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Effects of Polycyclic Aromatic Hydrocarbons on Marine and Freshwater Microalgae – A Review

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Abstract :

The first synthetic review of the PAHs effects on microalgae in experimental studies and aquatic ecosystems is provided. Phytoplankton and phytobenthos from marine and freshwaters show a wide range of sensitivities to PAHs, and can accumulate, transfer and degrade PAHs. Different toxicological endpoints including growth, chlorophyll a, in vivo fluorescence yield, membrane integrity, lipid content, anti-oxidant responses and gene expression are reported for both freshwater and marine microalgal species exposed to PAHs in culture and in natural assemblages. Photosynthesis, the key process carried out by microalgae appears to be the most impacted by PAH exposure. The effect of PAHs is both dose- and species-dependent and influenced by environmental factors such as UV radiation, temperature, and salinity. Under natural conditions, PAHs are typically present in mixtures and the toxic effects induced by single PAHs are not necessarily extrapolated to mixtures. Natural microalgal communities appear more sensitive to PAH contamination than microalgae in monospecific culture. To further refine the ecological risks linked to PAH exposure, species-sensitivity distributions (SSD) were analyzed based on published EC50s (half-maximal effective concentrations during exposure). HC5 (harmful concentration for 5% of the species assessed) was derived from SSD to provide a toxicity ranking for each of nine PAHs. The most water-soluble PAHs naphthalene (HC5 = 650 µg/L), acenaphtene (HC5 = 274 µg/L), and fluorene (HC5 = 76.8 µg/L) are the least toxic to microalgae, whereas benzo[a]pyrene (HC5 = 0.834 µg/L) appeared as the more toxic. No relationship between EC50 and cell biovolume was established, which does not support assumptions that larger microalgal cells are less sensitive to PAHs, and calls for further experimental evidence. The global PAHs HC5 for marine species was on average higher than for freshwater species (26.3 and 1.09 µg/L, respectively), suggesting a greater tolerance of marine phytoplankton towards PAHs. Nevertheless, an important number of experimental exposure concentrations and reported toxicity thresholds are above known PAHs solubility in water. The precise and accurate assessment of PAHs toxicity to microalgae will continue to benefit from more rigorously designed experimental studies, including control of exposure duration and biometric data on test microalgae.

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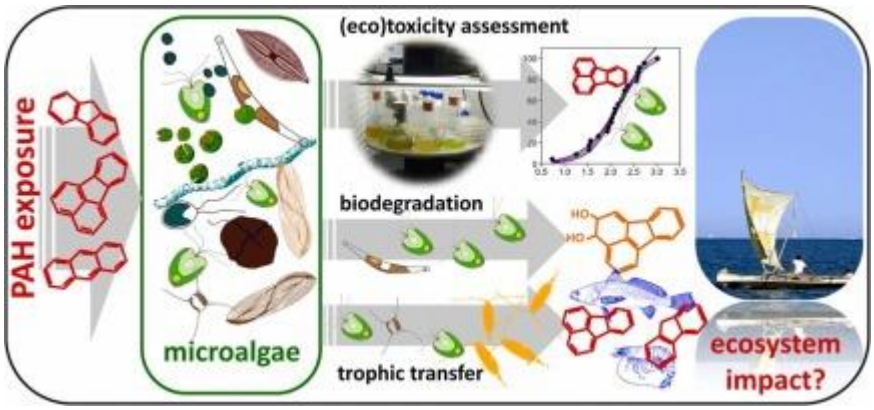
Graphical abstract



Highlights

► First review of Polycyclic Aromatic Hydrocarbons toxicity to microalgae. ► Population, biochemical and structural endpoints are highlighted. ► Species-sensitivity distribution analysis refine PAHs toxic thresholds for microalgae. ► Current knowledge often biased by experimental flaws. ► Further research necessary on these legacy contaminants still of concern.

Keywords : Polycyclic aromatic hydrocarbons, microalgae, phytoplankton, toxicity, species-sensitivity distributions, marine and freshwaters

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1. Introduction

The environmental fate and effects of polycyclic aromatic hydrocarbons (PAHs) have long attracted scientific interest because these pollutants are considered a serious potential threat for wildlife and human health due to their mutagenic, carcinogenic and toxic properties discovered almost a century ago (Cook 1940; Pryde 1934; USPHS 1990), and remain still of major concern (Weinstein et al. 2010). PAHs are a group of organic molecules composed of two or more fused aromatic rings, that comprise a major category of persistent organic pollutants contaminating the atmosphere (Kim et al. 2013; Zhang and Tao 2009), soils (Truskewycz et al. 2019), and both marine and freshwater environments (Alegbeleye et al. 2018; Behera et al. 2018; Hylland 2006;). They are considered priority pollutants in many countries, in particular in the USA and the European Union (Lerda 2011), where sixteen PAHs are listed (Fig. 1) as a major concern for environmental and human health (Samanta et al. 2002). The physical properties of PAHs vary according to their molecular weight and structure (Table 1), having low to very low water solubilities, and low to moderately high vapour pressures. Naphthalene and acenaphtylene (PAHs with two rings) are the most soluble in water (31.0 and 16.1 mg/L in distilled water respectively), and the most abundant compounds accounting for global athropogenic emissions (Shen et al. 2013).

PAHs can enter aquatic ecosystems from natural sources and human activities, the latter mainly by incomplete combustion of fossil and living fuels, industrial and urban runoff, petroleum processing and seepage or accidental oil spills (Boehm and Page 2007; Nizzetto et al. 2008; Van Metre et al. 2000; Yunker et al. 2002). Living biomass combustion can represent locally significant source of PAHs to ecosystems during forest and bush fires (Masclet et al. 1995) with records even from ancient fossil rocks (Boudinot and Sepúlveda, 2020), whereas purely biogenic sources of PAH contamination are limited, perylene being considered as a significant marker of natural origin (Varnosfaderany et al. 2014). In comparison, anthropogenic sources (industrial activities, waste incineration, automotive

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combustion, coal and natural gas exhausts, etc.) are much more important, depending on socio-economic trends at large temporal and spatial scales. During the 1980s, the annual direct release to aquatic environments was estimated as roughly 230 Gg of PAHs worldwide (Eisler 1987). Global historical and forecast trends were further refined by Shen et al. (2013), highlighting a PAHs peak emission to the atmosphere of 592 Gg in 1995, following a constant increase since the 1960's. These authors pointed out that 1) peak emission was delayed for more than twenty years in developing countries compared to developed ones, 2) 60% of the atmospheric emissions in 2007 were due to domestic and industrial biomass burning, and 3) naphthalene and acenaphthylene together accounted roughly for two-thirds of the total PAHs emissions (Shen et al. 2013). Diffuse inputs from atmosphere and terrestrial runoff result in low but measurable concentrations of PAHs in surface and deep waters even in the remote ocean at the ng/L level (Cai et al. 2016) whereas accidental pollution can cause total PAHs transient concentrations to exceed hundreds of µg/L in seawater for example (e.g. reviewed in Boehm and Page, 2007 and Mojiri et al., 2019), due to the high amounts of PAHs in crude and processed oils. These point-source inputs are combined with contamination through non-point source inputs by atmospheric transport and deposition, continental runoffs and river inputs into coastal margins, and subsequent off-shelf export (Castro-Jimenez et al. 2012; Yunker et al. 2002), making PAHs among the most common pollutants present at high concentrations in the global aquatic environment, both continental and marine (Mojiri et al. 2019; Zhang et al. 2021).

Because of their hydrophobic properties, PAHs tend to accumulate in sediments and the latter have traditionally been viewed as a sink for these contaminants in aquatic ecosystems (Douben 2003; Page et al. 1999; Roberts 2012). In sediment they persist and undergo very slow transformation, providing explicit records of human activities in the surrounding environments (Lv et al. 2020, Sutilli et al. 2020). Buried PAHs stocks in marine and freshwater sediments could hence constitute a subsequent source for water column contamination during resuspension (Chen et al. 2020, Jaglal 2020) or bioturbation (Tian et al. 2020), potentially threatening pelagic and benthic organisms. PAHs that spread and persist on water tend to adsorb on supended solids and organic matter (Accardi-Dey and Gschwend 2002), and their lipophilic characteristics enables them to be easily transfered to various compartments in food webs (Baumard et al. 1998) that can eventually contaminate human consumers (Grova et al. 2002; Smith et al. 2001). Uptake and accumulation of PAHs in ecosystem food webs has been frequently highlighted as a major environmental and health concern (Liu et al. 2006; Samanta et al. 2002; Zheng et al. 2016; Balgobin and Singh, 2019).

Elimination and removal of PAHs from aquatic ecosystem relies on both abiotic and biotic processes (Duran and Cravo-Laureau 2016), mainly by volatilization and photo-oxidation, and by bioaccumulation and biodegradation respectively. Photo-oxidation induced by ultraviolet light can be

an important pathway for PAHs elimination in aquatic ecosystems, whereas in sediments its importance is negligible, as it applies only to PAHs exposed to light in the sediment surface layers (Doick et al. 2005; Jones et al. 1989). The exposure of PAHs to sunlight, including UV, results in partially oxidized PAH species some of which can be directly cytotoxic and phototoxic (Duxbury et al. 1997; Huang et al. 1995). Final oxidation products such as quinones, hydroxylated quinones and benzoic acids, can be more toxic than parent PAHs (Huang et al. 1997; Marwood et al. 2003; McConkey et al. 1997; Sargian et al. 2005). Biodegradation by microorganisms also plays a significant role in PAH removal processes in aquatic ecosystems (González-Gaya et al. 2019), and microbial utilization of PAHs as carbon source was suspected early on (Sisler and Zobell 1947). Both prokaryotes and eukaryotes can express enzymatic capacities that allow them to oxidize aromatic and polyaromatic compounds (Cerniglia 1992), and persistent contamination of watersheds is likely to induce shiufts in microbial communities (Liu et al. 2021), making PAHs significant organic source in the aquatic environment (Vila-Costa et al. 2020). Furthermore, alcohols, aldehydes and carboxylic acids have been detected as PAH degradation products by freshwater microalgae (Warshawsky et al. 1990, 1995), and marine phytoplankton (Arias et al. 2016).

Comprehensive reviews regarding the ecotoxicity of PAHs in aquatic ecosystems remain scarce especially considering their ubiquitous occurrence, well-established toxicity (Alegbeleye et al. 2017; Douben 2003; Honda and Suzuki 2020; Hylland 2006; Tian et al. 2020; Wu et al. 2011). The relevance of these compounds for environmental risk assessment and regulation policies is nevertheless evident, and would benefit from the same interest as emerging contaminants in the recent years (e.g. Xin et al. 2021). To the authors' knowledge, there has been no specific review to date addressing the effects of PAHs on the main aquatic primary producers of aquatic ecosystems, namely microalgae[1]. However, a significant number of individual studies have reported the toxicity of PAHs for microalgal species and complex phytoplanktonic or phytobenthic communities, either of marine or freshwater origin, in the laboratory or in ecosystems (e.g. Okumura et al. 2003, Echeveste et al. 2010a, 2010b, 2011, Ben Othman et al. 2012, Su et al. 2022). This interest is warranted by the ecological importance of microalgae, and especially phytoplankton in the open ocean, which account for roughly half of the Earth's annual primary production (Field et al. 1998). Given their importance as a source of oxygen and food to upper trophic levels, as a major sink for anthrophogenic CO_2 as well as for nutrient cycling, microalgae are highly relevant to water pollution issues. Furthermore, the

[1] For convenience, in the present review the term "microalgae" is intended to encompass all the unicellular organisms able to perform oxygenic photosynthesis and sharing chlorophyll *a* as the main active photosynthetic pigment. Therefore, microalgae designate 1) eukaryotic organisms within their respective phyla and cyanobacteria, together with 2) planktonic and benthic organisms referred to as phytoplankton and phytobenthos respectively, when relevant.

global carbon cycle is highly dependant on the structure and diversity of marine phytoplankton communities (Tréguer et al. 2018). The fact that microalgae, mostly autotrophic and unicellular with rapid growth rates, are easily cultivated in the laboratory or manipulated as whole communities in the field has led to their widespread use as model organisms in research and environmental risk assessment (e.g. Maestrini et al. 1984; Nyholm and Källqvist 1989), including normalized standard assays (e.g. ISO technical guidances ISO 8692: 2012, ISO 10253: 2016, and ISO 14442: 2006). A drawback arise from the existence of numerous models of organisms (species, strains, complex communities…), PAH compounds (single molecule, mixtures…), environmental contexts (marine, freshwater, temperate…), together with numerous toxicity endpoints (population, physiology, -omics based…), which make generalization and comparisons difficult.

The focus of this review is to provide an unprecedented extensive and critical synthesis of the known effects of PAHs on microalgae from scientific literature and databases, including both culture model microorganisms and natural communities. The toxicity data gathered and analyzed were based on eco-toxicological studies on microalgal species in culture and on natural communities from marine, freshwater and coastal waters, including benthic and planktonic species. The toxicity of PAHs to population endpoints, physiological activities, enzymatic processes, and genomic markers are reported, together with the documented properties of bioaccumulation and biodegradation when available. For the first time, species sensitivity distributions (SSD) are provided using the available EC_{50} data on individual taxa are presented to conclude on relative toxicity of individual priority PAHs and compare sensitivities among various microalgae, as this approach can support ecological risk assessment of many chemicals (Altenburger et al. 2004, Larras et al. 2015). Finally, the relative toxicity of priority PAHs towards marine and freshwater phytoplankton is compared with knowledge gaps and future directions identified, since the extent of available knowledge remains limited in relation to the number of compounds, species, and environmental conditions encountered.

2. Toxicity of PAHs on microalgae in culture

2.1. Microalgal species-specific differences

Experimental studies are typically designed to measure dose-effect relationships, showing declines in growth or biomass as a function of increasing PAH concentration. The most commonly reported toxicological endpoint was population growth or biomass reached after a given time, measured in batch culture of a single species exposed to increasing doses of the chemical in comparison to controls. This experimental approach constitutes the core of standardized testing of chemicals using microalgae as model organism (OECD, 2006), including determined exposure duration (classically 96

h). Several effect endpoints addressing different biological responses such as photosynthesis, chlorophyll *a* content, fluorescence yield, membrane integrity, lipid content, anti-oxidant responses, protein synthesis and gene expression, have been used to determine their impact on microalgae at different observation levels, from gene regulation to population dynamics. Toxicities are most often reported as EC_{50}s (effective concentration, reducing growth of biomass by 50% relative to control – unexposed conditions) or equivalent, making expressions of toxicities comparable to each other when provided. Only a few PAHs compounds among the sixteen included in the priority list of USA and European Union have been tested for toxicity to microalgae (Table 2, Suppl. Table 1).

2.1.1. Population and biomass endpoints

A database search was performed using the ECOTOX Ver. 5 online database of the U.S. Environmental Protection Agency (Olker et al. 2022), using PAH as chemical family, algae as organisms target, and EC_{50} as reported values. To complete the set of data, a survey as exhaustive as possible of published literature was performed using the search engine provided by the Web of Science (Clarivate Analytics) with ((microalgae* OR phytoplankton) AND (PAH OR polycyclic*)) as search string, and the last query was performed on July, 3rd 2022. Complementary resources were retrieved through direct analysis of cited references (downward search) and articles subsequent citations (upward search). All curated data (n = 253) are reported as supplementary material (Suppl. Table 1).

We report for our literature review a total of 151 complementary evaluations of EC_{50}s for twelve PAHs compounds tested against over sixty different species of microalgae, and for readership's convenience we report on Table 2 only the species for which at least four EC_{50}s evaluations were retrieved (accounting for fifty-six EC_{50}s values). The whole recent literature dataset reports a total of fourteen species from freshwater and 25 from marine origins, exposed to PAHs as a single stressor (no interactions with other environmental parameters, see section 3.2.). Phenanthrene, anthracene and naphthalene were the two compounds for which there was the most data available (20, 19 and 19 respectively). Fluoranthene (17 values) and pyrene (14) are following. The three most studied microalgae in recent literature regarding PAH toxicity and EC_{50} assessment are the marine diatom *Phaeodactylum tricornutm* (n = 13), the marine prymnesiophyte *Isochrysis galbana* (n = 11), and the freshwater chlorophyte *Raphidocelis subcapitata*, recommended in standard testing (OECD, 2006), represented in a balanced manner (n = 12).

