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minireview

Polycyclic Aromatic Hydrocarbons in Environmental Matrices, Milk and Eggs: Occurrence, Legislation and Analytical Challenges[§]

Running title: PAHs in Water, Soil, Milk and Eggs

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SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds consisting of carbon and hydrogen atoms, with more than two fused benzene rings. PAHs are associated with risks to human health, particularly carcinogenesis. One exposure route is the ingestion of contaminated food, which can result from high-temperature preparation and processing (such as grilling, smoking, toasting, frying, and deep-frying), as well as from PAHs present in soil, air, and water due to environmental pollution, including incomplete combustion or pyrolysis of organic matter during industrial processing and motor vehicle exhaust. Deposition of PAHs from the air leads to their entry into soil, which is their main route into the environment – sediments and water. Analytical methods for determining PAHs in environmental samples include extraction, purification, and detection techniques. Sample preparation is a crucial step in the analysis of PAHs, especially in complex food matrices that require efficient extraction and clean-up procedures to isolate PAHs from co-extracted matrix components.

This review provides an overview of the available literature – from sample preparation procedures, extraction and clean-up methods, to instrumental analytical techniques in various matrices, including soil, water (wastewater, drinking water, surface water), and food of animal origin (egg, milk) – thus offering a comprehensive summary in one place. The paper also reviews the occurrence of PAHs in different types of matrices in the context of legislation.

Keywords: polycyclic aromatic hydrocarbons; environmental samples; food of animal origin, analytical methods

INTRODUCTION

The increase in industrial production, economic and technological development, urbanisation, and population growth has caused rapid and significant changes worldwide. Thermal processing, low-quality and incomplete combustion, exhaust fumes from motor vehicles, and space heating serve as primary sources of global pollution, generating highly toxic compounds that are dangerous to human health and ecosystems, most notably polycyclic aromatic hydrocarbons (PAHs) [1]. Chemically, PAHs are hydrophobic organic molecules composed exclusively of carbon and hydrogen atoms arranged in two or more fused benzene rings, exhibiting linear, angular, or clustered molecular geometries [2]. Depending on the number of rings, they are categorised as low molecular mass (LMM) PAHs (with two or three rings) and high molecular mass (HMM) PAHs (with four or more rings).

While modern ecosystems are vulnerable to various chemical stressors, PAHs represent a critical class of ubiquitous and toxic environmental and food contaminants that have been extensively

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studied due to their proven carcinogenic effects [3]. Dietary ingestion serves as the primary pathway of human exposure for non-smoking populations. When once discharged into atmosphere, these pollutants undergo deposition, inevitably accumulating in terrestrial and aquatic compartments, including soil and surface waters. Because of their strongly hydrophobic nature, environmental PAHs preferentially concentrate in the lipid-rich components of agricultural ecosystems. This facilitates their carry-over into the livestock food chain, resulting in bioaccumulation within dairy and poultry matrices. Consequently, food contamination can occur through both upstream environmental pathways during primary production and downstream thermal or industrial food processing techniques

Over the years, numerous reviews [3–9] have been published on the analysis of PAHs in food and environmental samples. However, given the vast number of generic publications, there remains a critical need for more integrated, conceptually focused resource that maps the specific continuity of contamination from environmental sinks into target animal products. To address this gap and establish a precise narrative focus, this review explicitly synthesizes current data on PAH occurrence and regulatory frameworks across interconnected compartments: soil, urban wastewater, surface water, drinking water, and specifically, milk and chicken eggs. The primary objective is to deliver a critical comparative analysis of modern extraction and isolation methodologies, alongside advanced instrumental chromatographic techniques utilized for their trace detection and quantification.

POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

Sixteen specific PAH compounds have been designated by the U.S. Environmental Protection Agency (EPA) as priority pollutants because of their high environmental toxicity [10]. These compounds, comprising naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene (Fig. 1), are mostly lipophilic, poorly soluble in water, unstable, and have a high tendency to adsorb to small organic substances (<2.5 µm) such as ash and soot [4]. Their aqueous solubility decreases with molecular mass and the number of aromatic rings in their structure. While structurally resistant to heat and chemical corrosion, PAHs remain highly sensitive to photo-induced degradation.

