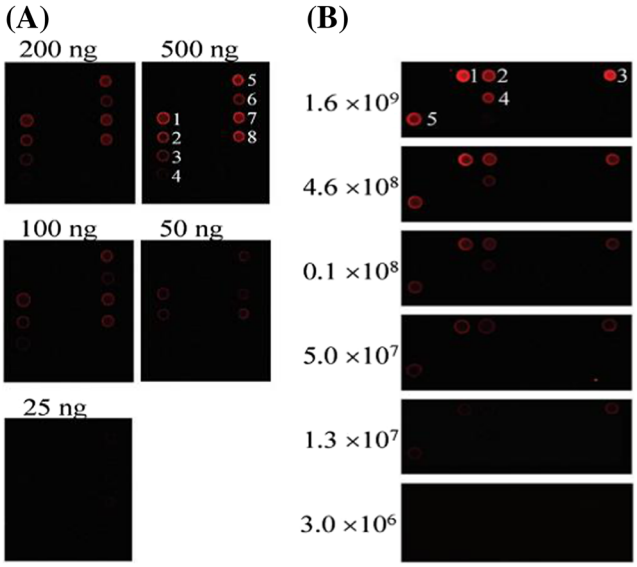

Fig. 4 Detection sensitivity of 50-mer FGA-based hybridization. **a** Fluorescence images showing DNA detection sensitivity with pure genomic DNA. The probes from the following genes indicated succinyl-CoA–benzylsuccinate CoA-transferase (1, gi9622538; 2, gi9622535; 3, gi9622533; 4, gi9622531), benzoyl-CoA reductase (5, gi3724172; 6, gi3724140; 7, gi3724168), and 6-oxocyclohex-1-ene-1-carbonyl-CoA hydratase (8, gi3724166). **b** Fluorescence images showing RNA detection sensitivities with total RNAs from known numbers of cells (on the left). RNA hybridization sensitivity randomly labeled with Cy5 using reverse transcriptase indicate 1, gi2246756 (1,2-dihydroxy-1,2-dihydronaphthalene dehydrogenase); 2, gi22000712 (hydroxymuconicsemialdehyde hydrolase); 3, gi17863959 (naphthalene dioxygenase); 4, gi551908 (salicylate hydroxylase); and 5, gi45703 (catechol 2,3-dioxygenase) (Rhee et al. 2004)

Polymerase chain reaction

The detection and degradation of contaminants in natural environments are critically dependent on the metabolic capabilities of the indigenous microbial communities. To enhance the knowledge of microbial communities, genomic tools have been employed to enrich the efficient microbes. The 16S rRNA gene-based PCR amplification has been extensively exploited to scrutinize microbial community structure that responds under stressful conditions (Liang et al. 2009b). Real-time PCR-based assays have been used to quantify Gram-positive and Gram-negative bacterial populations which are efficient in degrading the polycyclic aromatic hydrocarbons in soil and sediment samples. The specific and sensitive real-time PCR assays have been explored to quantify the environmental DNA samples that were used as bio-indicators for diagnosis of PAH-degrading bacterial population density. The primer of the gene that encodes the alpha subunit of the PAH ring-hydroxylating dioxygenases (PAH-RHDα), involved in the initial step of the aerobic metabolism of PAHs, was designed against the different allele types present in the database common to the Gram-negative and Gram-positive PAH degraders (Cébron et al. 2008). The alpha subunit genes of the initial PAH dioxygenases were used as targets for the PCR detection of PAH-degrading strains. Thus, the PCR analysis of *Pseudomonas* strains showed highly conserved sequences of alpha subunits (nahAc allele) (Moser and Stahl 2001). PCR based on the 16S rRNA genes of *Mycobacterium* sp. PYR-1 and *Mycobacterium* sp. PAH135 was capable of mineralizing [^{14}C]pyrene in contaminated soil (Wang et al. 1995). Differential detection of key enzymes of polyaromatic hydrocarbon-degrading bacteria was identified by PCR and gene probe techniques. The genes for PAH dioxygenase and active catechol 2,3-dioxygenase (C23O) that

catalyze PAH degradation were identified by PCR primers and internal oligonucleotide probes (Meyer et al. 1999). The degenerative oligonucleotide probes are to be used for the detection of highly conserved sequences of functional genes. The gene-encoding 2-naphthoylCoA reductase (Ncr) involved in the dearomatization of naphthalene as model of PAHs was screened out by PCR-based microbial assay (Morris et al. 2014). Thus, the catabolic gene of aliphatic and aromatic hydrocarbons involved in the biodegradation of *n*-alkanes and PAH biodegradation, respectively, was isolated from psychrotrophic bacteria of Arctic soils. Detection of the genes *alkB* and *nahAc/ndoB* in *Pseudomonas* sp. strains BI7 and BI8 was screened by PCR analysis with oligonucleotide primers specific for *alkB* and *nahAc/ndoB* as compared with known bacterial biodegradative pathways (Fig. 5a) (Whyte et al. 1997). The potential degraders of PAHs such as *Cycloclasticus*, *Pseudomonas*, *Pseudoalteromonas*, *Halomonas*, *Marinomonas*, and *Dietzia* were isolated, differentiated, and characterized on the basis of 16S rRNA for PAH degradation which was confirmed using Illumina high-throughput sequencing (IHTS), and the PCR-DGGE profile is shown in Fig. 5b (Dong et al. 2015). Meng et al. (2015) have used real-time immuno-PCR (RT-IPCR) assay based on a biotinylated reporter DNA system, for ultrasensitive detection of pyrene (PYR) and homologous PAHs in water. The linear range of the assay was measured from 500 fmol L^{-1} to 5 nmol L^{-1} with a detection limit of 450 fmol L^{-1}. The contents of PAHs in tap water, lake water, and mineral water were 1.12, 2.40, and 0.38 nmol L^{-1}, respectively (Meng et al. 2015). Thereafter, an improved competitive RT-IPCR based on pyrene-modified DNA for the detection of pyrene and its homologs was developed by Meng et al. (2016). The PAHs in

