Stockholm University

Algal Sensitivity to Chemical Pollution Expressed as Chemical Activity

Talles Bruno Oliveira dos Anjos

Doctoral Thesis in Environmental Sciences at Stockholm University, Sweden 2025

Algal Sensitivity to Chemical Pollution Expressed as Chemical Activity

Talles Bruno Oliveira dos Anjos

Academic dissertation for the Degree of Doctor of Philosophy in Environmental Sciences at Stockholm University to be publicly defended on Friday 21 March 2025 at 13.00 in DeGeersalen, Geovetenskapens hus, Svante Arrhenius väg 12 and online via zoom, public link is available at the department website.

Abstract

The presence of hydrophobic organic contaminant (HOC) mixtures in marine environments threatens aquatic life and ecosystem processes. With thousands of chemicals present in the environment, accurately estimating their potential effects remains a major challenge. Here, chemical activity is employed as a unified metric to link baseline toxicity with the overall chemical load of a polycyclic aromatic hydrocarbon mixture, which serve as model compounds for HOCs. In **Paper I**, exposure to the chemical mixture resulted in growth inhibition in the cryptophyte *Rhodomonas salina*, following a dose-response curve with an effective activity (Ea_{50}) of 0.078. Notably, chlorophyll *a* concentrations exhibited hormesis. Baseline toxicity impacted photosynthesis at the cellular level, which led to more pronounced effects at the population level. In **Paper II**, five phytoplankton species showed varying levels of vulnerability, with chemical activity explaining at least 74% of the growth inhibition. Adaptive mechanisms (e.g., increases in lipid content, Chl *a* hormesis) and demographic traits (e.g., species-specific growth rates) likely contributed to the unexplained variance. Natural variations in lipid content and profile, along with alterations in lipid composition due to stress, provided insights into distinct patterns for energy utilization and their connection to chemical stress. The diatom *Phaeodactylum tricornutum* (Ea_{50} = 0.184) was the least affected by chemical exposure, exhibiting low lipid content and a higher growth rate. In contrast, populations of *Prymnesium parvum* (Ea_{50} = 0.072) and *R. salina*, both with high lipid content and low growth rates, were more vulnerable. In **Paper III**, a natural phytoplankton and bacterioplankton community was exposed to the PAH mixture. Exposure to a chemical activity of 0.1, which caused approximately 50% growth inhibition in monocultured laboratory populations (**Paper II**), resulted in significant reductions in phytoplankton diversity (**Paper III**). Sensitive taxa, including the chlorophyte *Pseudoscourfieldia marina*, cryptophytes, and picocyanobacteria, declined by 40-94% (**Paper III**). Bacterial communities also showed reductions in both α- and β-diversity, with a shift toward dominance by tolerant Proteobacteria taxa (98% in exposed samples). To assess chemical exposure under more realistic environmental conditions, **Paper IV** experimentally demonstrated that passive samplers can be used to assess the uptake and toxicity of the PAH mixture in the red macroalgal species *Ceramium tenuicorne*. By combining passive sampler uptake data with water turbidity, a predictive model was developed to estimate the chemical activity in *C. tenuicorne*, providing a basis for estimating photosynthesis inhibition in the alga. This thesis advances the understanding of algal sensitivity to HOC mixtures by using chemical activity to link toxicity with chemical load. It also demonstrates the potential of passive samplers for estimating the chemical activity of HOC mixtures and assessing their ecological risks in ecologically relevant settings. The findings highlight the key physicochemical processes governing algal uptake, baseline toxicity, and the resulting effects on photosynthetic efficiency, population vulnerability, and community structure.

Keywords: *chemical activity, baseline toxicity, hydrophobic organic contaminants, aquatic ecotoxicology, chemical mixtures, community effects, lipids, biological traits, passive samplers, passive dosing, equilibrium partitioning, phytoplankton.*

Stockholm 2025
http://urn.kb.se/resolve?urn=urn:nbn:se:su:diva-239047

ISBN 978-91-8107-104-7
ISBN 978-91-8107-105-4

Department of Environmental Science

Stockholm University, 106 91 Stockholm

ALGAL SENSITIVITY TO CHEMICAL POLLUTION EXPRESSED AS CHEMICAL ACTIVITY

Talles Bruno Oliveira dos Anjos

Stockholm University

Algal Sensitivity to Chemical Pollution Expressed as Chemical Activity

Talles Bruno Oliveira dos Anjos

ISBN print 978-91-8107-104-7
ISBN PDF 978-91-8107-105-4

Front page artwork by Maryam Moghaddam, used with the artist's permission
Printed in Sweden by Universitetsservice US-AB, Stockholm 2025

To those who pursue
freedom through
education and science

“For apart from inquiry, apart from the praxis, individuals cannot be truly human. Knowledge emerges only through invention and re-invention, through the restless, impatient, continuing, hopeful inquiry human beings pursue in the world, with the world, and with each other.”

— Paulo Freire, Pedagogy of the Oppressed

Contents

List of Papers

Paper I: Dos Anjos, T.B.O., Abel, S., Lindehoff, E., Bradshaw, C., and Sobek, A., 2023. Assessing the effects of a mixture of hydrophobic contaminants on the algae *Rhodomonas salina* using the chemical activity concept. Aquatic Toxicology, 265, p.106742. https://doi.org/10.1016/j.aquatox.2023.106742

I took the lead in designing the study and carried out the exposure. I was actively involved in the chemical analysis and data processing, and I took the lead role in writing the manuscript.

Paper II: Dos Anjos, T.B.O., Nham, Q., Abel, S., Lindehoff, E., Bradshaw, C., and Sobek, A., 2024. Differences in Phytoplankton Population Vulnerability to Chemical Activity of Mixtures. Environmental Science: Processes & Impacts. https://doi.org/10.1039/D4EM00249K

I took the lead in designing the study and carried out the exposure. I performed the laboratory experiment, led the chemical analysis and data processing, and I took the lead role in writing the manuscript.

Paper III: Chemical Activity of a Chemical Mixture Induces Alterations in Natural Phyto- and Bacterioplankton Communities. Dos Anjos, T.B.O., Sobek, A., Bradshaw, C., Serrana, J., Abel, S., and Lindehoff, E., 2025. Submitted to Environmental Toxicology and Chemistry.

I took the lead in designing the study and carried out the exposure. I performed the laboratory experiment, led the chemical analysis and data processing, and I took the lead role in writing the manuscript.

Paper IV: Can Passive Samplers Replace Biota in Risk Assessment of Resuspended Contaminated Sediments? Chaumet, B., Lo, H., Azaroff, A., Jonsson, S., Dos Anjos, T.B.O., Bonaglia S., and Sobek, A.

I contributed to the medium preparation, collection of photosynthetic efficiency data and gathering samples for PAH analysis in biota and I was involved in data evaluation, visualization and in writing the manuscript.

Sammanfattning

Med tusentals kemikalier närvarande i miljön, är en korrekt uppskattning av deras potentiella effekter fortfarande en stor utmaning. Här används kemisk aktivitet som ett enhetligt mått för att koppla samman toxicitet med den totala kemiska belastningen från en PAH-blandning (polycykliska aromatiska kolväten), där PAH:er används som modellföreningar för HOCs. I **Artikel I** resulterade exponering för den kemiska blandningen tillväxthämning hos kryptofyten *Rhodomonas salina*, enligt en dos-responskurva med en effektiv aktivitet (Ea_{50}) på 0.078. Noterbart var att klorofyll *a* visade hormesis vid kemisk aktivitet på 0.08 och 0.1. I **Artikel II** visade fem olika populationer av fytoplankton varierande nivåer av sårbarhet, där den kemiska aktiviteten förklarade minst 74% av effekten. Adaptiva mekanismer (t.ex. ökningar i lipidinnehåll, klorofyll *a*- hormesis) och demografiska egenskaper (t.ex. art-specifika tillväxthastigheter) bidrog troligtvis till den oförklarade variansen. Diatomen *Phaeodactylum tricornutum* (Ea_{50} = 0.184) var minst påverkad av kemisk exponering, med lågt lipidinnehåll och högre tillväxthastighet, medan populationer av *Prymnesium parvum* (Ea_{50} = 0.072) och *R. salina*, båda med högt lipidinnehåll och låga tillväxthastigheter, var mer sårbara. I **Artikel III** utsattes ett naturligt fytoplankton- och bakterieplanktonsamhälle för PAH-blandningen. Exponeringen ledde till betydande reduktioner i fytoplanktons biodiversitet, där känsliga taxa som klorofyten *Pseudoscourfieldia marina*, kryptofyter och picocyanobakterier minskade med 40–94%. Bakteriesamhällen visade också reduktioner i både α- och β-diversitet, med en förskjutning mot dominans av Proteobacteria-taxonomier (98% i behandlade prover). I **Artikel IV** utvärderades användningen av en passiv provtagare för att bedöma upptag och toxicitet av kemikalieblandningen i den röda makroalgen *Ceramium tenuicorne* i ett experimentellt system med sediment och vatten. En modell uppskattade den kemiska aktiviteten i *C. tenuicorne* baserat på upptag i den passiva provtagaren och turbiditet, vilket gav en grund för att uppskatta fotosynteshämning i *C. tenuicorne*. Denna avhandling fördjupar förståelsen av algers känslighet för HOC-blandningar genom att använda kemisk aktivitet för att koppla ihop toxicitet med kemisk belastning. Resultaten belyser de fysikaliska och kemiska processerna som styr algers upptag, toxicitet orsakat av HOCs som fördelas till cellens membran och de resulterande effekterna på fotosynteseffektivitet, populationssårbarhet och samhällsstruktur. Forskningen visar också på potentialen hos passiva provtagare för att uppskatta den kemiska aktiviteten hos HOC-blandningar och bedöma deras ekologiska risker under miljörelevanta förhållanden.