To protect public health from dietary and environmental exposure to these carcinogenic compounds, global legislation—most notably within the European Union (EU) -establishes stringent thresholds, traditionally employing benzo[a]pyrene as a primary toxicological marker, frequently supplemented by the sum of four specific compounds (PAH4: benzo[a]pyrene, benz[a]anthracene,

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benzo[b]fluoranthene, and chrysene) [1,11,12]. In the context of dietary exposure, the regulation of PAHs in food is governed by Commission Regulation (EU) 2023/915 [12]. Interestingly, raw milk and chicken eggs do not have specific maximum levels (MLs) under Regulation (EU) 2023/915. Instead, safety compliance for these primary agricultural commodities relies on the “As Low As Reasonably Achievable” (ALARA) principle [1,10]. This regulatory omission at the food level is scientifically justified by strict EU feed regulations, specifically Directive 2002/32/EC on undesirable substances in animal nutrition, which establishes maximum thresholds for contaminants in livestock feed to prevent the carry-over and bioaccumulation of environmental PAHs from contaminated forage or soil into dairy and poultry products [13]. However, strict limits are enforced for highly vulnerable populations, setting a maximum of 1.0 µg/kg for both benzo[a]pyrene and the sum of PAH4 in infant formula and follow-on formula derived from milk.

The prevention of human exposure through environmental vectors is handled with equal stringency. For aquatic systems, Directive (EU) 2020/2184 regulates drinking water by setting a maximum limit of 0.01 µg/L for benzo[a]pyrene and a combined limit of 0.10 µg/L for the sum of four indicative PAHs (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene) [11]. Furthermore, the EU Water Framework Directive (2000/60/EC) and Directive 2013/39/EU [14] classify these compounds, alongside anthracene, fluoranthene, and naphthalene, as priority substances for surface waters. To prevent urban and industrial discharges from compromising these limits, municipal wastewater treatment is tightly controlled under Directive 91/271 [15]. Nationally, such as in Croatia, these requirements are translated into strict waste water emission limit values, restricting concentrations to 0.01 mg/L for anthracene, naphthalene, and fluoranthene, 0.005 mg/L for benzo[a]pyrene, 0.003 mg/L for benzo[b]fluoranthene and benzo[k]fluoranthene, and 0.0002 mg/L for benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene [16].

Finally, since soil serves as the primary environmental sink that facilitates the transfer of PAHs into the agricultural food chain via animal grazing, terrestrial monitoring has received significant legislative updates. The Soil Monitoring and Resilience Directive (known as the Soil Monitoring Law), which officially entered into force on 16 December 2025, mandates member states to establish localized operational trigger values to identify and remediate contaminated sites [17]. Point-source soil contamination is further abated via the REACH Regulation [18]. Regulations prohibit the sale of mulches or granules intended for synthetic turf infill, sports fields, or playground surfaces if the combined concentration of regulated PAHs exceeds 20 mg/kg (0.002 % by mass). On a national level, Croatian legislation complements these efforts by enforcing MRLs for the total sum of PAHs at 1 or 2

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mg/kg of dry matter, depending heavily on physical characteristics, light or skeletal characteristics of the soil matrix [19].

DETERMINATION OF PAHs

Analytical techniques for PAH characterisation in environmental and food samples requires a sequential approach combining robust extraction and high-selectivity detection methodologies. Within this analytical workflow, sample preparation represents the most critical phase, particularly when dealing with complex, fat-containing food matrices. Due to their highly lipophilic and hydrophobic properties, PAHs are systematically co-extracted with major lipid constituents, including bulk triglycerides, sterols, and free fatty acids. If not rigorously removed during a targeted clean-up step, these co-extracted lipids introduce severe chemical noise, cause signal quenching or enhancement during chromatographic separation, and rapidly degrade instrumental hardware. Consequently, modern sample preparation strategies must effectively isolate trace-level PAHs from these overwhelming matrix components, balancing optimal target recovery with exhaustive lipid removal. An overview of the diverse extraction and purification pathways routinely deployed for both environmental and food matrices is systematically categorized and summarized in Fig. 2 [20,21].

Extraction and clean up procedures

Water samples

To accurately measure low-level concentrations in water, samples require pre-treatment with enrichment and clean-up steps. This is typically achieved by liquid-liquid extraction (LLE) [22,23], in which PAHs are transferred from water into immiscible organic phases such as hexane or dichloromethane. For example, EPA Method 610 specifies dichloromethane as the standard solvent for removing these compounds by LLE [24]. However, the critical limitations of LLE -difficult automation, emulsion formation, use of large volumes of hazardous, high-purity solvents, low cost-effectiveness – have driven a shift towards alternative methodologies like solid phase extraction (SPE). SPE effectively overcomes these drawbacks by offering automation, higher reproducibility and reduced solvent consumption. While classical approaches rely heavily on standard C8, C18, and polystyrene/divinylbenzene sorbents [25], recent advancements in materials science have expanded the SPE landscape to include carbon nanotubes, molecularly imprinted polymers and metal-organic frameworks, significantly improving selectivity and enrichment factors for trace environmental analysis. To further adhere to 'green' chemistry standards, current developments focus on the miniaturization of extraction techniques. As a result, solid-phase microextraction (SPME) has become increasingly popular eliminating organic solvents by utilizing a coated fused-silica fiber. However,

