Environmental Research 249 (2024) 118394

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Review article

Metabolomics perspectives of the ecotoxicological risks of polycyclic aromatic hydrocarbons: A scoping review

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ARTICLE INFO

Keywords:
Polycyclic aromatic hydrocarbons (PAHs)
Environmental contaminants
Ecotoxicological effects
Metabolomics approaches
Multiple PAH interactions

ABSTRACT

Polycyclic Aromatic Hydrocarbons (PAHs) represent persistent environmental pollutants ubiquitously distributed in the environment. Their presence alongside various other contaminants gives rise to intricate interactions, culminating in profound deleterious consequences. The combination effects of different PAH mixtures on biota remains a relatively unexplored domain. Recent studies have harnessed the exceptional sensitivity of metabolomic techniques to unveil the significant ecotoxicological perils of PAH pollution confronting both human populations and ecosystems. This article furnishes a comprehensive overview of current literature focused on the metabolic repercussions stemming from exposure to complex mixtures of PAHs or PAH-pollution sources using metabolomics approaches. These insights are obtained through a wide range of models, including *in vitro* assessments, animal studies, investigations on human subjects, botanical specimens, and soil environments. The findings underscore that PAH mixtures induce cellular stress responses and systemic effects, leading to metabolic dysregulations in amino acids, carbohydrates, lipids, and other key metabolites (e.g., organic acids, purines), with specific variations observed based on the organism and PAH compounds involved. Additionally, the ecological consequences of PAH pollutants on plant and soil microbial responses are emphasized, revealing significant changes in stress-related metabolites and nutrient cycling in soil ecosystems. The complex interplay of various PAHs and their metabolic effects on several models, as elucidated through metabolomics, highlight the urgency of further research and the need for comprehensive strategies to mitigate the risks posed by these widespread environmental pollutants.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic persistent pollutants with two to seven fused aromatic rings (Lawal, 2017). These molecules derive from natural and anthropogenic sources, being classified as petrogenic (i.e., originated from fossil fuels); biogenic (i.e., derived from natural biological processes) or pyrogenic (i.e., derived from incomplete combustion of organic matter) (Abdel-Shafy and Mansour, 2016). The lipophilic and semi-volatile properties of PAHs facilitate their transport in the environment leading to their ubiquitous presence in the air, soil, water, and food (Zhang et al., 2015). Out of around 10,000 PAH compounds (Nowakowski et al., 2022; Parshintsev et al., 2017), the United States Environmental Protection Agency (EPA) has classified only sixteen of them as priority contaminants (EPA, 2014). This recognition is based on their pervasive presence, long-lasting impact on the environment, and the practicability, consistency and comparability of studies performed in this area. Although sixteen PAHs are still widely reported in the literature, some authors have suggested

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https://doi.org/10.1016/j.envres.2024.118394
Received 13 November 2023; Received in revised form 29 January 2024; Accepted 30 January 2024
Available online 1 February 2024

adding other relevant PAHs to the list (up to 40 PAHs, depending on the research aims) (Andersson and Achten, 2015).

The International Agency for Research on Cancer (IARC) categorized these sixteen PAHs into carcinogenic and non-carcinogenic chemicals based on their ability to induce carcinogenicity in humans and other organisms, as outlined in the 2012 classification (IARC, 2012). The chemical structures and IARC classifications of the sixteen PAHs are presented in Fig. 1. Group 1 comprises PAHs that have been confirmed as carcinogenic to humans. Those in groups 2A or 2B are deemed to be probable or possible human carcinogens, while group 3 consists of non-carcinogenic PAHs. For certain animals, particular PAHs such as naphthalene or anthracene can still pose carcinogenic risks through inhalation, even though they are not generally classified as carcinogenic (Dybing et al., 2013). da Silva Junior et al. (2021) have recently found compelling evidence indicating the genotoxicity, mutagenicity, and carcinogenicity resulting from non-priority PAHs. Other specific

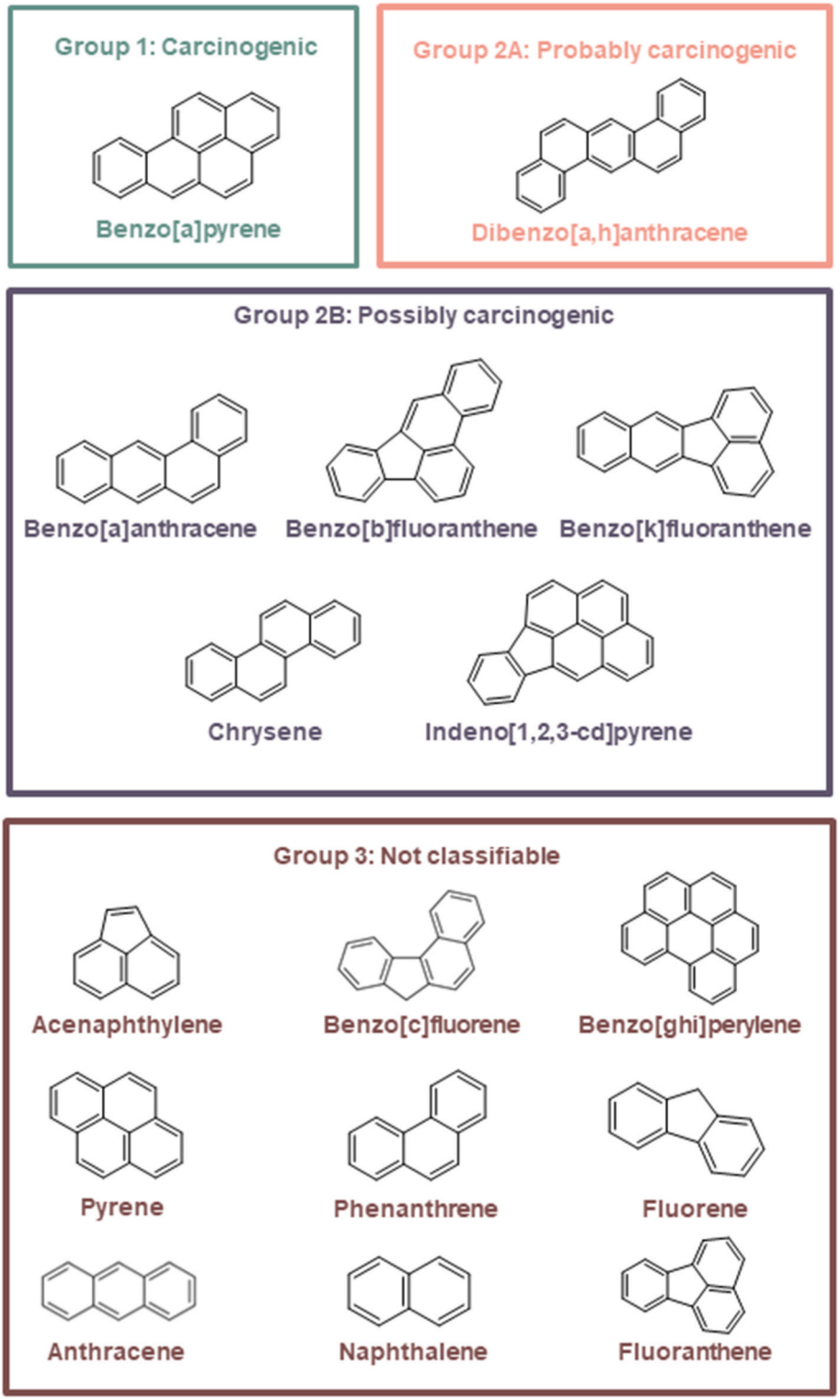

Fig. 1. List of 16 priority PAHs and their corresponding carcinogenic group according to the International Agency for Research on Cancer (IARC, 2012).

harmful effects of PAHs on humans and the environment include oxidative stress, endocrine disruptions, and teratogenic effects (Idowu et al., 2019). Given their widespread distribution and harmful effects on the environment (Idowu et al., 2019), it is crucial to focus on the presence of these substances in aquatic environments. This is especially important because coastal ecosystems are under significant human influence, and sediments can accumulate these harmful hydrophobic substances (Beyer et al., 2014).

Environmental quality guidelines for PAHs are consistently drawn up for individual substances, compromising risk assessment due to the lack of knowledge about PAH interaction mechanisms (McGrath et al., 2019; Panizzi et al., 2017; Sundt et al., 2012). Humans, animals, plants, and other organisms are often exposed to complex mixtures of chemical contaminants. When different or similar chemicals interact, they can cause toxic effects that are different in strength or type compared to the effects of each chemical individually. This phenomenon is attributed to the potential synergistic, additive, or antagonistic interactions (Altenburger et al., 2012; Beyer et al., 2014; Bramatti et al., 2022; Branco et al., 2021; Martins et al., 2015, 2016; Panizzi et al., 2017). Fully understanding the potential risks that these combinations pose to humans and the environment requires a comprehensive investigation into their interconnected mechanisms and biological impacts (Lovindeer et al., 2023). By doing so, we can provide effective and targeted preventive measures to mitigate these risks and preserve the well-being of the ecosystems.

Metabolomics, the large-scale study of low molecular weight compounds (metabolites) within cells, tissues, biofluids or organisms (Fiehn, 2002; Nicholson et al., 1999), has been increasingly used in the last decade to assess the impact of complex mixtures on biological systems and gain a more comprehensive understanding of the associated risks (Lankadurai et al., 2013; Martins et al., 2019). This approach is particularly valuable for detecting sub-acute toxicity and uncovering the underlying mechanisms of chemical exposures, such as those from complex mixtures (Martins et al., 2019; Meador and Nahrgang, 2019; Wilson et al., 2023). In line with this, the present article aims to review the metabolic responses associated with exposure to multiple PAHs spanning across various study models like *in vitro* models, animals, humans, plants, and microorganisms in soils. To the best of our knowledge, this review is the first to comprehensively synthesize the metabolic responses to PAH exposure in these distinct research models.