Overall, a broad scale of sensitivities to PAHs was reported, with EC_{50}s ranging over up to three orders of magnitude for a given compound (Table 2, Suppl. Table 1). For example, Echeveste et al. (2010a) reported EC_{50}s for pyrene exposure starting from 35 µg/L on the marine cyanobacterium

Prochlorococcus sp. up to 19640 µg/L for the marine diatom *Thalassiosira* sp. during the same study (Supl. Table 1). A similar range was observed for anthracene, with EC_{50}s from 1.62 µg/L on the freshwater cyanobacterium *Microcystis aeruginosa* to 7000 µg/L on the marine chlorophyte *Platymonas helgolandica* (Bi et al. 2015). The second most studied PAH regarding its population effects on microalgae, fluoranthene, exhibited a relatively narrow range of EC_{50}s, from 54 µg/L for the marine picochlorophyte *Picochlorum* sp. (Ben Othman et al. 2012) to 2838 µg/L for the marine diatom *Phaeodactylum tricornutum* (Tato and Beiras 2019).

Considering the sensitivity of the marine diatom *Phaeodactylum tricornutum* to single PAH exposure, the variability of the responses reported depending on the study is obvious. For fluoranthene, the lowest EC_{50} was 103 µg/L (Wang et al. 2008) and the highest 2838 µg/L (Tato and Beiras 2019). The second most studied microalga, the marine prymnesiophyte *Isochrysis galbana*, exhibited a similar range of sensitivities expressed as EC_{50}, from 112 µg/L for fluoranthene to 2220 µg/L for naphthalene (Pérez et al. 2010).

Among the factors that can partly explain differences in species responses to various PAHs, cell size has received particular attention. Surface to volume ratio is amongst the most prominent trait of microalgae (Litchman and Klausmeier 2008); smaller cells are more efficient in retrieving dissolved elements from their surroundings and less likely to sink, and this has been proposed as one key driver of phytoplankton uptake of persistent organic contaminants (e.g. Del Vento and Dachs 2002, Baho et al. 2019). From this biometrical framework, Ben Othman et al. (2012) hypothesized that smaller cells, having a higher surface to- volume ratio, exhibit higher uptake rates for PAHs resulting in higher sensitivities to exposure. Similarily, Echeveste et al. (2010a) concluded that the smallest taxa of the oceanic picoplankton (0.2- 2 µm) *Prochlorococcus* and *Synechococcus* were more sensitive to pyrene exposure compared to larger nanoplanktonic species (2- 20 µm).

A single study (Wang et al. 2020) addressed the influence of PAH exposure on the outcome of competition between two freshwater microalgae in culture. It was reported that, under pyrene exposure, the cyanobacterium *Microcystis aeruginosa* outcompetes the chlorophyte *Chlorella pyrenoidosa*. These authors concluded that PAH contamination in lakes was likely to favour cyanobacterial blooms, with an indirect impairing of aquatic ecosystem health by pollution (Wang et al. 2020)

It is worth underlining the fact that more than a third of the EC_{50}s values reported in Table 2 and Suppl. Table 1 exceed the known solubilities of the tested PAHs (signalled by an asterisk in Table 2, and by red color in Suppl. Table 1). The fact that all these studies nevertheless reported evidence of dose-response patterns might therefore suggest that unsoluble forms of PAHs (either particulate or

colloidal) could pose toxic threats to microalgae. However, this would require further research addressing uptake and adsorption of PAHs to microalgae together with more accurate determination of actual PAH exposure during testing, such as the recently developed passive dosing methodologies (Bragin et al. 2016, Niehus et al. 2018, Kreutzer et al. 2022).

A further enhancement of knowledge about PAHs ecotoxicity to microalgae will rely on determination of mixture toxicity, scarcely addressed to date for these chemicals. Several studies intended to evaluate the potential toxicity of PAHs mixtures on natural communitues (see section 3.2.) whereas only Niehus et al. (2018) and Kreutzer et al. (2022) reported convincing experimental assessment on culture models. The latter study focused on PAHs in sediment pore water, whose composition and concentrations were reproduced using passive dosing, revealing a high toxicity of mixtures compared to single molecule exposure (e.g. Rotondo et al. 2021).

2.1.2. Metabolic, biochemical and structural endpoints

Besides population growth endpoints such as growth rate or achieved biomass, several metabolic endpoints have been addressed upon PAH exposure in microalgal cultures (Table 3 to 5). Photosynthetic function among metabolic parameters (Table 3) and induction of enzymatic stress related mechanisms (Table 4) are the most studied metabolism in response to PAH toxicity, when compared to the limited data on respiration and dinitrogen fixation (a specifc trait of N_2-fixing cyanobacteria). Change in intracellular biochemical composition has also been considered as a suitable marker of PAH-induced stress in microalgae, either as direct evidence of oxidative stress (e.g. intracellular malondialdehyde content), or as a consequence of changes in biosynthesis (e.g. pigments and various metabolic compounds) and used as toxicity evaluation endpoint (Table 5).

Since photosynthesis is a key function of microalgae, its perturbation during exposure to chemicals has been extensively studied. The analysis of *in vivo* variable chlorophyll fluorescence, a non-invasive and inexpensive assessment of photosynthetic process, provides two methods of choice to reveal alteration of photosystem II (PSII) functioning under toxic stress including PAH exposure. The OJIP test (Stirbet and Govindjee 2011), based on the ultra-fast analysis of chlorophyll fluorescence kinetics upon saturating light exposure, showed photosynthetic impairment in the freshwater chlorophyte *Chlamydomonas reinhardtii* under anthracene exposure (Aksmann and Tukaj 2008). From analysis of *in vivo* fluorescence transients, these authors suggested that anthracene toxicity to *C. reinhardtii* was mostly due to cell membrane alteration around PSII, a non-specific mode of action that modified energy allocation and transmembrane proton balance during the photosynthetic process, partly counterbalanced by an increase in respiratory metabolism (Aksmann and Tukaj 2008). Pulse Amplitude Modulated (PAM) fluorescence provides an equivalent proxy of the photosynthetic

efficiency of PSII, expressed as the fluorescence yield F*v*/F*m* (where F denotes fluorescence, *v*: variable, and *m*: maximal; for a complete nomenclature and rationale see Maxwell and Johnson 2000) and is considered sensitive to environmental stress including many chemicals (Dorigo and Leboulanger 2001). Fluorescence yield is equivalent to the average photosynthetic performance of the studied organism (Maxwell and Johnson 2000) and any decrease in the measured yield is expected to indicate a decrease in photosynthetic potential and alteration of PSII integrity. Benzo(a)anthracene, fluoranthene, naphthalene, phenanthrene and pyrene were shown to induce a reduction in fluorescence yield in several species of marine phytoplankton (Table 3). The marine prymnesiophyte *Isochrysis galbana* exposed to four PAHs (pyrene, naphthalene, phenanthrene, and fluoranthene) suffered from photosynthetic shrinkage (Perez et al. 2010), fluoranthene being the most toxic with a significant effect threshold concentration of 112 µg/L (Table 3). Using the same approach, Ben Othman et al. (2012) compared the toxicity of benzo(a)anthracene and fluoranthene on seven marine phytoplankton species and showed that all strains suffered from a significant reduction in F*v*/F*m* at the highest concentrations tested. In their study, the two marine chlorophytes *Nannochloris* sp. and *Picochlorum* sp. were the most sensitive and fluoranthene appeared to have a greater effect on *in vivo* fluorescence than benzo(a)anthracene (Table 3). Depending both on the species and the PAH considered, the functional absorption cross-section of PSII showed either a decrease or increase relative to the controls (Ben Othman et al. 2012). This can be attributed to the differential size adaptation of the PSII antenna and further to the balance between photoautotrophy and heterotrophy as suggested by Aksmann and Tukaj (2008), both contributing to a compensating mechanism that may rescue photosynthetic functioning under moderate PAHs stress.

Photosynthetic activity eventually results in extracellular release of dioxygen O_2, which can be tracked in culture media to detect PAH exposure effects (Table 3). Anthracene exposure resulted in a decline of O_2 production rates in two freshwater chlorophytes *C. reinhardtii* (Aksmann and Tukaj 2008) and *Scenedesmus armatus*, whereas in contrast the latter species was shown by the same authors to increase its O_2 production when exposed to phenanthrene (Aksmann and Tukaj 2004) suggesting a difference in targeted metabolisms. Photosynthetic O_2 production by the marine diatom *Phaeodactylum tricornutum* was consistently lower under exposure to fluorene, naphthalene, and phenanthene (Kusk 1981b, 1981c). This was similarly the case for the freshwater unicellular cyanobacterium *Synechocystis* sp. PCC 6803 exposed to pyrene (Shao et al. 2010). The other facet of photosynthesis, CO_2 fixation, has been studied less often since the required use of stable or radioactive carbon isotopes is more resource and time-consuming, and requires dedicated facilities for radioisotope handling and waste disposal. For two marine diatoms, *Thalassiosira pseudonana* (Andersen et al. 1990) and *P. tricornutum* (Kusk 1981a), the exposure to naphthalene (2 and 10 mg/L

for both species respectively) resulted in decreased inorganic carbon fixation rates by half relative to control cultures.

As it has been suggested from analysis of *in vivo* chl *a* fluorescence transients, PAH exposure is likely to affect aerobic respiration in microalgae. Aksmann and Tukaj (2008) showed that *C. reinhardtii* respiration was enhanced when exposed to anthracene (Table 3), a pattern similarily reported for the diatom *P. tricornutum* exposed either to fluorene, naphthalene, or phenanthrene (Kusk 1981b).

Dinitrogen reduction by N_2-fixing cyanobacteria is another key trait contributing globally to the supply of bioavailable nitrogen in aquatic ecosystems (Canfield et al. 2010). As such, nitrogenase activity has been tested for its sensitivity to PAH exposure. Using the acetylene reduction assay, Bastian and Toetz (1985) showed that nitrogen fixation in the freshwater heterocystous cyanobacterium *Aphanizomenon flos-aquae* was repressed during the exposure to six different PAHs (Table 3).

The diversity of metabolic targets affected by PAHs provides relevant and often sensitive endpoints for exposure and risk assessment, and enzymatic markers of stress compensation were measured upon exposure to PAHs (Table 4). Superoxide dismutase (SOD) is a group of enzymes involved in defense mechanisms against Reactive Oxygen Species (ROS) and is present in all living organisms. As such, SOD induction has been examined in several microalgal species exposed to different PAHs resulting in contradictory results (Table 4). Anthracene, fluoranthene, fluorene and phenanthrene triggered SOD increase in five experiments out of eleven, whereas three occurrences of reduction were reported for the exposure of *Chlorella vulgaris* to 0.1 µg/L phenanthrene (Calderón-Delgado et al. 2020), 1000 µg/L pyrene (Lei et al. 2006) or 1000 µg/L fluoranthene (Tomar and Jajoo 2021). Similarly to SOD, catalases (CAT) are highly active and ubiquitous enzymes involved in ROS scavenging, for which responses to PAH exposure were variable: increases in CAT were reported for *C. vulgaris* under exposure to 10 mg/L fluorene (Ashgari et al. 2020) or 25 mg/L phenanthrene (Ashgari et al. 2018) whereas CAT activity reduction was reported by different authors (Table 4) even for the same species and PAH (Calderón-Delgado et al. 2020). The synoptic assessment of oxidative stress response in four freshwater chlorophytes exposed to pyrene performed by Lei et al. (2006), involving activity measurements of glutathione-S-transferase and glutathione reductase in addition to SOD and CAT, led to a cautionary statement: these authors concluded that the responses of the enzymatic activities studied, in extent and direction, were too variable depending on the species to provide a reliable indicator of pyrene stress in microalgae (Lei et al. 2006).

Induction of stress responses in PAH-exposed microalgae could result in changes in biochemical contents in parallel with enzymatic activities. Namely, glutathione involved in ROS reduction, and

malondialdehyde as a product of lipid peroxidation, are two typical biochemical markers of oxidative stress. Few studies have examined these compounds (Table 4) with conflicting results: glutathione was found unchanged in *C. vulgaris* and *S. quadricauda* exposed to pyrene, whereas an increase in cellular content was reported for the same PAH applied to *Scenedesmus platydiscus* and *Selenastrum capricornutum* (Lei et al. 2006). With a quite similar pattern, malondialdehyde content of *C. Vulgaris*, *S. platydiscus* and *S. capricornutum* was unaffected during pyrene exposure but reduced in *S. quadricauda* (Lei et al. 2006). Conversely, naphthalene was shown to increase malondialdehyde in *C. vulgaris* (Kong et al. 2010) as fluoranthene did for *Phaeodactylum tricornutum* (Wang and Zheng 2008). More unequivocal patterns were retrieved from studies on pigment content: fluoranthene, fluorene and phenanthrene were involved in a decrease in chlorophyll *a* in the marine diatom *Cyclotella caspia* and the chlorophyte *C. vulgaris* (Table 5). The latter species was also affected by a concomitant decrease in chlorophyll *b*, corroborating the overall sensitivity of microalgal photosynthetic apparatus to PAHs. By combining growth inhibition assay and various viability tests using flow cytometry on marine phytoplankton cultures, An et al. (2021) were able to decompose toxic effects in solid-liquid fractionated extracts of sediments, highlighting the prevailing of PAHs (benzo[a,c]anthracene and picene) in overall toxicity of contaminated sediments.

Another group of chemicals, phenolic compounds, have been considered in microalgae facing PAH stress. Total phenols and flavonoids, a class of polyphenols, were shown to increase in *C. vulgaris* cells exposed to phenanthrene or fluorene (Ashgari et al. 2018, 2020). These compounds are involved in the scavenging of ROS, and their increase has been reported during the exposure of microalgae to various chemicals (e.g. Fazelian et al. 2019).

Taking into consideration the hydrophobic character of PAHs, microalgal lipid content is also affected by exposure (Table 5) and can contribute to PAH partitioning in the cells. A single study by Croxton et al. (2015) highlighted the increase in total lipid content, detected using fluorochrome staining and flow cytometry, in the marine benthic diatom *Nitzschia brevirostris*. This increase was hypothesised to correspond either to an increased energy demand within cells, and / or a process allowing sequestration and dilution of toxic PAH in intracellular lipid bodies. Evidence for PAH bioaccumulation was provided by Shishlyannikov et al. (2017) for the freshwater diatom *Synedra acus* subsp. *Radians*, using epifluorescence microscopy on cultures spiked with crude oil, taking advantage of the blue autofluorescence of PAHs when excited with near-UV light. These authors suggested that linear hydrocarbons of crude oil enhanced the cell membrane permeability further favouring PAHs accumulation into intracellular lipid bodies. The extent of the PAH effects on microalgal membrane integrity have been studied by Croxton et al. (2015) and Aksmann and Tukaj (2008) on *N. brevirostris* and *C. vulgaris* respectively, with similar evidence of significant alterations

and increase in permeability during exposure. This deterioration of cellular structures is likely to translate into alterations of cell morphology (Table 5), eventually resulting in changes in cell size and shape.

2.1.3. Effects on gene expression and protein synthesis

To detect the toxicity of PAHs on phytoplankton cultures, omics methods have also been implemented aiming at various target genes and proteins. These approaches rely on the previous knowledge of genomic and proteomic structures, currently restricted to a few cyanobacteria and eukaryotic microalgae. The first photosynthetic organism fully sequenced was the freshwater unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (Kaneko et al. 1996). By exposing this strain to pyrene, Shao et al. (2010) showed up-regulation of the *psbA* gene coding for the photosystem II core protein D1, suggesting an increase of protein turnover in damaged PSII as evidenced by *in vivo* chl *a* fluorescence analysis in the same study. Other photosynthetic genes targeted during this study, *psbB*, *psbC* and *psbO* controlling PSII assemblage and functioning, were not significantly regulated in *Synechocystis* sp. PCC 6803 during PAH exposure.