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because the amount of sorbent in SPME is very small ($< 0.7 \mu\text{g}$), the absolute mass of extracted analytes remains restricted, which inherently limits its applicability for ultra-trace environmental monitoring. To address this capacity limitation, stir-bar sorptive extraction (SBSE) is another extraction procedure that has shown great potential for extracting PAHs from water [26]. The method is based on immersing a magnetic rod coated with a polymer layer – most often polydimethylsiloxane (PDMS) – directly into a water sample. SBSE successfully integrates sampling and high-capacity pre-concentration into a single solventless operation, making it highly effective for complex aqueous matrices. Notably, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach has also been adapted for screening surface and waste waters [27–29]. While partitioning into acetonitrile followed by d-SPE clean up (using PSA (to remove organic acids), C18 (to remove lipids/lipophilic substances), or GCB (for pigments) is highly effective for rapid high-throughput screening, a critical analytical challenge arises regarding regulatory compliance. For drinking water analysis where legal limits are extremely low (e.g. $0.01 \mu\text{g/L}$ for benzo[a]pyrene), the sensitivity and limit of detection (LOD) of the QuEChERS method is not sufficient [29]; even more sensitive methods such as SBSE or large-volume classical SPE (1 L) are often required to achieve the necessary sensitivity. Alternatively, dispersive liquid-liquid microextraction (DLLME) is a relatively new and very popular method due to its speed and low cost [30,31]. A mixture of extraction solvent (e.g. chloroform) and dispersive solvent (e.g. acetone) is rapidly injected into a water sample. This instantaneous injection forms a highly turbid solution, creating an exceptionally large contact surface area between the fine droplets and the water matrix. Consequently, equilibrium is reached almost instantaneously, allowing the PAH-containing droplets to be rapidly separated via centrifugation. The advantage of DLLME lies in its ability to achieve remarkably high enrichment factors within a few minutes while utilizing minimal solvent volumes.

Soil samples

Analysing PAHs in solid environmental samples, such as soil, requires their prior isolation, typically achieved through thermal or solvent-based extraction [2,32]. Ultrasonic treatment and mechanical agitation (stirring or shaking) are often used for this purpose, but the choice of technique depends on practical considerations, including operational costs, throughput requirements, and matrix complexity. In the late 20th century, Soxhlet extraction was the primary method, using organic solvents such as toluene, methanol, or hexane to separate these compounds. Soxhlet extraction offers maximum efficiency and does not require filtration after extraction, but it is extremely slow, consumes hundreds of millilitres of solvent, and uses a lot of energy. To circumvent these limitations,

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Ultrasonic Extraction (USE) utilizes high-frequency ultrasonic waves that mechanically break the bonds between soil particles and the PAH organic fraction. USE offers the advantage of shorter extraction times and allows parallel processing of multiple samples, although it is less effective for very "old" pollution where PAHs have migrated deep into inaccessible matrix micropores. Under high-pollution scenarios, however, comparative evaluations demonstrate that USE can yield higher recoveries than conventional Soxhlet protocols due to continuous acoustic cavitation [33,34].

Crucially, raw liquid soil extracts obtained via USE or classical shaking contain high concentrations of co-extracted soil constituents, such as humic and fulvic acids, which necessitate exhaustive clean-up prior to chromatographic analysis. SPE serves as the primary tool for this purification stage rather than direct matrix isolation. Beyond standard non-polar alkyl-modified silica (C-18 non-polar phase), modern soil analysis increasingly implements high-capacity polymeric sorbents, such as hydrophilic-lipophilic balanced (HLB) materials, molecularly imprinted polymers (MIPs), and ionic liquid-coated Fe_3O_4 magnetic nanoparticles, which vastly improve matrix cleanup capacities and analyte recoveries [35].

For automated, high-throughput monitoring, Pressurised Liquid Extraction (PLE), also designated as Accelerated Solvent Extraction (ASE) and microwave-assisted extraction (MAE) represent the leading advanced methodologies. As standardized in EPA Method 3545A, PLE operates at elevated temperatures (100–150 °C) and pressures (100–140 bar), which lower solvent viscosity to enhance pore penetration and overcome robust van der Waals interactions between PAHs and the soil organic carbon fraction [36]. This approach achieves up to a 90 % reduction in solvent volume compared to conventional techniques, with automated closed-cell configurations processing over 24 samples unattended, making it exceptionally effective for complex forest soils [37]. Another extraction procedure is Microwave-Assisted Extraction (MAE), in which a mixture of soil and solvent is heated with microwave energy in closed, pressurised vessels, as described in EPA Method 3546 [38]. Precise temperature control ensures high repeatability of results. While both methods are rapid and use elevated temperatures and pressures, the main difference is that ASE automatically filters the extract through a cell, while in MAE the sample remains in the solvent vessel, requiring an additional step of liquid-solid separation.