2. Chemical properties, exposure routes and biotransformation of PAHs in humans

PAHs are organic compounds that are colourless or have a white to pale yellow colour (Abdel-Shafy and Mansour, 2016). The term PAH is intended for compounds that consist only of carbon and hydrogen atoms (Rengarajan et al., 2015). These compounds have two or more fused aromatic rings, arranged in several structural configurations (angular, linear, or cluster arrangements). The structure of these compounds may result from angularly condensed aromatic rings, possibly due to chemical distortions in regions with maximal impact, termed as *fjord* or *bay* regions. PAHs that exhibit *fjord* regions (e.g., dibenzo[a,i]pyrene) are mainly reactive, non-planar and bind preferentially to adenine nucleotides. In comparison, PAHs with a *bay* region (e.g., benzo[a]pyrene) are less reactive, planar and bind to guanine nucleotides (Kim et al., 2013). The PAHs' structural configuration provides a wide range of chemical, physical, and toxicological characteristics. Normally, an increase in the angularity and size of a PAH is parallel to an increase in the electrochemical stability and hydrophobicity (Lawal, 2017). Low molecular weight PAHs contain up to four fused benzene rings (e.g., fluorene, naphthalene, and phenanthrene), while high molecular weight PAHs contain more than four fused benzene rings in their configuration (e.g., benzo[b]fluoranthene, benzo[a]pyrene, and chrysene), and are more toxic and persistent (Abdel-Shafy and Mansour, 2016; Lawal, 2017).

Due to their environmental ubiquity, human exposure occurs for an extended period and usually at low levels (Jarvis et al., 2014). The main routes of exposure are inhalation, ingestion, and dermal contact (especially in occupational settings) (Ravindra et al., 2008). The effects of exposure to PAHs on human health depend on the extent and route of exposure, the individual susceptibility, the concentration and number of PAHs to which the individual was exposed, and the particular toxicity of the compound (Rengarajan et al., 2015). Simultaneous exposures may occur through multiple routes, such as dermal and inhalation exposure to polluted air, increasing the total absorption dose (Lawal, 2017). Furthermore, significant exposure can arise from the consumption of grilled, smoked foods, and seafood (Gohlke et al., 2011; Sun et al., 2019; Yebra-Pimentel et al., 2015). PAH exposure can cause adverse outcomes, due to the carcinogenic, immunotoxic, clastogenic, and teratogenic properties of these molecules (Gangar et al., 2010; Miller and Ramos, 2001; Yang et al., 2010). Several studies have incidentally found a link between exposure to PAHs and an intensified risk of developing cancer from the skin, lung, bladder, kidney, breast, larynx, prostate, blood (leukaemia), brain, and colorectal (Clapp et al., 2008; Hamidi et al., 2016; Korsh et al., 2015; Lee and Choi, 2023; Mallah et al., 2022; Pan et al., 2005; Petit et al., 2019; White et al., 2016).

PAHs are not reactive and require metabolic activation to elicit their deleterious effects (Zhang et al., 2012). The metabolic activation of PAHs involves reactions of phase I (activation) and phase II (conjugation). After metabolic activation, PAHs produce reactive metabolites such as dihydrodiols, epoxide intermediates, quinones and phenols (Urbancova et al., 2016). The enzymes that are especially involved in the bioactivation of PAHs are Cytochrome P450 Family 1 (CYP1A1, CYP1A2, and CYP1B1) during phase I of detoxification (Ewa and Danuta, 2017). CYP1A1 and CYP1B1 are extremely inducible by PAHs through activation of aryl hydrocarbon receptor (AhR), which in turn contributes to the higher formation of PAH metabolites and subsequent DNA damage (Avilla et al., 2020; Baird et al., 2005). The reactive metabolites produced from PAH activation may form bulky DNA-adducts, and induce nucleobase oxidation by action of reactive oxygen species (Baird et al., 2005). PAH activation occurs through three pathways (Fig. 2) involving the formation of 1) radical cations by CYP-peroxidases; 2) *bay* and *fjord* region diol-epoxides by CYP-dependent monooxygenases; and 3) redox-active o-quinones by aldo-keto reductases (Jin and Penning, 2007; Xue and Warshawsky, 2005). These products may have mutagenic and tumorigenic activities, but how PAH co-exposure modulates these pathways remains unknown (Holme et al., 2023).

PAHs are often biotransformed into oxidation products that are more toxic than the original contaminants (Tian et al., 2018). The biotransformation of PAHs leads to the formation of DNA-reactive metabolites through the action of phase I enzymes and peroxidases, as well as the generation of highly polar conjugates (e.g., quinones, phenols, and dihydrodiols) via phase II enzymes (Gao et al., 2018a; Zhang et al., 2012). After interacting with DNA, PAH metabolites cause mutations that either activate oncogenes or deactivate tumour suppressor genes (Jarvis et al., 2014). If mutations in tumour suppressors occur, they can lead to uncontrolled regulation of the cell cycle, increase DNA damage, and eventually carcinogenesis (Jarvis et al., 2014; Martins et al., 2015). Hence, when the DNA sequence in genes responsible for cellular replication is disrupted, the likelihood of developing cancer and other diseases significantly rises (Moorthy et al., 2015). Moreover, the PAH metabolization process can also lead to the enhance of the production of reactive oxygen species (ROS), which in turn can exert direct effects on lipids, DNA, or proteins (Kafferlein et al., 2010). However, recent studies pointed to the capacity of the glutathione system as a central modulator of PAH toxicity in human liver cells (Branco et al., 2021).

3. A metabolomics-based understanding of PAHs ecotoxicology

3.1. Literature search

Ecotoxicology is a multidisciplinary field, which integrates

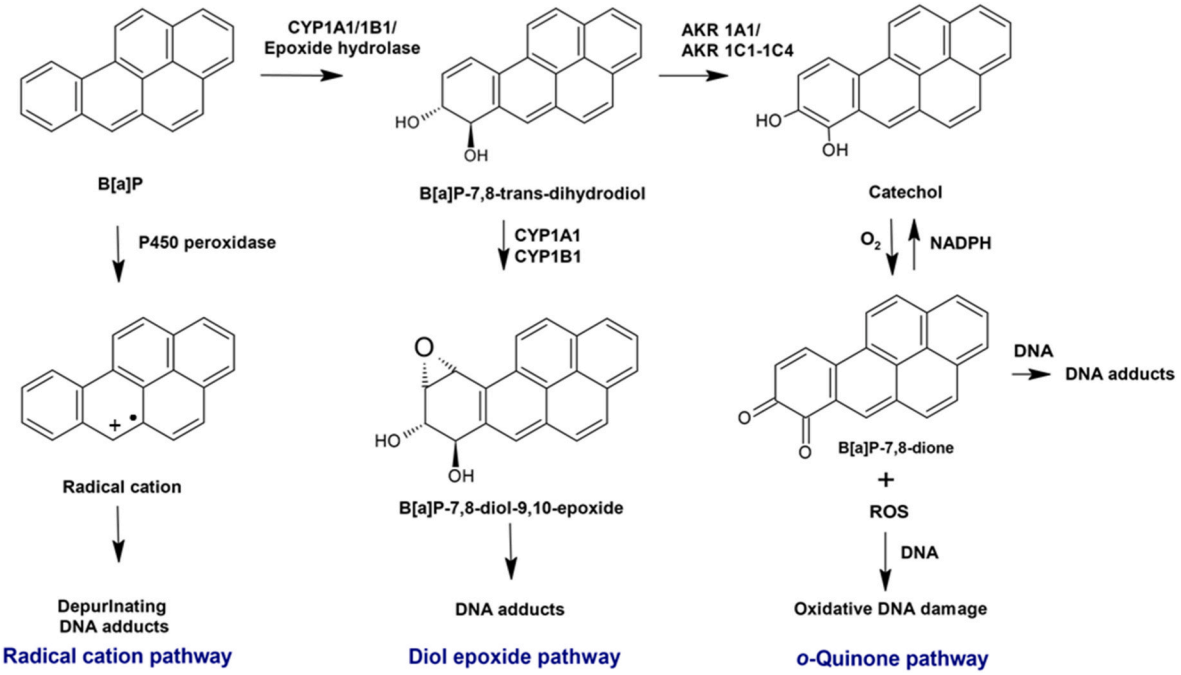

Fig. 2. Schematic representation of three pathways of metabolic activation of PAHs using benzo[a]pyrene (B[a]P) as an example. Adapted from Ran et al. (2008) and Zhang et al. (2012).

toxicology and ecology, in the study of the effects of toxic chemicals on biological organisms (Vasseur et al., 2021). In recent years, metabolomics-based approaches have been widely employed to address challenging ecotoxicological issues (Dumas et al., 2022; Gao et al., 2018a; Sun et al., 2022). In this review article, the literature search was carried out in PubMed (U.S. National Library of Medicine) and Scopus databases to access the studies that apply metabolomics approaches to identify the metabolic responses of biological organisms to PAH mixtures or PAH-pollution sources, considering the following keywords or expressions ("polycyclic aromatic hydrocarbons") AND (metabolomics). The search was carried out in January 2023, considering all literature published in English until the end of 2022. The search retrieved 120 papers in PubMed and 200 papers in Scopus that were screened according to the following inclusion criteria: 1) papers reporting original results; 2) papers evaluating the metabolic response to PAHs in biological systems (cells, animals, humans, plants, and soils); and 3) papers considering exposure to PAH mixtures or PAH-pollution sources. Papers reporting only exposure to individual compounds were excluded. After the screening of PubMed and Scopus results, 25 articles were considered for the present review which were organized by the biological model (*in vitro,* animal, human, plants, and soils) as shown in Fig. 3. The subchapters below summarize the main objectives and findings of the studies done so far and the metabolites affected by PAH exposure in different models. In studies that involve human cell lines and biological samples, the potentially disrupted metabolic pathways are emphasized. For the remaining organism models, we have made a conscious effort to avoid the misuse of pathway analysis tools (e.g., over-representation analysis (ORA), topology-based pathway enrichment analysis (TPEA)) based on human metabolic pathway databases (e.g., Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2023). This decision was influenced by the recent recommendation made by Wieder et al. (2022), who have highlighted the common misinterpretation of metabolite changes in environmental organisms when using human databases.