Resumo

Estimar os efeitos de milhares de produtos químicos presentes no ambiente é um grande desafio para os cientistas e sociedade. Nesse contexto, o uso da "atividade química total" (chemical activity) oferce uma métrica unificada para relacionar o potencial desses contaminantes, como os Hidrocarbonetos Aromáticos Policíclicos (HAPs) em causar toxicidade, como a narcose celular. No capítulo I, observou-se que a exposição a uma mistura de HAPs impediu o crescimento da criptófita *Rhodomonas salina*, gerando uma curva dose-resposta e uma "atividade química efetiva" (Ea_{50}) de 0.078. Além disso, foi observada uma reação positiva no teor de clorofila nas células expostas atividades químicas totais de 0.08 e 0.1. No capítulo II, cinco populações de diferentes espécies de fitoplâncton apresentaram diferentes níveis de vulnerabilidade, com a atividade química explicando pelo menos 74% da inibição do crescimento populacional. Mecanismos adaptativos, como aumento nos lipídios e hormese de chl *a*, e características demográficas como taxas de crescimento específicas de cada espécie, contribuíram para a variância não explicada. A diatomácea *Phaeodactylum tricornutum* (Ea_{50} = 0.184) foi a menos afetada pela exposição química. Essa espécie tinha o teor lipídico menor e uma taxa de crescimento maior que populações mais vulneráveis, como *Prymnesium parvum* (Ea_{50} = 0.072) e *R. Salina*. No capítulo III, uma comunidade natural de fitoplâncton e bacterioplâncton foi exposta à mistura de HAPs. A exposição uma atividade química total de 0.1 levou a reduções significativas na diversidade do fitoplâncton, com alguns grupos sensíveis, como a clorófita *Pseudoscourfieldia marina*, criptófitas e picocianobactérias, apresentando uma redução de densidade de 40 a 94%. A comunidade bacteriana também mostrou reduções na diversidade alpha e beta e Proteobacteria se tornou o grupo taxonômico mais dominante nas amostras expostas. No Capítulo IV, foi testado o uso de amostradores passivos na absorção e na toxicidade da mistura de HAPs na macroalga vermelha *Ceramium tenuicorne*. Um modelo preditivo foi criado para estiomar a "atividade química total" em *C. tenuicorne* com base na absorção pelo amostrador passivo e na turbidez da água. A relação entre o amostrador passivo e a alga foi usada para estimar a inibição da fotosíntese. Assim, esta tese contribui para o entedimento de como as algas respondem to COHs, através da "atividade química total" para relacionar toxicidade com a carga química total dessas misturas. Os resultados destacam os processos físico-químicos que governam a absorção de contaminantes pelas algas, assim como, efeitos resultantes da narcose celular na eficiência fotosintética, na vulnerabilidade das populações e na estrutura das communidades.

Abbreviations

HOC	Hydrophobic organic contaminant
PAH	Polycyclic aromatic hydrocarbon
Ace	Acenaphthene
Phe	Phenanthrene
Flu	Fluorene
FluO	Fluoranthene
PCB	Polychlorinated biphenyl
OCP	Organochlorine pesticide
C_{free}	Freely dissolved concentration in water
C_{biota}	Concentration in biota
$S_{L\ water}$	Subcooled liquid solubility in water
$S_{L\ biota}$	Subcooled liquid solubility in biota
a_{max}	Maximum chemical activity
$a_{\ biota}$ / $a_{\ Biota\ Mix}$	Chemical activity of the mixture in biota
$a_{\ PS\ Mix}$	Chemical activity of the mixture measured by the passive sampler
Ea_{50}	Effective activity 50% effect level
K_{OC}	Organic carbon – water partition ratio
PDMS	Polydimethylsiloxane
PE	Polyethylene
OECD	Organisation for Economic Co-operation and Development
PAM	Pulse amplitude modulation fluorometry
Fv/Fm	Ratio for maximum efficiency of photosystem II
chl *a*	chlorophyll *a*
POC	Particulate organic carbon
NTU	Nephelometric turbidity unit
HPLC-PDA	High-performance liquid chromatography coupled to a photodiode array detector
GC-MS	Gas chromatography-mass spectrometry
ASV	Amplicon sequence variant
BODIPY	Boron-dipyrromethene
GLM	Generalized linear model
IS	Labelled internal standard

1. Introduction

1.1. Global chemical pollution impact on aquatic ecosystems

Chemical pollution from human activities increasingly threatens natural processes that sustain human life on Earth. Scientists conclude that humanity has crossed a threshold at which the planet is pushed beyond a safe operational space for both human and environmental health (Persson et al., 2022). Currently, over 350 000 different chemicals are in use globally, and this number is expected to double in the coming years (Wang et al., 2020). A major concern for environmental health is our inability to fully understand and manage the effects of the growing number of chemicals being produced and which may eventually reach the environment.

The oceans and seas act as sinks for many chemical pollutants, where they can persist for decades, or even centuries. Persistent and hydrophobic organic contaminants (HOCs) such as polychlorinated biphenyls (PCBs), dioxins, and organochlorine pesticides (OCPs), are particularly notorious for accumulating in ocean deep water and sediments (Konat and Kowalewska, 2001; Lohmann et al., 2006; Ma et al., 2015). Polycyclic aromatic hydrocarbons (PAHs), a group of HOCs, are known for a long environmental stability, contributing to the overall toxicity exerted by HOCs (Neff et al., 2005; Nordberg et al., 2024). At the same time, emerging chemicals, such as pharmaceuticals, pesticides and industrial chemicals further stress aquatic ecosystems (Spaan et al., 2023; Tijani et al., 2015). Chemical pollution in marine ecosystems affects both environmental health and the vital services these ecosystems provide. Chemical pollution can degrade the quality and availability of food sources, damage critical habitats such as estuaries and coral reefs, weaken fish immune systems, and cause DNA damage in marine species (Landrigan et al., 2020).

Phytoplankton, along with cyanobacteria, microalgae and macroalgae, are fundamental to life on Earth. They play a crucial role in carbon cycling and contribute to global primary production in proportions comparable to those of terrestrial ecosystems (Häder et al., 2014). The diversity of phytoplankton is essential for maintaining the stability of marine ecosystems and supporting global nutrient cycling (Ptacnik et al., 2008). Phytoplankton are efficient in taking up HOCs from the water, as HOCs accumulate in the lipid-rich cell membranes (Sobek et al., 2004; Swackhamer and Skoglund, 1993). Macroalgae, or seaweeds, similarly contribute to primary production and provide essential habitats for other species (Duarte et al., 2022).

Both phytoplankton and macroalgae can be affected by HOCs, which disrupt their metabolic, biochemical, and community structures (Ben Othman et al., 2023; Echeveste et al., 2010, 2011; Zokm et al., 2022). Thus, phytoplankton and macroalgae have the potential to serve as real-time indicators of aquatic ecosystem health. Monitoring changes in species composition, abundance, and growth patterns can provide valuable insights into the overall condition of the aquatic ecosystems.

1.2. Chemical activity and baseline toxicity

Chemicals in the environment often occur as complex mixtures containing potentially thousands of compounds. Managing the great numbers and diversity of new chemicals introduced to the market presents a significant challenge to society. Current risk assessment frameworks are limited in their ability to protect both human health and the environment from the potential risk these chemicals pose (Fenner and Scheringer, 2021). As a result, many chemicals bypass regulatory frameworks and enter the environment, contributing to the complex mix of known and unknown substances that aquatic ecosystems face (European Commission, 2019; Fenner and Scheringer, 2021). Complex chemical mixtures can have significant ecological impacts, with biological membranes serving as the first barrier against chemical contaminants at the cellular level (Escher et al., 2002). When chemicals accumulate to sufficient levels in the cell membranes, they can disrupt membrane structure and function, a phenomenon known as narcosis or baseline toxicity (Van Wezel and Opperhuizen, 1995). Baseline toxicity is additive, and environmental chemical mixtures frequently follow a simple additive model (Landrum et al., 2003; Warne and Hawker, 1995). In this model, chemicals that individually would not cause baseline toxicity can still contribute to the overall baseline toxicity of the mixture (Hermens et al., 1984; Smith et al., 2013). The additive model is particularly relevant for neutral, hydrophobic chemicals, which primarily exert toxicity through baseline toxicity. While synergism can occur between chemical classes with specific modes of action, the nature of environmental mixtures – typically composed of multiple compounds at low concentrations – often leads to the convergence of synergistic and additive models (Martin et al., 2021; Warne and Hawker, 1995). Therefore, baseline toxicity and the assumption of additive toxicity can provide a useful starting point for characterizing the effects of environmental mixtures of neutral chemicals.