Lately, the QuEChERS protocol has been adapted as a low-cost, high-throughput alternative for terrestrial monitoring. While standard multi-residue methods rely on partition into acetonitrile assisted by magnesium sulfate and sodium chloride followed by dispersive SPE (d-SPE) cleanup [39,40], recent optimization strategies have substituted acetonitrile with an acetone:n-hexane (1:4

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V/V) binary mixture, yielding excellent recoveries (70.7–115.1 %) and outstanding precision (RSD 3.9 %) [41].

Comparative studies evaluate these extraction efficiencies, demonstrating that combining ultrasonic extraction with a QuEChERS cleanup step significantly enhances the recovery rates for high-molecular-mass (heavy) PAHs in soil, whereas standalone ultrasonic extraction with organic solvents offers a broader recovery across diverse PAH congeners [32]. To support these extraction methodologies within standardized monitoring frameworks, ISO 18287:2006 serves as the primary consensus protocol, defining the parameters for the determination of PAHs in terrestrial matrices using GC-MS [42].

Food of animal origin

Food products, particularly of animal origin, are highly susceptible to PAH contamination primarily through environmental pathways, as these lipophilic pollutants bioaccumulate along the food chain from contaminated air, soil, grass, and animal feed [43]. Due to their strongly hydrophobic nature, PAHs exhibit a high affinity for lipid-rich matrices [44]. Consequently, lactating livestock and poultry ingest contaminated biomass, leading to the direct transfer and accumulation of PAHs into milk and eggs – a critical pathway governed by strict feed regulations to mitigate human dietary exposure [44]. Analysing PAHs in complex food matrices is challenging, as these compounds frequently bind strongly to the matrix – particularly in high-fat products – making extraction difficult [45]. This challenge is particularly pronounced in high-fat dairy products like cream and whole milk, as well as in egg yolks, where the robust removal of fat is imperative.

As PAHs are highly fat-soluble, they are typically co-extracted with lipids, requiring a subsequent clean-up phase to remove interfering fatty acids and triglycerides [9]. Traditional isolation protocols often rely on rigorous alkaline saponification followed by LLE, Soxhlet, or USE. [46,47]. While traditional methods like Soxhlet or USE are effective, they are often labour-intensive and slow. To isolate the target analytes, purification by SPE is commonly used, employing sorbents such as C18, silica, Florisil, or PS-DVB. For example, silica cartridges can retain triglycerides while allowing PAHs to elute with hexane-dichloromethane mixtures [9]. More recently, QuEChERS methodology has emerged as a rapid, high-throughput alternative for food of animal origin, including dairy and egg matrices [48-50]. In these applications, the addition of specialized d-SPE sorbents, specifically C18 or enhanced matrix removal (EMR-lipid) phases to freeze out and retain lipids, is vital to achieve adequate extract purification without losing low-molecular-mass PAHs. When focusing specifically on complex egg matrices, traditional extraction boundaries have been overcome by adopting these

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tailored green analytical platforms. Recent protocols validate that coupling modified QuEChERS extractions with pass-through lipid-removal sorbents dramatically minimizes sample treatment duration from days to hours, ensuring sharp baseline separations. Alternatively, advanced solvent-free configurations, such as cold fiber-solid-phase microextraction (CF-SPME) combined with GC-MS, have proven exceptionally robust for trace-level PAH determination in egg yolks, providing high sensitivity and limits of detection ranging between 0.0002 and 0.046 µg/g [51].

Separation and detection

Following extraction and purification, PAH analytes undergo instrumental quantification [22,44]. Selecting the optimal technique requires a critical evaluation of sensitivity, selectivity, matrix effects, and practical applicability. While high-performance liquid chromatography with fluorescence detection (HPLC-FLD) offers exceptional selectivity for natively fluorescent PAHs and is less susceptible to matrix-induced enhancement effects, gas chromatography-mass spectrometry (GC-MS) provides structural mass-spectral confirmation, which is indispensable for identifying non-fluorescent derivatives and co-eluting isomers.