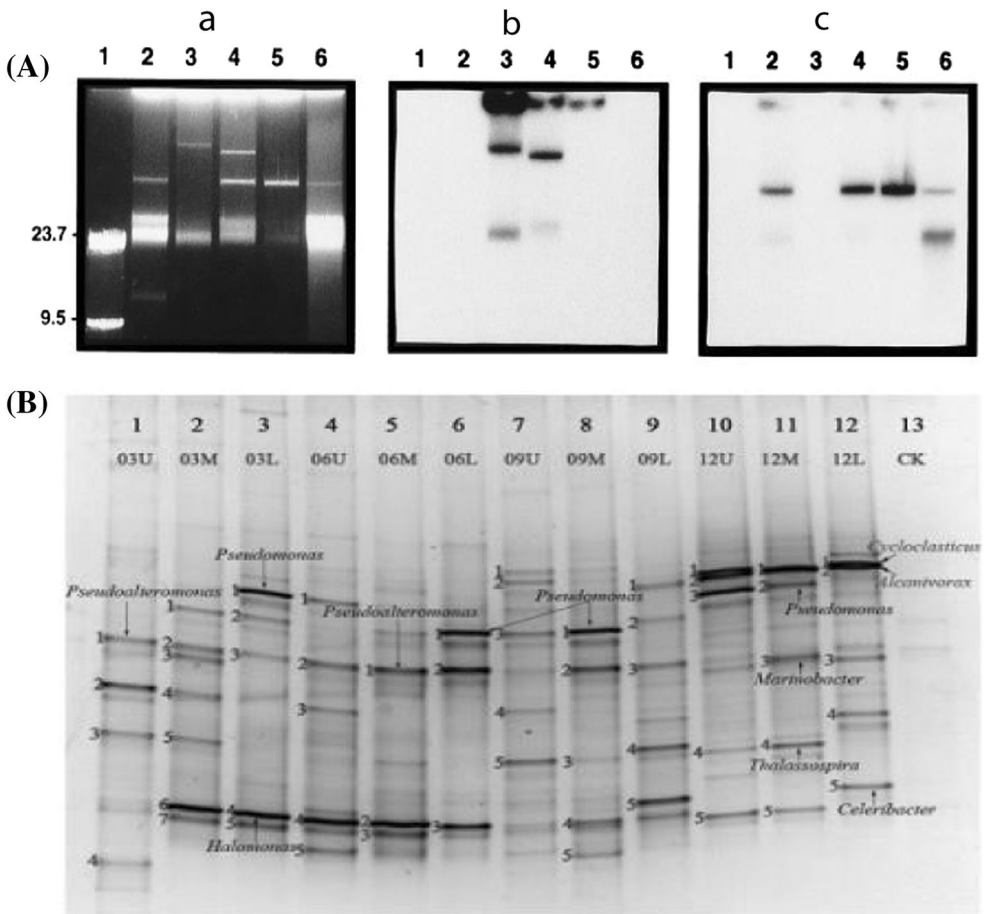

Fig. 5 Plasmid analysis and localization of *alkB* and *nahAc/ndoB* in *Pseudomonas* sp. strains BI7 and BI8 by Southern analysis. **a** Total DNA (chromosomal and plasmid DNAs) from psychrotrophic biodegradative *Pseudomonas* sp. strains BI7, BI8, and BI9 was isolated and examined by gel electrophoresis and Southern analysis for the localization of *alkB* and *nahAc/ndoB*. Agarose gel electrophoresis showing the presence of large plasmids in the psychrotrophic isolates (a). Southern hybridization analysis of chromosomal and plasmid DNAs shown in **a** transferred to a nylon membrane and probed with the 870-bpalkB gene probe derived from *P. oleovorans* ATCC 29347 (b). Southern hybridization analysis of chromosomal and plasmid DNAs shown in **a** transferred to a nylon membrane and probed with the 642-bp*ndoB* gene probe derived from *P. Putida* ATCC 17484 (c). Lanes: 1, lambda (HindIII) ladder; 2, *P. putida* ATCC 17484 (NAH3 plasmid); 3, *P. oleovorans* OCT plasmid; 4, *Pseudomonas* sp. strain BI7; 5, *Pseudomonas* sp. strain BI8; 6, *Pseudomonas* sp. strain BI9 (Whyte et al. 1997). **b** PCR-DGGE profiles of 12 PAH-degrading consortia that were enriched from different sites are shown in different lanes, and the last lane indicates negative control (Dong et al. 2015)

samples compete with the pyrene-modified DNA to bind with the monoclonal antibody (McAb) coated on PCR plate. The use of PCR system for rapid and ultrasensitive detection of pyrene and its homolog assay showed good linear range from 5 pmol L^{-1} to 5 nmol L^{-1} with a detection limit of 3.5 pmol L^{-1} (Meng et al. 2016). Sun et al. (2017) have designed a whole-cell bioreporter using PCR tool that could effectively metabolize naphthalene and evaluate its presence in water and soil samples. The whole-cell bioreporter showed high specificity and sensitivity and positively responded to naphthalene even at 0.01 mM (Sun et al. 2017).