Chemical activity can be used to assess the baseline toxicity exerted by mixtures of chemicals on aquatic organisms (Gobas et al., 2018; Reichenberg and Mayer, 2006). First introduced by Lewis (1901, 1907), chemical activity is widely used in chemical engineering, medicine, and more recently in toxicology and ecotoxicology (Gobas et al., 2018; Reichenberg and Mayer, 2006; Schmidt and Mayer, 2015). Chemical activity provides a measure of the "chemical load" in the environment and correlates with the degree of membrane saturation in organisms, which is a reliable indicator of baseline toxicity, regardless of the chemical mixture's composition (Engraff et al., 2011; Schmidt and Mayer, 2015). The chemical activity of a compound in water can be approximated by calculating the ratio of its freely dissolved concentration in water (C_{free}), to its solubility (liquid or sub-cooled liquid, if the chemical is a solid at room temperature) (Gobas et al., 2018). The (hypothetical) pure liquid state at 298 K (1 atm) is used for solids when calculating chemical activity because, in the absence of crystalline lattice energies, the compound will behave as if it were in the liquid-state when dispersed in non-aqueous phases (e.g., lipids, organic carbon) (Schwarzenbach et al., 2016). C_{free} refers to the fraction of molecules in an aqueous solution that are not bound to particles or dissolved organic carbon, with the reference state being "dissolved in pure water" (Reichenberg and Mayer, 2006). The ratio results in a dimensionless metric that ranges from 0 to 1, which represents the fraction of chemical saturation relative to the compound's pure liquid state, equivalent to 0 - 100%. The total chemical activity of a mixture is obtained by summing the individual compounds' activities.

The use of chemical activity is particularly valuable when combined with polymers for passive dosing and passive sampling (Gobas et al., 2018). The passive dosing polymer acts as a source of the test chemical or chemical mixture in exposure tests, controlling the chemical activity or C_{free} through equilibrium partitioning (Ribbenstedt et al., 2017; Smith et al., 2010). When organisms are exposed to a constant C_{free} of a non-metabolizing chemical in a given environment, they accumulate the chemical over time until equilibrium is reached. At equilibrium, concentrations in the organism stabilize, and the chemical activities across compartments are equal. Passive sampling involves the placement of polymers in environmental media to assess the bioavailable fraction (C_{free}) in different compartments, such as water and sediment (Jonker et al., 2015; Vrana et al., 2005). In this context, passive samplers have become valuable tools for estimating the uptake and bioaccumulation of contaminants in aquatic organisms (Allan et al., 2013; Hajeb et al., 2022).

1.3. Applicability of chemical activity to understand effects of mixtures in aquatic systems

Several studies have shown that chemical activity and biological effects follow a dose-response curve. For green freshwater phytoplankton species such as *Pseudokirchneriella subcapitata* and *Raphidocelis subcapitata*, the effective activity that caused a 50% effect (Ea_{50}) typically falls within the range of 0.01 – 0.1 (Schmidt and Mayer, 2015). This range is consistent with the baseline toxicity observed in various organisms, such as the water flea *Daphnia magna* (Smith et al., 2013), the springtail *Folsomia candida* (Mayer and Holmstrup, 2008) and the brine shrimp *Artemia franciscana* (Rojo-Nieto et al., 2012). Recently, chemical activity and passive dosing were applied to assess the toxicity of PAH mixtures to the marine diatom *Phaeodactylum tricornum* (Niehus et al., 2018), and the green algae *Raphidocelis subcapitata* (Kreutzer et al., 2022), revealing significant variability in the dose-response data for individual PAHs. Previous studies did not directly measure chemical activity in algae but assumed it to be equivalent to the chemical activity in the surrounding media.

Building on the established link between chemical activity and baseline toxicity (Niehus et al., 2018; Smith et al., 2010), it is theoretically possible to predict effects caused by a certain level of membrane saturation in unicellular organisms by assuming that a specific level of chemical activity corresponds to a particular cellular effect. However, these effects propagate to the population level, influenced by the varying sensitivity of different species and the vulnerability of their populations (Rubach et al., 2011; Van Straalen, 1994). The vulnerability of phytoplankton populations to chemical pollution, at sub-lethal toxicity levels, varies across species in both concentration-based and chemical activity-based assessments (Niehus et al., 2018; Reichenberg and Mayer, 2006; Schmidt and Mayer, 2015). Population-level endpoints, such as growth inhibition in phytoplankton species, are influenced by biological and ecological traits. These traits — including lipid content, lipid profile, and growth rate — may influence phytoplankton responses to chemical mixtures (Bretherton et al., 2020; Häder and Gao, 2015). However, the specific ways in which these traits contribute to variations in population vulnerability remain unclear.

The diversity and structural composition of phytoplankton communities, as well as their interactions with organisms like bacteria, are crucial for ecosystem functions, nutrient cycling and productivity (Buchan et al., 2014; Litchman and Klausmeier, 2008; Tréguer et al., 2017). However, community-level studies remain scarce.

For example, ecotoxicological studies on chemical stress in aquatic systems at community level accounted for only 10 ± 5% of all relevant studies reviewed by Schuijt et al. (2021). In a recent review, Ben Othman et al. (2023) attributed the lack of studies on mixtures of PAHs — extending to other HOCs — in natural phytoplankton communities to the complexities inherent in such research. A persistent challenge, discussed at both individual and population levels, and also pertinent to community-level experiments, is that the toxicity of mixtures is often not adequately addressed. Additionally, toxicity thresholds for certain species can vary depending on the structure and composition of the natural phytoplankton communities (Ben Othman et al., 2023). Within these communities, the ecological context and the specific organisms present are key determinants of community-level effects (Schäfer et al., 2023; Thompson et al., 2018).

Assessing exposure and toxicity in environment requires methods capable of accounting for environmental dynamics. In natural systems, sediments often serve as a source of HOCs, which can be released as C_{free} into the water column, contributing to exposure and baseline toxicity in aquatic organisms. Passive samplers are assumed to mimic the uptake of HOCs into lipids in organisms, and as a result, they have been used to measure the chemical activity and to study the biological uptake of contaminants in various organisms including polychaetes, oligochaetes, mussels, aquatic insects, crustaceans, gastropods, and fish (Allan et al., 2025; Joyce et al., 2016; Lydy et al., 2014). Although some studies have combined passive samplers with algae — for example, to investigate sewage effluents (Vermeirssen et al., 2010) and herbicide uptake (Escher et al., 2006) — aquatic plants remain less studied. Furthermore, no research has specifically addressed the uptake and toxicity of HOC mixtures released from sediments, during environmentally relevant turbid conditions, in alga using passive samplers and chemical activity as a measure of pollution.

2. Aim and objectives

This thesis aims to advance the understanding of chemical mixtures and their effects at the base of the aquatic food webs, spanning from baseline toxicity at the cellular level to impacts at population and community levels. Chemical activity was employed to quantify the chemical burden, providing a measure of the overall chemical load in the environment, independent of the specific composition of the mixture.

The following objectives were outlined:

I. To enhance the understanding of the applicability of chemical activity for studying the effects of chemical mixtures on phytoplankton.

II. To evaluate how biological traits contribute to the differences in phytoplankton population vulnerability to chemical activity of mixtures.

III. To assess the impact of a chemical mixture on natural (field-collected) phyto- and bacterioplankton communities, accounting for interactions between populations and associations among species within the community.

IV. To evaluate the use of a polyethylene passive sampler to estimate the toxicity of a chemical mixture in the red macroalga *Ceramium tenuicorne* under sediment resuspension.

3. Material and methods

3.1. Overview

Paper I applied the concept of chemical activity to assess the effects of a HOC mixture on the cryptophyte alga *Rhodomonas salina.* The study examined how growth inhibition and photosynthesis-related endpoints correlated with the mixture chemical activity (Fig. 1). The HOC mixture, composed of four PAHs, had the same composition across all four papers. In **Paper II**, four additional phytoplankton species were exposed to the same mixture (Fig. 1). The study assessed differences in population vulnerability based on adaptive mechanisms (e.g., lipid production) and demographic traits (e.g., growth rate). Both **Papers I** and **II** used monocultures in 20 ml vials. **Paper III** investigated the impact of the chemical mixture on a natural phytoplankton and bacterioplankton community collected from the Baltic Sea. This study used a 10-fold larger volume while applying similar experimental principles (e.g., passive dosing) as in **Papers I** and **II** (Fig. 1). **Paper IV**, explored the use of passive samplers to estimate accumulation and toxicity in the red algae *C. tenuicorne*. The experimental setup included artificial sediment spiked with the PAH mixture, brackish water and a magnetic stir bar to increase turbidity (as a proxy for resuspension) and enhance the release of contaminants into the water (Fig. 1). **Paper IV** also evaluated the use of a diffusive gradient passive sampler to assess methylmercury (MeHg) in *C. tenuicorne* (not mentioned in Fig. 1); however, the focus of this thesis will be on the PAH mixture.