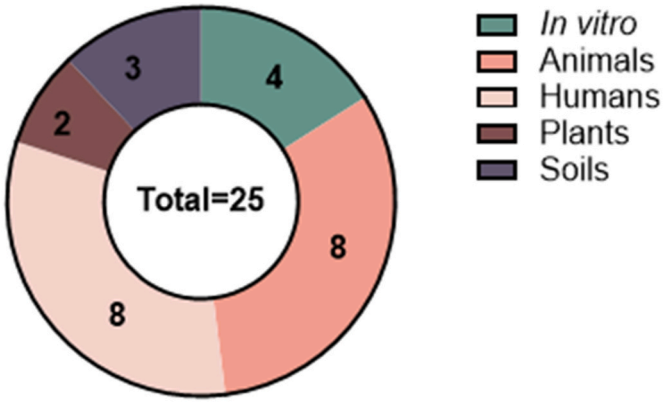

Fig. 3. Doughnut chart illustrating the number of metabolomic-based studies (published from 2010 up to 2022) investigating the metabolic responses of different research models to PAH mixtures or PAH-polluted geographic areas.

3.2. Results and discussion

3.2.1. In vitro models

In vitro models have been largely applied to evaluate the combined effects of exposure to PAHs since they have a high-throughput performance and the possibility of investigating a specific mode of action (MoA) (Beyer et al., 2014; Bopp et al., 2015; Bramatti et al., 2022; Branco et al., 2021). In the metabolomics field, these models offer valuable insights into the metabolic pathways affected by PAH exposure, along with novel biomarkers for exposure. This not only enhances our understanding of the subject but also contributes to the reduction of animal testing through improved methodology refinement. In the past decade, numerous studies have been conducted using fish, mouse, and human immortalized cell lines to investigate the combined effects of PAHs *in vitro*. Table 1 provides a comprehensive summary of the studies conducted so far, presenting the key findings obtained.

Replacing marine ingredients with plant ingredients in the salmon feeds has introduced a new cocktail of plant-oil-derived contaminants

Table 1
In vitro metabolomic studies focused on the effects of exposure to PAH mixtures.

Tested PAHs	Concentration(s)/ exposure duration	Cell type (sample size)	Analytical platform	Main results	Reference
Phenanthrene Benzo[a]pyrene Both PAHs combined with pesticides (chlorpyrifos, endosulfan)	1 μM 100 μM (for each PAH and pesticide) /24h	Atlantic salmon primary hepatocytes ($n = 5$ per group)	^{1}H NMR (polar intracellular metabolites) FT-ICR-MS (intracellular lipids by lipidomics)	PAHs combined with pesticides: ↑ Cholesta-8-en-3β-ol; zymosterol; lathosterol; cholesta-7,24-dien-3β-ol; vitamin D3; 7-dehydrocholesterol; calcidiol; cholesterol; desmosterol ↓ γ-Linolenate; dihomo-γ-linolenate; arachidonate; linoleate; 9-cis, 11-*trans*-octadecadienoate; crepenynate; arachidonate	Søfteland et al. (2014)
PAH mixture containing 16 congeners (1:1) Short-chain chlorinated paraffins (SCCPs) mixture (1:1)	160 μg/L (total PAHs concentration) 100 μg/L (total SCCPs concentration) /24 h	Human hepatoma cell line (HepG2, $n = 6$ per group)	UPLC-MS (intracellular extract)	PAHs + SCCPs: ↑ Phosphatidylcholines; lysophosphatidylcholines; phosphatidylethanolamine; lysophosphatidylethanolamine; sphingomyelin; lysosphingomyelin ↓ TCA cycle metabolites; short-chain acylcarnitines; putrescine; glycolysis Affected metabolic pathways after PAHs + SCCPs exposure: glycerophospholipid (up-regulation of phospholipids), linoleic acid, α-linolenic acid, and arachidonic acid metabolisms	Wang et al. (2018)
Binary PAH mixture (1-methylcholanthrene and fluoranthene 1:1)	15 μM 40 μM /24 h	Mouse lung epithelial cell line (C10, $n = 4$ per group)	LC-MS (intracellular lipids by lipidomics)	293 significantly altered metabolites after exposure to the binary PAH mixture, including several classes of phospholipids Affected metabolic pathways: glycerophospholipid metabolism, phosphatidylinositol signalling pathways, sphingolipid and glycerolipid metabolisms	Siegrist et al. (2019)
Oil sand extract containing polyaromatic compounds (including PAHs)	10 % and 20 % of lethal concentration (cytotoxicity experiment) /24 h	Human lung carcinoma cell line (A549, $n = 6$ per group) Human hepatoma cell line (HepG2, $n = 6$ per group) Human neuroblastoma cell line (SK-N-SH, $n = 6$ per group)	HPLC-MS (polar intracellular metabolites)	A549 cells exposed to polyaromatic compounds: ↑ Cysteine; leucine; glutamine; phenylalanine; 4-hydroxyphenylpyruvic acid; tyrosine; gamma-glutamylcystein; estrone; 17-beta-estradiol; testosterone; progesterone; leukotriene A4; 18-hydroxyarachidonic acid; arachidonic acid 5-hydroperoxide; corticosterone; prostaglandin F1a; aldosterone; 18-hydroxycorticosterone; sphingosine 1-phosphate; sphinganine 1-phosphate; leukotriene D4; phosphoadensine phosphosulfate; protoporphyrin IX; biliverdin; bilirubin; leukotriene C4; coproporphyrinogen III ↓ Lysine; 3-sulfinoalanine; histidine A549 metabolic dysregulations: steroid hormone biosynthesis; aminoacyl-tRNA biosynthesis; arachidonic acid metabolism HepG2 cells exposed to polyaromatic compounds: ↑ Diacetylspermine; lipoxin B4; 20-hydroxy-leukotriene E4 ↓ 3-Methylcytosine; myristic acid; 11-*cis*-retinol; dehydroepiandrosterone; glycerol tributanoate; 5-androstene-3b,16b,17a-triol; leukotriene C4; farnesylcystein; 17-hydroxydocosahexaenoic acid; resolving E1; cortisone; dehydroepiandrosterone sulfate; 7-O-acetylsalutaridinol; 7-a,27-dihydroxycholesterol; chenodeoxycholic acid glycine conjugate; glycocholic acid; deoxycytidine triphosphate; leukotriene D4; uridine diphosphate glucuronic acid; biliverdin; leukotriene C4; 1-diphosinositol pentakisphosphate; bisdiphosphoinositol tetrakisphosphate; triiodothyronine glucuronide; geranylgeranyl diphosphate HepG2 metabolic dysregulations: primary bile acid biosynthesis; arachidonic acid metabolism; steroid hormone biosynthesis SK-N-SH cells exposed to polyaromatic compounds: ↑ Queuine; dehydroepiandrosterone; 19-hydroxyandrost-4-ene-3,17-dione; 18-hydroxy-icosapentaenoate; Resolvin E1; nicotine glucuronide; 17-hydroxydocosahexaenoic acid; corticosterone; 18-hydroxycorticosterone; sphingosine 1-phosphate; inositol trisphosphate; folic acid; thymidine 5'-triphosphate; cytidine triphosphate; uridine triphosphate; chlordecone alcohol; leukotriene D4; maltotriose; sulfolithocholylglycine; taurohyocholate; protoporphyrin IX; protoporphyrinogen IX; adenosine tetraphosphate; acetyl-CoA; butanoyl-CoA; glutaconyl-CoA ↓ All-trans-retinoic acid SK-N-SH metabolic dysregulations: fatty acid metabolism; pyrimidine metabolism; steroid hormone biosynthesis	Sarma et al. (2019)

FT-ICR-MS: Fourier-transform ion cyclotron resonance-mass spectrometry; ^{1}H NMR: proton nuclear magnetic resonance spectroscopy; UHPLC-MS: Ultra-high performance liquid chromatography-mass spectrometry; LC-MS: liquid chromatograph-mass spectrometry; ↑ metabolites found up-regulated (significantly increased levels); ↓ metabolites found down-regulated (significantly decreased levels).

(e.g., PAHs and pesticides). To evaluate the interaction effects of these contaminants, Søfteland et al. (2014) performed an *in vitro* study using Atlantic salmon primary hepatocytes. This study integrated the knowledge obtained from three omics approaches, including metabolomics, lipidomics, and transcriptomics. The hepatocytes were exposed to PAHs (benzo(a)pyrene and phenanthrene) and pesticides (chlorpyrifos and endosulfan) individually and in different mixtures of all contaminants. The mixtures of PAHs and pesticides showed the strongest cell viability reduction effect among the tested conditions. However, no notable distinctions were detected in the metabolomics analysis (polar metabolites) between the control and exposed groups. According to lipidomics analysis, a branch of metabolomics, exposure to mixtures of PAHs and pesticides resulted in increased levels of lipid species including cholesta-8-en-3β-ol, zymosterol, lathosterol, cholesta-7,24-dien-3β-ol, vitamin D3, 7-dehydrocholesterol, calcidiol, cholesterol, and desmosterol. Conversely, there was a decrease in several lipids such as γ-linolenate, dihomo-γ-linolenate, arachidonate, linoleate, 9-cis, 11-trans--octadecadienoate, crepenynate, and arachidonate. These findings revealed a clear impact of exposure to PAHs and pesticides on the hepatic lipid metabolism of Atlantic salmon. The authors concluded that exposure to PAH and pesticide mixtures has largely addictive effects due to the overlap between the lipidome perturbations induced by the individual contaminants and mixtures. Nevertheless, at high concentrations, synergistic effects were claimed.