Impact of nanoparticles

Nano-technology is an emerging branch of science, and the use of nanoparticle in analytical and biological systems enhances the sensitivity and detection limit of PAHs to many folds as compared to the traditional methods using bulk materials. The detection limit of analytical methods is hardly reported up to the nanometer level and is unable to determine pico- and femto-level detections while nanoparticles have specific affinity towards various chemicals that increase the sensitivity power and signal amplification of analytical and biological techniques. Therefore, we have focused on exploring the efficacy of nanoparticles as compared to conventional bulk materials used for the processes of detection, extraction, and quantification of PAHs. The selective and efficient removal of PAHs is a major challenge to the industrial sector. The use of nano- and microdimension-based particles in the remediation of PAHs has emerged a new horizon over conventional support materials because of their unique physical and chemical characteristics. The large surface-to-volume ratio, adsorptivity, and the unique optical, electronic, and magnetic properties of each nanoparticle which has eminent sensitivity varying with its surrounding environment are the fundamental ideas for sensing the pollutant (Liu et al. 2011; Acevedo et al. 2010; Wang 2006). The bioavailability of PAHs for microbes is limited due to their low solubilization in the aqueous phase. The use of nanoparticles made from a poly(ethylene) glycol-modified urethane acrylate (PMUA) precursor chain enhanced the bioavailability for phenanthrene. The rate of mineralization of phenanthrene in the presence and absence of PMUA nanoparticles is evident in that the bacteria population utilizes nanoparticles linked to phenanthrene faster than the system without the nanoparticles (Tungittiplakorn et al. 2005). The role of nano clay-immobilized MnP obtained from *Anthracophyllum discolor* was compared with free MnP in order to degrade pyrene (> 86%), anthracene (> 65%), and to a lesser extent fluoranthene (< 15.2%) and phenanthrene (< 8.6%). The immobilized enzyme on nano clay enhanced the enzymatic transformation of anthracene as compared to free MnP which could be a valuable option for in situ bioremediation purposes (Acevedo et al. 2010). Yanasinee Suma et al. examined the enzymatic decomposition of aromatic hydrocarbon intermediates using dioxygenase enzyme immobilized onto single-walled carbon nanotube (SWCNT). To enhance the catalyst efficiency of the enzyme, homogeneous dispersion of the surfactant-activated carbon nanotube has been employed to retain 40% activity after 7 cycles of enzyme reusability (Suma et al. 2016). Hassink et al. (1993) reported that the size of nanopores in soil which was of less than 100-nm diameter has been reduced due to the persistence of PAHs influencing the positive correlation of bacterial biomass and physiology of soil (Hassink et al. 1993; Cornelissen et al. 2005). However, the risk assessment of nanoparticles in biological ecosystem is a challenge to estimate the impact of nanoparticles because of its capability to alter cell membrane properties and intracellular proteins resulting in broad impacts on bacterial carbon cycling. The biodegradation of polycyclic aromatic hydrocarbon by *Mycobacterium* sp. RJGII-135 was inhibited in the presence of silver nanoparticle (AgNP) due to its antibacterial properties (Mueller-Spitz and Crawford 2014). The adsorption of PAHs on silica nanopores was characterized by free surface area, free volume fraction, mean square displacement, and adsorption energy which correlated the adsorption of small PAHs with nanopore. The adsorption value of small PAHs was more sensitive to the changes in pore diameters, while the tie-up of large PAHs with silica pores was more suited to the number of aromatic rings (Fig. 6a) (Sui et al. 2016). The surface characteristics of Phen-loaded and Phen-non-loaded nanoparticles were investigated by means of root elongation (RE) tests (Yang and Watts 2005). The study of the sorption of tricyclic aromatic hydrocarbons with analogous ionization potential on microporous alumino silicate zeolite (HZSM-5 zeolite) allowed only anthracene to pass through void space which illustrates the effect of the size and shape of the sorbate upon the ionization yield. Conversely, phenanthrene and dimethylanthracene were limited due to steric constraints and weak ionization yield (Moissette et al. 2002). Salahi et al. prepared nanoporous membrane using polyacrylonitrile (PAN) polymer with a nominal pore size of 10 nm and surface area of 66 cm^2 for the treatment of petroleum effluent waste water. The results showed that the nanoporous membrane efficiently removed the content of total suspended solids (TSS), total dissolved solids (TDS), oil and grease content, and chemical and biochemical oxygen demands to 100, 44.4, 99.9, 80.3, and 76.9%, respectively (Salahi et al. 2013). The role of nanoporosity and hydrophobicity in sequestration of PAHs was correlated with silica beads (2.5–15 nm), 3-aminopropyl-bonded silica beads (6 nm), diatomite beads (5.4 μm), and polystyrene beads without pores extending the mineralization of phenanthrene. The sequestration and reduced bioavailability occur when hydrophobic compounds enter into nanopores having hydrophobic surfaces (Nam and Alexander 1998). A non-biological method for photocatalytic degradation of pyrene (PYRE), phenanthrene (PHE) and BaP in the presence of nanoparticle (TiO_2) was also reported under UV light (Zhang et al.

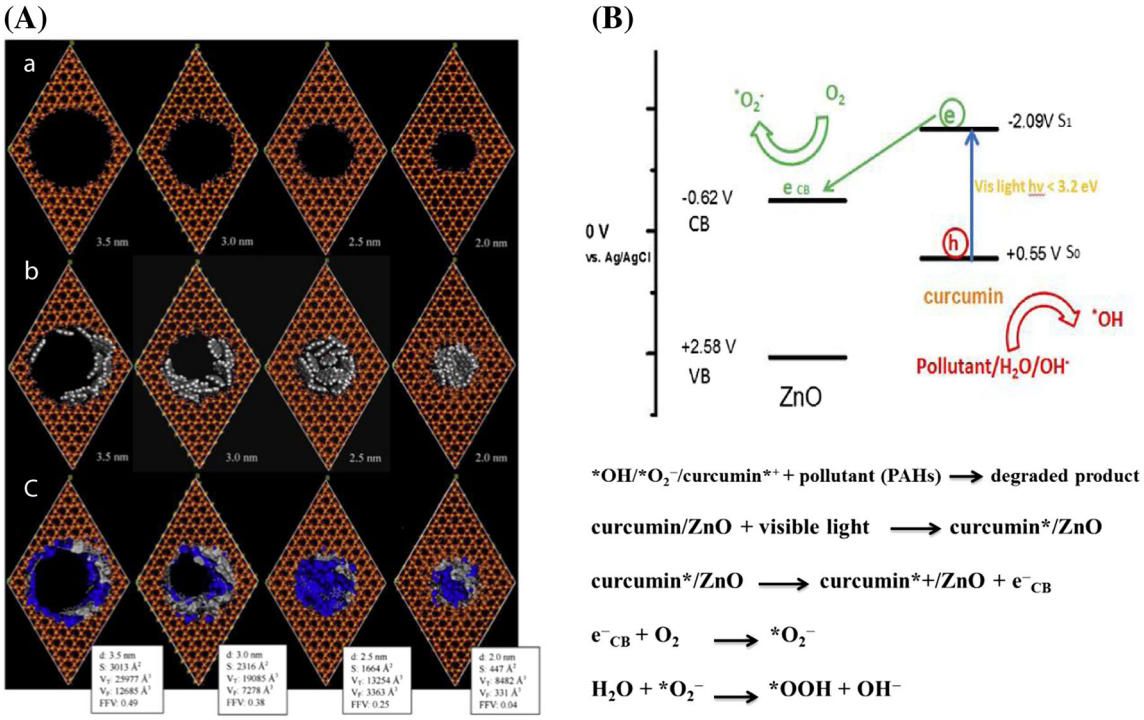

Fig. 6 **a** The geometric distribution of adsorbate molecules largely depended on the atomic arrangement of inner surface in silica nanopores affecting the distribution form of the next layer. PAHs preferred to adhere to the inner wall of the nanopores. When the aperture is small (2 and 2.5 nm), PAHs tended to stack together by rotation and fill to the pore center showing snapshots of the initial silica pores (a), PAHs adsorption in the silica pores (b), and the free volume and surface area after adsorption (c) (*d*, pore diameter; *S*, free surface area; VT, total volume; VF, free volume; FFV, free volume fraction) (Sui et al. 2016). **b** Schematic representation of the electron and hole transfer processes in the ZnO/curcumin systems and the working mechanism (Moussawi et al. 2016)