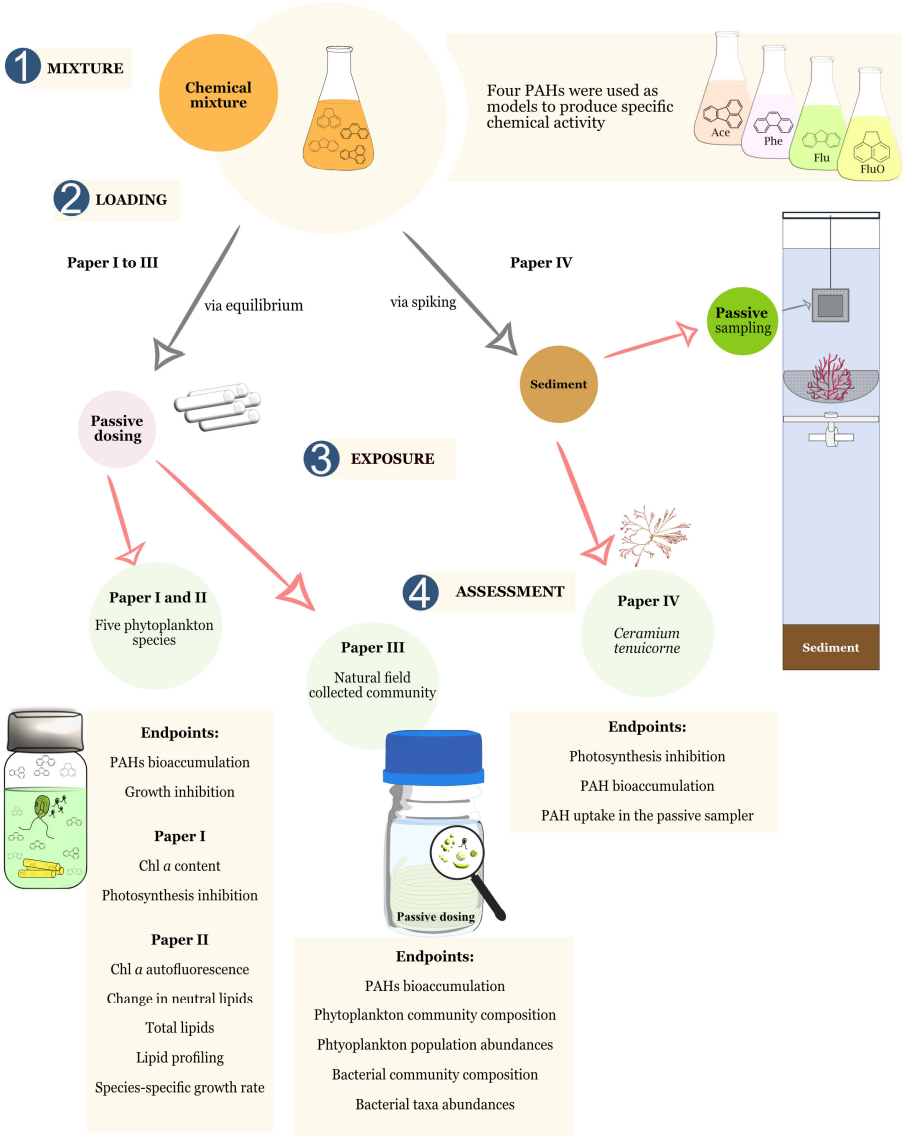

Figure 1: Conceptual summary diagram of the experimental work showing the progression of experiments across the four papers. The mixture of four PAHs had the same composition across all four papers: Monoculture experiment setup (**Paper I**) with *Rhodomonas salina* exposed to the chemical mixture in 20 mL vials; Multiple species exposure (**Paper II**) using the setup as in **Paper I;** Community-level exposure setup (**Paper III**) with a natural Baltic Sea phytoplankton and bacterioplankton community; Sediment exposure setup (**Paper IV**) with sediment, passive samplers and the red algae *C. tenuicorne*.

3.2. Chemicals and materials

PAHs have frequently been used and demonstrated to be appropriate as model compounds for HOCs in chemical activity-based research (Engraff et al., 2011; Smith et al., 2013). **Papers I-IV** used four PAHs to achieve varying levels of chemical activity (Fig. 1). The selected PAHs were: acenaphthene, phenanthrene, fluorene, and fluoranthene. All PAHs were procured from Sigma-Aldrich, Copenhagen, Denmark. For the preparation of saturated solutions, we utilized methanol (HPLC-grade) and n-hexane was employed for the extraction and analysis of the PAHs from algae. Translucent silicone cords made of polydimethylsiloxane (PDMS) (3 mm diameter, AlteSil^{TM}, Altec®, England) were used as passive dosing donors in **Papers I**, **II** and **III**. The silicone rods were cleaned following the procedures described by Birch et al. (2018), then cut into one-gram (**Paper I** and **II**) and ten-gram pieces (**Paper III**). Polyethylene (PE) was used as passive sampler in **Paper IV**. PE sheets (2 cm x 1.8 cm, 35 µm thick, 11.9 mg) were cleaned by soaking in methanol, acetone, and n-hexane for 24 hours each, then stored in n-hexane. Stainless steel nets, custom-made to hold the PE sheets, were rinsed with ethanol and acetone. The passive sampler-net assemblies were prepared on the day of the experiment, no more than an hour before exposure began. Artificial sediment in **Paper IV** was prepared following the OECD guideline 218 to create sediment with 70% of fine (< 63µm) and 30% of coarse particles (> 63µm) simulating, the sediment particle size distribution in the Baltic Sea.

3.3. Mixture preparation and chemical loading

In **Papers I** – **III**, PAH-methanol suspensions of the four PAHs were prepared by oversaturating methanol with PAH crystals in amber scotch glass bottles (Smith et al., 2010). The chemical activity in the PAH solutions was estimated based on the compound's melting point, with the entropy of melting approximated as 56 J mol^{-1} K^{-1} (Walden's rule) using equation (1):

$$a_{max} = exp\ (6.8\ [1 - \frac{Tm}{T}\ ]) = \frac{Ss}{SL} \qquad \text{Eq. (1)}$$

where a_{max} is the maximum chemical activity in solution (unitless), T_m is the melting point (K) of each PAH, and T is the exposure temperature (290 K) (Rojo-Nieto et al., 2012; Smith et al., 2010). Alternatively, the maximum chemical activity can be estimated by the ratio of the solubility of the PAH compound (S_s) and its aqueous subcooled liquid solubility (S_L), at the same temperature (Gobas et al., 2018; Mayer and Holmstrup, 2008). The saturated solvent solutions were used to prepare PAH mixtures with varying chemical activities. In **Paper I**, chemical activity ranged from 0.008 to 0.135, and in **Paper II**, an additional high level (0.17) was added with the range from 0.02 to 0.17. The chosen range in both papers aligns with observed baseline toxicity levels (Schmidt et al., 2013). In **Paper III**, a target chemical activity of 0.1, which corresponds to the Ea_{50} of all five phytoplankton species combined in **Paper II**, was set in order to ensure detectable effects for proof of concept. The cleaned silicone pieces were placed in amber scotch bottles with the PAH mixture and allowed to equilibrate at room temperature (25 °C) for at least 48 h at a bench shaker. Afterward, the silicone rods were washed with Milli-Q water. The loaded silicone pieces were then distributed into scintillation vials: 1 g silicone per vial with 20 ml Milli-Q for **Papers I** and **II**, or 10 g silicone per flask with 100 ml Milli-Q for **Paper III**. The vials were stored at -20 °C until exposure.

In **Paper IV**, a mixture of the same four PAHs was prepared in acetone. A target activity of 0.14 was set for the sediment to ensure sufficient levels of exposure through freely dissolved concentrations in the medium. PAH spiking was performed on Fontainebleau sand prior to mixing all components, following OECD 218 guidelines. The sand was manually mixed and left to dry under the fume hood, covered with aluminum foil, for 2 to 3 hours before adding the mineral components and mixing with the wet peat.

The artificial sediment was then immediately added to the cores, and brackish water was introduced. The cores were kept in the dark at 10°C for stabilization for 9 days to stabilize before initiating exposure with *C. tenuicorne*.

3.4. Test organisms and exposure medium

In **Paper I**, exposure to *Rhodomonas salina* (Cryptophyceae) (KAC 30) was tested, while **Paper II** examined four species: *Monoraphidium minutum* (Chlorophyceae) (KAC 90, sphere 5-10 µm), *Prymnesium parvum* (Haptophyta, Coccolithophyceae) (KAC 39, oval shape 10-12 x 5 µm), *Phaeodactylum tricornutum* (Bacillariophyta) (CCMP 2928, elongated oval, 10 x 3 µm), and a picoeucaryote species (KAC119, sphere 2µm), closely related to *Nannochloris* sp. (Trebouxiophyceae). These phytoplankton species represent different eukaryotic groups, each with distinct growth rates and variations in lipid composition. All cultures were isolated from the Baltic Sea and belong to the Kalmar Algae Collection (KAC), Linnaeus University, Sweden. The stock cultures were maintained in f/2 medium (Guillard, 1975) at 7 psu at a light intensity of 80 - 100 µmol photons m^{-2} s^{-1}, 16:8 light-dark cycle and at 17°C. In the case of *P. tricornutum*, silica was added to the medium. In **Paper III**, surface seawater with natural phytoplankton and bacterioplankton communities was collected from a coastal Baltic Sea site near Kalmar, Sweden. The water was transported, and stored at 4 °C, and the exposure began within three hours of collection. In **Paper IV**, the red algae *C. tenuicorne* was used as a model species. The monoculture (isolated from the Baltic Sea) was cultured in filtered, autoclaved brackish water (salinity 6.8 ± 0.1 ‰) with added nutrients, vitamins, and trace elements (Bruno and Eklund, 2003). Female clones were kept at 22 ± 1 °C in sterilized Petri dishes under a 14:10 light/dark cycle (35 ± 5 µmol $m^{-2}s^{-1}$). Actively growing algae were maintained by weekly transfer to fresh medium.

3.5. Exposure, sampling and endpoint assessments

Before the start of the exposure in **Papers I** and **II**, the stock cultures were prepared 3-4 days in advance to ensure the phytoplankton were in the exponential growth phase at the time of exposure to the chemical mixture. The algae growth inhibition test followed the OECD guideline 201, lasting for 72 hours, with one deviation:160 mg/L of sodium bicarbonate was added to minimize the growth inhibition due to CO_2 limitation in closed systems.