The combined toxicity assessment of PAHs and short-chain chlorinated paraffins (SCCPs) raises significant concern since relatively high levels of both chemicals were reported in human milk and blood (Li et al., 2017; Santonicola et al., 2017; Singh et al., 2008; Xia et al., 2017). In this regard, Wang et al. (2018) applied a metabolomics strategy to assess the combined effects of a mixture of PAHs and SCCPs (PAHs + SCCPs), at the levels found in milk and blood, on the human hepatocellular carcinoma (HepG2) cells. Their findings indicated that exposure to PAHs + SCCPs resulted in the highest number of distinct metabolites, with 91 identified in comparison to 43 for PAHs alone and 61 for SCCPs. Among these 91 differential metabolites, there was an up-regulation of metabolites such as phosphatidylcholines, lysophosphatidylcholines, phosphatidylethanolamine, and lysophosphatidylethanolamine. Conversely, there was a down-regulation of metabolites including sphingomyelins, lysosphingomyelins, TCA cycle metabolites, short-chain acylcarnitines, putrescine, and metabolites participating in glycolysis. The combined exposure to PAHs and SCCPs induced a synergistic up-regulation of phospholipid metabolism, an additive up-regulation of fatty acid metabolism, a down-regulation of TCA cycle and glycolysis, and an antagonistic effect on purine metabolism. This study highlights that disturbance of lipid metabolism caused by exposure to a combination of PAHs and SCCPs should be an important concern for human health.

Based on previous studies suggesting that low molecular weight PAHs induced critical cell signalling events that contribute to pulmonary disease (Osgood et al., 2014, 2017), Siegrist et al. (2019) performed a lipidomics study to elucidate the mechanism and potential pathological events associated with the toxicity of a representative binary mixture (1:1) of 1-methylanthracene and fluoranthene. The exposure was performed with and without the p38 mitogen-activated protein kinase (MAPK) and cytosolic phospholipase A2 (cPLA2) inhibitors in a mouse lung epithelial cell line (C10 cells). p38 MAPK has vital roles in the signal transduction pathways leading to inflammation involved in pulmonary diseases, while cPLA2 (cytosolic phospholipase A2) is a key enzyme in eicosanoid biosynthesis. Their results revealed that 293 metabolites were significantly different in response to the binary PAHs mixture, including several phospholipid classes. Based on their results, the binary mixture induced alterations in the phosphatidylinositol signalling and metabolism of glycerophospholipids, sphingolipids, and glycerolipids, providing evidence that the plasma membrane is supplying substrates for the p38 MAPK/cPLA2 pathway. Overall, these results proved that exposure to PAHs can lead to an early lipid signalling mechanism in lung epithelial cells.

Oil sand deposits are also a source of PAHs and other chemicals for the environment. Little is known regarding the toxicity of oil sand extracts to humans and most of the information refers to the individual chemicals isolated from the sediments. To improve human health risk assessment, Sarma et al. (2019)studied the effects of oil sand extracts (OSE) in the viability and metabolic pathways of HepG2, human lung carcinoma (A549), and human neuroblastoma (SK-N-SH) cell lines. The three cell lines were exposed to polyaromatic compounds extracted from oil sands (including PAHs) at concentrations inducing 10 and 20 % mortality (as assessed by the MTT assay). The untargeted metabolomic analysis revealed 33 altered metabolites showing increased levels of several amino acids, steroid-related metabolites, and leukotrienes in A549 cells exposed to polyaromatic compounds. These alterations were putatively associated with dysregulations in various metabolic pathways, specifically in steroid hormone biosynthesis, aminoacyl-tRNA biosynthesis, and arachidonic acid metabolism. In HepG2 cells, the exposure to polyaromatic compounds induced alterations in the levels of 28 metabolites. Most of these altered metabolites were down-regulated, including several leukotrienes. Additionally, three metabolites, diacetylspermine, lipoxin B4 and 20-hydroxy-leukotriene E4, were found up-regulated in HepG2 cells upon exposure. Based on this, results from HepG2 cells were associated with putative dysregulations in primary bile acid biosynthesis, arachidonic acid metabolism, and steroid hormone biosynthesis. Finally, the SK-N-SH cells revealed significant alterations in the levels of 27 metabolites, including increased levels of metabolites involved in fatty acid, butanoate, and pyrimidine metabolisms, and steroid hormone biosynthesis. The results of this study showed that polyaromatic compounds may induce toxicity in multiple target organs, such as the human liver, lung, and brain.

3.2.2. *Animal studies*

Animal studies in this area have been focused on the effects of PAH exposure (individually or in mixture), and exposure to water contaminated with PAHs, as summarized in Table 2. The studies are organized by type of exposure, including studies conducted in laboratory settings to understand the effects of PAH exposure (standards and water contaminated), mesocosm studies addressing the impact of PAHs on animals kept in controlled environments, and studies discussing the effects of PAH pollution on fish living in their natural habitats. It is important to highlight that these studies have exclusively focused on aquatic organisms, including water fleas, rainbow trout alevins, mussels, topsmelt, zebrafish, and Atlantic killifish. *Daphnia magna* is a very popular model in ecotoxicology and environmental toxicology research. This planktonic crustacean possesses a remarkable sensitivity to various chemicals. Its small size, short life cycle, and ease of cultivation make it an invaluable tool for studying the potential effects of environmental contaminants on other aquatic organisms (Vandenbrouck et al., 2010). Mussels and other bivalve molluscs are models commonly used as sentinel species in ecotoxicological research and environmental monitoring programs. The widespread utilization of these organisms is due to their high prevalence in marine, estuarine, and freshwater environments, their ability to filter-feed, and their capacity to accumulate significant levels of contaminants in tissues (Martins et al., 2012). Moreover, mussels have been established as a valuable asset in active biomonitoring studies (Cappello et al., 2013, 2017). Zebrafish have also become pivotal in ecotoxicology and environmental toxicology research, with their extensive use aimed at understanding the impact of

Table 2
Studies on animal metabolism focused on the effects of exposure to mixtures of PAHs and water contaminated with PAHs.

Experimental system	Exposure under study	Concentration(s)/ Exposure duration	Organism (scientific name)/ Biological matrix	Analytical platform	Main results	Reference
Laboratory	Pyrene Fluoranthene Binary mixtures of pyrene and fluoranthene	0.062, 0.125, 0.187 and 0.250 toxic units /96 h	Water flea *(Daphnia magna)* /*Daphnia* juveniles ($n = 3$ per group, 200 juveniles per replicate)	^{1}H NMR (polar extract) GC-MS (lipid extract)	Pyrene exposure: ↑ Aminomalonic acid; glucofuranoside; glucopyranose; glucuronolactone; glutamine; heptanedioic acid; lysine; succinate; turanose ↓ Alanine; aspartic acid; gluconic acid; ornithine Fluoranthene exposure: ↑ Alanine; asparagine; ornithine; phenylalanine; tyrosine; proline ↓ Galactose; maltose; palmitic acid Binary mixture exposure: ↑ Alanine; aspartic acid; glutamine; glycine; myo-inositol; turanose ↓ Asparagine; cadaverine; galactose; glucose; glucuronolactone; lactate; malic acid; nonanoic acid; putrescine; SH purine-2-amine; uridine	Vandenbrouck et al. (2010)
Laboratory	Retene Fluoranthene Binary mixture of retene and fluoranthene	32 μg/L retene 50 μg/L fluoranthene /14 days	Rainbow trout alevins (*Oncorhynchus mykiss*) /Heart ($n = 4$ per group, 45 hearts per replicate)	GC-MS (polar extract)	Retene exposure: no alterations Fluoranthene exposure: ↑ Glucuronic acid; arabitol Binary mixture exposure: ↓ Methionine; putrescine; hypotaurine; phenylalanine	Eriksson et al. (2022)
Laboratory	Water-accommodated fraction (WAF) and chemically-enhanced WAF (CEWAF) of Prudhoe Bay crude oil	Adult topsmelt exposure: WAF - 1.56, 3.13, 6.25, 12.5, and 25 g/L of total hydrocarbon content CEWAF: 0.063, 0.125, 0.25, 0.5, and 1.0 g/L of total hydrocarbon content Direct embryonic exposure: WAF - 1.56, 3.13, 6.25, 12.5, and 25 g/L of total hydrocarbon content CEWAF: 0.25, 0.5, 1.0, 2.5, and 7.5 g/L of total hydrocarbon content /96 h exposure	Topsmelt (*Atherinops affinis*)/ muscle of adult topsmelt ($n = 3$ per concentration) and three day old topsmelt embryos ($n = 3$ per concentration)	^{1}H NMR (polar extract)	Adult topsmelt exposed to WAF: ↓ Lactate Adult topsmelt exposed to CEWAF: ↓ Phosphocreatine Topsmelt embryos directly exposed to WAF: ↑ Glutamate; taurine ↓ Valine; lactate; alanine; glutamine; succinate Topsmelt embryos directly exposed to CEWAF: ↑ Glutamine ↓ Glutamate	Van Scoy et al. (2012)
Laboratory	Sediment-derived water-soluble fraction of diluted bitumen (SDWSF)	10 % and 100 % SDWSF (14.6 ng/μL of total PAH and alkyl-PAH)/120 h post-fertilization	Zebrafish (*Danio rerio*)/ embryos ($n = 6$ exposures, ~100 per exposure)	^{1}H NMR (polar extract)	Exposure to 10 % SDWSF vs. control: ↑ Glycerol; tyrosine ↓ Taurine Exposure to 100 % SDWSF vs. control: ↑ Glycerol; inosine; threonine; tyrosine ↓ Alanine; betaine; lysine/ glutamine; taurine	Fujita et al. (2021)
Mesocosms	Polluted seawater at the "Augusta-Melilli-Priolo" petrochemical area, Italy	NR/30 days	Mussels (*M. galloprovincialis*) /digestive gland ($n = 15$ per site)	^{1}H NMR (polar extract)	Mussels caged at polluted site vs. mussels caged at reference site: ↑ Valine; lysine; phenylalanine; acetoacetate; thymidine; adenine	Fasulo et al. (2012)
Mesocosms	Polluted seawater (PAHs and mercury) at the "Augusta-Melilli-Priolo" petrochemical area, Italy	NR/30 days	Mussels (*Mytilus galloprovincialis*) /Gill tissues ($n = 12$ per site)	^{1}H NMR (polar extract)	Mussels caged at polluted site vs. mussels caged at reference site: ↑ Isoleucine; leucine; valine; alanine; arginine; glutamate; glutamine; aspartate; glycine; acetate; acetoacetate; succinate; malonate; glucose; ATP; ADP; hypotaurine; taurine; betaine; homarine ↓ Tyrosine; glycogen; acetylcholine	Cappello et al. (2013)