2008). Photoelectronic and photocatalytic sensitivities of nanoparticles could be tuned by either coupling with catalyst or modifying the surface functional group. The curcumin-conjugated ZnO nanostructures enhanced the removal efficiency to a far extent for perylene, fluoranthene, and chrysene. The removal was found to be faster for chrysene than perylene and extraordinary for fluoranthene (Moussawi and Patra 2016). The photodegradation mechanism and removal efficiency of PAHs are summarized in Fig. 6b. The photoexcitation of the ZnO/curcumin system at $\lambda \geq 445$ nm results in consequent generation of electrons (e) in the conduction band (CB) of curcumin; these excited electrons are transferred to the semiconductor's conduction band of ZnO and further to the molecular oxygen leading to the formation of a series of active oxidizers. The organic contaminant having higher reduction potential is to be oxidized by the excited oxidizer generated by the ZnO/curcumin system (Moussawi and Patra 2016). The magnetic multi-walled carbon nanotubes (mMWCNTs) have been explored for determination of polycyclic aromatic hydrocarbons followed by GC-MS. The values of LODs and LOQs were ranged at 0.10–0.88 and 0.34–2.9 ng g^{-1}, respectively, with 87.8–122.3% recovery. Carbon nanotubes (CNTs) have π-π electrostatic stacking property and electron-rich, hydrophobic nanomaterial with large specific area offering good adsorbent for determination of PAHs (Zhao et al. 2011). The comparative efficacy of non-modified multi-walled carbon nanotubes (MWCNTs) and modified-MWCNTs was evaluated as sorbents for the extraction of PAHs. The results demonstrated that the high extraction capability of modified MWCNTs has recoveries between 83 and 92%. (Paszkiewicz et al. 2017). A synergistic mesoporous silica nanoparticle–sodium deoxycholate (mPS-NaDC), named LPMS1 and LPMS2, with pore sizes of 3.05 and 3.83 nm, was developed for room temperature phosphorimetry. The values of LOD in the presence of LPMS2-NaDC for five PAHs, naphthalene (NAP), PHE, ANT, PYR, and benzo(k)fluoranthene (Bkf), were measured to be 500, 5.0, 2.0, 10, and 15 nM, respectively (Qin et al. 2017).

Conclusion

Each technique has its own advantages and limitation depending upon the interest of outcome. PAHs having a class of conjugated π electron are an elementary principle for the molecular-level characterization. Therefore, the qualitative aspects of spectroscopy are dependent on the distribution of energy inherited by molecules at a particular moment. Spectroscopy techniques determine the functional groups of molecules and differentiate meta- and para-derivatives. Chromatographic techniques are mainly concerned in the separation of molecules. GC-MS is the most frequently used

technique for the detection of PAHs due to the non-ionic and unpolar property of PAHs whereas HPLC-FLD is extensively used for selectivity and sensitivity. Advancement of analytical techniques with various detectors overcomes the operational limitation of instrument. Thus, integrated approaches of all these techniques are to be used for the determination of unknown structures. However, analytical techniques are expensive, sophisticated, and not suitable for on-site field monitoring. Like analytical techniques, biological assays are impotent to discriminate and segregate the molecules having similar molecular weight of different chemical structure along with enantiomers and chiral compounds. However, the biological assay has its own advantages offering higher sensitivity, specificity, and immediate recognition of PAHs in real-time aspect. In most cases, the result yields a good correlation and high precision for the existence of molecules. Screening of PAH genes closely related to PAH-degrading microbes has been distinguished through probe-based microarray and PCR methods. Microarray and PCR are powerful and high-throughput tools used for the isolation and characterization of catabolic genes involved in PAH degradation. The uses of antibodies against PAH recognition showed great promise as a field instrument for the instant and on-site monitoring of PAH contaminant. It could be extended for the detection of a wide spectrum of organic pollutants. For this reason, immunoassays/biosensor has been explored for on-site and real-time assessments of PAH contaminants.

Funding information The author gratefully acknowledges the financial support from the Council for Scientific and Industrial Research (CSIR), New Delhi, in the form of fellowship.

References

Acevedo F, Pizzul L, González ME, Cea M, Gianfreda L, Diez MC (2010) Degradation of polycyclic aromatic hydrocarbons by free and nanoclay-immobilized manganese peroxidase from *Anthracophyllum discolor*. Chemosphere 80(3):271–278

Alarie JP, Bowyer JR, Sepaniak MJ, Hoyt AM, Vo-Dinh T (1990) Fluorescence monitoring of a benzo [a] pyrene metabolite using a regenerable immunochemical-based fiber-optic sensor. Anal Chim Acta 236:237–244

Banni M, Sforzini S, Arlt VM, Barranger A, Dallas LJ, Oliveri C, Readman JW (2017) Assessing the impact of benzo [a] pyrene on marine mussels: application of a novel targeted low density microarray complementing classical biomarker responses. PLoS One 12(6):e0178460

Bentivegna CS, DeFelice CR, Murphy WR (2016) Excitation–emission matrix scan analysis of raw fish oil from coastal New Jersey menhaden collected before and after hurricane Sandy. Mar Pollut Bull 107(2):442–452

Berset JD, Ejem M, Holzer R, Lischer P (1999) Comparison of different drying, extraction and detection techniques for the determination of priority polycyclic aromatic hydrocarbons in background contaminated soil samples. Anal Chim Acta 383(3):263–275

Bortolato SA, Arancibia JA, Escandar GM (2008) Chemometrics-assisted excitation−emission fluorescence spectroscopy on nylon membranes. Simultaneous determination of benzo [a] pyrene and dibenz [a, h] anthracene at parts-per-trillion levels in the presence of the remaining EPA PAH priority pollutants as interferences. Anal Chem 80(21):8276–8286

Cann PM, Spikes HA (2005) In-contact IR spectroscopy of hydrocarbon lubricants. Tribol Lett 19(4):289–297

Carabajal MD, Arancibia JA, Escandar GM (2017) Excitation-emission fluorescence-kinetic data obtained by Fenton degradation. Determination of heavy-polycyclic aromatic hydrocarbons by four-way parallel factor analysis. Talanta 165:52–63