The desired inoculum was determined using a cell counter (**Paper I**) or flow cytometer (**Paper II**) to achieve an initial density of 4-6 x 10^4 cells/mL in each 20 mL vial. The vials were incubated in a climate-controlled room under the same conditions as the stock cultures. At the end of the trials, growth rates were assessed for all five phytoplankton species. In **Paper I,** chlorophyll *a* (chl *a*) was extracted and quantified, and photosynthetic inhibition measured using Pulse Amplitude Modulation Fluorometry (PAM). In **Paper II**, in addition to autofluorescence of chl *a* obtained with flow cytometry, changes in neutral lipids were assessed through BODIPY fluorescence. Lipid profile characterization and total lipid quantification were performed on non-exposed algae in **Paper II**. In **Paper III**, the natural community was exposed to the chemical mixture for 72 h, using the same methodology as in the phytoplankton tests. After exposure, samples were taken and phytoplankton larger than 5 µm (nano- and microphytoplankton) were counted using inverted light microscopy, while cells in the range of 0.5-3 µm (picophytoplankton) were identified via flow cytometry (Beckman-Coulter CytoFLEX®). Bacterioplankton abundance was analyzed using a modified protocol from Gasol et al., (2016), and DNA was extracted for a bacterial community composition analysis based on 16S rRNA. In **Paper IV**, *C. tenuicorne* was exposed for 7 days at 21 °C with a 10:14 dark/light cycle. The performance of PSII was assessed using PAM. Samples were dark-adapted at ambient temperature for 30 minutes prior to measurement, following the protocol of Burritt and Mackenzie (2003). Turbidity, ranging from 0.5 to 4.5 NTU (Nephelometric Turbidity Unit), was assessed on day 0 and on day 7 using a turbidity meter (HACH 2100NIS).

3.6. Exposure confirmation

Chemical activity in water

After exposure in **Papers I** to **III**, the medium was discarded, and vials containing loaded silicone were rinsed, refilled with Milli-Q water, and equilibrated for 48 hours at room temperature (20 °C). PAH concentrations were then analyzed using high-performance liquid chromatography coupled to a photodiode array detector (HPLC-PDA) with a HALO 90 Å PAH Column (particle size of 2.7 µm, 2.1 x 50 mm), using Milli-Q water/acetonitrile as mobile phase. Quantification was carried out using an external calibration curve. Chemical activities during exposure was calculated based on the ratio between freely dissolved PAH concentrations (C_{free}) in Milli-Q water and their respective subcooled solubilities (S_L) in the water (Eq. 2). Both C_{free} and S_L were corrected for the exposure temperature (17 °C) (See details in **Paper I** and **II**).

$$a = C_{free} / S_L \quad \text{Eq. (2)}$$

Chemical activity in biota

To estimate PAH concentrations in the phytoplankton (**Papers I** and **II**) or in the phyto-bacterioplankton community (**Paper III**), subsamples of the exposed medium were analyzed for particulate organic carbon (POC) using an elemental analyzer. From a separate set of subsamples, PAHs were extracted with n-hexane using ultrasonic processing and quantified via GC-MS (Trace 1310, Thermo Scientific), with 30 m × 0.25 mm × 0.25 µm column (Thermo Scientific) under electron impact ionization mode. PAH amounts were quantified from a calibration curve and their associated internal labelled standards (IS) and the concentrations normalized to the particulate organic carbon content of the samples. The chemical activity in the biota (a_{biota}) was calculated as the ratio of the PAH concentrations in the organic carbon (C_{biota}) to the subcooled liquid solubility in the biota ($S_{L\ biota}$). The $S_{L\ biota}$ was derived as the product of the $S_{L\ water}$ and the partition ratio for the studied matrix, in the case of **Papers I** to **III**, the organic carbon – water partition ratio (K_{OC}), as shown below (Eq. 3):

$$a_{biota} = C_{biota} / (S_{L\ water} * K_{oc}) \quad \text{Eq. (3)}$$

For these calculations, three different values for K_{oc}, as reported in literature (Burgess et al., 2012; Di Toro, 1985; Karickhoff, 1981; Szabo et al., 1990), were used to convert the $S_{L\ water}$ to represent $S_{L\ biota}$.

In **Paper IV**, *C. tenuicorne* was extracted using accelerated solvent extraction (ASE) with a 50:50 (v:v) mixture of acetone /n-hexane at 100°C for 3 cycles. The extract was concentrated to a volume of 1-2 mL, and a subsample of 200 µL was used for GC-MS analysis. The chemical activity in the biota (a_{biota}, referred to as $a_{Biota\ Mix}$ in **Paper IV**) was calculated by estimating C_{free} from the PAH concentrations in biota normalized to lipid content (C_{biota}) divided by the partition ratio between organic carbon and water (K_{OC}) reported by Szabo et al. (1990).

$$C_{free} = C_{matrix} / K_{OC} \quad \text{Eq. (4)}$$

The estimated C_{free} was then applied in Eq. (2) to calculate the chemical activities in *C. tenuicorne.*

Chemical activity in passive samplers

In **Paper IV**, PE samplers were rinsed with Milli-Q-water, cleaned of biofilm, and placed in 2 mL vials, then filled with n-hexane and quantification standards. The vials were placed on a roller mixer overnight to ensure thorough extraction. The samplers were extracted twice (2 x 700 µL = 1400 µL + internal labelled standards). The extracts were dried, centrifuged, and 900 µL of the supernatant was used for GC-MS analysis. The chemical activities in the PE samplers were calculated in manner similar to that for *C. tenuicorne* (Eq. 4). However, instead of *Koc*, the partition ratio between the passive sampler polymer and water (K_{PE}) was used.

3.7. Data analysis

In summary, **Papers I** and **II** employed a sigmoidal dose-response model using log-transformed chemical activities to assess the relationship between chemical activity and growth inhibition, calculating the effective chemical activity (Ea_{50}). For this thesis, an additional dose-response model was derived, combining data from all five phytoplankton species to assess the level of variance explained by the chemical activity, treating the five datasets as a single entity (see Fig. 3-F). In **Paper I**, a one-way analysis of variance (ANOVA) was applied to compare the effects of the treatment on chl *a* concentration and the F_v/F_m ratio.

In **Paper II**, an unpaired t-test was used to compare differences in growth rates between exposed and non-exposed cultures. A one-way ANOVA, followed by Tukey's post-hoc test, was performed to assess differences in total lipids among non-exposed cultures and different species. Another one-way ANOVA was performed to evaluate changes in chl *a* autofluorescence per cell, neutral lipid content, particulate organic carbon (POC) per cell, and mortality rates caused by the mixture. In **Paper III**, multiple unpaired t-tests were performed to compare the mean delta densities of various taxonomic groups of phytoplankton between the control and treatment groups. The diversity and structure of the bacterial community were based on the extracted DNA, 16S rRNA amplification, and processing the amplicon sequence variants (ASVs) using bioinformatic tools (detailed in **Paper III)**. In **Paper IV**, an exponential decay function was used to quantify turbidity, expressed as the area under the curve ($turbidity_{AUC}$). This method enabled the assessment of turbidity changes over time, with time on the x-axis and turbidity (in NTU) on the y-axis. A linear model function was applied to identify a model for $a_{Biota\ Mix}$, using two predictors: total chemical activity in the passive sampler ($a_{PS\ Mix}$) and $turbidity_{AUC}$. The purpose of this model was to understand the relationship between the chemical activity of the chemical mixture in the passive sampler, turbidity, and bioaccumulation in *C. tenuicorne*. A more detailed description of the model evaluation is available in **Paper IV**.

4. Results and discussion

4.1. From baseline toxicity to population level effects

A linear and positive correlation was observed between the calculated chemical activities in the phytoplankton and chemical activity in the medium (**Paper I** and **II**). Most chemical activity predictions for the tested species closely followed the 1:1 line (Fig. 2) indicating that the system was in equilibrium, and thus that chemical activity in the algae mirrored that of the silicone and the exposure medium. The lower chemical activities in *P. parvum* compared to equilibrium levels are uncertain due to low amounts of POC available for the analysis. However, given the 72-hour exposure in a closed system and previous data showing that equilibrium is reached for other single-cell species and HOCs within 25 hours (Gerofke et al., 2005), we assume *P. parvum* was near equilibrium.