(*continued on next page*)

Table 2 (*continued*)

Experimental system	Exposure under study	Concentration(s)/ Exposure duration	Organism (scientific name)/ Biological matrix	Analytical platform	Main results	Reference
Mesocosms	Polluted seawater (PAHs and mercury) at the "Augusta-Melilli-Priolo" petrochemical area, Italy	NR/60 days	Mussels (*Mytilus galloprovincialis*) /Posterior adductor muscle (n = 15 per site)	^{1}H NMR (polar extract)	Mussels caged at polluted site vs. mussels caged at reference site: ↑ Isoleucine; leucine; proline; alanine; glycine; hypotaurine; homarine; ↓ Arginine; aspartate; glycogen; glucose; uracil	Cappello et al. (2017)
Natural habitat	Highly PAH-contaminated site at the Elizabeth River system, Virginia, USA	Contaminated site: 295,903.9 ng/g total PAHs/dry sediment Reference site: 953 ng/g total PAHs/dry sediment	Atlantic killifish (*Fundulus heteroclitus*) /Intestine (n = 10 per site)	LC-MS/MS (polar and lipid extracts)	Contaminated vs. reference site: ↑ Sphingolipids; glycerophospholipids ↓ Acylcarnitines; amino acids	Redfern et al. (2021)

^{1}H NMR: proton nuclear magnetic resonance spectroscopy; GC-MS: gas chromatograph-mass spectrometry; LC-MS/MS: liquid chromatograph-mass spectrometry; NR: Not reported; ↑ metabolites found up-regulated (significantly increased levels); ↓ metabolites found down-regulated (significantly decreased levels).

environmental exposures on human health and disease (Bambino and Chu, 2017). Their rapid development, transparent embryos, and genetic similarity to humans make them ideal for studying the effects of pollutants and chemicals on biological processes.

Daphnia Magna was selected as the animal model for the first metabolomics study to assess the effects of exposure to PAH mixtures (Vandenbrouck et al., 2010). The authors exposed juvenile daphnids to two PAHs (pyrene and fluoranthene) and their binary mixture, adopting an acute exposure approach (96 h) to measure endpoints such as gene expression (transcriptomics), metabolic profiling, and energetic content. The results revealed significant alterations in the levels of 40 metabolites after exposure to PAHs individually and/or in a mixture. Specifically, exposure to pyrene led to a decrease in the levels of various metabolites, including one amino acid and several organic acids. Additionally, there was an up-regulation of several carbohydrates, amino acids and organic acids. Fluoranthene exposure resulted in decreased levels of two carbohydrates, and one fatty acid, along with an increase in the levels of several amino acids. Moreover, the combined exposure to the binary mixtures led to a down-regulation in one amino acid and pyrimidine, and several organic acids and amines. Conversely, there was an up-regulation of several amino acids, one carbohydrate and one alcohol. These metabolic responses revealed that the combined effects of the mixture can exhibit both similarities and differences compared to the effects of the individual compounds. Furthermore, the observed metabolic changes may indicate a disruption in aminosugar metabolism attributed to exposure to PAHs.

PAH toxicity has been associated with alterations in the heart structure and function of fish larvae during early life development. Therefore, Eriksson et al. (2022) investigated how the cardiac metabolome of newly hatched rainbow trout alevins *(Oncorhynchus mykiss)* responded to retene and fluoranthene exposure, either individually or as a mixture. These PAHs have different toxicity mechanisms, while retene is an aryl hydrocarbon receptor 2 agonist, fluoranthene is a weak aryl hydrocarbon receptor 2 agonist and a cytochrome P450 inhibitor. Rainbow trout alevins were exposed to retene, fluoranthene, or the binary mixture of the two PAHs for 14 days. Only fluoranthene impacted the cardiac metabolome inducing a significant increase in glucuronic acid and arabitol. On the other hand, the binary mixture induced a significant decrease in the levels of methionine, putrescine, hypotaurine, and phenylalanine. These findings suggested an altered energy metabolism in the heart of alevins exposed to fluoranthene and altered amino acid catabolism after exposure to the binary mixture, as corroborated by proteome analysis.

Topsmelt (*Atherinops affinis*) are a vital fish species in the exosystems of California's coastal bays, estuaries, and near-shore coastal waters. The transportation of crude oil between California and Alaska has raised concerns regarding the heightened risk of an accidental marine oil spill. This poses a potential threat to the bays and estuaries and can have a profound impact on the spawning Topsmelt and their developing offspring. Van Scoy et al. (2012) conducted a study to investigate the metabolic effects of the water-accommodated fraction (WAF; physically-dispersed) versus the chemically-enhanced WAF (CEWAF) of weathered Prudhoe Bay Crude Oil (PBCO) on adult and embryonic topsmelt after a 96-h exposure. CEWAF had higher PAH concentrations when compared to WAF. Exposure to WAF and CEWAF resulted in a greater number of significant changes in embryos directly exposed compared to adult muscle tissue. Embryonic exposure to WAF affected several amino acids and organic acids, whereas CEWAF impaired the levels of glutamine and glutamate. Adult muscle tissue only exhibited a significant decrease in lactate and phosphocreatine levels when exposed to WAF and CEWAF, respectively. Based on these findings, physically- and chemically-dispersed oiled seawater may affect the energy production pathways of topsmelt.

The discharge of diluted bitumen (dilbit) from pipelines poses a threat to the health of aquatic biota (Alderman et al., 2020). Limited understanding persists regarding the consequences of dilbit exposure to evaporative weathering and sediment binding, as well as its toxic mechanisms. To address this, Fujita et al. (2021) delved into the mechanisms of zebrafish embryotoxicity following exposure to the water-soluble fraction of dilbit derived from sediment (SDWSF) using a metabolomics approach. Zebrafish embryos exposed to the SDWSF revealed several developmental malformations. In addition, embryos exposed to SDWSF experienced significant alterations in the concentrations of nine different metabolites. These metabolites consisted of seven amino acids, one purine nucleoside, and one sugar alcohol. These results indicated potential perturbations in amino acid metabolism caused by exposure to dilbit which can be key events in the development of zebrafish embryos.PAHs and mercury (Hg) were found in concentrations exceeding international regulatory guidelines on sediments collected from coastal zones near industrial installations (Di Leonardo et al., 2007). Fasulo et al. (2012) carried out a study to assess the metabolic responses of the digestive gland tissue of mussels (*Mytilus galloprovincialis*) caged in anthropogenic-impacted areas and reference sites along the Augusta coastline in Sicily, Italy. Chemical analysis revealed elevated levels of PAHs in the digestive gland of mussels from the industrial area compared to the control group. Mussels caged in the polluted site exhibited a significant increase in the levels of several amino acids, two nucleotides (thymidine and adenine) and one organic acid (acetoacetate) compared to mussels caged in the reference site. Changes in the metabolites associated with energy metabolism suggested a shift towards anaerobic fermentation, possibly resulting in reduced utilization of metabolites in the TCA cycle. Furthermore, the rise in acetoacetate levels indicated a potential disruption in lipid metabolism. Cappello et al. (2013) also conducted a study to investigate the metabolic responses of gill tissues of mussels (*Mytilus galloprovincialis*) caged in at the same petrochemical area and reference site.

Metabolite analysis on gill tissues demonstrated significant changes in the levels of several amino acids (e.g., alanine, glycine), metabolites involved in energy metabolism (e.g., glucose, glycogen, ATP, and ADP), osmolytes (hypotaurine, taurine, betaine, and homarine) and a neurotransmitter (acetylcholine). These alterations indicated potential disturbances in energy metabolism, osmotic regulation, and neurotransmission. Later, Cappello et al. (2017) performed a study to elucidate the biological effects of petrochemical contamination on the posterior adductor muscle of mussels (*Mytilus galloprovincialis*) caged in the same eastern Sicily petrochemical area. Similar to the previous study on gill tissues, the posterior adductor muscle revealed significant changes in several amino acids, osmolytes (hypotaurine, betaine, taurine, and homarine), metabolites participating in the energy metabolism (malonate, glycogen, glucose, and ATP/ADP), and nucleotides (inosine, and uracil). This metabolomics study highlighted disturbances in energy metabolism, amino acids metabolism, and osmoregulatory processes after environmental petrochemical contamination, demonstrating the need for an active biomonitoring strategy for environmental risk assessment.

Atlantic killifish (*Fundulus heteroclitus*) from the Elizabeth River system (Virginia, USA) has evolved resistance to high concentrations of PAHs present in the creosote sediment. Reported adaptations to PAH exposure in Elizabeth River killifish subpopulations include modifications in the aryl hydrocarbon receptor pathway and up-regulation of antioxidant defence systems (Di Giulio and Clark, 2015). Redfern et al. (2021) conducted a study on the Atlantic killifish to evaluate a potential relationship between shifts in the commensal microbiome and organismal physiology associated with evolved resistance to PAHs. Adult killifish were collected from a highly PAH-contaminated site and a reference site in Virginia, USA. Metabolomic analysis of the killifish intestine samples from the PAH-contaminated site revealed higher levels of sphingolipids and glycerophospholipids. In contrast, a trend for significantly higher levels of acylcarnitines and amino acids was observed in the fish collected at the reference site. These findings suggested a down-regulation in the protein metabolism and alterations in signalling and lipid metabolism in the intestines of fish subpopulations exposed to high concentrations of PAHs. Moreover, changes in sphingolipid levels were possibly linked to shifts in the gut microbial community of PAH-resistant fish.