The marine diatom *Thalassiosira pseudonana* was the first eukaryotic marine phytoplankton submitted to whole genome sequencing (Armbrust et al. 2004), allowing the analysis of gene expression under PAH or crude oil water-accomodated fraction (WAF) exposure (Table 6). Pyrene, fluoranthene and benzo(a)pyrene were shown to induce over-expression of *lacsA* (a gene coding for long chain acyl-coA synthetase, involved in lipid metabolism) whereas *sil3* (a gene coding for silaffin protein contributing to silica frustule formation in diatoms) was repressed (Bopp and Lettieri 2007). Similar results were reported by Carvalho et al. (2011b) during exposure of *T. pseudonana* to PAHs mixture obtained from WAF preparation: a down regulation of expression was reported for several genes, *sil3*, *sit1*, *diox* and *hsf* (Table 6) whereas three genes were up-regulated, *lacsA*, *sil1* and *timb*. Eventually, PAHs exposure affects the process of silica uptake in diatoms by a down-regulation of *sit1* gene and by a decreased uptake of silica, resulting in a reduction in the intracellular silica pools. This would likely impair the formation of diatom frustules, resulting in the inhibition of cell division and a reduction in growth rates together with an increasing sinking rate. As reported for the cyanobacterium *Synechocystis* sp. PCC 6803, photosynthetic metabolism of *T. pseudonana* was affected by PAH exposure. Bopp and Lettieri (2007) detected in this species a down-regulation of the expression of *3HfcpA* and *3HfcpB* genes, both encoding for PSII proteins, that eventually result in decreased pigment content and photosynthetic activity.

The potentially toxic freshwater cyanobacterium *Microcystis aeruginosa* responded to anthracene exposure by modifications of the microcystin gene cluster expression (Bi et al. 2016). Two genes

(*mcyD*, *mcyH*) were up regulated, whereas one (*mcyB*) was repressed; after twelve days of exposure, a significant increase in microcystin content was reported, suggesting that PAH contamination in aquatic ecosystems could result in unexpected changes in harmful algal blooms toxicity. In a similar vein, Breterthon et al. (2019) showed an increase in domoic acid production (a complex aminoacid neurotoxin responsible for the Amnesic Shellfish Poisoning) by the marine diatom *Pseudo-nitzschia* sp. When exposed to a crude oil WAF containing 157 µg/L of mixed PAHs.

The aforementioned studies on *T. pseudonana* (Carvalho et al. 2011a, 2011b, Carvalho and Lettieri 2011) provided a focus on the expression of genes and the synthesis of proteins related to stress responses. Depending on the targeted genes (Table 6), regulation of expression appeared enhanced (e.g. *tmbi*) or repressed (e.g. *hsf*, *diox*) making difficult any generalization regarding the use of stress-related gene expression as a suitable marker of PAH toxicity on microalgae. Furthermore, a whole transcriptome study by Hook et al. (2014) on the benthic marine diatom *Ceratoneis closterium* exposed to WAF (PAH content was not provided in the study) showed a repression of transcription of genes involved in photosynthesis, respiration, nutrient cycling and purine metabolism, whereas stress responses such as heat-shock proteins remained unaffected.

2.2. Interactions of PAHs effects with other stressors

Toxic effects of PAHs can be modulated by other environmental parameters, such as light (intensity and nature), variables linked to global change (temperature, pH and pCO_2) or to eutrophisation (dissolved nutrients).

2.2.1. Interactions of PAHs and ultraviolet light

It has been known for almost a century that co exposure to ultra-violet radiation and tar favored skin cancer (Findlay, 1928), and polycyclic aromatic hydrocarbons were rapidly pointed out as responsible for a significant part of toxicity of petroleum compounds (Mottram and Doniach, 1938). Since then, considerable attention has been paid to the influence of visible light and UV radiation on the toxicity of PAHs to various organisms (Ankley et al. 1994, Arftsen et al. 1996). Two different mechanisms can be involved to explain light-induced toxicity: the photomodification of PAH generally producing new oxidized compounds more toxic than parent chemicals, and photosensitization by which oxygen singlet compounds can be produced resulting in enhanced direct oxidative damage to organisms.

Several studies have addressed the influence of UV radiation and overall light quality on the PAH toxicity against microalgae (Table 7). Differences in the light quality providing photosynthetically available radiations (PAR) necessary for photosynthetic growth have been addressed, by comparing tungsten-filament bulbs or fluorescent tubes with spectra peaking in orange visible spectum (“gold

light") to regular white fluorescence tubes. Wang et al. (2008) found that the toxicity of single and mixture PAHs on the marine diatom *Phaeodactylum tricornutum* increased in the presence of UV radiation (Table 7). The relative toxicity ranking was fluoranthene> pyrene> anthracene> phenanthrene without UV whereas when UV radiation was applied during PAH exposure, the toxic ranking changed to anthracene> fluoranthene> pyrene> phenanthrene (Table 7). This was confirmed by the EC_{50}s value of each PAH in the absence of UV, which was considerably higher than that in the presence of UV (Table 7). On the same species, Okay and Karacik (2007) reported that pyrene, fluoranthene, phenanthrene and chrysene in presence of UV radiation have an increased toxicity. Specifically, chrysene alone did not alter growth in the absence of UV-A exposure (Table 7). Similarly, the growth rate inhibition of the freshwater chlorophyte *Raphidocelis subcapitata* (designed as *Selenastrum capricornutum*) by anthracene was elicited under UV-A exposure (Gala and Giesy 1992). Other researchers have examined the response of the zeaxanthin/violaxanthin ratio in the presence of UV radiation. Southerland and Lewitus (2004) showed that *Ankistrodesmus* sp., a benthic estuarine chlorophyte, responded by an increase in the intracellular zeaxanthin/violaxanthin ratio when exposed to both UV and fluoranthene (Table 7). These authors explained that, similar to the response to photoproduction of oxygen radicals, zeaxanthin might divert singlet energy produced by photo-activated PAHs and reduce chemical stress (Southerland and Lewitus 2004). The influence of light quality on the toxicity of PAHs was modeled by Grote et al (2005) while emphasizing the importance of the structural characteristics of individual compounds: the gap between the highest occupied and lowest unoccupied orbitals (reffered to as "HOMO-LUMO gap") was proposed as the main mechanistic explanation for toxicity induction by PAHs on microalgae.

Many studies have demonstrated that photomodifed PAHs are also often more reactive and acutely toxic than parent compounds (Grote et al. 2005; Marwood et al. 1999, 2003; Miller et al. 2001). McConkey et al. (1997) suggested that the toxicity of PAHs in the presence of UV radiation may increase probably due to the production of ROS, including superoxide anions ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radicals ($HO^{\bullet}$) in the membranes of the organism following uptake into the tissue, which may in turn damage cell constituents. Actually, ROS can interact with lipids, proteins and nucleic acids, causing lipid peroxidation, protein denaturation, DNA mutation and inducing cell death (Bowler et al. 1992; Sigaud-Kunter et al. 2005). Spehar et al. (1999) suggested that phototoxicity occurs once energy is transferred from the activated triplet state of the PAH molecule to molecular oxygen, thereby creating singlet oxygen, which can react with biomolecules (e.g., amino acids, fatty acids) to cause cell damage (Larson and Berenbaum 1988; Newsted and Giesy 1987). To prevent such damage, plant cells have developed defense strategies against ROS production. These strategies include non-enzymatic systems (carotenoids, ascorbate,

glutathione, etc.) antioxidant enzymes (SOD, peroxidases, glutathione-S-transferase GST), and repair systems (DNA repair systems, oxidized protein and phospholipid turnover) (Halliwell and Gutteridge 1999).

2.2.2. Effecs of temperature, pH, and pCO_2 on PAHs toxicity

Temperature appears to be important in affecting the toxicity of PAHs, with toxicity increasing with temperature (Table 7). A 5°C rise in culture conditions significantly increased the toxicity of all the PAHs tested on the marine chlorophyte *Tetraselmis chuii* (Vieira and Guilhermino 2012). At 20°C the EC_{50}s were 3.326, 1.813 and 1.316 mg /L but decreased at 25°C to 2.145, 0.992 and 0.262 mg /L for anthracene, naphthalene and phenanthrene respectively. Together with temperature rise, oceanic pCO_2 is likely to increase significantly in the 21st century; as mentioned above for silica metabolism, exposure of the marine diatom *Skeletonema costatum* to benzo(a)pyrene in CO_2-supplemented culture media resulted in a decrease of biogenic silica content and overall photosynthetic efficiency (Li et al. 2021).

2.2.3. Interactions of dissolved nutrients and PAHs toxicity to microalgae

Effects of PAH exposure to microalgae can also be modulated by nutrient supply (Table 7). Djomo et al. (2004), reported that with high levels of nitrate (200 mg L^{-1}) in the culture medium, the growth inhibition caused by individual PAHs on *Scenedesmus subspicatus* could have been partly explained by the hydroxylation of PAHs by reactive nitrate. Indeed, Zeep et al. (1987), reported that nitrate ions favored the reactivity of hydroxylated radicals with PAHs when dissolved in water, suggesting a synergic mechanism. However, the pattern of effects interaction remains difficult to decipher in more complex systems, such as multi-trophic mesocosms, where top-down and bottom-up control result in unpredictable effects on microbial primary producers (Sundbäck et al. 2010).

2.3. Accumulation and biodegradation of PAHs by microalgae

PAHs uptake by microalgae was considered relevant in the context of trophic transfer of contaminants in aquatic food chains (Fan and Reinfelder 2003, He et al. 2021, Wang et al. 2021), to compare dissolved and dietary routes of exposure in marine organismes including symbiotic corals (Ashok et al. 2020), or as a way to remediate contaminated water and soil slurries (Subashchandrabose et al. 2017, Machado Marques et al. 2021). Hydrophobicity of PAHs makes these compounds likely to accumulate in intracellular lipid bodies of microalgae (Subashchandrabose et al. 2014, Shishlyannikov et al. 2017). No general rules can apply to PAHs trophic transfer in aquatic food webs, depending on the length of the food chain considered. Short pathways including primary producers, zooplankton grazers and planktivourous fishes were shown consistent with trophic

accumulation and transfer (Wang and Wang 2006, Wang et al. 2021), whereas low assimilation efficiency together with increased metabolic transformation in consumers was reported explaining PAHs trophic dilution (Wan et al. 2006). Microalgae are also considered as efficient transporters of hydrophobic contaminants, including PAHs, to the benthic ecosystem (Ding et al. 2021, He et al. 2021) due to their bioaccumulation potential, contributing to the sediment burden of PAHs.

In their review of PAHs degradation by microorganisms, Ghosal et al. (2016) highlighted the fact that archaea, bacteria and fungi, have received more attention than microalgae. Degradation rates and overall efficiency depend on the uptake and bioconcentration of PAHs, the nature of enzymatic apparatus (Méndez García and García de Llasera 2021), the type of microalgae considered and appears further controlled by biotic (possible presence of bacteria, e.g. Kahla et al. 2021) and abiotic (typically light and UV) environmental factors. Biotechnological applications are considering both the accumulation and the degradation potential, therefore tolerance of microalgal models to PAHs toxicity and pathways identification are paramount to achieve efficient processes (Subashchandrabose et al. 2017, García de Llasera et al. 2021, Machado Marques et al. 2021).

Since the early work of Cerniglia and colleagues (Cerniglia 1992; Cerniglia et al. 1980; Cerniglia and Gibson 1979) numerous microalgal species have been shown to degrade PAHs in culture, including cyanobacteria, diatoms, and chlorophyceae. Juhasz and Naidu (2000) reported from a literature review that 17 algal species, such as the cyanobacteria *Oscillatoria* sp., *Nostoc* sp. and *Anabaena* sp., the chlorophyta *Dunaliella tertiolecta* and *Chlamydomonas angulosa*, and the diatoms *Nitzschia* sp., and *Navicula* sp., among others, were able to degrade naphthalene. However, only two species were able to degrade benzo[a]pyrene and fluoranthene, the cyanobacteria *Oscillatoria* sp. and *Agmenellum quadruplicatum* (currently regarded as *Merismopedia quadruplicata*). In a comparable work, Ghanbarzadeh et al. (2022) elected the cyanobacteria *Nostoc calcicola* as the most efficient species to remove phenanthrene compared to two other cyanobacteria and two chlorophytes. The authors highlighted the increase in antioxidant enzymatic activity together with enhanced growth rate in *N. calcicola*, tolerance to the PAH being a key characteristic to allow biodegradation.

The list of microalgal candidates for PAH biodegradation was early estimated to more than fourty strains by Ghosal et al. (2016), and several more species have been found to degrade fluoranthene. For example, the chlorophyte *Raphidocelis subcapitata* (*Selenastrum capricornutum*) was shown to remove 96% of phenanthrene, 100% of fluoranthene, and 100% of pyrene from culture media (Chan et al. 2006). In the latter study, PAH removal efficiency was reduced by less than half when cell densities decreased from 10^7 to 5 10^4 cells /mL. At least nine metabolites of phenanthrene were detected in the freshwater chlorophyte *Scenedesmus subspicatus* (Šepič et al. 2003), whereas Lei et

al. (2007) reported *Chlorella vulgaris* as the least efficient species in removing and transforming PAHs (fluoranthene and pyrene) compared to *S. capricornutum* which was the most effective species and suggested that the more the biomass the higher the removal percentages. The same authors suggested that the removal efficiency of PAHs in mixture was similar or higher than for a single compound. Hong et al. (2008) showed that the ability of the diatom *Nitzschia* sp. to accumulate and degrade PAH was higher than that of another diatom, *Skeletonema costatum*. The same authors indicated that both diatoms showed comparable or higher efficiency to remove phenanthrene and fluoranthene in mixture than separately. Glutathion-S-transferase may play an important and crucial role in biodegradation of pyrene by microalgae, with species-dependent enzyme activity (Lei et al. 2003). Comparing several chlorophyte species, Lei et al. (2007) indicated that *Raphidocelis subcapitata* (*Selenastrum capricornutum*), *Chlorella miniata*, *Chlamydomonas* sp., *Scenedesmus quadricauda*, *S. platydiscus*, and *Synechocystis* sp., could biodegrade 0.1mg /L of pyrene from 34 to 100% in seven days. It was recently demonstrated that microalgal degradation of PAHs could be partly attributed to exocellular enzymes (García de Llasera et al. 2022): the freshwater chlorophyte *Selenastrum capricornutum* (*Monoraphidium capricornutum* according to current consensus) was able to initiate the formation of dihydrodiols metabolites, the first degradation process of benzo[a]pyrene, in the culture medium.

Several studies have examined the relative importance of live versus dead cells in the degradation of PAHs (Takáčová et al. 2014). Luo et al. (2014) studied the removal and biodegradation of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, Indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene, under darkness or light in live and dead *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*) cells. The presence of dead algal cells in test medium increased the photodegradation rates of PAHs, whereas live microalgal cells actively removed the PAHs from the medium. The transformation efficiency of PAHs by live and dead *R. subcapitata* under different light sources was dependent on the nature of PAH compound. Under incandescent and fluorescent lightning (referred to by the authors as “gold” and “white”), only benzo(a)anthracene and benzo(a)pyrene were highly transformed in both live and dead cell assays. Benzo(g,h,i)perylene, dibenzo(a,h)anthracene were the most stable and recalcitrant PAHs. In fact, dibenzo[ah]anthracene with more ‘bends’, presented less transformation than benzo(a)anthracene and benzo(g,h,i)perylene that have more stable configurations (a cluster PAH with one ring surrounded and five benzene rings on five sides, Blumer 1976). Some studies concluded that cell walls provide many potential binding sites for organic and inorganic pollutants and additional binding sites likely become more available in dead cells compared to live cells (Avery et al. 1998). It has been proposed as more advantageous to use dead microbial cells for PAH bioremediation because dead

organisms are not further affected by toxic wastes, and do not require any continuous nutrient supply (Aksu 2005, Ke et al. 2010).

Accompanying bacteria are known to mitigate microalgal sensitivity to toxic chemicals and metals (Fouilland et al. 2018), mostly by degrading or sequestering the toxicants reducing their bioavailability to microalgae. Only a few recent studies have specifically addressed the xenic status of microalgal cultures during PAHs biodegradation experiments. Ghosal et al. (2016) suggested that bacteria / microalga co-metabolism could enhance overall biodegradation potential, as shown by diatom-bacteria consortia isolated from polluted coastal environments exposed to benzo(a)anthracene or fluoranthene (Kahla et al. 2021) or to complex PAH mixtures (Ben Garali et al. 2021). Relying on the cooperative metabolisms of microalgae-bacteria consortia appears as a promising perspective to provide bioremediation solutions (Ahmad 2021), that would be implemented to reduce the PAH contamination of the environment (Dai et al. 2022) or in industrial processes based on photobioreactors for example (García de Llasera et al. 2018, Machado Marques et al. 2021). Metabolic pathways that are involved in PAHs degradation by microalgae are still relatively poorly known, and the use of mass-spectrometry and proteomics would allow a better identification of metabolites and enzymes involved (a recent extensive review is provided in Mendéz García and García de Llasera 2021).