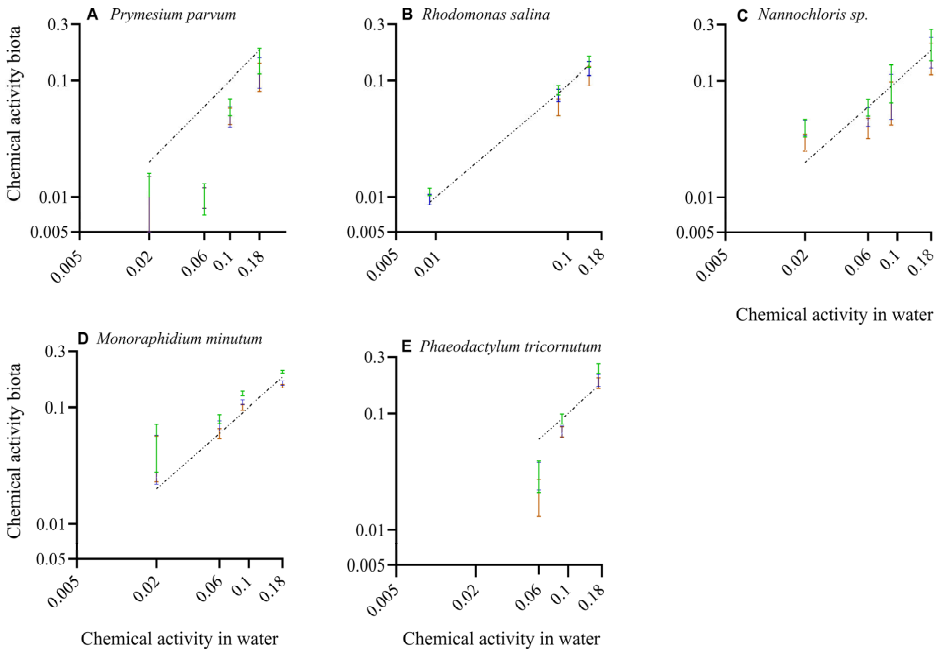

Figure 2: Correlation between PAH chemical activities in tested algae and in the exposure medium. Colored bars represent the mean chemical activities and their respective standard deviations in the phytoplankton. Three different organic carbon-water partition ratios (K_{oc}) from literature were used for calculation of chemical activity in the algae. Green: Szabo et al., 1990. Blue: Di Toro, 1985; Orange: Burgess et al., 2012. The dashed line represents the 1:1 line assuming equilibrium. (Figure 3 reprinted from **Paper II**: Dos Anjos et al. (2024), Differences in phytoplankton population vulnerability in response to chemical activity of mixtures. Environmental Science: Processes & Impacts, 26(11), pp.2062-2075)

Thermodynamically, and considering the well-established correlation between chemical activity and baseline toxicity (Niehus et al., 2018; Smith et al., 2010), it is expected that all five algae species would reach similar levels of membrane saturation, with comparable effects at the cellular level. However, the response of the five phytoplankton populations varied in relation to the chemical activity exerted by the PAH mixture (Fig. 3). The vulnerability order, from most to least vulnerable population, was as follows: *P. parvum* (Ea_{50} = 0.072), *R. salina* (Ea_{50} = 0.080), *Nannochloris* sp. (Ea_{50} = 0.103), *M. minutum* (Ea_{50} = 0.110), and *P. tricornutum* (Ea_{50} = 0.184). The difference in the Ea_{50} values between the most vulnerable population, *P. parvum*, and the least vulnerable, *P. tricornutum*, was a factor of 2.5 (Fig. 3).

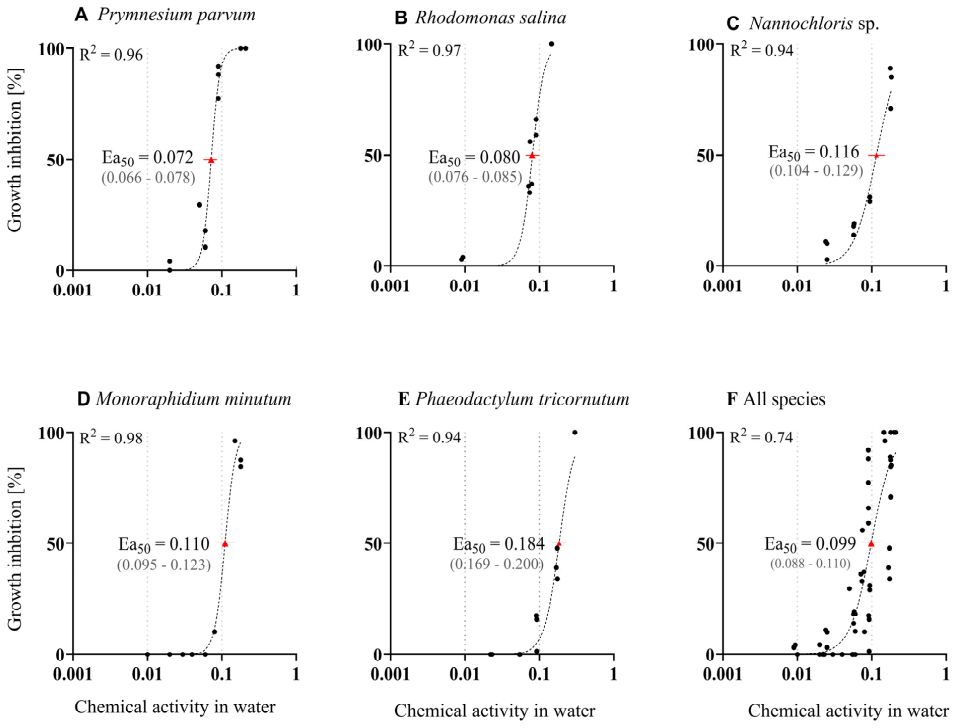

Figure 3: Dose-response curves illustrating the effects of a PAH mixture (chemical activity in water) on the growth of five algae species. The black solid line represents a sigmoidal curve on the relative percentage of growth inhibition over three days, determined by cell abundance measured with a flow cytometer (except for *Nannochloris* sp. and *Rhodomonas salina*, for which optical density was used). The response curve for *P. tricornutum* was extrapolated to a 100% effect at a chemical activity of 0.3. Effective activity that causes 50% of growth inhibition (Ea_{50}) was calculated (red triangle) with their respective 95% confidence interval. Dotted gray lines indicate the range of commonly observed baseline toxicity. (Figure 4 modified from **Paper II**: Dos Anjos et al. (2024), Differences in phytoplankton population vulnerability in response to chemical activity of mixtures. Environmental Science: Processes & Impacts, 26(11), pp.2062-2075).

When the data from the five phytoplankton species were fitted into a single dose-response curve, chemical activity accounted for 74% of the variation in the biological response (Fig. 3-F). This indicates that growth inhibition at population level was primarily driven by spontaneous physicochemical processes, such as the partitioning and diffusion of contaminants into membranes.

The remaining variability in the data set likely accounts for biological and ecological traits, including lipid content and profile, changes in neutral lipid content (Fig. 4-A), increases in chl *a* (Fig. 4-B) or other pigments, and species-specific growth rates. For example, at chemical activity of 0.06 and 0.09, neutral lipid fluorescence per cell increased by 6% and 7.5%, respectively, for *P. parvum*. At a chemical activity of 0.17 fluorescence per cell increased by 3.4% for *P. tricornutum*, and by 11% for *Nannochloris* sp. (Fig. 4-A). These results indicate that the synthesis of neutral lipids was an active mechanism during exposure, with varying intensities across species at different levels of chemical activity. Previous studies support the notion that increases in lipid content are linked to cellular repair mechanisms, membrane reconstitution, and a reduction in oxidative stress (Croxton et al., 2015; Morales et al., 2021).

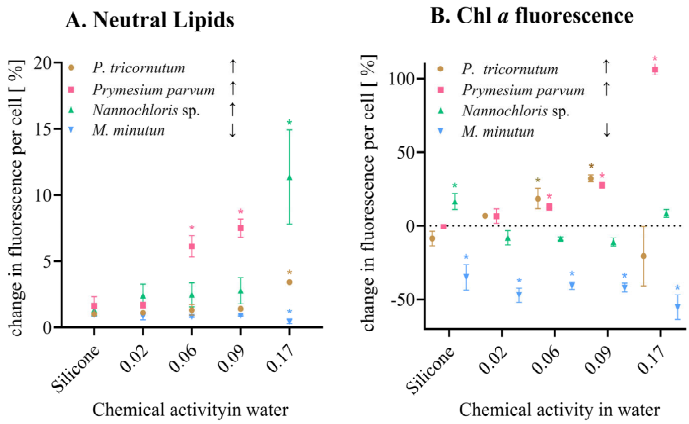

Figure 4: **A**: Change in mean chlorophyll *a* autofluorescence (670 nm) per cell relative to the control **B**: Neutral lipid content of different algae species measured indirectly via 525 nm fluorescence of lipids stained with BODIPY, expressed as a change in fluorescence per cell relative to the control as a function of the mixture chemical activity. (Figure 6 modified from **Paper II**: Dos Anjos et al. (2024), Differences in phytoplankton population vulnerability in response to chemical activity of mixtures. Environmental Science: Processes & Impacts, 26(11), pp.2062-2075).

Chl *a* fluorescence per cell, relative to the control, showed distinct responses across species at varying chemical activity levels. For *P. parvum*, fluorescence increased by 13% (± 1.7), 28% (± 1.5), and 106% (± 3.1) at chemical activities of 0.06, 0.09, and 0.17, respectively.

For *P. tricornutum*, fluorescence increased by 18% (± 5.7) at 0.06 and 32% (± 1.96) at 0.09 chemical activities (Fig. 4-B). For *R. salina*, chl *a* content per cell increased by 51% (± 3.8) at chemical activity of 0.08 and 58% (± 1.0) at 0.1 (Fig. 5-A). Interestingly, for *R. salina*, significant reductions in the photosynthetic efficiency were only detected at the highest chemical activity applied (0.13) (Fig 5-B). These results showed that the chlorophyll stimulation occurred at lower chemical activities up to a certain threshold of chemical exposure. The overall photosynthetic efficiency per unit of chl *a* decreased (Fig. 5-C), indicating that the elevated chl *a* levels contributed to maintain baseline photosynthetic activity during chemical stress. These results underscore that the PAH mixture affects not only cell membranes but also the membranes of chloroplasts and thylakoids. The energy allocated to adaptive and repair processes in the phytoplankton cells (toxicodynamic), such as increases in neutral lipids and chl *a* (Figs. 4 and 5), likely reduced population growth, as suggested by previous studies (Fields et al., 2014; Schuhmann et al., 2012). Species such as *P. parvum* being the most sensitive species, not only had the highest lipid content and the lowest growth rate (Table 1), but also displayed the earliest response in increasing lipid content when exposed to the chemical mixture (Fig. 4-A).