3.2.3. Human studies

Humans are frequently exposed to intricate combinations of PAHs and various chemicals through different routes, which renders it impracticable to evade their exposure. Sources of exposure to these compounds are assorted and include breathing indoor/outdoor air, foods contaminated with natural or anthropogenic PAHs, cigarette smoking, inhaled smoke from open fireplaces, and volatile fossil fuels, among others (Zhang et al., 2015). Typically, humans are also exposed to a wide range of other deleterious chemicals, including dibenzo-*p*-dioxins (PCDDs), polychlorinated biphenyls (PCBs), dibenzofurans (PCDFs) and naphthalenes (PCNs), heavy metals, and pesticides (Jarvis et al., 2014). Continued exposure to these complex mixtures can result in common complex diseases and additive/synergistic health effects (Chan et al., 2006; Chen et al., 2019; Nadal et al., 2004). Multiple studies have established a significant connection between human exposure to mixtures of PAHs and the onset of various health conditions, such as respiratory disorders, lung cancer, and cardiovascular diseases (Chen et al., 2017, 2019). Table 3 demonstrates how various studies have applied metabolomics techniques to identify the association between exposure to different sources of PAH-pollution and the potential impact on human well-being.

Living close to petrochemical complexes can negatively affect the health of nearby residents. These facilities release a variety of harmful pollutants that can contaminate the air, water, and soil, leading to a multitude of health issues such as respiratory illnesses, cardiovascular diseases, and even cancer (Yuan et al., 2018, 2020a, 2020b). Wang et al. (2015) investigated, for the first time, the human metabolic responses to chronic environmental PAH exposure. The study focused on a cohort of healthy volunteers divided into exposed and control groups. The exposed group comprised volunteers living in a polluted area near a large coking plant, whereas the control group comprised individuals from a nonpolluted area. The analysis of PAH exposure biomarkers in urine samples confirmed higher levels of PAH pollutants in the exposed group compared with the control. The metabolomic analysis revealed significant alterations in the levels of urinary metabolites participating in the amino acid, purine, lipid, and glucuronic acid metabolisms in the exposed group compared with control. The authors suggested that chronic environmental exposure to PAHs leads to oxidative stress-related effects in humans.

Several studies investigated simultaneously the urinary levels of biomarkers of exposure to industrial pollutants and metabolome perturbations in individuals living in the vicinity of a large petrochemical complex (No. 6 Naphtha Cracking Plant) in Taiwan (Chen et al., 2017, 2019, 2021; Yuan et al., 2016). In this context, Yuan et al. (2016) considered a cohort of individuals living within a 10 km radius of the petrochemical complex (high-exposure group) and gender- and age-matched individuals living farther than the 10 km radius. The results revealed higher urinary levels of exposure and stress biomarkers in the high-exposure group compared with the low-exposure group. On the other hand, the metabolomic analysis showed that the high-exposure group had significantly lower levels of amino acids and carbohydrates in their blood plasma compared to the low-exposure group. This decline could potentially be attributed to the activation of peroxisome proliferator-activated receptor (PPAR) and insulin signalling and the occurrence of oxidative/nitrosamine stress. Later, Chen et al. (2017) assessed the potential health impact of air pollutants in the urine metabolome of children and elderly individuals living near the same petrochemical complex in Taiwan. They found age-dependent dysregulations in metabolic pathways, including tryptophan and phenylalanine metabolisms in children, and glycine, serine, and threonine metabolism in elderly individuals. Through the analysis of urinary levels of different biomarkers of exposure and early health effects, the authors were able to link these metabolic dysregulations with multiple air toxics and oxidative stress. Chen et al. (2019) conducted an additional study to identify the disruptions in blood serum metabolism that arise from exposure to carcinogens in the same petrochemical complex. This study focused on a group of children and adolescents, who resided in the petrochemical area for over 5 years. The urinary concentrations of biomarkers related to carcinogen exposure, oxidative stress and early health effects were also measured. The authors found alterations in several metabolic pathways related to exposure to carcinogens (IARC group 1 and 2), increased oxidative stress and early health effects. These metabolic pathways included the purine metabolism, TCA cycle, fatty acid metabolism, and glutathione metabolism. In another study, Chen et al. (2021) investigated the lipidome alterations in the serum of children and adolescents living near the petrochemical complex. The researchers identified 15 potential lipid features associated with multiple industrial pollutants, as well as increased oxidative stress and early health effects. These lipid features included several lysophosphatidylcholines (LPCs), phosphatidylcholines (PCs) sphingomyelins (SMs), and phosphatidylinositols (PIs) that were found dysregulated in the high-exposure group compared to the low-exposure group. The four studies collectively present compelling new evidence that sheds light on the profound effects of air pollution on human health and the intricate metabolic mechanisms associated with environmental exposure.

Superfund sites in the United States are locations that have been severely impacted by hazardous waste and require immediate attention for extensive cleanup efforts by EPA (Kiaghadi et al., 2021). The list of contaminants of concern at Superfund sites across the United States includes PAHs. Based on this, Suter et al. (2019) investigated a potential relationship between high exposure to PAHs and an increased rate of preterm birth in women living near Superfund sites. The researchers

Table 3
Human metabolomic studies focused on exposure to PAH-pollution sources.

PAH exposure source	Groups of individuals under study	Cohort size	Biological matrix	Analytical platform	Main results	Reference
Coking industry	- Residents in an area polluted by the coking industry (exposed group) - Residents in a nonpolluted area (control)	Exposed group: n = 142 elderly non-smokers n = 79 elderly smokers n = 148 children Control group: n = 96 elderly non-smokers n = 35 elderly smokers n = 66 children	Urine	LC-MS	Exposed group vs. control: ↑ 3-Methylhistidine; pyroglutamic acid; 2-isopropylmalic acid; azelaic acid; decenedioic acid; hydroxytetradecanedioic acid; decenedioylglucuronide; heptenedioylcarnitine; octenedioylcarnitine; nonenedioylcarnitine; 3-hydroxydecanoylcarnitine; dodecanedioylcarnitine; nonanoylcarnitine; decadienylcarnitine; hydroxydodecenoylcarnitine; dodecadienylcarnitine; dodecenoylcarnitine ↓ Uric acid Exposed group showed putative alterations in amino acid, purine, lipid, and glucuronic acid metabolisms	Wang et al. (2015)
Petrochemical complex	Residents near a petrochemical complex: - High-exposed individuals living within a 10 km radius of the petrochemical complex and with high urinary levels of vanadium and 1-hydroxypyrene - Low-exposed individuals living farther than a 10 km radius	High-exposed n = 80 Low-exposed n = 80	Plasma	^{1}H NMR	High-exposed vs. low-exposed: ↑ Isoleucine ↓ Alanine; glutamine; phenylalanine; α-glucose; β-glucose; N-acetyl glycoprotein; –CH=CH- resonance of lipids -High-exposed group revealed putative alterations in amino acid and carbohydrate metabolisms potentially associated with elevated oxidative/nitrosative stress responses	Yuan et al. (2016)
Petrochemical complex	Residents near a petrochemical complex: - High exposed individuals living close to the complex with urine concentrations of vanadium and 1-hydroxypyrene in the top 60 % - Low-exposed individuals living further away, with urine concentrations of vanadium and 1-hydroxypyrene in the bottom 40 %	High-exposed: n = 40 children (ages 9–15) n = 71 elderly (ages >55) Low-exposed: n = 70 children n = 71 elderly	Urine	GC × GC-TOF-MS	High-exposed vs. low-exposed children: ↑ Hydrocarbons; lipids; organonitrogen compounds; phenylpropanoids and polyketides ↓ Benzenoids; organic acids; organoheterocyclic compounds; organooxygen compounds High-exposed children showed putative alterations in tryptophan metabolism and phenylalanine metabolism High-exposed vs. low-exposed elderly individuals: ↑ Benzenoids; hydrocarbons; lipids; organic acids; organoheterocyclic compounds; organonitrogen compounds; organooxygen compounds; organosulfur compounds High exposed elderly individuals revealed putative perturbations in glycine, serine, and threonine metabolism	Chen et al. (2017)
Petrochemical complex	Residents near a petrochemical complex: - High-exposed individuals living close to the complex with urine concentrations of vanadium and 1-hydroxypyrene in the top 60 % - Low-exposed individuals living further away, with urine concentrations of vanadium and 1-hydroxypyrene in the bottom 40 %	High-exposed: n = 37 children and adolescents (ages 9–15) Low-exposed: n = 70 children and adolescents (ages 9–15)	Serum	UHPLC-MS	High-exposed vs. low-exposed: ↑ Ketoleucine; carnitine; isovalerylcarnitine; aspartic acid: octenoyl-L-carnitine ↓ Pyroglutamic acid; adenosine monophosphate; inosinic acid; oxoglutaric acid; malic acid High exposure was associated with putative alterations in the TCA cycle, purine, fatty acid, and glutathione metabolisms	Chen et al. (2019)
Petrochemical complex	Residents near a petrochemical complex: - High-exposed individuals living close to the complex with urine concentrations of vanadium and 1-hydroxypyrene in the top 60 % - Low-exposed individuals living further away, with urine concentrations of vanadium and 1-hydroxypyrene in the bottom 40 %	High-exposed: n = 37 children and adolescents (ages 9–15) Low-exposed: n = 70 children and adolescents (ages 9–15)	Serum	UHPLC-MS lipidomics	High-exposed vs. low-exposed: ↑ Hexanoylcarnitine; lysophosphatidylcholines (LPC 18:1); sphingomyelins [SM (d18:1/22:0); SM (d18:1/25:0)]; phosphatidylcholines (PCs) [PC (16:0/20:1); PC(18:2/20:5); PC(18:2/14:0); PC (18:2/17:1)] ↓ Dodecanoylcarnitine; tetradecanoylcarnitine; pentadecanoylcarnitine; hexadecenoylcarnitine; linoleylcarnitine; pristanoylcarnitine; phosphatidylinositols [(PI(32:1)); PI(34:3)]	Chen et al. (2021)
Superfund site	Women living near superfund sites in Harris County	Preterm birth (<37 weeks) n = 22 Term birth (≥37 weeks) n = 20	Placental tissue (polar extracts)	UHPLC-MS	Preterm birth vs term birth: Alterations in the levels of 81 metabolites including several carbohydrates and carbohydrate conjugates; phenols; lipids and lipid-like compounds; tryptamines and derivatives; steroids and steroid derivatives; benzenoids Preterm birth was associated with putative alterations in pentose phosphate pathway, inositol phosphate metabolism, and starch and sucrose metabolism	Suter et al. (2019)
Air pollution	Healthy young adults who were exposed to elevated PAHs after	n = 26 young adults	Serum	UHPLC-MS	Metabolites associated with the urinary levels of monohydroxylated phenanthrenes:	Lu et al. (2021)