Gas chromatography (GC) is a widely used technique for identifying PAHs in food samples, primarily because these compounds have thermal stability and sufficient volatility for vaporisation. A critical limitation in standard GC protocols is the reliance on conventional capillary columns, such as 5 %-phenyl)-methylpolysiloxane (HP-5MS) or methyl arylene polymers (DB-5MS). These standard phases frequently fail to achieve baseline separation for critical isomeric pairs, such as chrysene/triphenylene or the benzo[fluoranthene] isomers (b, k, and j), which share identical mass fragmentation patterns under electron ionization (EI). To resolve these co-elution challenges – particularly in complex, high-fat matrices like egg yolks – the application of specialized, high-selectivity PAH-specific GC capillary columns (such as DB-EUPAH or Rxi-PAH) is imperative.

These GC systems generally employ electron ionisation (EI) sources and single quadrupole (Q) analysers, which are most effective when operating in selective single ion monitoring (SIM) mode [52]. While most studies rely on SIM mode, alternative analysers such as the triple quadrupole (QqQ) or ion trap are less frequently used. When these instruments are employed, they typically operate in selected-reaction monitoring or product ion scan modes [22]. High-resolution mass spectrometry (HRMS), such as time-of-flight (TOF), has also been implemented; however, its widespread adoption is limited by high operational costs. Although advanced analysers and tandem MS could offer greater selectivity, the use of single-stage MS and Q-type analysers remains the industry standard, largely due to the inherent structural stability of PAH molecules.

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Liquid chromatography has long been the primary technique for PAH analysis and is included in several official protocols from the US EPA and the International Organization for Standardization [53]. However, standard reversed-phase C18 columns packed with 3–5 μm particles often lack the structural resolution necessary to separate the 16 priority EPA PAHs under standard gradient profiles. Consequently, modern liquid chromatography has shifted toward specialized, polymerically bonded PAH-specific HPLC stationary phases. These phases feature unique, high-density C18 bonding styles that maximize shape selectivity, enabling the precise separation of planar and non-planar PAH stereoisomers. Due to its exceptional selectivity, fluorescence detector (FLD) is most commonly used for analysing food matrices. Other detectors, such as diode-array (DAD), UV-Vis, or fast-scanning fluorescence (FSFD), are also used, but generally serve as auxiliary tools for result verification [22,44]. Specifically, FSFD captures the outflow spectrum from the column and uses high-speed wavelength scanning to generate complex three-dimensional chromatograms. Nevertheless, a practical drawback of HPLC-FLD compared to GC-MS/MS is its inability to detect non-fluorescent compounds (such as cyclopenta[c,d]pyrene) and its lack of mass-spectral confirmation, making a multi-platform approach highly advantageous. To ensure high data comparability and strict compliance with global food safety and environmental legislation, analysis is heavily standardized under official consensus methodologies. The European Committee for Standardization established EN 16619:2015 as the official protocol for the determination of regulated toxicological markers (PAH4) in foodstuffs via GC-MS, while EN 15851:2010 details the determination of benzo[a]pyrene using HPLC-FLD [54]. Similarly, international aquatic monitoring strictly applies ISO 17993:2002 for HPLC-FLD determination of 15 priority PAHs in water matrices, and ISO 28540:2020 for mass-spectrometric confirmation of the 16 US EPA priority pollutants in drinking, surface, and waste waters [55,56].

PAHs OCCURRENCE

Natural phenomena, such as volcanic eruptions and forest or steppe fires, contribute to the presence of PAHs in the environment. However, human activities are the main cause of elevated PAH concentrations in soil. These pollutants are released into the atmosphere through ash and exhaust gases, then disperse and accumulate in both surface water and soil. Once in the environment, PAHs may undergo processes such as chemical oxidation, photolysis, volatilisation, or adsorption. The most ecologically sustainable method for neutralising contaminated sites is microbial transformation [57]. The efficiency of this biodegradation depends on several factors, including the chemical properties of the specific PAH, the type and density of the microbial population, and the availability of nutrients. In

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addition to environmental exposure, humans are also exposed to these compounds through tobacco smoke and the consumption of contaminated food.

Occurrence in environmental matrices

Thermal treatments, such as home cooking or industrial drying and heating, are significant sources of PAH formation in food products. These contaminants are frequently detected in fats, oils, and smoked, grilled, or fried items; high concentrations can also result from the roasting of tea leaves and coffee beans. Once these compounds reach the soil, they tend to adhere strongly to small particles and organic matter, which hinders their degradation and promotes long-term environmental persistence [20]. The distribution of PAHs in the soil matrix is governed by several interacting factors, including temperature, soil composition, the presence of other pollutants, and local microbial activity. Globally, regional monitoring programs reveal profound variations in topsoil PAH burdens, reflecting local industrialization and urbanization intensities. In agricultural soils across China, comprehensive surveys have detected the entire suite of 16 EPA priority PAHs—ranging from highly volatile LMW structures like naphthalene, acenaphthene, fluorene, phenanthrene, and anthracene, to heavy HMW isomers, including fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene, with a combined mean concentration of 388 ng/g [2,58]. In Europe, topsoil screening in southeastern Romania indicated a similar broad-spectrum contamination profile, yielding a mean total PAH concentration of 214 ng/g [58]. In stark contrast, pristine rural forest soils in China's Pearl River Delta exhibited minimal background levels of 35.86 ng/g [51], whereas urban soils in Indianapolis, USA, reached extreme anthropogenically driven peaks of 36,500 ng/g.