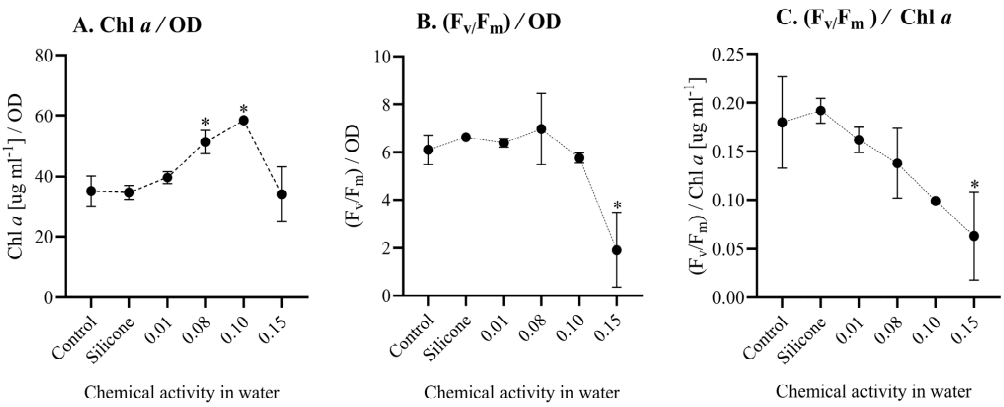

Figure 5: Photosynthetic efficiency and chlorophyll *a* (chl *a*) dynamic in *R. salina* exposed to a PAH mixture: **A**: Chl *a* (µg ml^{-1}) normalized to optical density (OD). **B**: photosynthetic efficiency rate F_v/F_m normalized to OD. **C**: F_v/F_m normalized to chl *a* concentration. Significant differences of the treatments were assessed by one-way ANOVA. Stars indicate significant differences ($p < 0.05$) between treatments and the control. (Figure 3 modified from **Paper I**: Dos Anjos et al. (2023), Assessing the effects of a mixture of hydrophobic contaminants on the algae *Rhodomonas salina* using the chemical activity concept. Aquatic Toxicology, 265, p.106742).

Table 1: Growth rate and lipid content in exposed and non-exposed algae

Species	Non-exposed (mean ± s.d., n=3)		Exposed (mean ± s.d., n=3)	
	Lipid Content [%]	μ	μ Control	μ Passive Dosing
P. parvum	32.8 ± 1.70^{a}	0.44 ± 0.02^{c}	0.53 ± 0.15	0.45 ± 0.14
R. salina	22.1 ± 1.23^{b}	0.48 ± 0.02^{c}	0.42 ± 0.02	0.45 ± 0.03
M. minutum	19.7 ± 0.43^{bc}	0.56 ± 0.01^{b}	0.49 ± 0.06	0.69 ± 0.13
P.tricornutum	18.3 ± 0.89^{c}	0.68 ± 0.02^{a}	0.56 ± 0.19	0.47 ± 0.05
Nannochloris sp.	14.8 ± 0.43^{d}	0.58 ± 0.00^{b}	0.43 ± 0.01	0.49 ± 0.01*

(μ) The specific growth rate d^{-1}. (*) Significant difference between control and Passive dosing. Different letters indicate significant differences ($p<0.05$) between treatments according to the Tukey´s post-hoc test.

Beyond individual sensitivity, the species' life history influences the population's intrinsic recovery potential (Van Straalen, 1994). For example, *P. parvum* and *R. salina* exhibited high lipid content (Table 1) and diverse fatty acids before exposure (See **Paper II**), indicating a natural tendency to allocate significant energy to lipid storage and synthesis rather than growth. In contrast, *P. tricornutum* directed more energy toward population growth than lipid synthesis and storage (Table 1, Fig. 4-B), which may have conferred an advantage in sustaining populations during the algae toxicity test. The species-specific growth rate also factored into the overall growth inhibition analysis, where growth rates in the treatments were normalized to the control. Populations of *P. parvum* and. *R. salina*, with their lower growth rates, were more vulnerable to the chemical mixture than *P. tricornutum*, which exhibited higher growth rates (Table 1). The results indicate that lipid content and profile are closely linked to species-specific growth rates — a key trait for intrinsic recovery and population dynamics.

4.2. From baseline toxicity to community level effects

Aside from the study on soil bacteria by (Winding et al., 2019), this study is among the first to assess community-level exposure to HOC mixtures using passive dosing and chemical activity as a metric. For the exposure of a natural bacterio- and phytoplankton community (**Paper III**), the total chemical activity in the medium was 0.1, while in the POC, it ranged from 0.068 to 0.093, depending on the K_{oc} values used. Exposure to a chemical activity of approximately 0.1 led to a significant decline in phytoplankton diversity (Fig. 6).

Sensitive taxa, including the green algae *Pseudoscourfieldia marina*, small cryptophytes, picocyanobacteria (*Synechococcus* sp.) and photosynthetic picoeukaryotes, experienced reductions ranging from 40 to 94% (Fig. 6). The diatom *Chaetoceros* sp. emerged as the dominant species in the treated group. The ability of less sensitive taxa like diatoms to maintain membrane integrity has been suggested as a mechanism for mitigating baseline toxicity (Croxton et al., 2015). Despite a 45% reduction in the total density of phytoplankton cells in the 0.5-3 µm size range, the representation of picophytoplankton increased in the exposed samples. This shift was attributed to the decline in flagellates and the subsequent dominance of phycocyanin-rich *Synechococcus* sp. (Fig. 6). Flagellate populations, which were the most abundant group in the control treatment, decreased from 84% to 33% in the chemical mixture treatment. Previous research has highlighted the heightened sensitivity of flagellates to organic contaminants and heavy metals (Goni-Urriza et al., 2018). Among the five tested phytoplankton species, the flagellates *P. parvum* and *R. salina* (**Paper II**) were the most sensitive to the chemical mixture. The partitioning of contaminants such as HOCs can disrupt critical membrane functions, like electrical conductance and membrane channels that are essential for cell motility in flagellates (Bretherton et al., 2020; Zheng et al., 2014). In contrast, diatoms generally exhibit greater tolerance to HOCs (Cohen et al., 1991; Croxton et al., 2015). For example, the diatom *P. tricornutum* showed a 50% growth inhibition (Ea_{50}) at chemical activity of 0.18 (Fig. 3-E), which is more than double the Ea_{50} values observed for the flagellates *R. salina* (Ea_{50} = 0.08) and *P. parvum* (Ea_{50} = 0.07) (**Paper I** and **II**). Furthermore, natural populations tend to be more sensitive than laboratory cultures (Echeveste et al., 2010; Moore and Harriss, 1974).

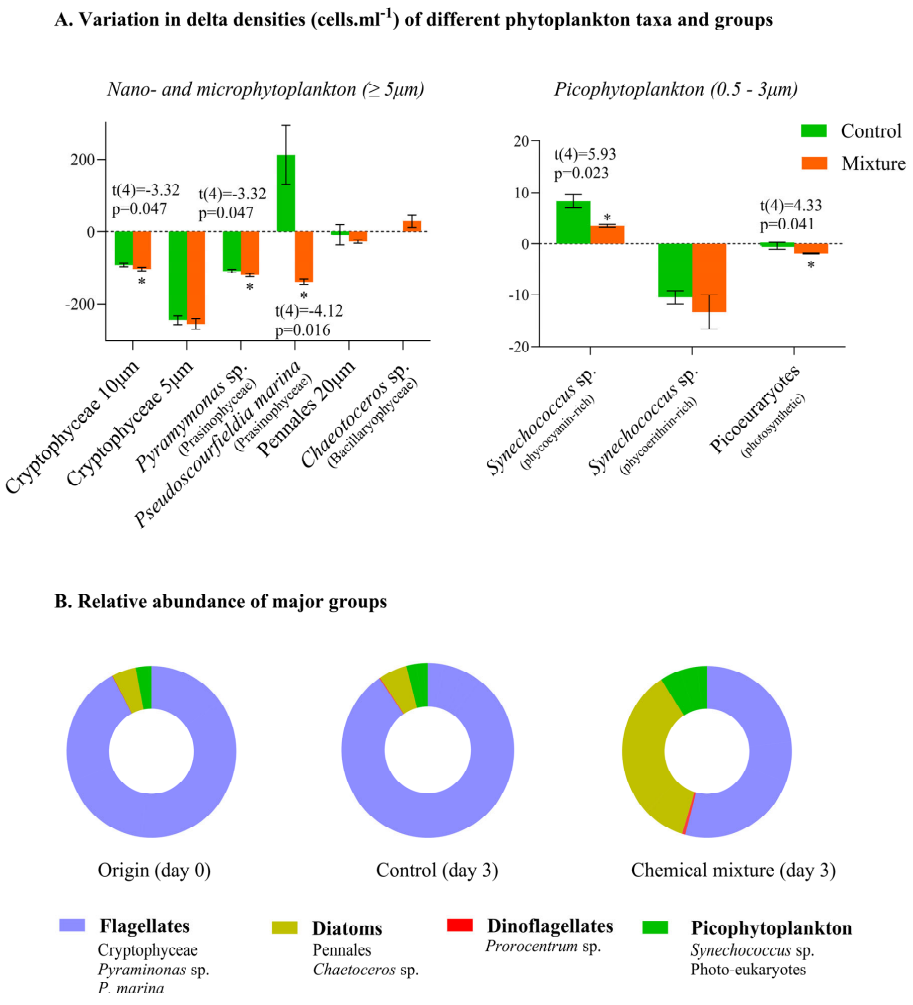

Figure 6: Impact of a chemical mixture on the phytoplankton community. **A**: Delta densities (changes in density compared to Day 0) of identified phytoplankton taxa larger than 5 µm, as observed under a microscope, and picophytoplankton groups (0.5-3 µm), measured using a flow cytometer. **B**: Relative abundance of major phytoplankton taxa/groups, showing reductions in specific taxa due to chemical exposure. Asterisks (*) indicate statistically significant reductions in populations between the control and treatment on Day 3, with p-values ≤ 0.05 based on unpaired t-tests comparing the control and treatment groups. (Figure 3 from **Paper III**).