(*continued on next page*)

Table 3 (*continued*)

PAH exposure source	Groups of individuals under study	Cohort size	Biological matrix	Analytical platform	Main results	Reference
	travelling from Los Angeles to Beijing (air air-polluted site)	Three-time points of sample collection: LA-before – 1–3 weeks before departing LA During – 6–8 weeks after the arrival in Beijing LA-after – 4–7 weeks after returning to LA			↑ Inosine; ↓ Pyroglutamic acid; taurine; lysophosphatidylcholine (O-16:0); methionine; tryptophan Metabolites associated with the urinary levels of and 2-phenanthrenecarboxylic acid: ↓ Pyroglutamic acid The metabolic dysregulations were associated with an oxidative homeostasis-related response and delayed enzymatic deinduction response	
Motor vehicle exhaust	Urban underground parking garages (UPG) workers exposed to particle-bound PAHs and non-UPG workers	UPG workers n = 20 (ages 29–63) Non-UPG workers n = 20 (ages 22–73)	Urine	UHPLC- MS	UPG workers vs. non-UPG workers: ↑ *p*-Cresol; stearic acid; palmitic acid; 2-phenylbutyric acid; ↓ Lactose; galactonic acid; statil; 2(1H)-pyridinone; butyric acid; pyruvaldehyde; histidylvaline; biopterin; lactate; aminoadipic acid; 5-methylcytosine UPG workers revealed dysregulations in urinary metabolites putatively participating in the metabolism of amino acids, nucleotides, carbohydrates, lipids, and cofactors and vitamins	Wu et al. (2021)

[1]H-NMR: proton nuclear magnetic resonance spectroscopy; LC-MS: liquid chromatography-mass spectrometry; GC × GC-TOF-MS: two-dimensional gas chromatography-time-of-flight mass-spectrometry; UHPLC-MS: ultra-performance liquid chromatography–mass spectrometry; UPG: underground parking garages; ↑ metabolites found up-regulated (significantly increased levels); ↓ metabolites found down-regulated (significantly decreased levels).

measured the levels of PAHs and PAH-DNA adducts in the placenta from preterm and non-preterm deliveries. The metabolomic analysis was also performed in the placental tissue. Indeed, the placenta of preterm deliveries showed higher levels of PAHs (benzo[a]pyrene, benzo[b]fluorene, dibenz[a,h]anthracene) and PAH-DNA adducts compared with term deliveries for women living near Superfund sites. Preterm placental tissues also revealed alterations in pentose phosphate, inositol phosphate and starch and sucrose pathways in women living near Superfund sites. These findings suggested that residing close to Superfund sites can potentially increase the risk of preterm birth.

Different countries have significant differences in the level of PAH pollution, with China being identified as the leading global emitter (Zhang and Tao, 2009). Lu et al. (2021) identified a unique opportunity for a natural experiment involving healthy young adults who experienced elevated PAHs for 10 weeks after travelling from Los Angeles (LA) to Beijing. Serum and urine samples were collected from the same individuals before, during and after the travel. Urine was analysed for the detection of both unsubstituted and alkylated PAHs, while an untargeted metabolomics analysis was carried out on serum samples. Several metabolites changed significantly 6−8 weeks after the travel to Beijing. From these, four amino acids, one lysophosphatidylcholine, and one purine nucleoside were found to be linked to the levels of monohydroxylated phenanthrene molecules in urine. Additionally, one amino acid was found to be associated with the urinary levels of 2-phenanthrenecarboxylic acid. These findings indicate that unsubstituted and alkylated PAHs have different subacute effects. Although no significant association was found with urinary PAH levels, significant changes in the levels of other metabolites were observed between the three collection time points. The changes in the serum metabolic profiles of these travellers indicate a response related to oxidative homeostasis and a delayed enzymatic deinduction response.

Vehicle exhaust is a significant cause of air pollution that has been associated with a variety of adverse health effects on humans, including diabetes (Riva et al., 2018). Particle-bound PAHs may contribute to the development of diabetes, but the exact mechanisms are still not fully understood. Wu et al. (2021)investigated the potential pathological mechanism underlying diabetes attributed to vehicle air pollution by examining the levels of particulate matter and PAHs in three underground parking garages (UPG), along with the urinary metabolome and unmetabolized PAHs of both UPG and non-UPG workers. The urine samples of UPG workers showed significantly higher levels of unmetabolized 5–6 ring PAHs compared to those of non-UPG workers. The UPG workers also exhibited significant dysregulations in the levels of metabolites involved in several metabolic pathways, including amino acid, nucleotide, carbohydrate, lipid, and cofactors and vitamins metabolisms. Functional enrichment analysis revealed that most dysregulated metabolites participate in carbohydrate and lipid metabolisms. Scientists discovered a connection between disturbances in carbohydrate metabolism and the hypoxia-inducible factor 1 (HIF-1) signalling pathway, although further investigation is necessary. These results suggested that particle-bound PAHs from vehicle air pollution could be a contributing factor to diabetes among garage staff, road workers, and drivers.

3.2.4. Plants

Plants possess an extraordinary capacity to generate a diverse range of metabolites that surpasses that of any other organism group. With their unique features, they are capable of synthesizing secondary metabolites to adapt to both abiotic and biotic stressors (Feizi et al., 2020; Sivaram et al., 2019), and effectively remediating contaminated soils (Sivaram et al., 2020). Metabolomics has been employed for the rapid and reproducible identification of plant metabolites and their associated metabolic dysregulations upon exposure to PAHs. Table 4 displays the outcomes of two metabolomic studies carried out on plants, which aimed at investigating the effects of exposure to PAHs.

Phytoremediation, the use of plants to degrade PAHs and other contaminants, is widely acknowledged as a sustainable and cost-effective approach (Kuppusamy et al., 2017; Sivaram et al., 2020). The uptake of PAHs and similar organic compounds by plants involves two crucial steps (Collins et al., 2006). Initially, there is an equilibrium established between the chemicals in the aqueous solution and the plant roots. Subsequently, the chemicals are absorbed by lipophilic root solids, such as cell membranes and walls. Studies using fluorescent microscopy have revealed that PAHs have a particular affinity for the lipid bodies within the cells (Subashchandrabose et al., 2014). Nevertheless, the potential influence of PAHs on the regulation of plant metabolism has yet to be fully investigated. Sivaram et al. (2019), examined the impact of exposure to high molecular weight PAHs (benzo[a]pyrene and

Table 4
Plant metabolomic studies focused on the effects of exposure to PAH mixtures.

PAH under study	Concentration(s)/ Exposure duration	Plant (scientific name)	Analytical platform	Main results	References
Benzo[a]pyrene Pyrene Binary mixture of benzo[a] pyrene and pyrene (1:1)	9.8 mg L^{-1} benzo[a] pyrene 9.7 mg L^{-1} pyrene /7 days	Maize (*Zea mays* L)	GC-MS (polar metabolites)	Benzo[a]pyrene exposure vs. control: ↑ Tyrosine; tryptophan; acetylcholine Pyrene exposure vs. control: ↑ Glutathione; proline ↓ Methionine; 2-oxosuccinamate Binary mixture exposure vs. control: ↑ Phenylalanine; proline; glycolic acid; tryptamine; glutamyl-threonine; glutamyl-glycine; methionine; 2-oxosuccinamate ↓ Tryptophan; hydroxypyruvate; pyrimidine; cysteamine	Sivaram et al. (2019)
Mixture of pyrene, anthracene, acenaphthylene, and acenaphthene	0, 10, 50, and 100 μgL^{-1} of four PAHs /39 days	Lettuce (*Lactuca sativa* L)	^{1} H NMR (polar metabolites)	PAH mixture exposed vs. control: Alterations in the levels of organic acids (methylmalonic acid; gluconic acid; 3-methyl-2-oxovaleric acid), and sugars (galactose; mannose; arabinose; glucose; mannose; melibiose; ribofuranose; sucrose; sorbose; tagatose; xylose; trehalose; glycerol; meso-erythritol; ribitol)	Feizi et al. (2020)

GC-MS: gas chromatograph-mass spectrometry; ^{1}H NMR: proton nuclear magnetic resonance spectroscopy; ↑ metabolites found up-regulated (significantly increased levels); ↓ metabolites found down-regulated (significantly decreased levels).

pyrene), both individually and when combined in a mixture, on the metabolome of maize (Zea mays L.). Two amino acids (tyrosine and phenylalanine) and acethylcholine were found significantly up-regulated after benzo[a]pyrene exposure compared with the control. Contrasting, pyrene exposure induced a significant up-regulation of glutathione and proline, and down-regulation of methionine and 2-oxo-succinamate. These results revealed an adaptation behaviour of maize to survive the stress conditions caused by PAH exposure. The binary mixture exposure resulted in a greater number of noteworthy changes, with a significant increase in the levels of various amino acids, while the levels of hydroxypyruvate, tryptophan, and pyrimidine were significantly decreased. The findings demonstrated that the combined PAH mixture is significantly more toxic than its individual compounds. Moreover, the results provided compelling evidence of the detrimental impact of PAHs on the growth and development of plants. This knowledge has the potential to greatly impact the field of phytoremediation.

In the field of molecular ecotoxicology of plants, it is also crucial to address the issue of bioaccumulation of PAHs in vegetables, particularly when they are cultivated in fields irrigated with treated wastewater. Feizi et al. (2020) conducted a comprehensive metabolomic analysis to assess the impact of four PAHs (pyrene, anthracene, acenaphthylene, and acenaphthene) present in irrigation water on lettuce (*Lactuca sativa* L*)*. Their objective was to ascertain the specific metabolites that were influenced by these pollutants. The exposure to PAH influenced the levels of various organic acids and sugars. However, the statistical significance of each metabolite and their direction of variation were not reported. These results suggested that PAH exposure may influence the nutritional composition of lettuce.