3. Effects of individual PAH and mixtures on natural phytoplankton communities

Few studies have examined the impact of PAHs on natural phytoplankton assemblages (Table 8). The interpretation of field studies is more complicated in comparison to the impact of PAH on phytoplankton in pure cultures. Under natural conditions, PAHs are typically present as a mixture of compounds and natural phytoplankton communities are highly diverse. The main complications are: (i) the toxicity thresholds may change when considering a natural phytoplankton community including different taxonomic units and species of varying size (Hjorth et al. 2007, 2008), (ii) the toxic effects induced by single PAHs are not necessarily the same when PAHs are added simultaneously as a mixture which is the typical situation in aquatic environments (Echeveste et al. 2010b) and (iii) the interaction with other abiotic and biotic factors (i.e nutrients, UV, predators, etc) can also result in unpredictable effects. Risk assessment of PAHs in oceanic waters is particularly useful in predicting the effects of accidental oil spills, where various hydrocarbons of different nature and toxicity are released in the water column (Softcheck 2021, Putzeys et al. 2022). In such a context, studies addressing the toxicity of water-accomodated fractions (WAF) of crude oil, obtained by mixture equilibrium with seawater, were considered.

3.1. Effect of individual PAHs

Several studies have addressed the effect of single PAH on phytoplankton communities, whereas others have investigated the consequences of PAH exposures in combination with additional stressors (nutrients, UV radiation, etc). Hjorth et al. (2007) investigated the effects of pyrene exposure on marine phytoplankton communities in association with bacteria and zooplankton using *in situ* microcosms (Isefkjord, Denmark). Planktonic chl *a* concentrations decreased over the short term in a dose-dependant manner under exposure (Table 8). At low and medium exposures, changes in phytoplankton community structure were observed with a reduction in diatoms, cryptophytes, and prymnesiophytes densities over time. Prasinophyceae densities increased over the medium term. In case of exposure to high concentrations (250 nmol /L, 50.6 µg/L), prymnesiophytes and cryptophytes were present in lower proportions compared to diatoms, Cyanophyceae, Chlorophyceae and Euglenophyceae (Hjorth et al 2007; Table 8). However, no effects on photosynthetic activity were reported on the pyrene-exposed communities. In contrast, in a study of biofilms from an Arctic coastal system (Petersen et al. 2008), primary production rates decreased under pyrene exposure concentrations as low as 0.81 µg /L and toxic effects increased when biofilms were exposed to natural sunlight UV radiation.

The toxicities of pyrene and phenanthrene have been compared, by exposing natural phytoplankton communities sampled from several marine ecosystems (Table 8). In Atlantic Ocean and Mediterranean Sea *in situ* experiments (Echeveste et al 2010a; Table 8), pyrene was found more toxic than phenanthrene as expected based on the higher number of aromatic rings. In this study, cell size was emphasized as an important parameter determining the sensitivity to PAHs, with the smallest pico-cyanobacteria, *Prochlorococcus* and *Synechococcus*, being the most sensitive (Table 8), whereas geographical origin did not affect sensitivity to PAHs. For example, the EC_{50} for phenathrene was similar between the Atlantic and Mediterranean both for *Prochlorococcus* (36.3 vs. 30.2 µg /L) and for *Synechococcus* (42.5 µg /L vs 42 µg /L). Echeveste et al. (2010a) suggested that contamination with PAHs would have a greater effect in oligotrophic waters because of the dominance of more sensitive small sized phytoplankton. During a global survey cruise in three oceans (Atlantic, Indian and Pacific) Cerezo and Agustí (2015a) demonstrated that PAH exposure resulted in a delayed cell division of the picophytoplankton *Prochlorococcus*. The time lag was dose dependant, and DNA synthesis (S-phase) was likely to be the main sensitive step on exposure to a mixture of 8 PAHs (Cerezo and Agustí 2015a). The same authors demonstrated that at the microbial pelagic level, food-web structure was modified upon PAH exposure together with reduced growth of picoplanktonic primary producers (Cerezo and Agustí 2015b).

Using five days exposure to naphthalene of pristine planktonic communities in microcosms, Bouvy et al. (2021) reported by contrast an increase in the density of *Prochlorococcus* and *Synechococcus* components of picophytoplankton without significant impact on larger phytoplankton, using microcosms enclosures to expose plankton communities from a pristine tropical lagoon. These authors highlighted the fact that naphthalene, initially spiked at 24 µg/L, disappeared totally from the experimental systems, and dit not caused observable effects on phytoplankton photosynthetic efficiency.

The interactions between nutrients supply and pyrene exposure in toxicity (Table 8) were studied by Hjorth et al. (2008) on phytoplankton communities experimentally exposed in *in situ* mesocosms including bacteria and zooplankton. Results showed that with or without nutrients primary production was significantly lowered by 50 % under 50 nMol (102 µg/L) pyrene exposure compared to control communities. The effects of UV and fluoranthene on the growth and pigment composition were addressed by Southerland and Lewitus (2004) on natural phytoplankton communities sampled from Murrells Inlet estuary (Table 8). These authors showed that biomass expressed as chl *a* was not affected by the combination of fluoranthene and visible light but decreased under UV and fluoranthene co-exposure. Moreover, the zeaxanthin/violaxanthin ratios increased in natural phytoplankton communities under UV + fluoranthene conditions. Southerland and Lewitus (2004) suggested that like the response to photo-production of oxygen radicals, zeaxanthin accepted singlet energy produced by photo-activated PAHs, in which case cellular zeaxanthin content increased in response to UV-induced PAH toxicity. Photosynthetic electron transport in natural assemblages of phytoplankton from Lake Erie (Marwood et al. 1999) was inhibited in the presence of anthracene or its photomodified product, 1,2-dihydroxyanthraquinone (1,2-dhATQ). Results showed that anthracene (EC_{50}= <0.2mg /L) was more toxic on photosynthetic potential expressed as Fv/Fm than 1,2-dhATQ (EC_{50}=2 mg/L) and this impact was not due to aqueous solubility because 1,2-dhATQ is the most water-soluble. 1,2-dhATQ caused an inhibition of ΔF/F'm in phytoplankton in the dark (Marwood et al. 1999). It has been suggested that molecules similar in structure to 1,2-dhATQ might inhibit electron transport directly by blocking PSII, or between PSII and PSI at cytochrome-b/ f (Karukstis et al. 1990; Oettmeier et al. 1998). Photomodified anthracene can produce metabolites and radicals that inhibit photosynthesis (Huang et al. 1997; Mallakin et al. 1999). In a subsequent study on Lake Erie phytoplankton Marwood et al. (2003) examined the inhibition of photosynthesis (Fv/Fm and ΔF/F'm) for natural assemblages exposed to anthracene, phenanthrene, fluoranthene, and photomodified PAHs (anthraquinone ATQ, phenanthrenequinone PHEQ and 1,2dhATQ) in the laboratory and concluded that anthracene, fluoranthene, and PHEQ were more toxic to phytoplankton (Table 8) than ATQ, 1,2dhATQ and phenanthrene. Several studies have previously

demonstrated that oxidized PAHs have greater aqueous solubility and toxicity towards bacteria and aquatic vascular plants (Babu et al. 2001; McConkey et al. 1997; Huang et al. 1997).

3.2. Effect of PAH mixtures

As mentioned previously, PAHs are typically present under natural conditions as a mixture of compounds. However, few studies have addressed the effects of PAH mixtures on natural phytoplankton assemblages. Echeveste et al. (2010b) tested the impact of mixture of two (phenanthrene and pyrene) and 16 PAHs on natural phytoplankton sampled from the subtropical Atlantic Ocean (Table 8). These authors showed that the open ocean phytoplankton communities, dominated by *Prochlorococcus* sp., *Synechococcus* sp. and small photosynthetic eukaryotes, were strongly affected by the exposure to PAHs. PAH mixtures were more toxic to phytoplankton than single compounds and a greater population decline occurred when the complexity of the contaminant mixture added increased (Table 8; Echeveste et al. 2010b), by as much as 103 times the toxicity indicated for a single PAH. In a subsequent study, Echeveste et al. (2011) compared the impact of 16 PAHs phototoxicity on natural marine phytoplankton from the Mediterranean Sea, Atlantic, Arctic and Southern oceans. Results showed that PAH mixtures induced a decrease in natural phytoplankton for all the taxonomic groups, oceanic provinces, and treatments tested. Moreover, treatments with PAH in mixtures and in presence of UV were the most toxic to phytoplankton. For example, in the case of Mediterranean Sea, the EC_{50}s for population growth were 4.6 and 1.97 µg /L in the absence and presence of UV respectively. In oligotrophic waters, commonly dominated by picophytoplankton (below 3 µm in size), the joint action of UV and PAHs mixtures is likely highly significant. Larger cells usually more abundant in eutrophic waters appeared less sensitive to PAH phototoxicity (Echeveste et al. 2011).

The impact of a 16 PAHs mixture on natural phytoplankton communities was addressed using 5-days microcosms exposure in two Mediterranean coastal ecosystems, Bizerte (Southwestern) and Thau (Northwestern) lagoons (Ben Othman et al. 2018). This study showed that the endpoint of chl *a* was the most sensitive to exposure to PAH mixtures (EC_{50}s were 1.21 and 2.04 µg /L in Bizerte and Thau lagoon respectively) compared to the Fv/Fm proxy of photosynthetic potential (EC_{50}s were >75 and 420 µg /L in Bizerte and Thau lagoon respectively) in both ecosystems. Moreover, dramatic changes in the taxonomic composition occurred at the onset of exposure: pico-, nano- and micro-phytoplankton were all negatively affected at high concentrations of PAHs. However, picophytoplankton in Bizerte communities eventually recovered contrary to the other fractions at the end of the experimental exposure. In both ecosystems, the large diatom *Entomoneis paludosa* was favoured under exposure to PAHs cocktail, whereas autotrophic flagellates and dinophytes were

negatively affected, smaller cells appearing more tolerant to PAHs. In this study, sensitivity was not related to phytoplankton cell size, and authors suggested a side-toxicity of PAHs on grazers, resulting in changes in top-down control (Ben Othman et al. 2018). This higher sensitivity of natural phytoplankton communities, compared to single-species tests, may result from synergistic or additive effects of environmental stressors (e.g. UV, nutrient deficiencies) and the extent of trophic interactions. In addition, the simultaneous contamination by mixed PAHs can induce combined effects (additive, synergistic and antagonistic) on phytoplankton, which are different from those caused when PAHs are used separately. In summary, phytoplankton exposed to PAHs in natural communities appears generally more sensitive than monospecific cultures exposed in the laboratory, and PAH contamination could result in changes in the phytoplankton and biofilm communities' composition. Ecological functions such as primary production can be impaired under exposure, whereas indirect effects could be expected on other biotic components.

To date, the toxic interactions between PAHs and other contaminants are still poorly addressed, Nevertheless, the increasing worldwide aquatic pollution motivates new research, such as for example the combined effects of PAHs and microplatics (Su et al. 2022, Zhang et al. 2022).

3.3. Effect of oil inputs

Studies have reported that PAHs are one of the major components released from oil spills (Yamada et al. 2003; Albers 1995; Kennish 1997). For example, González et al. (2009) found that crude oil contained highly toxic PAHs, dominated by naphthalene and its alkylated derivates (89% of total PAH concentrations). The latter are typically found in the oil water-soluble fraction. This fraction was tested on two different natural assemblages of primary producers in microcosms (González et al. 2009, Table 8). The results showed that Fv/Fm, chl *a* and primary production decreased rapidly following addition of PAHs in water, in agreement with previous results obtained during mesocosm experiments (Sargian et al. 2005; Siron et al. 1995). In contrast, after an oil addition, a rise in phytoplankton abundance and primary production has often been reported depending on the time frame of sampling following additions (Carman et al. 1997; Kelly et al. 1999; Vargo et al. 1982). Oceanic picophytoplankton was more sensitive than coastal picophytoplankton to PAHs exposure, with subsequent changes in the structure of the plankton community (Table 8). González et al. (2009) highlighted that stimulation of selected size-fractions of phytoplankton (such as large diatoms under low PAH concentration exposure) may be due to: (i) increase in nutrient regeneration, resulting from the breakdown of sensitive plankton exposed to PAHs (Hjorth et al. 2007, 2008), and (ii) reduction in predation pressure due to the negative effect of oil on the abundance of heterotrophic grazers. Kelly et al. (1999) reported an increase in phytoplankton abundance that they attributed to a decrease in

predation pressure and not to any stimulatory effect of oil on phytoplankton. In a subsequent experiment, González et al. (2013) used larger mesocosms (> 1 m^3) to evaluate the effect of PAHs from oil spills on coastal marine phytoplankton assemblages exposed for 8 days and reported that chl *a*, primary production and communities composition were not strongly affected by PAHs (20-60 µg /L of chrysene equivalents). In the latter study, no indirect trophic cascading effects were detected, contrary to what was previously reported in microcosm experiments (González et al. 2009), and the PAH concentration decay rates, higher in mesocosms than in microcosms, was suggested by the authors as an explanation for the reduced effects (González et al. 2013).

Some studies have examined the effect of crude oil accompanied by other stressors. Sargian et al. (2005) used microcosms to determine the effects of the water- accomodated fraction (WAF) of crude oil dominated by dissolved naphthalene and its analogs (Total PAHs= 18 598 µg /L) also using two levels of UVBR (Ultraviolet-B radiation) defined as “natural” and “high”. The effects were assessed on a natural phytoplankton assemblage isolated from the lower St. Lawrence Estuary. Strong negative effects on the natural plankton assemblage (growth rates and cell division) were reported in the absence of UV-radiation. These authors suggested that soluble petroleum hydrocarbons could completely mask the effects induced by UVBR on marine microorganisms.

4. PAHs risk assessment to aquatic ecosystems using Species-Sensitivity Distributions for microalgae

A key issue in environmental risk assessment is the confrontation of environmental exposure data (the concentrations of chemicals in the ambient, namely aquatic ecosystems for microalgae) to the known toxicity of chemicals, with documented hazard thresholds. Formally this implies to provide risks quotients (Meng et al. 2019) ideally based on environmental monitoring and a quantitative toxicity evaluation of any relevant chemical (exposure concentration in the realm / toxicity value). The latter is often lacking since chemicals are in huge numbers with increasing uses and release, including PAH derivatives (Idowu et al. 2018), and several models are proven useful to help in determining the unharmful concentrations, such as PNECs (predicted no-effect concentrations). For that purpose, a paramount use of multiple single-species toxicity data is the derivation of HC_5, the harmful concentration (during exposure) for 5% of the tested species, and one of the most common way to evaluate HC_5 is the construction of species-sensitivity distribution curves.

The available data on PAH effects on microalgae indicate an extremely large range of toxic thresholds and effects, making difficult any general statement on actual ecotoxic consequences of aquatic pollutions by PAHs. To better capture the ecotoxicological threats linked to PAH exposure, and extrapolate single dose-effect experiments, a species sensitivity distribution (SSD) analysis was

performed using EC_{50}s values from various databases and previously published studies. Several models and statistical approaches have been used to address the question of construction and interpretation of SSD curves (Posthuma et al. 2002). For simplicity a logistic model was adjusted here to available EC_{50} and comparable data (listed as IC_{50} or LC_{50} namely), in order to derive harmful concentrations to 5% of the tested species (HC_5). This HC_5 concentration can be considered as the environmental limit that is protective for organisms and ecosystems (Wheeler et al. 2002).

Log-normal distribution models were fitted to the available data using Past 3.23 software for OS X (Hammer et al. 2001), after data checking for normality (Shapiro-Wilkinson), then processed using ssdtools web app based onto dedicated R package (Thorley and Schwarz 2018, Dalgarno 2020) to calculate HC_5 and confidence limits of hazardous concentrations. Bootstrap samples (n=10,000) were performed for each set of data according to the recommendation of the authors (https://bcgov-env.shinyapps.io/ssdtools/).