Significant regional variations are also well-documented within South-eastern Europe. In Croatia's Istrian region, the Trget site near the port of Bršica was identified as heavily polluted, recording total PAH values of 14.29 µg/g, while further heavy contamination was also observed near the Raša-Štalije railway and the Vlačka power plant [59]. Comparative investigations around the Plomin Thermal Power Plant confirmed the presence of all 16 EPA priority congeners; total concentrations peaked at 13,535 ng/g in the immediate vicinity of the plant and declined monotonically to background levels of 31 ng/g at distances exceeding 10 km [60], illustrating a clear point-source deposition gradient. Parallel urban studies in Serbia highlight these spatial discrepancies: park soils in Kruševac averaged a relatively low burden of 55 µg/kg [61], whereas urban topsoils in Novi Sad exhibited a mean concentration of 363 µg/kg, spanning a wide operational range from 22 to 2247 µg/kg [62]. Notably, while dibenz[a,h]anthracene was entirely absent in the Serbian cohorts [61,62],

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it was consistently quantified at trace levels in Croatian samples, where benz[a]anthracene emerged as the most dominant high-molecular-mass congener [59,60].

Due to increased anthropogenic activities and industrialisation, water pollution with persistent compounds is rising. In aquatic environments, PAHs are mainly introduced through various pathways, including atmospheric transport, rainfall, runoff, and industrial discharge [63]. Comprehensive environmental occurrence data demonstrate that aqueous matrices consistently exhibit a significantly higher proportion of lower-molecular-mass (light) PAHs compared to high-molecular-mass (heavy) congeners [23]. This distribution pattern is fundamentally governed by the higher water solubility and lower octanol-water partition coefficients (K_{ow}) of 2- and 3-ring PAH structures, whereas the highly hydrophobic 4- to 6-ring compounds rapidly partition out of the water column into solid phases such as suspended matter and sediments.

In the surface waters of China's Pearl River Delta, various PAHs were detected with concentrations of 25.63 ng/L during the summer and slightly lower levels of 25.15 ng/L in the spring [58]. Pronounced monthly fluctuations are further evidenced in European surface systems, such as the Donji obodni kanal HE Čakovec in Štefanec, Croatia [64]. At this location, *fluoranthene* concentrations shifted from below the limit of quantification ($<0.0009 \mu\text{g/L}$) in June 2017 to $0.001 \mu\text{g/L}$ in October. Furthermore, the concentration of indeno[1,2,3-c,d]pyrene in August was $0.0296 \mu\text{g/L}$, but in December this value was significantly lower at $0.0007 \mu\text{g/L}$. It can be assumed that concentration values are higher during the summer period due to increased temperature and evaporation, as temperatures exceed 23°C during the summer months. This leads to evaporation of water from rivers, lakes, and the sea, reducing the amount of water while PAHs are retained as stable compounds. In colder regions, PAH levels in the atmosphere increase during winter due to increased domestic heating (coal/wood) and higher stability of the particulate phase. Wet deposition during rainy or flood seasons can significantly increase soil PAH concentrations as pollutants are washed from the atmosphere into the ground. Beyond surface waters, monitoring across Europe and North America urban areas has identified PAH concentrations in wastewater ranging from less than 1 to $625 \mu\text{g/L}$ [20]. Baseline studies show distinct concentrations among environmental compartments, measuring $2.5\text{--}3.5 \mu\text{g/L}$ in dam water, $2.0\text{--}10.4 \mu\text{g/L}$ in rivers, and $0.071\text{--}2.7 \mu\text{g/L}$ in wastewater. In India, environmental monitoring has shown that PAH contamination extends beyond surface waters to groundwater, with total PAH and BaP concentrations varying from 0.05 to $75,570 \text{ ng/L}$ [65]. For instance, a comprehensive evaluation of 120 groundwater samples across six districts in Jharkhand revealed significant contamination by the 16 priority PAHs.

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Occurrence in milk and egg matrices

The carry-over of environmental contaminants into the agricultural food chain has prompted extensive global monitoring of milk matrices. Because PAHs are highly lipophilic, they preferentially partition into lipid fractions, meaning their accumulation rates vary drastically depending on regional industrialization, farming practices, and the specific type of milk.