Cébron A, Norini MP, Beguiristain T, Leyval C (2008) Real-time PCR quantification of PAHs-ring hydroxylating dioxygenase (PAHs-RHD α) genes from gram positive and gram negative bacteria in soil and sediment samples. J Microbiol Methods 73(2):148–159

Cerezo MI, Linden M, Agustí S (2017) Flow cytometry detection of planktonic cells with polycyclic aromatic hydrocarbons sorbed to cell surfaces. Mar Pollut Bull 118(1):64–70

Chen J, Huang YW, Zhao Y (2015) Characterization of polycyclic aromatic hydrocarbons using Raman and surface-enhanced Raman spectroscopy. J Raman Spectrosc 46(1):64–69

Cheng CN, Lai JH, Huang MZ, Oung JN, Shiea J (2012) Analysis of polar components in crude oil by ambient mass spectrometry. In crude oil emulsions—composition stability and characterization, InTech

Cho JH, Lee DY, Lim WK, Shin HJ (2014) A recombinant Escherichia Coli biosensor for detecting polycyclic aromatic hydrocarbons in gas and aqueous phases. Prep Biochem Biotechnol 44(8):849–860

Christensen JH, Hansen AB, Mortensen J, Andersen O (2005) Characterization and matching of oil samples using fluorescence spectroscopy and parallel factor analysis. Anal Chem 77(7):2210–2217

Cornelissen G, Gustafsson Ö, Bucheli TD, Jonker MT, Koelmans AA, van Noort PC (2005) Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environ Sci Technol 39(18):6881–6895

Costa JCS, Sant'Ana AC, Corio P, Temperini MLA (2006) Chemical analysis of polycyclic aromatic hydrocarbons by surface-enhanced Raman spectroscopy. Talanta 70(5):1011–1016

da Silva SA, Sampaio GR, da Silva Torres EAF (2017) Optimization and validation of a method using UHPLC-fluorescence for the analysis of polycyclic aromatic hydrocarbons in cold-pressed vegetable oils. Food Chem 221:809–814

Danyi S, Brose F, Brasseur C, Schneider YJ, Larondelle Y, Pussemier L, Scippo ML (2009) Analysis of EU priority polycyclic aromatic hydrocarbons in food supplements using high performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector. Anal Chim Acta 633(2):293–299

Del Carlo M, Di Marcello M, Perugini M, Ponzielli V, Sergi M, Mascini M, Compagnone D (2008) Electrochemical DNA biosensor for polycyclic aromatic hydrocarbon detection. Microchim Acta 163(3–4):163–169

Díaz-Moroles NE, Garza-Ulloa HJ, Castro-Ríos R, Ramírez-Villarreal EG, Barbarín-Castillo JM, Waksman-de Torres N (2007) A comparison of the performance of two chromatographic and three extraction techniques for the analysis of PAHs in sources of drinking water. J Chromatogr Sci 45(2):57–62

Dijkmans T, Van Geem KM, Djokic MR, Marin GB (2014) Combined comprehensive two-dimensional gas chromatography analysis of polyaromatic hydrocarbons/polyaromatic sulfur-containing hydrocarbons (PAHs/PASH) in complex matrices. Ind Eng Chem Res 53(40):15436–15446

Diletti G, Scortichini G, Scarpone R, Gatti G, Torreti L, Migliorati G (2005) Isotope dilution determination of polycyclic aromatic hydrocarbons in olive pomace oil by gas chromatography–mass spectrometry. J Chromatogr A 1062(2):247–254

Dionex Corporation Application note 95 (1994) polycyclic aromatic hydrocarbon determination by reversed-phase high-performance liquid chromatography. Scientific, Thermo

Dong C, Bai X, Sheng H, Jiao L, Zhou H, Shao Z (2015) Distribution of PAHs and the PAHs-degrading bacteria in the deep-sea sediments of the high-latitude Arctic Ocean. Biogeosciences 12(7):2163–2177

European Commission (2002) Opinion of the scientific committee on food on the risks to human health of polycyclic aromatic hydrocarbons in food SCF/CS/CNTM/PAF/29/final,

European Commission. Commission Regulation (EC) No. 836/ 2011 of 19 August 2011 amending regulation (EC) no. 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3- MCPD and benzo(a)pyrene in foodstuffs

Fähnrich KA, Pravda M, Guilbault GG (2003) Disposable amperometric immunosensor for the detection of polycyclic aromatic hydrocarbons (PAHs) using screen-printed electrodes. Biosens Bioelectron 18(1):73–82

Fan Z, Keum YS, Li QX, Shelver WL, Guo LH (2012) Sensitive immunoassay detection of multiple environmental chemicals on protein microarrays using DNA/dye conjugate as a fluorescent label. J Environ Monit 14(5):1345–1352

Fery-Forgues S, Lavabre D (1999) Are fluorescence quantum yields so tricky to measure? A demonstration using familiar stationery products. J Chem Educ 76(9):1260

Forbes MD (Ed.). (2010) Carbon-centered free radicals and radical cations: structure, reactivity, and dynamics (Vol. 2). John Wiley & Sons

Fromberg A, Højgård A, Duedahl-Olesen L (2007) Analysis of polycyclic aromatic hydrocarbons in vegetable oils combining gel permeation chromatography with solid-phase extraction clean-up. Food Addit Contam 24(7):758–767

García-Delgado C, Yunta F, Eymar E (2013) Methodology for polycyclic aromatic hydrocarbons extraction from either fresh or dry spent mushroom compost and quantification by high-performance liquid chromatography–photodiode array detection. Commun Soil Sci Plant Anal 44(1–4):817–825. https://doi.org/10.1080/00103624.2013.749439

Goulas A, Louvel B, Waterlot C (2015) Analytical method for determining polycyclic aromatic hydrocarbon pollutants using ultrafast liquid chromatography with fluorescence detection and the recent column packed with the new 5 μm Kinetex-C18 core-shell particles. Can J Chem 93(5):564–571

Gu X, Tian S, Zhou Q, Adkins J, Gu Z, Li X, Zheng J (2013) SERS detection of polycyclic aromatic hydrocarbons on a bowl-shaped silver cavity substrate. RSC Adv 3(48):25989–25996