To cope with the scarcity of available data and published studies for the 16 priority PAHs, a global analysis of PAH toxicity to all aquatic organisms (including microalgae, phytoplankton, vascular plants, zooplankton, invertebrates and vertebrates) was first performed (last query on October, 4th 2021), extracted using the US Environmental Protection Agency ECOTOX database search engine (https://cfpub.epa.gov/ecotox/) and compared to toxicity data for phytoplankton and microalgae taxa from marine and freshwater origins completed with further bibliographic analysis.

4.1. PAHs SSD for all aquatic organisms

A total of 878 data were retrieved from the US EPA database, selected after cleaning of duplicate entries (same species, same PAHs, same EC_{50} value), taxonomic mismatches and doubtful data (mostly uncertain concentration units or undefined values). The most frequently tested compound was fluoranthene (n=243), followed by naphthalene (n=194) and phenanthrene (n=126). All the 16 priority PAHs were found documented in the database, and 66.2% of the available EC_{50}s were for freshwater species. No distinction between toxicity endpoint or test conditions was done for the overall evaluation.

Algae (benthic, planktonic) accounted for only 10.5% of all the raw data, with crustacean (45.9%) and fishes (19.7%) representing the two most documented groups of aquatic organisms regarding PAH toxicity assessments. EC_{50}s values ranged from 0.0001 to 1300 mg /L, with a median of 0.3535 mg /L and a mean of 0.3073 mg /L, and passed the normality test (Shapiro-Wilkinson, W = 0.9966, p(normal) = 0.05259). Interestingly, fluoranthene was both the most toxic (LC_{50} of 0.001 mg /L on 28-days aged winter flounder, a marine fish, under 96 h UV exposure, Spehar et al. 1999) and the least

toxic (LC_{50} of 1300 mg /L on 24 h aged water fleas, freshwater zooplankton, exposed for 24 h, LeBlanc 1980) across the twenty reported PAHs toxicity values.

A global SSD curve was obtained using the 878 EC_{50}s validated data, regarding all aquatic species sensitivity to all PAHs (Suppl. Fig. S1). Despite the combining of different chemicals not being conventional, the log normal cumulative distribution model was a good fit to the data and provided an aquatic HC_5 value of 2.76 µg /L (Table 9).

4.2. PAHs SSDs for microalgae

Dataset was established using the abovementioned ECOTOX database (last query on July, 3rd 2022) completed with collected data from literature as previously stated, using EC_{50} as evaluation endpoint of toxicity. Duplicate values (same strain, same PAH, same EC_{50} value) were removed from the dataset.

4.2.1. Single-PAH SSDs

ECOTOX database retrieval and compilation of bibliographic data yielded 251 EC_{50}s values for phytoplankton both in culture and in natural communities (Table 2, 3 and Suppl. Table S1), among which 137 were of freshwater and 114 of marine origin. Among the total available data, only 158 published EC_{50} were below 100% of the known water solubility value (S_w) for the corresponding PAH, which were further considered except for benzo[a]anthracene and benzo[a]pyrene. For the two latter PAHs, significant numbers of EC_{50}s were published almost exclusively above water solubility. A first SSD curve was drawn for all the data (Fig. 2A), providing an overall evaluation of HC_5 of 4.72 µg/L whatever the microalga or chemical considered. A clear difference in sensitivity of all freshwater species (n=79, Fig. 2B) and marine species (n=79, Fig. 2C) was observed; the HC_5 for marine species was one order of magnitude higher than that for freshwater ones (26.3 and 1.09 µg /L respectively, Table 9).

Due to the limited available data, species-sensitivity distribution curves (Fig. 3) were derived for only nine polycyclic aromatic hydrocarbons, and HC_5 calculated for each single compound (Table 9). The PAHs in alphabetical order and their HC_5 are summarized here.

- Acenaphthene: Only seven EC_{50}s were available for acenaphthene, from 322 to 1400 µg /L. The freshwater chlorophyte *Raphidocelis subcapitata* showed both the highest (Japanese Ministry of Environment, 2015) and lowest values (USEPA, 1978). Species-sensitivity distribution (Fig. 3A) provided an HC_5 value of 274 µg /L.
- Anthracene: The most sensitive species to anthracene was the freshwater Chlorophyceae *Monoraphidium capricornutum* (formerly *Selenastrum capricornutum*) with an EC_{50} of 3.3 µg

/L (Gala and Giesy 1992), and the most tolerant one the cyanobacterium *Anabanea fertilissima* with an EC_{50} of 5000 µg /L (Patel et al. 2015) one order of magnitude larger than expected solublity. Considering a S_w value of 434 µg /L for anthracene, 35 values were retained for further analysis, and SSD fitting (Fig. 3B) gave an HC_5 value of 2.37 µg /L.

- Benzo[a]anthracene: Twelve values were retrieved for benzo[a]anthracene toxicity against microalgae, all but one being above S_w. The most sensitive species was the Chlorophyceae *Chlorella fusca* var. *vacuolata* (EC_{50} of 2.62 µg /L, Grote et al. 2005) and the least sensitive was the marine/brackish water Chlorophyceae *Dunaliella tertiolecta* (EC_{50} of 788 µg /L, Ben Othman et al. 2012). An SSD curve drawn with all values (Fig. 3C) led to an HC_5 of 4.23 µg /L for benzo[a]anthracene.
- Benzo[a]pyrene: Twenty-two values were retrieved for benzo(a)pyrene toxicity against microalgae, with only eleven being above S_w. The most sensitive species was the freshwater Chlorophyceae *Chlorella fusca* var. *vacuolata* (EC_{50} of 0.63 µg /L, Grote et al. 2005) and the least sentitive was the Chlorophyceae *Chlorobion braunii* (formerly *Ankistrodesmus braunii*, EC_{50} of 1300 µg /L, Schoeny et al. 1988). A SSD curve drawn with all values (Fig. 3D), even above S_w, gave an HC_5 of 0.834 µg /L for benzo[a]pyrene.
- Fluoranthene: Twenty-one EC_{50}s values were gathered for fluoranthene toxicity to microalgae below S_w. The most sensitive species was *Chlorella fusca* var. *vacuolata* (EC_{50} of 5.38 µg /L, Grote et al. 2005) and the least one was the marine diatom *Skeletonema costatum* (66800 µg /L, Syracuse Research Corporation 1978). The SSD drawn for $EC_{50} < S_w$ (Fig. 3E) gave an HC_5 value of 15.5 µg /L.
- Fluorene: Twelve EC_{50}s values were used to draw SSD curve for fluorene (Fig. 3F) with an HC_5 of 76.8 µg/L. The most sensitive species was the marine prymnesiophyte *Pavlova lutheri* (Okumura et al. 2003) whereas the marine chlorophyte *Dunaliella bioculata* was the most tolerant to fluorene exposure (Heldal et al., 1984).
- Naphthalene: Eighteen EC_{50} values were reported for naphthalene, from 660 µg /L (prymnesiophyte *Pavlova lutheri*, Okumura et al. 2003) to 68200 µg /L (Chlorophyceae, *Desmodesmus subspicatus* formerly named *Scenedesmus subspicatus*, Djomo et al. 2004), Sw value in water being 31200 µg /L. The calculated HC_5 for naphthalene, based on the SSD for all the EC_{50} values below solubility (Fig. 3G), was 650 µg /L.
- Phenanthrene: Thirty-nine EC_{50} values below Sw were reported for phenanthrene, ranging from 20.8 (cyanobacteria from a natural Mediterranean assemblage of *Synechococcus* sp., Echeveste et al. 2010a) to 945 µg /L (freshwater chlorophyte *Chlamydomonas angulosa*,

Hutchinson et al. 1980). The calculated HC_5 for phenanthrene, based on the SSD (Fig. 3H), was 24.3 µg /L.

- Pyrene: Seventeen EC_{50} values below Sw were reported for pyrene, ranging from 7.03 (chlorophyte *Chlorella fusca* var. *vacuolata*, Grote et al. 2005) to 135 µg /L (cyanobacteria from a natural Mediterranean assemblage of *Synechococcus* sp., Echeveste et al. 2010a). The calculated HC_5 for pyrene, based on the SSD (Fig. 3I), was 11.7 µg /L.

4.2.2. Relationship between PAH HC_5s for microalgae, solubility in water and log K_{ow}

Published data on PAH solubility often rely to only a few numbers of publications (see references in Table 1), and in most cases only available for pure water as solvent, whereas salinity increase is expected to reduce PAH solubility in estuarine and marine waters (Kreutzer et al. 2022). Plotting all EC_{50}s against the water solubility constants S_w for each PAH (Fig. 4A) nevertheless suggested a significant contribution of solubility to observed toxicity expressed as EC_{50} (n=158, r^2=0.687, p=3.29*10^{-14}). Importance of PAH solubility was more obvious when considering the harmful concentrations to 5% of species HC_5, determined for each compound. By plotting calculated HC_5 for the nine PAH processed through SSD fitting against S_w, a log-log relationship (Fig. 4B) show that the most water-soluble PAHs are the least toxic to microalgae (n=9, r^2=0.926, p=0.00003). This illustrates the fact that compounds with low solubility are more prone to adsorb and enter the exposed cells, which is much clearer when considering the octanol-water partition coefficient expressed as K_{ow}. A robust linear relationship (n=9, r^2=0.757, p=0.0022) can be drawn (Fig. 5A) between log HC_5s and log K_{ow}; anthracene appears as an outlier in this correlation, with a mean toxicity about one order of magnitude higher (i.e. HC_5 reduced by ten) than could be expected from K_{ow}. Overall, the most hydrophobic PAHs, of high molecular weight and low solubility (Table 1) are the most toxic to microalgae, as uptake and bioconcentration factors predicted can vary over three orders of magnitude (Del Vento and Dachs 2002).

Similarly, a linear correlation (r^2= 0.595, p = 0.015, Fig. 5B) can also be highlighted between microalgae HC_5s and PAH toxicity assessment using predicted no effect concentrations (PNECs) from toxicity data on other living organisms or quantitative structure-activity relationship modelling (Wang et al. 2016). This would suggest that microalgae testing could be valuably performed in further PAHs ecotoxicity assessment, both as robust representative of all aquatic living organisms, and as cost-effective, easy to handle, and fast-response experimental models. The fact that anthracene again appears as an outlier when evaluated using experimental toxicity data (closed triangles in Fig. 5B) compare to QSAR-based predictions (open blue circles in Fig. 5B), highlight the limits of *in silico*

environmental risk assessment and the persistent need for experimental testing, both complementing each other.

4.2.3. Relationship between PAH toxicity and cell size

The estimation of microalgae biovolume is often difficult since phenotypic variations are observed on the same species depending on strain, culture conditions, nutrient status, etc. Methods were provided to calculate the cell biovolumes from microscopic examination including recently user-friendly equations and available spreadsheets (Borics et al. 2021), but actual biovolume of model microalgae are too scarcely provided in ecotoxicology literature as a whole.

The dataset (Suppl. Table S1) comprised 137 occurences where biovolume was available for a given EC_{50}, below solubility in water of the considered PAH, either as proposed by the authors of the published study, or provided by Borics et al. (2021). No effect of biovolume was apparent (Suppl. Figure S2A), most likely 1) because most of the biovolume estimates were from theoretical calculations (Borics et al. 2021, Olenina et al. 2006), and 2) because of the non-normal distribution of biovolume data. To test wether microalgae biovolume could influence the sensitivity of a given strain for a single PAH, we calculated the quotient EC_{50}/HC_5, for each data and tested species, assuming HC_5 as the most consistent illustration of the toxicity level to microalgae for a given PAH. The higher the EC_{50}/HC_5 value, the lesser the considered taxon is sensitive relative to all species tested for the same compound. No relationship arose between EC_{50}/HC_5 and cell biovolume (Suppl. Fig. S2b). This highlights the fact that more than biovolume itself, the surface-to-volume ratio needs to be considered, as it was proven to control the uptake and toxicity of various chemicals, mostly hydrophobic pollutants (Del Vento and Dachs, 2002) but also more soluble such as methyl-mercury (Tada and Marumoto 2020) or copper ions (Joonas et al. 2021) for example. As for testing with animals and higher plants, experimental evaluation of toxic chemicals on microalgae would be greatly enhanced if accompanied with consistent data on the size and shape of the model organisms.

4.2.4. Relationship between PAH toxicity and microalgae phylogeny

Since phylogenetic groups are not equally represented within the data set, SSDs were traced for the two most studied phyla, i.e. Chlorophyceae (n=80) and diatoms (n=34), regardless of the PAH compound (Fig. 6). Resulting HC_5 were 1.21 and 39.7 µg /L for chlorophyceae (Fig. 6A) and diatoms (Fig. 6B), respectively. Further analysis separated the marine chlorophyceae (n=8, not shown) from their freshwater counterparts (n=79, Fig. 6C) with HC_5 of 9.45 and 0.754 µg /L, respectively (Table 9). On first impression, it would appear that diatoms are obviously more tolerant than chlorophytes however this may be partly the result of differences in marine vs. freshwater origin and biases in the number of species tested in each categories (e.g. only 2 freshwater diatoms). Furthermore,

freshwater chlorophyceae were found to be much more sensitive to PAH exposure than marine species of this family. This could in turn suggest that differences in sensitivities could be linked to the marine or freshwater environment of the tested microalgae, since only two freshwater diatoms species were tested for PAH sensitivity in the dataset. PAH toxicity could not be predicted from phylogenetic membership of microalgae without extended testing to other species and further comparative analysis of marine and freshwater taxa sensitivity to PAHs.

5. Conclusion

Microalgae exposure to PAHs (as single compounds or in mixture) can result in a reduction in growth rates and biomass, and these effects are dose-dependent. These pollutants can also alter the fluorescence yield and the functional absorption cross-section of PSII, both significant proxies for photosynthetic activity. Membrane integrity is impacted under PAH exposure, as the ultrastructure of cells (like diatoms), chloroplast and thylakoids can be impaired. PAH exposure often results in increases in lipid cellular content and antioxidant-response. The expression of some genes can be up-regulated (such as the genes *LacsA*, *psbA*, *tmbi*) but other are down-regulated (such as the genes *sil3*, *3HfcpA*, *3HfcpB*). Environmental factors including UV radiation and temperature can increase the toxicity of PAHs to algal species. In response to this toxicity, several phytoplankton species were shown capable of removing PAHs from media. Degradation and accumulation of PAHs are species-dependent and are different between live and dead cells. The PAHs removal depends also on cell concentrations or biomass, light, the type of PAH, lipid content, enzymatic potential and cell composition.

Natural algal communities, interacting and highly diverse, appear to be more sensitive to PAH contamination or to sporadic oil discharges than algal populations in culture. PAHs are typically present as mixture of compounds in natural water and they can induce several effects (additive, synergistic or antagonistic) on natural phytoplankton. Changes in algal composition have been observed in both marine (Kottuparambil and Agustí 2018) and freshwaters (Rimet et al. 2004) and combined effects of PAHs with UV radiation and nutrients have been reported in several cases (Echeveste et al. 2011).

For nine out of the 16 priority PAHs, the decreasing order of toxicity ranking, deduced from SSD analysis and HC_5 determination, was as follows: benzo[a]pyrene > anthracene > benzo(a)anthracene > pyrene > fluoranthene > phenanthrene > fluorene > acenaphthene > naphthalene, highly dependent on chemical solubility and K_{ow}. A complete lack of ecotoxicity data is obvious for the remaining PAHs listed as priority compounds. The link between increasing microalgal individual cell biovolume and greater tolerance to PAHs is not supported by EC_{50}s data and SSD analysis, mostly

because relevant information such as biovolume and surface-to-area ratio during exposure are not routinely measured during testing. The fact that marine microalgae appear more tolerant than freshwater microalgae to PAHs calls for further research, taking into account that salinity can modulate physical properties of PAHs (Xie et al. 1997). Improving knowledge on PAHs ecotoxicity towards marine species will be beneficial since coastal and open ocean ecosystems are increasingly exposed to pollution worldwide (Zhang et al. 2021, Zhang et al. 2022, Zhou et al. 2022), whereas pollution remains overlooked among the anthropic drivers of both marine and freshwater biodiversity erosion (Mazor et al. 2018).