3.2.5. *Microorganisms in soils*

Due to their hydrophobicity, PAHs can accumulate and persist in soil, sediments and rhizosphere (Martins et al., 2008). This pollution not only poses a serious threat of cancer but also exposes organisms to potential birth defects and genetic abnormalities (Gao et al., 2018b; Yan et al., 2004). Soil quality can be assessed by the presence of microorganisms since these are the most delicate and promising indicators (Li et al., 2019). Metabolomic techniques have been used to identify how microorganisms react to soil conditions when exposed to PAHs. Table 5 showcases three studies that have been conducted concerning this subject.

Table 5
Soil metabolomic studies focused on the effects of exposure to PAH mixtures.

PAHs under study	Concentration(s)	Treatment groups	Analytical platform	Main results	References
Ternary mixture of phenanthrene, pyrene and benzo[a]pyrene	50 mg kg^{-1} phenanthrene 50 mg kg^{-1} pyrene 10 mg kg^{-1} benzo [a]pyrene	PAH-contaminated soils	GC-MS	Ternary mixture exposure: ↑ Cholestan-3beta-ol; 3-hydroxybutyric acid; 2-ketoadipate ↓ Melbiose; isomaltose; *p*-anisic acid;	Li et al. (2019)
Ternary mixture of phenanthrene, pyrene and benzo[a]pyrene	50 mg kg^{-1} phenanthrene 50 mg kg^{-1} pyrene 10 mg kg^{-1} benzo [a]pyrene	**R:** rhizosphere soil; **BC-R:** biochar amended rhizosphere soil; **BC:** non-rhizosphere soil. $n = 3$ per group	GC-MS	BC-R after exposure to the ternary mixture: ↑ 9-Fluorenone; benzoin; *p*-anisic acid; 2-ketobutyric acid; monostearin; glucose 1-phosphate	Li et al. (2020a)
Ternary mixture of phenanthrene, pyrene and benzo[a]pyrene	10 mg kg^{-1} phenanthrene 10 mg kg^{-1} pyrene 5 mg kg^{-1} benzo[a] pyrene	**C-NR:** absence of rhizosphere **B-NR:** absence of rhizosphere and addition of biochar **C–R:** presence of rhizosphere **B–R:** presence of rhizosphere and biochar amend $n = 3$ per group	GC-MS	C–R after exposure to the ternary mixture: ↑ Carbohydrates; benzoic acids B–R after exposure to the ternary mixture: ↑ Carbohydrates; benzoic acids; saturated fatty acid; lipid-like compound; ↓ Organic acids B-NR after exposure to the ternary mixture: ↑ Saturated fatty acid; lipid-like compound; uridine; amino acids ↓ Organic acids	Li et al. (2020b)

GC-MS: gas chromatograph-mass spectrometry; ↑ metabolites found up-regulated (significantly increased levels); ↓ metabolites found down-regulated (significantly decreased levels).

Soil microorganisms can degrade PAHs, effectively transforming these potentially harmful compounds into less toxic substances. This process is known as biodegradation, and it relies on the utilization of enzymes capable of breaking down the aromatic rings within PAH molecules (Kadri et al., 2017). However, soil microorganisms may be negatively impacted by exposure to high levels of PAHs. Li et al. (2019) studied the response of soil microorganisms to fresh PAH stress in PAH-contaminated soils. The study showed that high concentrations of PAHs can reduce microbial diversity and activity, and alter the composition of microbial communities in soil. Soil metabolomics additionally revealed decreased levels of melbiose, isomaltose, and *p*-anisic acid, and increased levels of cholestan-3beta-ol, 3-hydroxybutyric acid, and 2-ketoadipate upon exposure. These findings revealed that PAH exposure can induce cascading effects on soil health and ecosystem functioning.

Some studies have demonstrated that biochar possesses the potential to effectively combat the detrimental effects of pollutants on soil (Lehmann et al., 2011). Biochar is a special type of charcoal created by heating organic matter without the presence of oxygen. The process turns the material into a stable form of carbon that has many advantages when used as a soil amendment. Biochar has been shown to improve soil quality, nutrient retention, and water-holding capacity, among other benefits. A study was conducted by Li et al. (2020a) to investigate the effect of biochar and plant roots (rhizosphere) on bacterial responses to fresh PAH stress in agricultural soils. The authors employed a combination of enzyme activity tests, high-throughput sequencing, and soil metabolomics. This study indicated that combining biochar with plant roots can significantly boost the ability of soil bacteria to handle the stress caused by PAH contamination. In addition, considerable changes in the metabolomic profiles of the soil were found, with particularly noticeable alterations occurring in amino acids, starch, and sucrose. Overall, the study indicated that when biochar is combined with plant roots, it can create a favourable environment for soil bacteria. As a result, the bacteria become more resilient and effective in breaking down PAH. Later, Li et al. (2020b) conducted another study aiming to characterize the degradation of PAHs by combining direct PAH degradation with soil carbon cycling. The study tested if the combination of rhizosphere and biochar could improve PAH degradation and affect soil carbon metabolism. Three treatment conditions were considered: non-rhizosphere soil with no biochar amendment (C-NR), non-rhizosphere soil with biochar amendment (B-NR), rhizosphere soil without biochar amendment (C–R), and rhizosphere soil with biochar amendment (B–R). The study found that B-NR affected the levels of glycerophospholipids and glycerolipids, while C–R influenced the levels of amino acids. On the other hand, B–R affected both amino acid and lipid levels. Furthermore, plant roots had a substantial impact on carbohydrate levels in biochar-amended soil. Overall, the study results suggested that the joint action of biochar and plant roots can accelerate carbon metabolism contributing to PAH biodegradation.

4. Concluding remarks

Metabolomic studies have the potential to provide a plethora of information for ecotoxicological research that cannot be obtained through traditional methods. This review provides crucial insights into the multifaceted impacts of PAH exposure in cells, animals, humans, plants, and microorganisms in soils. These findings collectively demonstrate that PAH mixtures can elicit cellular stress responses through alterations in cellular metabolites related to lipid, amino acid, steroid, and energy metabolisms. Furthermore, they manifest systemic effects on host physiology, disrupting the levels of amino acids, carbohydrates, energy metabolites, lipids, osmolytes, and metabolites associated with the gut microbial community in aquatic organisms. The specific dysregulations varied depending on the organism species and the PAH compounds involved in each study. The detection of dysregulated metabolites in human samples from individuals exposed to different PAH-pollution sources offers crucial insights into possible health hazards. The evidence found in these studies indicates that PAH-induced metabolic dysregulations in humans include changes in amino acid, carbohydrate, and lipid profiles potentially associated with cellular damage, oxidative stress mechanisms, and disruption of signalling pathways. Plants exposed to PAH mixtures undergo shifts in metabolites involved in stress responses and defence mechanisms against oxidative damage. Soil metabolomic studies revealed significant changes in the metabolism of microbial communities and the cycling of nutrients, emphasizing the impact of PAH contaminants on the essential functions of soil microorganisms and the overall processes of the ecosystem. Overall, this study underscores the crucial role of monitoring and mitigating PAH pollution for the protection of environmental, animal and public health. Comprehending the metabolic and ecological impact of PAH exposure is crucial for developing precise interventions and policies to minimize the harmful effects of these pollutants.

While the reviewed studies offer valuable insights, it is crucial to acknowledge their limitations. The use of human tumour cell lines in *vitro* studies has the potential to limit the applicability of findings to primary cells. The current research has mainly concentrated on aquatic organisms (water fleas, rainbow trout alevins, mussels, topsmelt, zebrafish, and Atlantic killifish). However, it is crucial to extend these investigations to include a broader range of species such as rats, birds, and insects. By adopting this broader approach, we can gain a more comprehensive understanding of the ecological consequences of these contaminants. In addition, the utilization of a single analytical platform for metabolomics analysis imposed inherent limitations, which restricted the number of detectable metabolites and hindered a comprehensive understanding of the metabolome. Furthermore, the application of databases for annotating metabolites can give rise to concerns regarding the accuracy of the identification process. As a result, the reliability of the identified metabolic disruptions may be compromised. Moreover, the published studies were constrained by limited sample sizes, impacting the robustness of their findings. These limitations should be considered in the interpretation of PAH-induced metabolic dysregulations. Furthermore, the lack of standardized protocols and methodologies across different studies introduces variability and creates challenges when comparing and integrating the findings from different research groups. Therefore, future studies must establish standardized procedures that ensure reproducibility and comparability of results, thus enhancing the reliability and validity of the observed metabolic dysregulations in the context of PAH exposure. In addition, by integrating additional “omics” technologies, such as proteomics and genomics, we can strengthen the relationship between exposure to PAHs and the resulting biological effects. Enhancing our understanding of the impact of PAH exposures on metabolic disruptions is crucial for developing effective risk assessment strategies and environmental remediation efforts.

Funding

This research was funded by national funds from FCT-Fundação para a Ciência e a Tecnologia, I.P., in the scope of the PAHMIX project - Mixtures of Environmental Carcinogens: a molecular approach to improve environmental risk assessment strategies (PTDC/CTA-AMB/29,173/2017), and the Research Unit on Applied Molecular Biosciences–UCIBIO (projects UIDP/04378/2020 and UIDB/04378/2020), the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2020) and the MARE - Marine and Environmental Sciences Centre Strategic Project (UIDB/04292/2020) and the Associate Laboratory ARNET (LA/P/0069/2020). PGP is supported by a research contract (under Scientific Employment Stimulus) CEECINST/00108/2021/CP2794/CT0001.

CRediT authorship contribution statement

Vânia Monteiro: Writing – original draft, Investigation, Conceptualization. **Diana Dias da Silva:** Writing – review & editing, Supervision. **Marta Martins:** Writing – review & editing, Funding acquisition. **Paula Guedes de Pinho:** Writing – review & editing, Supervision. **Joana Pinto:** Writing – review & editing, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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