Guerrini L, Garcia-Ramos JV, Domingo C, Sanchez-Cortes S (2009) Nanosensors based on viologen functionalized silver nanoparticles: few molecules surface-enhanced Raman spectroscopy detection of polycyclic aromatic hydrocarbons in interparticle hot spots. Anal Chem 81(4):1418–1425

Hassink J, Bouwman LA, Zwart KB, Brussaard L (1993) Relationships between habitable pore space, soil biota and mineralization rates in grassland soils. Soil Biol Biochem 25(1):47–55

Hsu CPS (1997) Infrared spectroscopy. Handbook of instrumental techniques for. Anal Chem 249

Hsu CS (2003) Analytical advances for hydrocarbon research. Springer

Izawa MRM, Applin DM, Norman L, Cloutis EA (2014) Reflectance spectroscopy (350–2500nm) of solid-state polycyclic aromatic hydrocarbons (PAHs). Icarus 237:159–181

Jung KH, Kim JK, Noh JH, Eun JW, Bae HJ, Kim MG, Park WS (2013) Characteristic molecular signature for the early detection and prediction of polycyclic aromatic hydrocarbons in rat liver. Toxicol Lett 216(1):1–8

Kacmaz S, Zelinkova Z, Wenzl T (2016) Rapid and sensitive method for the determination of four EU marker polycyclic aromatic hydrocarbons in cereal-based foods using isotope-dilution GC/MS. Food Addit Contam: Part A 33(4):631–638

Kim D, Kumfer BM, Anastasio C, Kennedy IM, Young TM (2009) Environmental aging of polycyclic aromatic hydrocarbons on soot and its effect on source identification. Chemosphere 76(8):1075–1081

Kumar S, Negi S (2015) Transformation of waste cooking oil into C-18 fatty acids using a novel lipase produced by *Penicillium chrysogenum* through solid state fermentation. 3. Biotech 5(5):847–851

Kumar S, Mathur A, Singh V, Nandy S, Khare SK, Negi S (2012) Bioremediation of waste cooking oil using a novel lipase produced by *Penicillium chrysogenum* SNP5 grown in solid medium containing waste grease. Bioresour Technol 120:300–304

Kumari R, Chaturvedi P, Ansari NG, Murthy RC, Patel DK (2012) Optimization and validation of an extraction method for the analysis of polycyclic aromatic hydrocarbons in chocolate candies. J Food Sci 77(1)

Lamani X, Horst S, Zimmermann T, Schmidt TC (2015) Determination of aromatic amines in human urine using comprehensive multidimensional gas chromatography mass spectrometry (GCxGC-qMS). Anal Bioanal Chem 407(1):241–252

Liang Y, Li G, Van Nostrand JD, He Z, Wu L, Deng Y, Zhou J (2009a) Microarray-based analysis of microbial functional diversity along an oil contamination gradient in oil field. FEMS Microbiol Ecol 70(2):324–333

Liang Y, Van Nostrand JD, Wang J, Zhang X, Zhou J, Li G (2009b) Microarray-based functional gene analysis of soil microbial communities during ozonation and biodegradation of crude oil. Chemosphere 75(2):193–199

Lin YY, Liu G, Wai CM, Lin Y (2007) Magnetic beads-based bioelectrochemical immunoassay of polycyclic aromatic hydrocarbons. Electrochem Commun 9(7):1547–1552

Liu Y, Su G, Zhang B, Jiang G, Yan B (2011) Nanoparticle-based strategies for detection and remediation of environmental pollutants. Analyst 136(5):872–877

Lu W, Li Y, Li R, Shuang S, Dong C, Cai Z (2016) Facile synthesis of N-doped carbon dots as a new matrix for detection of hydroxy-polycyclic aromatic hydrocarbons by negative-ion matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. ACS Appl Mater Interfaces 8(20):12976–12984

Ma Y, Cai C, Yang H, Luo L, Chen X (2014) The amplified resonance light scattering signal detection of DNA hybridization using polycyclic aromatic hydrocarbons as a probe. Spectrosc Lett 47(3):184–191

Mahmoudpour M, Mohtadinia J, Mousavi MM, Ansarin M, Nemati M (2017) Application of the microwave-assisted extraction and dispersive liquid–liquid microextraction for the analysis of PAHs in smoked rice. Food Anal Methods 10(1):277–286

Malawska M, Wiołkomirski B (2001) An analysis of soil and plant (Taraxacum officinale) contamination with heavy metals and polycyclic aromatic hydrocarbons (PAHs) in the area of the railway junction Iława Główna, Poland. Water Air Soil Pollut 127(1):339–349

Matschulat D, Deng A, Niessner R, Knopp D (2005) Development of a highly sensitive monoclonal antibody based ELISA for detection of benzo [a] pyrene in potable water. Analyst 130(7):1078–1086

Meimaridou A, Haasnoot W, Noteboom L, Mintzas D, Pulkrabova J, Hajslová J, Nielen MW (2010) Color encoded microbeads-based flow cytometric immunoassay for polycyclic aromatic hydrocarbons in food. Anal Chim Acta 672(1):9–14

Meng XY, Li YS, Zhou Y, Sun Y, Qiao B, Si CC et al (2016) An improved RT-IPCR for detection of pyrene and related polycyclic aromatic hydrocarbons. Biosens Bioelectron 78:194–199

Meng XY, Li YS, Zhou Y, Zhang YY, Qiao B, Sun Y et al (2015) Real-time immuno-PCR for ultrasensitive detection of pyrene and other homologous PAHs. Biosens Bioelectron 70:42–47

Meyer S, Moser R, Neef A, Stahl U, Kämpfer P (1999) Differential detection of key enzymes of polyaromatic-hydrocarbon-degrading bacteria using PCR and gene probes. Microbiology 145(7):1731–1741

Miège C, Dugay J, Hennion MC (1998) Optimization and validation of solvent and supercritical-fluid extractions for the trace-determination of polycyclic aromatic hydrocarbons in sewage sludges by liquid chromatography coupled to diode-array and fluorescence detection. J Chromatogr A 823(1):219–230

Moissette A, Marquis S, Gener I, Brémard C (2002) Sorption of anthracene, phenanthrene and 9, 10-dimethylanthracene on activated acid HZSM-5 zeolite. Effect of sorbate size on spontaneous ionization yield. Phys Chem Chem Phys 4(22):5690–5696

Morris BE, Gissibl A, Kümmel S, Richnow HH, Boll M (2014) A PCR-based assay for the detection of anaerobic naphthalene degradation. FEMS Microbiol Lett 354(1):55–59