The review presented here is the first synthesis of current knowledge about PAHs toxicity to microalgae, and highlights the fact that a significant number of published and archived EC_{50} values for PAH toxicity are not fully reliable due to water solubility not considered in the experimental procedures. Furthermore, accurate estimation of exposure is often out of reach due to the fugacity of tested PAHs in experimental systems. This begs for further ecotoxicity analysis of PAHs using microalgae as models, combining accurate chemical analyses (including actual exposure concentrations, kinetic decays, and even bioconcentration). Innovations could help in resolving exposure accuracy issues, such as the passive dosing method (Kreutzer et al. 2022). Simple but explicit dose-response data such as EC_{50}s, often overlooked in recent scientific literature because of the apparent lack of novelty, compared to biochemical or genomic endpoints or toxicokinetic studies are still needed to ensure a more accurate risk assessment of PAHs in aquatic environments.

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Glossary

PAH : polycyclic aromatic hydrocarbons

UV / UV-A: ultraviolet light radiation with wavelength below visible range.

SSD: species-sensitivity distribution

HC5: harmful concentration for 5% of the species tested, an output of SSD analysis.

EC50: effective concentration reducing by half a fitness endpoint relative to unexposed control

ISO: international standard organization

OJIP: a model for the fluorescence induction kinetics based on the step measured during the fast rise of chlorophyll a fluorescence upon light exposure

PSII: photosystem two

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PAM: pulse-amplitude modulated fluorescence

SOD: superoxide dismutase

ROS: reactive oxygen species

CAT: catalase

WAF: water-accommodated fraction

PAR: photosynthetically available radiation

GST: glutathione-S transferase

PSI: photosystem one

PNEC: predicted no-effect concentration

Sw: solubility in water for a given compound, usually in pure water and standard temperature conditions

Kow: octanol-water partition coefficient of a given compound

QSAR: quantitative structure-activity relationship

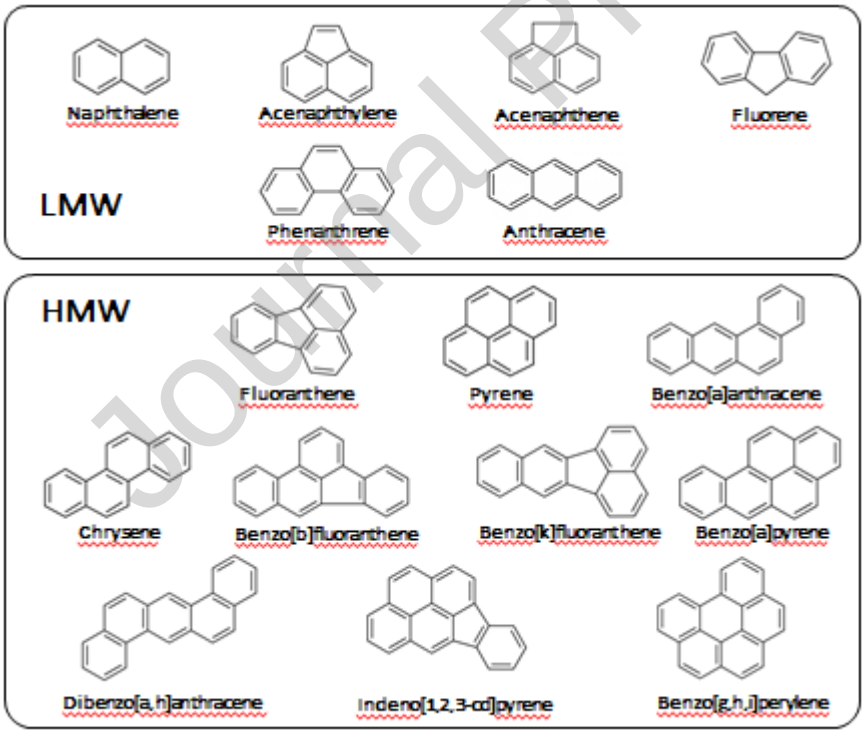

Figure 1. Semi-developed formulae of the 16 priority pollutants PAHs according to U.S. Environmental Protection Agency (USEPA, 1984); HMW: High molecular weight; LMW: Low molecular weight.

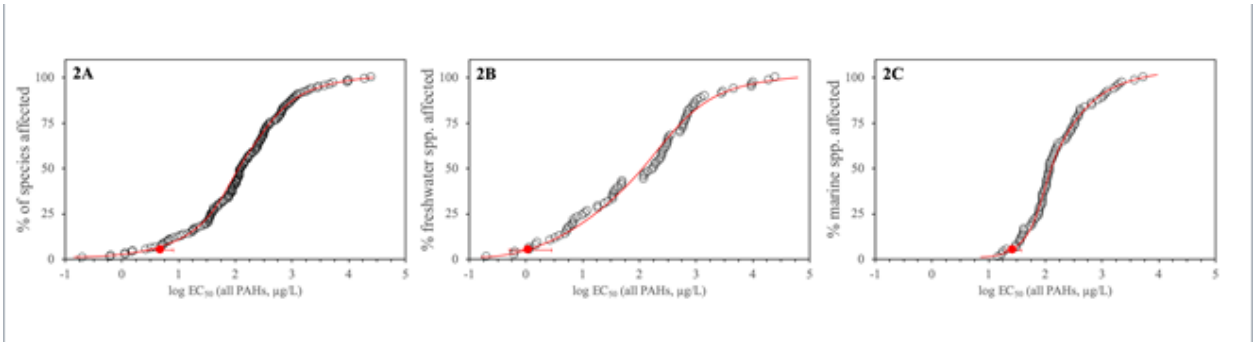

Figure 2. Species sensitivity distributions for all PAHs toxicity values (EC_{50}s, below or equal to water solubility of individual PAH) against all microalgae, fitted to a lognormal cumulative probability distribution (red curves). A: all EC_{50} data (n=158). B: all EC_{50} data for freshwater microalgae (n=79). C: all data for marine microalgae (n=79). HC_5 levels are indicated by closed red circles with 95% confidence limits.

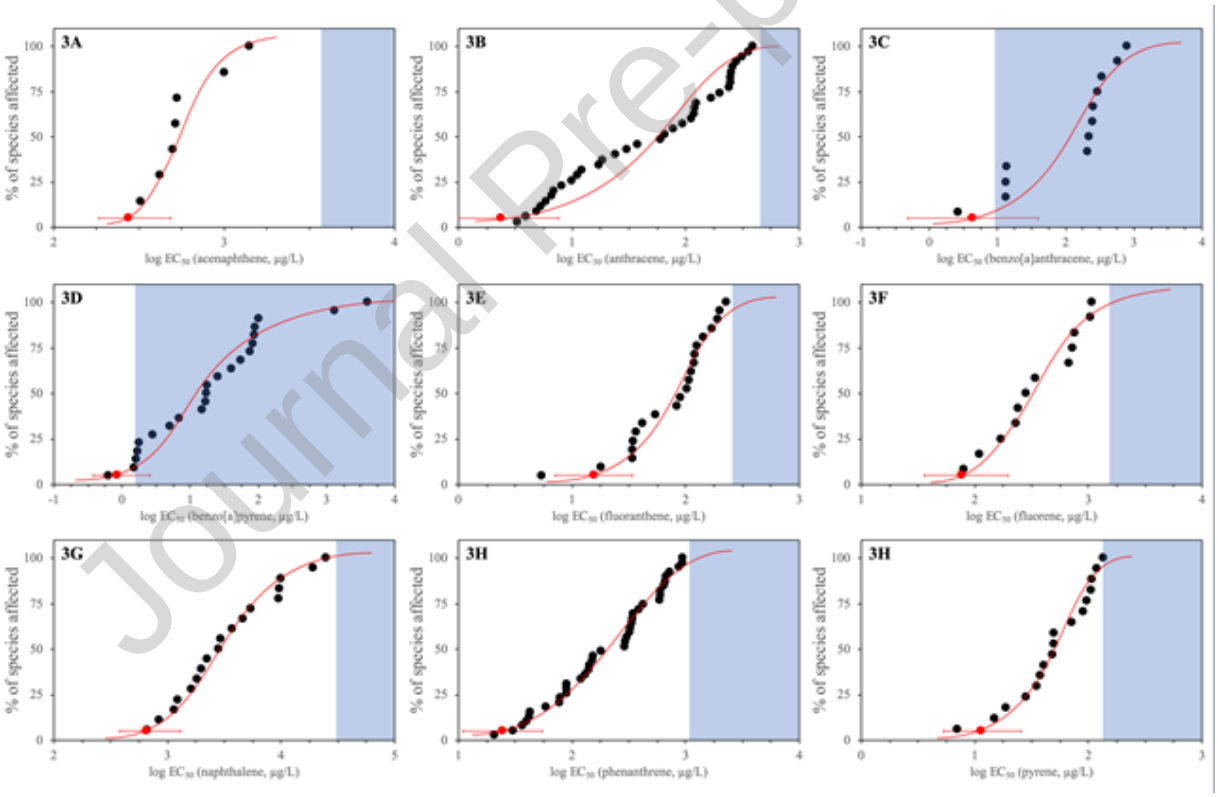

Figure 3. Species sensitivity distributions for single PAHs toxicity values (EC_{50}s) against all microalgae, fitted to a lognormal cumulative probability distribution (red curves). A: acenaphthene (n=7). B: anthracene (n=35). C: benzo(a)anthracene (n=12). D: benzo(a)pyrene (n=22). E: fluoranthene (n=21). F: fluorene (n=12). G: naphthalene (n=18). H: phenanthrene

(n=39). I: pyrene (n=17). Colored area indicates the limit of each PAH solubility in water. HC_5 levels are indicated by closed red circles with 95% confidence limits.

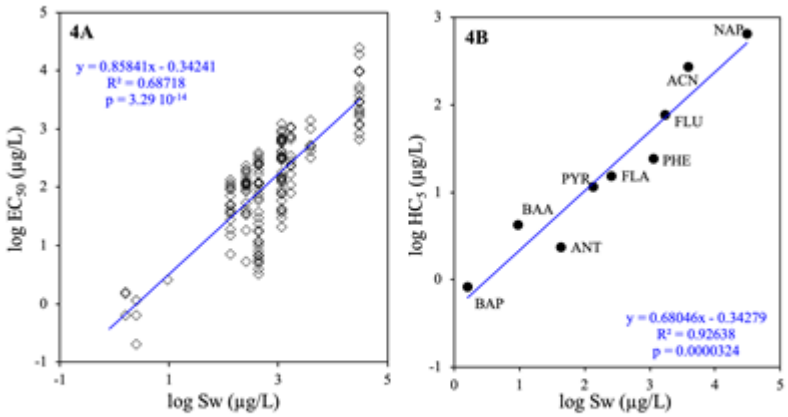

Figure 4. Relationship between PAH ecotoxicity values and solubility in water. A) Log-log regression of all EC_{50} values for microalgae against individual PAH solubility Sw (n=158, r^2=0.85841, p=3.29*10^{-14}). B) Log-log regression of calculated HC_5 to microalgae for the nine PAHs against solubility in water (n=9, r^2=0.6805, p=0.00004). The linear regression is figured by the blue line. BAP: benzo(a)pyrene; ANT: anthracene; BAA: benzo(a)anthracene; PYR: pyrene; FLA: fluoranthene; PHE: phenanthrene; FLU: fluorene; ACN: acenaphthene; NAP: naphthalene.

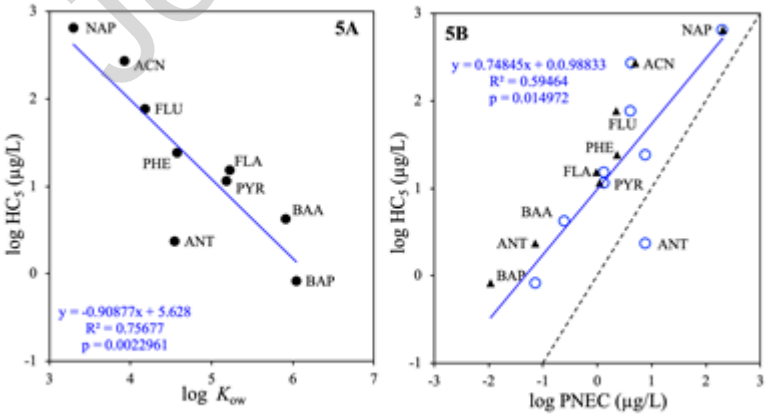

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Figure 5. A) Log-log regressions of HC_5 for all PAHs to octanol-water partition coefficient. B) Predicted No Effect Concentrations from Wang *et al.* (2016) calculated from QSAR modelling (open blue circles) or experimental data on various freshwater organisms (closed triangles). Blue line indicates regression between HC_5 (this study) and experimental PNEC (Wang et al. 2016). Black dotted line is the 1:1 correspondence. BAP: benzo(a)pyrene; ANT: anthracene; BAA: benzo(a)anthracene; PYR: pyrene; FLA: fluoranthene; PHE: phenanthrene; FLU: fluorene; ACN: acenaphthene; NAP: naphthalene.

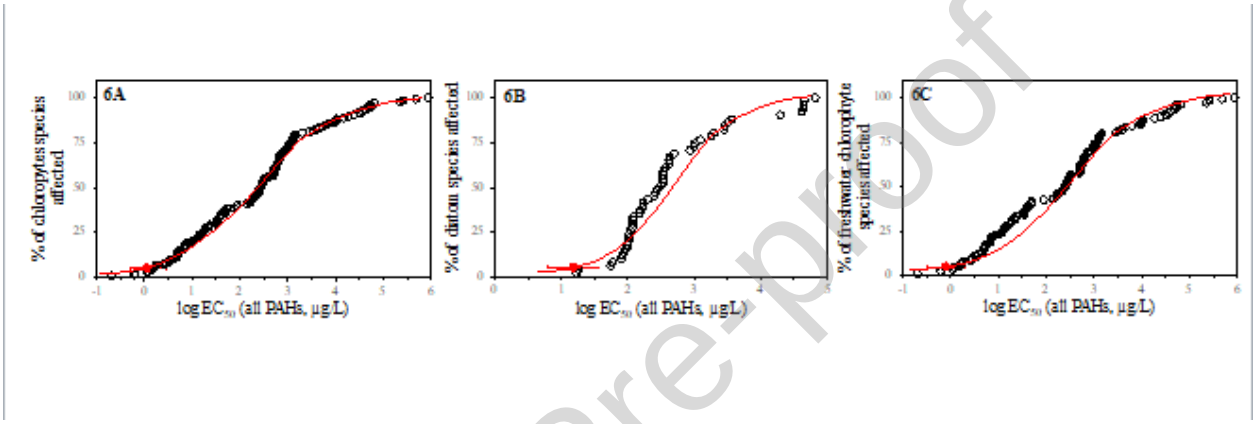

Figure 6. Species sensitivity distributions for all PAHs toxicity values (EC_{50}s below individual PAH solubility in water) against A) chlorophyceae (n=80); B) diatoms (n=34); and C) freshwater chlorophyceae (n=72) fitted to a lognormal cumulative probability distribution (red curves). HC_5 levels are indicated by closed red circles with 95% confidence limits.

Table 1. Summary of the physical and chemical properties of the sixteen priority PAHs in order of increasing benzene rings number.