In the commercial dairy sector, stark geographical variations have been documented. Commercial milk brands in Nigeria showed concentrations between 15.6 and 1711.8 µg/kg, while raw, UHT, and pasteurised milk in Italy contained significantly lower levels, ranging from 5.43 to 7.75 µg/kg [66]. Global profiles for powdered milk infant formulas further reflect these industrial discrepancies; while samples from Sri Lanka showed minimal baseline levels of 0.13 ng/g dry mass (dm), products sourced from heavily industrialized zones in Egypt reached maximum contamination peaks of 8200 ng/g dm [58].

Beyond bovine dairy, human breast milk monitoring underscores the widespread environmental exposure of maternal populations. In Spain, analysis of human milk samples successfully quantified 15 of the 16 EPA priority pollutants, with pyrene being the sole absent congener [58]. Crucially, the excretion profile of PAHs fluctuates significantly across lactation stages; lipid-dense colostrum exhibits higher total burdens (2.113 ng/g) compared to mature breast milk (1.34 ng/g). Comprehensive screening in Ghana further highlighted this exposure pathway, revealing the presence of 18 distinct PAH compounds in breast milk, with total concentrations reaching up to 15,936.6 ng/g lipid mass and a mean accumulation of 1105.63 ng/g lipid mass [66]. Within these cohorts, acenaphthene was detected in nearly 98 % of the maternal participants, while benzo[a]pyrene – the most potent regulated carcinogen – was quantified in 76 % of the samples. Mirroring environmental deposition profiles, volatile light PAHs dominated the structural distribution, with naphthalene alone accounting for over 77 % of the total maternal PAH burden.

Crucially, chicken eggs represent another major poultry matrix heavily susceptible to environmental PAH carry-over, a pathway directly driven by avian foraging behavior and soil-ingestion habits. Laying hens raised in free-range or environmentally exposed systems routinely ingest contaminated topsoil, grass, and ambient dust, which leads to the rapid absorption and metabolic mobilization of hydrophobic pollutants. Due to their strict properties, accumulated PAHs partition exclusively into the lipid-dense egg yolk, while remaining practically undetected in the aqueous egg white. Analytical monitoring programs confirm that lower-molecular-mass congeners (specifically naphthalene, phenanthrene and fluorene) represent the dominant structural fractions found in commercial and private egg samples.

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CONCLUSIONS

PAHs represent critical environmental and dietary contaminants with significant long-term implications for human health, particularly regarding their carcinogenic potential. This review underscores the continuous transfer pathway of these hydrophobic pollutants from environmental sinks, such as contaminated soil and water, into the agricultural food chain. Livestock grazing on polluted forage and the soil-ingestion habits of free-range poultry serve as primary vectors for the bioaccumulation of PAHs into fat-rich matrices, specifically milk and egg yolks.

Although the baseline concentrations typically quantified in dairy and poultry products remain relatively low and comply with the general ALARA principle, chronic low-dose dietary exposure poses a persistent public health concern. To achieve rigorous regulatory compliance and safeguard consumer safety, future research must shift from traditional, solvent-intensive extraction methods toward automated, high-throughput, and 'green' analytical workflows, such as advanced SBSE and optimized QuEChERS protocols. Furthermore, addressing current analytical challenges, such as achieving lower detection limits for drinking water and ensuring robust fat removal in complex lipid matrices without losing volatile low-molecular-mass congeners, remains imperative. Systematic longitudinal monitoring of both environmental substrates and livestock feed regulations is essential to effectively mitigate the entry of PAHs into the human diet.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTION

M. Denžić Lugomer wrote, edited and revised the manuscript. D. Pavliček and M. Đokić oversaw the process of writing, participated in conception and design, and later of revision of the manuscript. V. Jaki Tkalec, S. Furmeg, N. Bilandžić, A. Končurat, Ž. Cvetnić, I. Kvaternjak, I. Kmetič, T. Murati, D. Mijač Dretar and T. Novosel provided suggestions and critical revision for the manuscript. All authors read and approved the final manuscript.