Moser R, Stahl U (2001) Insights into the genetic diversity of initial dioxygenases from PAHs-degrading bacteria. Appl Microbiol Biotechnol 55(5):609–618

Moussawi RN, Patra D (2016) Nanoparticle self-assembled grain like curcumin conjugated ZnO: curcumin conjugation enhances removal of perylene, fluoranthene, and chrysene by ZnO. Sci Rep 6

Mueller-Spitz SR, Crawford KD (2014) Silver nanoparticle inhibition of polycyclic aromatic hydrocarbons degradation by mycobacterium species RJGII-135. Lett Appl Microbiol 58(4):330–337

Nam K, Alexander M (1998) Role of nanoporosity and hydrophobicity in sequestration and bioavailability: tests with model solids. Environ Sci Technol 32(1):71–74

Nassar HF, Tang N, Kameda T, Toriba A, Khoder MI, Hayakawa K (2011) Atmospheric concentrations of polycyclic aromatic hydrocarbons and selected nitrated derivatives in greater Cairo, Egypt. Atmos Environ 45(39):7352–7359

Negi S, Kumar S (2012) Evaluation of techniques used for parameters estimation: an application to bioremediation of grease waste. Appl Biochem Biotechnol 167(6):1613–1621

Paitan Y, Biran I, Shechter N, Biran D, Rishpon J, Ron EZ (2004) Monitoring aromatic hydrocarbons by whole cell electrochemical biosensors. Anal Biochem 335(2):175–183

Pakarinen JM, Teräväinen MJ, Pirskanen A, Wickström K, Vainiotalo P (2007) A positive-ion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry study of Russian and North Sea crude oils and their six distillation fractions. Energy Fuel 21(6):3369–3374

Paszkiewicz M, Caban M, Bielicka-Giełdoń A, Stepnowski P (2017) Optimization of a procedure for the simultaneous extraction of polycyclic aromatic hydrocarbons and metal ions by functionalized and non-functionalized carbon nanotubes as effective sorbents. Talanta 165:405–411

Pejcic B, Boyd L, Myers M, Ross A, Raichlin Y, Katzir A, Mizaikoff B (2013) Direct quantification of aromatic hydrocarbons in geochemical fluids with a mid-infrared attenuated total reflection sensor. Org Geochem 55:63–71

Perera FP, Rauh V, Whyatt RM, Tsai WY, Tang D, Diaz D, Kinney P (2006) Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. Environ Health Perspect:1287–1292

Piskonen R, Itävaara M (2004) Evaluation of chemical pretreatment of contaminated soil for improved PAHs bioremediation. Appl Microbiol Biotechnol 65(5):627–634

Qin J, Li X, Feng F, Pan Q, Bai Y, Zhao J (2017) Room temperature phosphorescence of five PAHs in a synergistic mesoporous silica nanoparticle-deoxycholate substrate. Spectrochim Acta A Mol Biomol Spectrosc 179:233–241

Raters M, Matissek R (2014) Quantitation of polycyclic aromatic hydrocarbons (PAH4) in cocoa and chocolate samples by an HPLC-FD method. J Agric Food Chem 62(44):10666–10671

Ratola N, Herbert P, Alves A (2012) Microwave-assisted headspace solid-phase microextraction to quantify polycyclic aromatic hydrocarbons in pine trees. Anal Bioanal Chem 403(6):1761–1769

Ré N, Kataoka VMF, Cardoso CAL, Alcantara GB, de Souza JBG (2015) Polycyclic aromatic hydrocarbon concentrations in gas and particle phases and source determination in atmospheric samples from a semiurban area of Dourados, Brazil. Arch Environ Contam Toxicol 69(1):69–80

Rein A, Adam IK, Miltner A, Brumme K, Kästner M, Trapp S (2016) Impact of bacterial activity on turnover of insoluble hydrophobic substrates (phenanthrene and pyrene)—model simulations for prediction of bioremediation success. J Hazard Mater 306:105–114

Rhee SK, Liu X, Wu L, Chong SC, Wan X, Zhou J (2004) Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. Appl Environ Microbiol 70(7):4303–4317

Rivera-Figueroa AM, Ramazan KA, Finlayson-Pitts BJ (2004) Fluorescence, absorption, and excitation spectra of polycyclic aromatic hydrocarbons as a tool for quantitative analysis. J Chem Educ 81(2):242

Rose M, White S, Macarthur R, Petch RG, Holland J, Damant AP (2007) Single-laboratory validation of a GC/MS method for the determination of 27 polycyclic aromatic hydrocarbons (PAHs) in oils and fats. Food Addit Contam 24(6):635–651

Rozentale I, Zacs D, Perkons I, Bartkevics V (2017) A comparison of gas chromatography coupled to tandem quadrupole mass spectrometry and high-resolution sector mass spectrometry for sensitive determination of polycyclic aromatic hydrocarbons (PAHs) in cereal products. Food Chem 221:1291–1297

Salahi A, Noshadi I, Badrnezhad R, Kanjilal B, Mohammadi T (2013) Nano-porous membrane process for oily wastewater treatment: optimization using response surface methodology. J Environ Chem Eng 1(3):218–225

Saleh A, Yamini Y, Faraji M, Rezaee M, Ghambarian M (2009) Ultrasound-assisted emulsification microextraction method based on applying low density organic solvents followed by gas chromatography analysis for the determination of polycyclic aromatic hydrocarbons in water samples. J Chromatogr A 1216(39):6673–6679

Simon R, Palme S, Anklam E (2006) Single-laboratory validation of a gas chromatography–mass spectrometry method for quantitation of 15 European priority polycyclic aromatic hydrocarbons in spiked smoke flavourings. J Chromatogr A 1103(2):307–313

Spier CR, Vadas GG, Kaattari SL, Unger MA (2011) Near real-time, on-site, quantitative analysis of PAHs in the aqueous environment using an antibody-based biosensor. Environ Toxicol Chem 30(7):1557–1563

Steffens J, Landulfo E, Courrol LC, Guardani R (2011) Application of fluorescence to the study of crude petroleum. J Fluoresc 21(3):859–864

Sugumaran V, Biswas H, Yadav A, Christopher J, Kagdiyal V, Patel MB, Basu B (2015) Molecular-level characterization of refinery streams by high-resolution mass spectrometry. Energy Fuel 29(5):2940–2950