CAS	PAH name	Formula	MW	V.P. at 25°C (Pa)	Sw (mg L^{-1})	MP (°C)	BP (°C)	Log K_{ow}	Log K_{oc}	*H* at 25°C (Pa m^{-3} mol^{-1})
91-20-3	Naphthalene	$C_{10}H_8$	128.18	33	31.0	81	218	3.30	3.3	43.01
208-96-8	Acenaphthylene	$C_{12}H_8$	152.20	1.35	16.1	93	280	3.93	4.07	12.17
83-32-9	Acenaphthene	$C_{12}H_{10}$	154.20	4.14	3.9	95	279	3.92	3.98	8.4

86-73-7	Fluorene	$C_{13}H_{10}$	166.23	$4.5\ 10^{-2}$	1.69	117	294	4.18	6.58	7.87
85-01-8	Phenanthrene	$C_{14}H_{10}$	178.24	$5.7\ 10^{-2}$	1.15	101	338	4.57	4.45	3.61
120-12-7	Anthracene	$C_{14}H_{10}$	178.24	$5.2\ 10^{-2}$	0.434	216	340	4.54	4.45	3.96
206-44-0	Fluoranthene	$C_{16}H_{10}$	202.26	$5.6\ 10^{-3}$	0.26	111	383	5.22	4.9	1.037
129-00-0	Pyrene	$C_{16}H_{10}$	202.26	$4.1\ 10^{-3}$	0.135	156	393	5.18	4.88	$9.2\ 10^{-1}$
56-55-3	Benzo(a)anthracene	$C_{18}H_{12}$	228.30	$2.3\ 10^{-4}$	0.0094	162	435	5.91	5.61	$5.8\ 10^{-1}$
218-01-9	Chrysene	$C_{18}H_{12}$	228.30	$4.8\ 10^{-5}$	0.002	256	441	5.86	5.16	5.86
205-99-2	Benzo(b)fluoranthene	$C_{20}H_{12}$	252.32	-	0.0015	168	481	5.80	6.04	-
207-08-9	Benzo(k)fluoranthene	$C_{20}H_{12}$	252.32	$4.1\ 10^{-6}$	0.0008	217	481	6.00	6.06	$1.6\ 10^{-2}$
50-32-8	Benzo(a)pyrene	$C_{20}H_{12}$	252.32	$3.2\ 10^{-6}$	0.0016	177	496	6.04	6.06	$4.6\ 10^{-2}$
53-70-3	Dibenzo(a,h)anthracene	$C_{22}H_{14}$	278.36	$8.1\ 10^{-8}$	0.0002	270	-	6.75	6.84	$1.7\ 10^{-4}$
193-39-5	Indeno(1,2,3-cd)pyrene	$C_{22}H_{12}$	276.34	$1.1\ 10^{-12}$	0.0025	278	-	6.50	6.5	$7.5\ 10^{-2}$
191-24-2	Benzo(g,h,i)perylene	$C_{22}H_{12}$	276.34	-	0.0003	-	-	7.04	6.58	-

Note: MW, molecular weight; V.P., vapor pressure; Sw, water solubility; MP, melting point; BP, boiling point; K_{oc}, organic carbon-water partition coefficient; K_{ow}, octanol-water partition coefficient; *H*, Henry's law constant. -: not provided. Data compiled from Allen (1997), Dabestani and Ivanov (1999), Ferreira (2001), Mackay et al. (2006), Mackay and Shiu (1992), Paraiba et al. (2011), Shiu and Mackay (1997), and USEPA (1984).

Table 2. Published EC_{50}s of various PAHs against the six most frequently tested microalgal species, based on **population endpoints** (growth rate or biomass in laboratory cultures). Species with less than 4 occurences together with data retrieved from the US EPA Ecotox database are provided as Supplementary Table 1. EC_{50} values marked with * are above known water solubility of the corresponding PAH, whereas § indicate exposure by passive dosing (see text). NB: *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum* are

referred to as *Raphidocelis subcapitata* according to currently accepted valid taxonomy (www.algaebase.org).

Species	Origin	PAH	EC_{50} (µg/L)	Reference
Chlorella vulgaris	freshwater chlorophyte	Fluoranthene	>5000*	Tomar and Jajoo 2021
		Naphthalene	4603	Yang and Wang 2017
			50000 < > 100000*	Kong *et al.* 2010
		Phenanthrene	11000*	Otero-Paternina *et al.* 2013
			143	Yang and Wang 2017
		Pyrene	164	
Dunaliella tertiolecta	marine chlorophyte	Benzo(a)anthracene	788*	Ben Othman *et al.* 2012
		Fluoranthene	387*	
		Fluorene	1070	Okumura *et al.* 2003
		Naphthalene	> 13800	
		Phenanthrene	> 460	
Isochrysis galbana	marine prymnesiophyte	Anthracene	65	Jian *et al.* 2000
		Benzo(a)anthracene	255*	Ben Othman *et al.* 2012
		Benzo(a)pyrene	88*	Jian *et al.* 2000
		Fluoranthene	112	Perez *et al.* 2010
			144	Ben Othman *et al*. 2012
		Fluorene	110	Okumura *et al.* 2003
		Naphthalene	840	
			2220	Perez *et al.* 2010
		Phenanthrene	140	Okumura *et al.* 2003
			389	Perez *et al.* 2010
		Pyrene	> 120	
Phaeodactylum tricornutum	marine diatom	Acenaphthene	420[§]	Niehus *et al*. 2018
		Anthracene	123	Wang *et al*. 2008
		Benzo(a)anthracene	>1825*	Ben Othman *et al.* 2012
		Fluoranthene	2838*	Tato and Beiras 2019
			372*	Ben Othman *et al.* 2012
			120[§]	Niehus *et al*. 2018
			103	Wang *et al.* 2008
		Fluorene	280[§]	Niehus *et al*. 2018
		Naphthalene	1240[§]	
		Phenanthrene	420[§]	

			154	Wang *et al*. 2008
		Pyrene	119	
			3195*	Echeveste *et al*. 2010a
Raphidocelis subcapitata	freshwater chlorophyte	Acenaphthene	1400	Japanese Ministry of Environment 2015
		Benzo(a)anthracene	290*	Kusk *et al.* 2018
		Benzo(a)pyrene	1.7	
			1.6[§]	Kreutzer *et al*. 2022
		Fluorene	720[§]	
			1045[§]	Bragin *et al.* 2016
			760	Japanese Ministry of Environment 2015
		Indeno[1,2,3-cd]pyrene	0.2	
		Naphthalene	10000	Kusk *et al.* 2018
		Phenanthrene	640	Japanese Ministry of Environment 2015
			120[§]	Kreutzer *et al.* 2022
		Pyrene	128[§]	
Scenedesmus vacuolatus	freshwater chlorophyte	Anthracene	506.2*	Grote *et al*. 2005
		Benzo(a)anthracene	13.22	
		Benzo(a)pyrene	17.7	
		Benzo(b)fluoranthene	22.3*	
		Benzo(k)fluoranthene	4.57*	
		Fluoranthene	34.0	
		Indeno[1,2,3-cd]pyrene	1.16	
		Phenanthrene	595.5	
		Pyrene	49.7	

Table 3. Summary of reported PAHs exposure effects on physiology and metabolism of various microalgal species. Concentration thresholds resulting in significant modification of metabolic responses compare to control conditions are given (not provided marked as nd).

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Values marked with * are above known water solubility of the corresponding PAH, as listed in Table 1.

Metabolism	Endpoint	PAH	Species	Observed effect	Effect threshold (µg/L)	Reference
Photosynthesis	*in vivo* chl *a* fluorescence	Anthracene	*Chlamydomonas reinhardti* cw92	fluorescence signal altered	125	Aksmann and Tukaj 2008
		Benzo(a)anthracene	*Alexandrium catenella*	reduction of photosynthetic yield	575*	Ben Othman *et al*. 2012
			Chaetoceros muelleri		575*	
			Dunaliella tertiolecta	no observed effect	> 2000 *	
			Isochrysis galbana	reduction of photosynthetic yield	186*	
			Nannochloris sp.		575*	
			Phaeodactylum tricornutum	no observed effect	> 2000 *	
			Picochlorum sp.	reduction of photosynthetic yield	575*	
		Fluoranthene	*Alexandrium catenella*		160	Pérez *et al*. 2010
			Chaetoceros muelleri		160	
			Dunaliella tertiolecta		506*	
			Isochrysis galbana		112	
			Nannochloris sp.		506*	Ben Othman *et al*. 2012
			Phaeodactylum tricornutum		50.6	
			Picochlorum sp.		506	
		Naphthalene	*Isochrysis galbana*		50.6	Pérez *et al*. 2010
		Phenanthrene			2220	
		Pyrene			389	
					120	

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			Synechocystis sp. PCC 6803		3125 *	Shao *et al*. 2010
	O_2 production	Anthracene	*Chlamydomonas reinhardti* cw92	reduction	500*	Aksmann and Tukaj 2008
			Desmodesmus armatus	reduction	nd	Aksmann and Tukaj 2004
		Fluorene	*Phaeodactylum tricornutum*	reduced to 64% of control	1000	Kusk 1981b
		Naphthalene		reduced to 33% of control	15000	Kusk 1981c
				reduced to 75% of control	1000	Kusk 1981b
		Phenanthrene	*Desmodesmus armatus*	increase	nd	Aksmann and Tukaj 2004
			Chlorella vulgaris (marine medium)	reduction	10	Jiang *et al.* 2022
			Skeletonema costatum	increase	1	
		Pyrene	*Synechocystis* sp. PCC 6803	reduction	625*	Shao *et al*. 2010
	CO_2 fixation	Naphthalene	*Thalassiosira pseudonana*	reduced to 50% of control	2000	Andersen *et al*. 1990
			Phaeodactylum tricornutum	reduced to 49% of control	10000	Kusk 1981a
Respiration	dark O_2 consumption	Anthracene	*Chlamydomonas reinhardti* cw92	increase	250	Aksmann and Tukaj 2008
		Fluorene	*Phaeodactylum tricornutum*	increased to 148% of control	1000	Kusk 1981b
		Naphthalene		increased to 144% of control	15000	
				increased to 148% of control	1000	
		Phenanthrene	*Chlorella vulgaris* (marine medium)	increase	1	Jiang *et al.* 2022
			Skeletonema costatum	increase	1	
Nitrogen fixation	acetylene reduction assay	Benzo(a)anthracene	*Anabaena flos-aquae*	reduction	29.9 *	Bastian and Toetz 1985
		Fluoranthene			434*	
		Fluorene			612	
		Naphthal			2071	

	ene				
	Phenanthrene			485	
	Pyrene			220.5*	

Table 4. Summary of reported PAHs exposure effects on enzymatic activities linked to stress response of various microalgal species. Concentration thresholds resulting in significant modification of metabolic responses compare to control conditions are given, if not available marked as nd. Concentration values marked with * are above known water solubility of the corresponding PAH, as listed in Table 1.

Endpoint	**PAH**	**Species**	**Observed effect**	**Effect threshold (µg/L)**	**Reference**
ascorbate peroxidase	Fluorene	*Chlorella vulgaris*	increase	10000*	Asghari *et al*. 2020
	Phenanthrene			10000*	Asghari *et al*. 2018
catalase	Fluorene	*Chlorella vulgaris*	increase	10000*	Asghari *et al*. 2020
	Phenanthrene			25000*	Asghari *et al*. 2018
			reduction	1	Calderón-Delgado *et al.* 2020
				1000*	Lei *et al*. 2006
	Pyrene	*Scenedesmus platydiscus*	reduction	100	
		Scenedesmus quadricauda	reduction	100	
		Monoraphidium capricornutum	no observed effect	>1000*	
glutathione peroxidase	Pyrene	*Scenedesmus platydiscus*	no observed effect	>1000*	
		Monoraphidium capricornutum	reduction	100	
		Chlorella vulgaris	increase	1000*	
		Scenedesmus quadricauda	increase	1000*	
		Monoraphidum	increase	1000*	

		capricornutum			
glutathione-S transferase	Pyrene	*Chlorella vulgaris*	no observed effect	>1000 *	
		Scenedesmus platydiscus	increase	100	
		Scenedesmus quadricauda	reduction	100	
		Monoraphidum capricornutum	increase	1000*	
superoxide dismutase	Anthracene	*Desmodesmus armatus*	increase	nd	Aksmann and Tukaj 2004
		Tetradesmus obliquus *Desmodesmus microspina* *Desmodesmus subspicatus*	transient increase, interaction with Cd^{2+}	250	Tukaj and Pokora 2006
	Fluoranthene	*Chlorella vulgaris*	reduction	1000*	Tomar and Jajoo 2021
		Cyclotella caspia	increase	150	Liu *et al*. 2006
	Fluorene	*Chlorella vulgaris*	increase	25000 *	Asghari *et al*. 2020
				25000 *	Asghari *et al*. 2018
	Phenanthrene		reduction	0.1	Calderón-Delgado *et al.* 2020
	Pyrene	*Desmodesmus armatus*	increase	nd	Aksmann and Tukaj 2004
		Chlorella vulgaris	reduction	1000*	Lei *et al*. 2006
		Scenedesmus platydiscus	no observed effect	>1000 *	
		Scenedesmus quadricauda	no observed effect	>1000 *	
		Monoraphidium capricornutum	no observed effect	>1000 *	

Table 5. Summary of reported PAHs exposure effects on biochemical content and cell morphology of various microalgal species. Concentration thresholds resulting in significant modification of metabolic responses compare to control conditions are given, if not available

marked as nd. Concentration values marked by * are above known water solubility of the corresponding PAH, as listed in Table 1.

Endpoint	PAH	Species	Observed effect	Effect threshold (µg/L)	Reference
glutathione	Pyrene	*Chlorella vulgaris*	no observed effect	>1000*	Lei *et al*. 2006
		Scenedesmus platydiscus	increase	1000*	
		Scenedesmus quadricauda	no observed effect	>1000*	
		Monoraphidium capricornutum	increase	1000*	
malondialdehyde content	Fluoranthene	*Phaeodactylum tricornutum*	doubled relative to control	200	Wang and Zheng 2008
	Naphthalene	*Chlorella vulgaris*	increase	nd	Kong *et al*. 2010
	Pyrene	*Chlorella vulgaris*	no observed effect	>1000*	Lei *et al*. 2006
		Scenedesmus platydiscus	no observed effect	>1000*	
		Scenedesmus quadricauda	reduction	1000*	
		Monoraphidium capricornutum	no observed effect	>1000*	
chlorophyll *a* content	Fluoranthene	*Cyclotella caspia*	reduction	100	Liu *et al*. 2006
		Chlorella vulgaris	reduction	1000*	Tomar and Jajoo 2021
	Fluorene			25000*	Asghari *et al*. 2020
				25000*	Asghari *et al*. 2018
	Phenanthrene			10	Calderón-Delgado *et al.* 2020
		Chlorella vulgaris (marine medium)	no observed effect	> 10	Jiang *et al*. 2022
		Skeletonema costatum	reduction	1	
chlorophyll *b* content	Fluorene	*Chlorella vulgaris*	reduction	25000*	Asghari *et al*. 2020

	Phenanthrene			10	Calderón-Delgado *et al.* 2020
				5000*	Asghari *et al*. 2018
carotenoids	Phenanthrene	*Chlorella vulgaris* (marine medium)	increase	10	Jiang *et al*. 2022
		Skeletonema costatum	reduction	1	
lipid content	Benzo(a)pyrene	*Nitzschia brevirostris*	increase	10	Croxton *et al*. 2015
	Naphthalene			10	
	Pyrene			10	
total flavonoids	Fluorene	*Chlorella vulgaris*	increase	2500*	Asghari *et al*. 2020
	Phenanthrene			2500*	Asghari *et al*. 2018
total phenolic compounds	Fluorene	*Chlorella vulgaris*	increase	2500*	Asghari *et al*. 2020
	Phenanthrene			2500*	Asghari *et al*. 2018
membrane integrity	Anthracene	*Chlamydomonas reinhardti* cw92	membrane disrupted	250	Aksmann and Tukaj 2008
	Benzo(a)pyrene	*Nitzschia brevirostris*	membrane disrupted	10	Croxton *et al*. 2015
	Naphthalene			10	
	Pyrene			10	
morphology	Fluorene	*Chlorella vulgaris*	cell shape modified	5000*	Asghari *et al*. 2020
	Phenanthrene			5000*	Asghari *et al*. 2018
	Fluoranthene	*Cyclotella caspia*	cell size and shape modified	200	Liu *et al*. 2006
	Phenanthrene	*Chlorella vulgaris*	cell diameter reduced	1	Calderón-Delgado *et al.* 2020

Table 6. Summary of genomics and proteomics approaches for assessment of PAH toxicity on microalgae. (+) denotes an increase in gene transcription / protein expression, (-) a decrease, (Ns) denotes non significant differences compare to non exposed controls. A

correspondence between genes and related encoded proteins and putative metabolic pathways is summarized.