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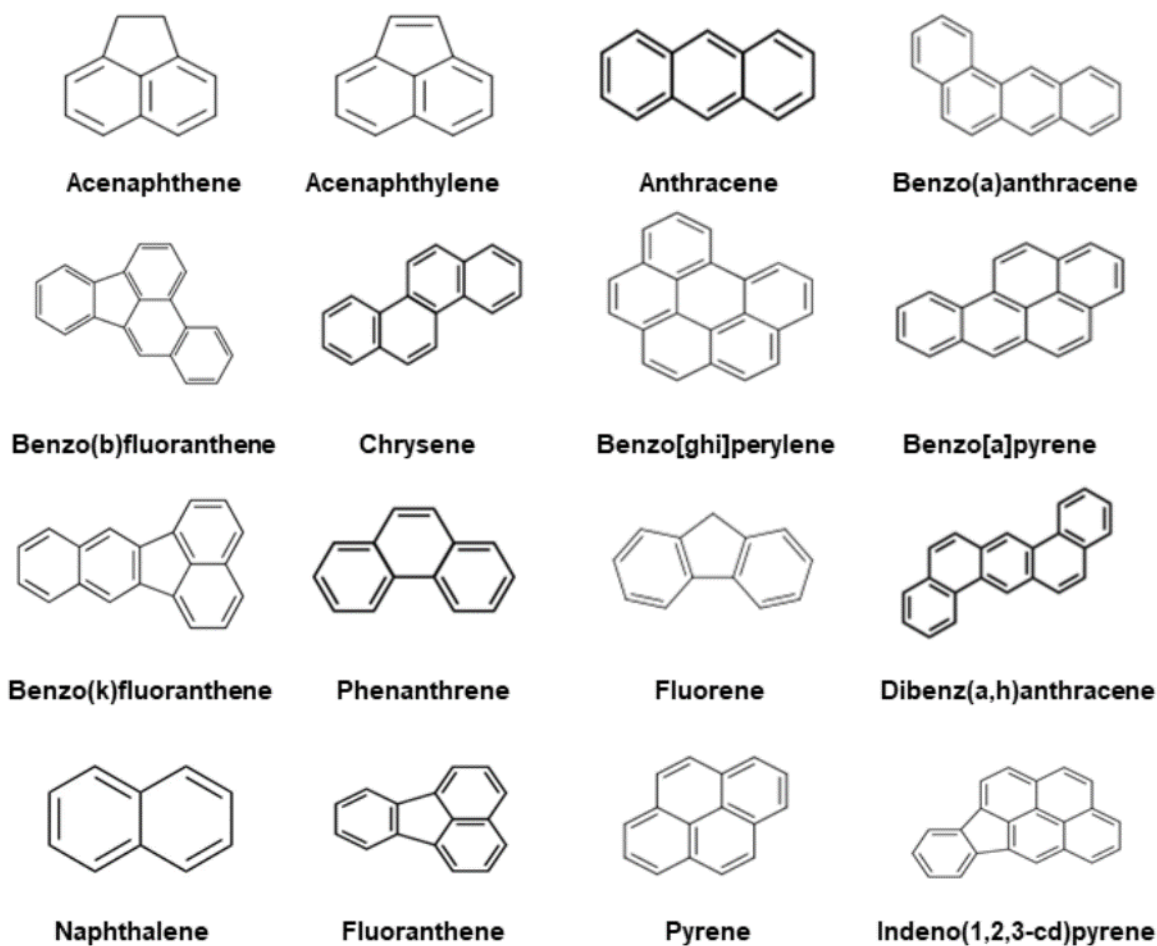


Fig. 1. 16 PAH compounds considered as important pollutants by the US EPA

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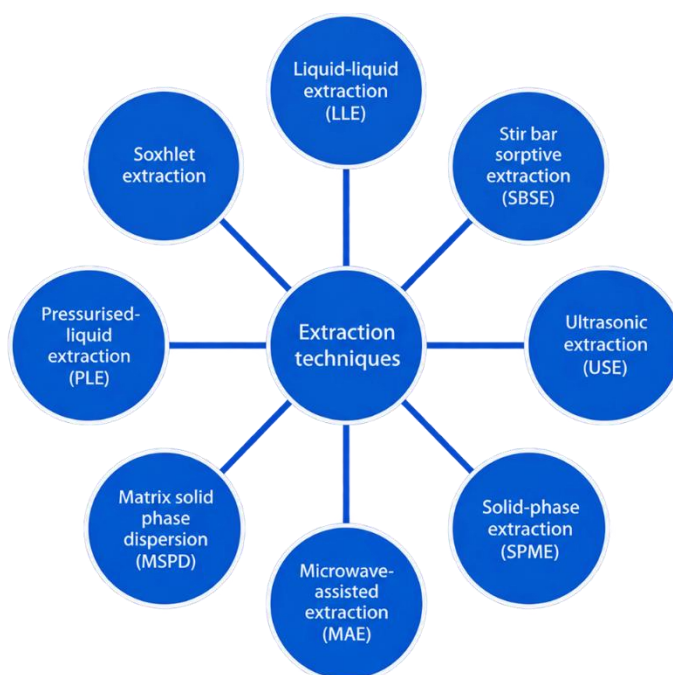


Fig. 2. Techniques for the extraction techniques of PAHs