Sui H, Li L, Zhu X, Chen D, Wu G (2016) Modeling the adsorption of PAHs mixture in silica nanopores by molecular dynamic simulation combined with machine learning. Chemosphere 144:1950–1959

Suma Y, Lim H, Kwean OS, Cho S, Yang J, Kim Y, Kim HS (2016) Enzymatic degradation of aromatic hydrocarbon intermediates using a recombinant dioxygenase immobilized onto surfactant-activated carbon nanotube. Bioresour Technol 210:117–122

Sun, Y., Zhao, X., Zhang, D., Ding, A., Chen, C., Huang, W. E., & Zhang, H. (2017). New naphthalene whole-cell bioreporter for measuring

and assessing naphthalene in polycyclic aromatic hydrocarbons contaminated site. ***Chemosphere***

Tarpani L, Vocci A, Selvaggi R, Pellegrino R, Ruspolini F, Taglieri L, Latterini L (2011) Solid-phase analysis of polycyclic aromatic hydrocarbons by Fluorimetric methods. Appl Spectrosc 65(12):1342–1347

Thomas F, Lorgeoux C, Faure P, Billet D, Cébron A (2016) Isolation and substrate screening of polycyclic aromatic hydrocarbon degrading bacteria from soil with long history of contamination. Int Biodeterior Biodegrad 107:1–9

Tian M, Qu J, Han X, Zhang M, Ding C, Ding J et al (2013) Microarray-based identification of differentially expressed genes in intracellular Brucella abortus within RAW264 7 cells. Plos One 8(8):e67014

Tizzard AC, Lloyd-Jones G (2007) Bacterial oxygenases: in vivo enzyme biosensors for organic pollutants. Biosens Bioelectron 22(11):2400–2407

Tommasini M, Lucotti A, Alfè M, Ciajolo A, Zerbi G (2016) Fingerprints of polycyclic aromatic hydrocarbons (PAHs) in infrared absorption spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc 152:134–148

Tungittiplakorn W, Cohen C, Lion LW (2005) Engineered polymeric nanoparticles for bioremediation of hydrophobic contaminants. Environ Sci Technol 39(5):1354–1358

Vásquez V, Báez ME, Bravo M, Fuentes E (2013) Determination of heavy polycyclic aromatic hydrocarbons of concern in edible oils via excitation–emission fluorescence spectroscopy on nylon membranes coupled to unfolded partial least-squares/residual bilinearization. Anal Bioanal Chem 405(23):7497–7507

Veyrand B, Brosseaud A, Sarcher L, Varlet V, Monteau F, Marchand P, Le Bizec B (2007) Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil samples using gas chromatography coupled to tandem mass spectrometry based on an isotope dilution approach. J Chromatogr A 1149(2):333–344

Wang P (2006) Nanoscale biocatalyst systems. Curr Opin Biotechnol 17(6):574–579

Wang JH, Guo C (2010) Ultrasonication extraction and gel permeation chromatography clean-up for the determination of polycyclic aromatic hydrocarbons in edible oil by an isotope dilution gas chromatography–mass spectrometry. J Chromatogr A 1217(28):4732–4737

Wang RF, Luneau A, Cao WW, Cerniglia CE (1995) PCR detection of polycyclic aromatic hydrocarbon-degrading mycobacteria. Environ Sci Technol 30(1):307–311

Wei MY, Wen SD, Yang XQ, Guo LH (2009) Development of redox-labeled electrochemical immunoassay for polycyclic aromatic hydrocarbons with controlled surface modification and catalytic voltammetric detection. Biosens Bioelectron 24(9):2909–2914

Whyte LG, Bourbonniere L, Greer CW (1997) Biodegradation of petroleum hydrocarbons by psychrotrophic pseudomonas strains possessing both alkane (alk) and naphthalene (nah) catabolic pathways. Appl Environ Microbiol 63(9):3719–3723

Yang L, Watts DJ (2005) Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicol Lett 158(2):122–132

Yang L, Chen B, Luo S, Li J, Liu R, Cai Q (2010) Sensitive detection of polycyclic aromatic hydrocarbons using CdTe quantum dot-modified TiO_2 nanotube array through fluorescence resonance energy transfer. Environ Sci Technol 44(20):7884–7889

Yang R, Zhao N, Xiao X, Yu S, Liu J, Liu W (2016) Determination of polycyclic aromatic hydrocarbons by four-way parallel factor analysis in presence of humic acid. Spectrochim Acta A Mol Biomol Spectrosc 152:384–390

Ye QY, Zhuang HS, Zhou C (2009) Detection of trace anthracene in soil samples with real-time fluorescence quantitative immuno-PCR using a molecular beacon probe. Environ Toxicol Pharmacol 28(3):386–391

Young CR, Menegazzo N, Riley AE, Brons CH, DiSanzo FP, Givens JL, Mizaikoff B (2011) Infrared hollow waveguide sensors for simultaneous gas phase detection of benzene, toluene, and xylenes in field environments. Anal Chem 83(16):6141–6147

Zachara A, Gałkowska D, Juszczak L (2017) Method validation and determination of polycyclic aromatic hydrocarbons in vegetable oils by HPLC-FLD. Food Anal Methods 10(4):1078–1086

Zhang L, Li P, Gong Z, Li X (2008) Photocatalytic degradation of polycyclic aromatic hydrocarbons on soil surfaces using TiO 2 under UV light. J Hazard Mater 158(2):478–484

Zhang X, Hou H, Xiong W, & Hu Q (2015) Development of a method to detect three monohydroxylated polycyclic aromatic hydrocarbons in human urine by liquid chromatographic tandem mass spectrometry J Anal Methods Chem, 2015

Zhao B, Zhang S, Zhou Y, He D, Li Y, Ren M, Fang J (2015) Characterization and quantification of PAH atmospheric pollution from a large petrochemical complex in Guangzhou: GC–MS/MS analysis. Microchem J 119:140–144

Zhao Q, Wei F, Luo YB, Ding J, Xiao N, Feng YQ (2011) Rapid magnetic solid-phase extraction based on magnetic multiwalled carbon nanotubes for the determination of polycyclic aromatic hydrocarbons in edible oils. J Agric Food Chem 59(24):12794–12800

Zhou L, Geng J, Liu B (2013) Graphene quantum dots from polycyclic aromatic hydrocarbon for bioimaging and sensing of Fe^{3+} and hydrogen peroxide. Part Part Syst Charact 30(12):1086–1092