Endpoint	PAH	Species	Observed effect	Reference
gene	Pyrene	*Thalassiosira pseudonana*	*lacsA* (+) *sil3* (-) *desB* (Ns) *sil1* (Ns) *3HfcpA* (-) *3HfcpB* (-) *rbj* (Ns)	Bopp and Lettieri 2007
	Fluoranthene			
	Benzo(a)pyrene			
	crude oil (WAF)		*lacsA* (+) *sil1* (+) *tmbi* (+) *sil3* (-) *sit1* (-) *diox* (-) *hsf* (-)	Carvalho *et al*. 2011b
	Pyrene	*Synechocystis* sp.	*psbA* (+) *psbB* (Ns) *psbC* (Ns) *psbO* (Ns)	Shao *et al*. 2010
	Anthracene	*Microcystis aeruginosa*	*mcyB* (-) *mcyD* (- then +) *mcyH* (- then +)	Bi *et al*. 2016
	Benzo(a)pyrene	*Thalassiosira pseudonana*	*sil1* (-)	Carvalho *et al*. 2011b
			lacsA (+) *sil3* (-) *diox* (-) *tmbi* (+) *hsf* (-) lacsA (+)	Carvalho *et al*. 2011a
protein			6 proteins up-regulated 7 proteins down-regulated	Carvalho and Lettieri 2011
whole transcri	crude oil (WAF)	*Ceratoneis closterium*	photosynthesis (-) respiration (-)	Hook *et al*. 2014

pt		purine metabolism (-) nutrient cycling (-) stress response (ns)	

Gene	Main process	Encoded protein
3HfcpA	photosynthetic pathway	fucoxanthin-chlorophyll a/c light harvesting protein
3HfcpB		fucoxanthin-chlorophyll a/c light harvesting protein
psbA		photosystem II protein D1
psbB		photosystem II CP47 reaction center protein
psbC		photosystem II CP43 reaction center protein
psbO		photosystem II manganese-stabilizing protein
rbj	signalling / transcription	GTP-binding protein
sil1	diatom frustule synthesis	silaffin precursor 1
sil3		silaffin precursor 3
sit1		silicon transporter
tmbi	stress signalling / apoptosis	anti-apoptotic BAX inhibitor
hsf	stress response	heat shock transcription factor
desB	stress signalling / lipid metabolism	sphingolipid delta-8 desaturase
diox	general metabolism / degradation pathway	4-hydroxyphenylpyruvate dioxygenase
lacsA	respiration pathway / lipid metabolism	long chain acyl-coA synthetase
mcyB	microcystin synthesis	peptide synthase
mcyD		polyketide synthase
mcyH		transporter

Table 7. Effects of environmental parameters applied to microalgae cultures on the reported toxicity of PAHs.

Environmental parameter	PAH	Species	Observed impact	Reference
pCO_2	Benzo(a)pyrene	*Skeletonema costatum*	exposure to 10 µg/L reduced the photosynthetic	Li *et al*. 2021

			efficiency and biogenic silicate content at high pCO_2	
Temperature	Anthracene, naphthalene, phenanthrene	*Tetraselmis chuii*	5% increase in temperature increased the toxicity of the PAHs	Vieira and Guilhermino 2012
pH	Phenanthrene	*Chlorella salina*	pH decrease from 9 to 6 resulted in a decrease of the EC_{50} by a factor of 8	Chen *et al*. 2018
Nitrate	5 different tested	*Scenedesmus subspicatus*	Nitrate ions in culture medium increased the radical formation with PAHs, increasing toxicity	Djomo *et al*. 2004
Light quality and UV intensity	Benzo(a)pyrene	*Selenastrum capricornutum*	fluorescent light peaking around 580 nm did not allowed the induction of benzo(a)pyrene toxicity contrary to cool white and "black light" artificial solar light resulted in increased toxicity of PAHs to the microalgae, UV-filtered light resulted in the lower toxicity	Cody *et al*. 1984
	14 different tested	*Scenedesmus vacuolatus*		Grote *et al*. 2005
	Anthracene, fluoranthene,	*Phaeodactylum tricornutum*	UV radiation during PAH exposure increased the	Wang *et al.* 2008

	phenanthrene, pyrene		toxicity	
	Fluoranthene	*Ankistrodesmus* sp.	UV radiation modified the microalga pigment content, mainly increasing the zeahanthin/violaxanthin ratio	Southerland and Lewitus 2004
	Fluoranthene, phenanthrene, pyrene	*Phaeodactylum tricornutum*	Both UV-A and UV-B radiations resulted in increased toxicity of each tested PAH	Okay and Karacik 2007
	Anthracene	*Selenastrum capricornutum*	EC_{50} for athracene was inversely related to UV-A intensity during exposure	Gala and Giesy 1992

Table 8. Studies reporting the toxicity of PAHs on experimentally exposed natural phytoplankton communities and photosynthetic biofilms. N.C.: nominal concentration. LOEC: lowest observed effect concentration, NOEC: no observed effect concentration.

Ecosystem	Location	PAH	Endpoint measurement	Main effects reported	Significant concentrations	Reference
Freshwater	Lake Erie, Canada	Anthracene 1,2dhATQ[§]	photosynthetic potential (variable chl *a* fluorescence)	toxicity only during daylight period toxicity not modulated by light	EC_{50} < 200 µg/L EC_{50} = 200 µg/L	Marwood *et al*. 1999
Freshwater	Lake Erie, Canada	Anthracene	photosynthetic potential	small differences in toxicity	314 < EC_{50} < 357 µg/L	Marwood *et al*. 2003

		Phenanthrene	(variable chl *a* fluorescence)	reported for daylight and dark samples	EC_{50} > 2000 µg/L	
		Fluoranthene			118 < EC_{50} < 125 µg/L	
		ATQ§			654 < EC_{50} < 684 µg/L	
		PHEQ§			90 < EC_{50} < 104 µg/L	
		1,2dhATQ§			1096 < EC_{50} < 1299 µg/L	
Freshwater	Rollingerbaach stream, Luxembourg	Fluoranthene	diatom diversity in biofilms	Community structure modified by PAH exposure, teratological effects at the higher concentration	2 and 200 µg/L (N.C.)	Rimet *et al*. 2004
Estuary	Murells Inlet, South Carolina	Fluoranthene	biomass (chl *a*) and accessory pigments	fluoranthene was toxic only wher combined with UV exposure; phytoplankton from polluted site tolerant to fluoranthene; xanthophyll cycling involved in phytoplankton response	95 µg/L (single dose exposure)	Southerland and Lewitus, 2004
Estuary	St Lawrence, Canada	Crude oil extract (98% naphthalene)	biomass (chl *a*) and cells < 20 µm	UV-B enhanced the toxicity	18.6 µg/L ΣPAHs	Sargian *et al*. 2005

Lagoon	Berre Lagoon, France	complex mixtures of metals and PAHs	photosynthetic oxygen production (biofilms)	PAHs contributed in the reduction of photosynthetic activities	LOEC 143 ng/L ΣPAHs	Pringault *et al*. 2015
Lagoon	Thau Lagoon, France Bizerte Lagoon, Tunisia	16 PAHs mixture	biomass (chl *a* and cell density), photosynthetic potential (variable fluorescence)	decrease in cell densities of pico-, nano-, and microphytoplankton, and reduction of photosynthetic potential decrease of cell densities of nano- and microphytoplankton, increase in picophytoplankton	EC_{50} 2.04 for biomass, 240 µg/L for photosynthetic potential EC_{50} 1.21 for biomass, > 75 µg/L for photosynthetic potential	Ben Othman *et al*. 2018
Lagoon	Juan de Nova Lagoon, Mozambique Channel	Naphthalene	biomass (chl *a* and cell density), taxonomic diversity, photosynthetic potential (variable fluorescence)	no change in phytoplankton biomass and activity, relative increase in picophytoplankton densities	23 µg/L (single dose exposure)	Bouvy *et al.* 2021
Coastal	Isefjord, Denmark	Pyrene	biomass (chl *a*), diversity (accessory pigments) and photosynthesis (^{14}C-incorpora	transient effects: decrease in biomass compensated by increase in productivity; changes in final community composition;	50.6 µg/L (higher exposure)	Hjorth *et al*. 2007

			tion)	relative increase in chlorophyta and cyanobacteria		
Coastal	Isefjord, Denmark	Pyrene	biomass (chl *a*), diversity (accessory pigments) and photosynthesis (^{14}C-incorporation)	transient effects: decrease in biomass compensated by increase in productivity; changes in final community composition; pyrene exposure compensated nutrient supply regarding biomass increase	two PAH injections of 11.2 µg/L at beginning and after 7 days incubation	Hjorth *et al*. 2008
Coastal	Sisimiut, Greenland	Pyrene	biomass (chl *a*) and photosynthesis (^{14}C-incorporation)	decrease in biomass and production at the lowest exposure concentration, increase in toxicity under natural sunlight UV exposure	LOEC 0.8 µg/L	Petersen *et al.* 2008
Coastal	Riá de Vigo, Galicia, Spain	Crude oil extract	biomass (chl *a* and cell density), taxonomic diversity, photosynthetic potential (variable	transient effects on biomass and photosynthetic potential; eventually changes in community structure; phytoplankton of oceanic	LOEC 8.6 µg/L expressed as chrysene equivalents	González *et al*. 2009

			fluorescence)	origin more sensitive to exposure		
Coastal	Riá de Vigo, Galicia, Spain	Crude oil extract	biomass (chl *a* and cell density), taxonomic diversity, photosynthetic potential (variable fluorescence)	"subtle" effects, not comparable to previous studies, suggested to result from the experimental set-up (buffering chracatreistics of mesocosms)	NOECs 19-58 µg/L expressed as chrysene equivalents	González *et al*. 2013
Coastal	Gulf of Mexico, Texas	Crude oil extract	photosynthetic potential (variable fluorescence)	no significant change in photosythetic parameters of phytoplankton exposed to crude oil water soluble fraction	NOEC > 70 µg/L ΣPAHs	Bera *et al.* 2020
Coastal	Loch Creran, Scotland	Crude oil	biomass (chl *a*) and microscopy cell count	disappearance of phytoplankton biomass exposed to crude oil	n.a.	Thompson *et al.* 2017
Coastal	Mallorca, Spain	Pyrene Phenanthrene	Dose-response on biomass (chl a), size classes of phytoplankton (flow cytometry), and live /	total populations declined at the lowest exposure concentration for both PAHs; picophytoplankton more sensitive than nanophytopl	EC_{50} from 20.8 to 179.5 µg/L	Echeveste *et al*. 2010a

			dead cells	ankton		
Oceanic	Central Eastern Mediterranean Southwestern Mediterranean Atlantic Ocean, South Canary Islands	Pyrene Phenanthrene Pyrene Phenanthrene		phytoplankton sensitivity to both PAHs was related to cell zize more than geographic origin	EC_{50} from 14.8 to 165.7 µg/L	
Oceanic	Northeastern subtropical Atlantic Ocean	16 PAHs mixture	Dose-response on biomass (chl a), size classes of phytoplankton (flow cytometry), and live / dead cells	exposure to PAHs mixture 20-fold the ambient level resulted in toxic effects	toxic concentrations expressed relative to ambient oceanic PAH levels	Echeveste *et al*. 2010b
Oceanic	Arctic Ocean, off Svalbard, Norway Southern Ocean, Bellingshausen Sea	16 PAHs mixture	Dose-response on biomass (chl a), size classes of phytoplankton (flow cytometry), and live / dead cells	synergistic effects of PAHs and UV radiation; picophytoplankton in oligotrophic water is the most sensitive	significant effects below 1 µg/L	Echeveste *et al*. 2011
Oceanic	Red Sea, off Saudi Arabia	Pyrene Phenanthrene	Dose-response on biomass	pyrene more toxic than phenanthrene,	LOEC pyrene 10 µg/L for	Kottuparambil and Agusti 2018

			and size classes of phytoplankton (flow cytometry)	picoeucaryotes more sensitive than picocyanobacteria	picoeukaryotes	
Oceanic	Atlantic, Indian and Pacific Oceans	4 to 12 PAHs mixture	Biomass (chl *a*) and cell density (flow cytometry)	two levels of exposure; picophytoplankton growth was repressed in all locations due to delay in DNA synthesis	LOEC 0.5 µg/L ΣPAHs	Cerezo and Agustí 2015a, 2015b

Table 9. Summary of SSD output for PAH toxicity to aquatic organisms and microalgae. Harmful concentrations HC_5 are derived from log-normal cumulative distribution modeling using ssdtools (see text). Values above known PAH solubility in water (S_w) are marked with an asterisk. LL: lower limit of HC_5, 95% confidence; UL: upper limit of HC_5, 95% confidence.

					risk assessment (µg/L)		
Data set	**n**	**lower value**	**median**	**upper value**	**HC_5**	**LL**	**UL**
all PAHs*, all aquatic organisms	878	0.1	n.a.	1300000*	2.76	2.08	3.72
all PAHs, all microalgae	158	0.2	127.5	25000	4.72	2.84	7.84
all PAHs, freshwater microalgae	79	0.2	180	25000	1.09	0.513	2.77
all PAHs, marine microalgae	79	14.8	120	5390	26.3	19.8	37.3
all PAHs, chlorophyceae	80	0.2	155.5	25000	1.21	0.499	2.79
all PAHs, diatoms	34	18	185	3730	39.7	25.1	68.6
all PAHs, marine chlorophyceae	8	17.2	187	5390	9.45	3	81.2
all PAHS, freshwater chlorophyceae	72	0.2	155.5	25000	0.754	0.433	2.76
acenaphthene	7	322	520	1400	274	184	485

anthracene	35	3.3	65	387	2.37	0.919	7.69
benzo[a]anthracene	12	2.625	235*	788*	4.23	0.486	39.3*
benzo[a]pyrene	22	0.631	17.5*	4000*	0.834	0.378	2.56*
fluoranthene	21	5.38	103	229	15.5	7.12	33.8
fluorene	12	80	310	1070	76.8	34.3	197
naphthalene	18	660	2890	25000	650	383	1310
phenanthrene	39	20.8	290	945	24.3	10.9	54.6
pyrene	17	7.04	49.7	135	11.7	5.28	25.6

Declaration of interests

☐ 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.

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Environmental Implication

Polycyclic Aromatic Hydrocarbons are toxic for ecosystems and living organisms, including humans. Dose-effect experiments of PAHs against microalgae are well represented. This is the first review about ecotoxicity data as EC50s, from literature and database. Several studies are flawed by exposure issues, weakening the assessment of PAHs toxicity using microalgae. Threshold environmental concentrations using species-sensitivity distributions will compensate this weakness, together with future well-designed studies. This would contribute to a reliable evaluation of microalgae PAHs degradation potential, which is considered as a promising bioremediation path. The current concern about emerging contaminants should not neglect the PAHs which remain significant pollutants.

Graphical Abstract

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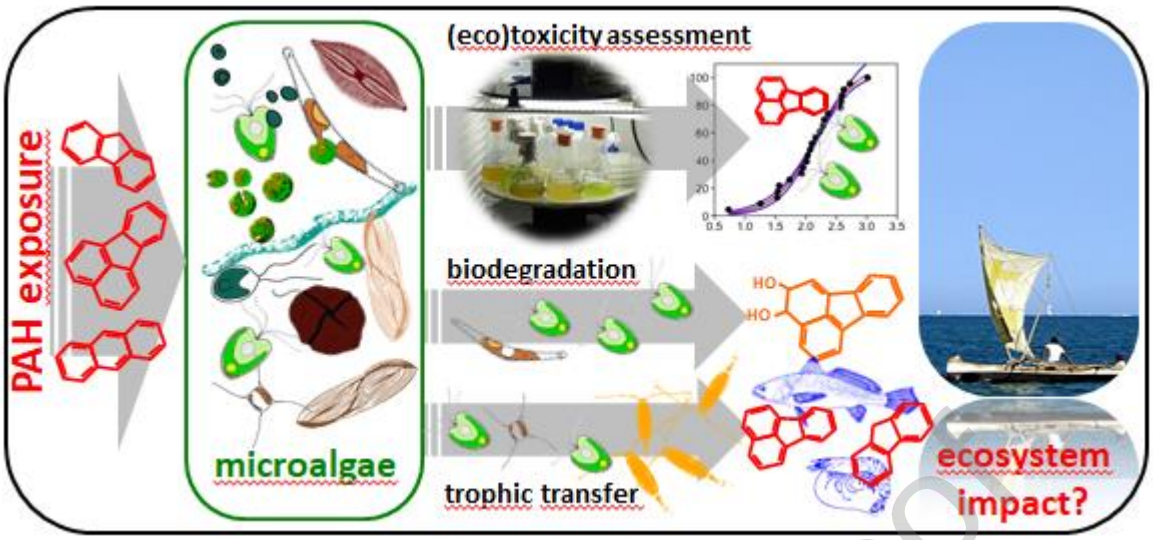

Highlights

- First review of Polycyclic Aromatic Hydrocarbons toxicity to microalgae
- Population, biochemical and structural endpoints are highlighted
- Species-sensitivity distribution analysis refine PAHs toxic thresholds for microalgae
- Current knowledge often biased by experimental flaws
- Further research necessary on these legacy contaminants still of concern

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