The exposed bacterial community composition exhibited significant shifts, characterized by a reduction in both α- and β-diversity, as well as an increased dominance of Proteobacteria, which comprised 98% of the bacterial taxa in the treated samples (Fig. 7). Key genera within the Proteobacteria, such as *Shewanella*, *Limnobacter*, *Sphingorhabus*, and *Paraperlucidibaca* became more abundant, underscoring the resilience of Proteobacteria to chemical stress. In contrast, the abundance of more sensitive phyla, including Campylobacterota and Bacteroidota, decreased significantly (Fig. 7). Alpha-, Delta-, and Gamma-Proteobacteria are commonly dominant in PAH- and PCB-contaminated environments (Chen et al., 2017; Quero et al., 2015; Ren et al., 2015), with many PAH-degrading bacteria belonging to this phylum (Tauler et al., 2016). Members of Bacteroidota and Actinobacteria have also been found in contaminated sediments, with a reduction in Actinobacteria linked to PAH-contaminated soils (Mukherjee et al., 2014; Quero et al., 2015; Ren et al., 2015).

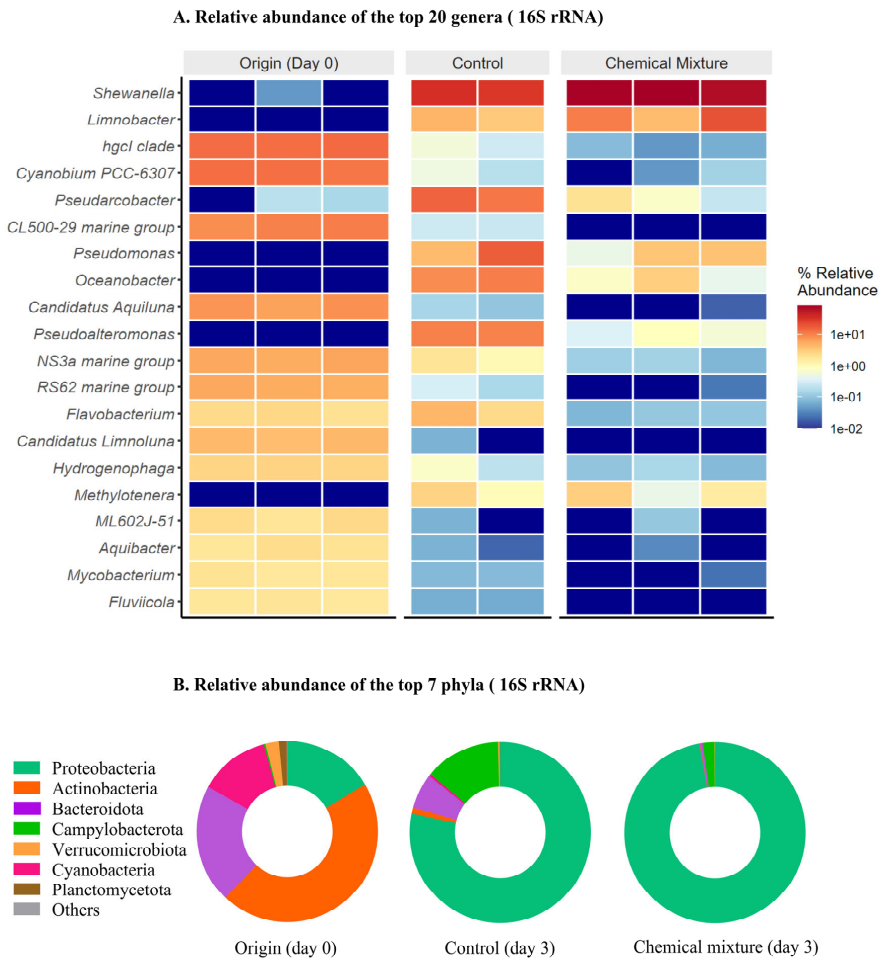

Figure 7: Effects of a chemical mixture on bacterioplankton populations. **A:** Density assessed based on the fluorescence of stained bacterial cells with SYTOX green. Effects of a chemical mixture on bacterioplankton taxa. **B**: Relative abundance (%) of the top 20 genera. **C:** Relative abundance (%) of the top 7 phyla. (Figs. 4 and 5 from **Paper III**).

4.3. Passive samplers to assess bioaccumulation and toxicity of chemical mixtures

Paper IV evaluates the use of a polyethylene passive sampler to estimate the bioaccumulation and toxicity of the PAH mixture (expressed as chemical activity) in the macroalga *C. tenuicorne,* with sediment resuspension as the source of contaminants. First, we investigated the relationship between the contaminant uptake by the passive sampler and bioaccumulation in *C. tenuicorne.* Next, we examined whether sediment resuspension influenced the passive sampler-biota relationship and assessed how bioaccumulation levels estimated from the passive sampler (estimated $a_{Biota\ Mix}$) correlate with toxicity, as measured by photosynthesis inhibition in *C. tenuircorne.*

The chemical activities of the PAH mixture in biota ranged from 0.001 to 0.03, while in the passive sampler, they ranged from 0.06 to 0.11. Unlike in **Papers I** to **III**, equilibrium-partitioning between the biota and the polymer was not observed. In dynamic systems, a nonequilibrium condition between the sampler and other environmental phases, such as biota in sediment resuspension, represents a more realistic scenario. The dynamic environment, with resuspended sediments and the time required for PAHs to transfer from the sediment to the water column may have contributed to the extended period needed for *C. tenuicorne* to reach equilibrium in the experimental setup. While equilibrium was observed within three days for the phytoplankton species in **Papers I** and **II**, achieving equilibrium may take several weeks for multi-cellular organisms in a dynamic system (Jonker et al., 2015). Additionally, biodegradation of PAHs by C. *tenuicorne* cannot be ruled out (**Paper IV**), as metabolic oxidation of benzo[a]pyrene has been reported in both green algae and charophytes (Kirso and Irha, 1998).

A comparison between the linear relationships of chemical activities of the PAH mixture in the two polymer-biota systems: passive sampler – *C. tenuicorne* (**Paper IV**) and passive dosing – phytoplankton (**Papers I** and **II**) revealed no statistically significant difference between the two slopes (Fig. 8). The similar patterns of PAH accumulation and consistent slopes across the two systems indicated that the accumulation of C_{free} PAHs by *C. tenuicorne* are similar to that in phytoplankton, and thus primarily governed by physicochemical properties of the chemicals and the cells' capacity to dissolve and store them (Sobek et al., 2004).

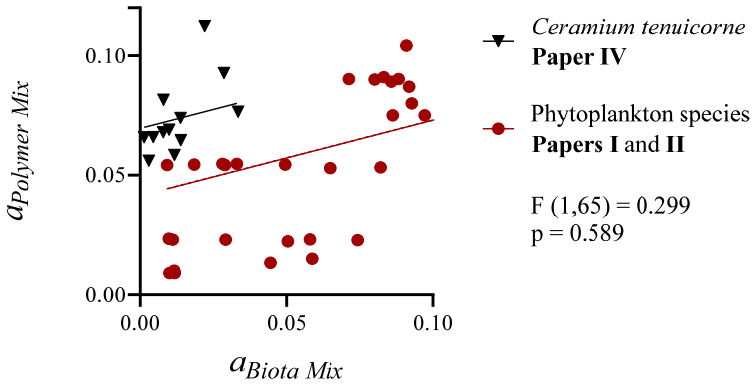

Figure 8: Relationship between $a_{Biota\ Mix}$ and activities in the passives sampler (**Paper IV**) and passive dosing polymer (**Papers I** and **II**) ($a_{Polymer\ Mix}$). The $a_{Biota\ Mix}$ values were derived from two datasets: black triangles represent chemical activities for *C. tenuicorne* (**Paper IV**), and red points represent predicted chemical activities from phytoplankton species (**Papers I** and **II**). A comparison of the slopes between the two datasets was performed using an extra sum-of-squares F-test, indicating that the slopes are not significantly different. (Modified figure 4 from **Paper IV**).

To further explore the relationship between contaminant uptake in the passive sampler and bioaccumulation in *C. tenuicorne* under sediment resuspension, a linear model (adjusted R^2 = 0.56) was established to estimate the total chemical activity in the algae ($a_{Biota\ Mix}$) (Eq. 5).

$$a_{biota\ Mix} = 0.347*(a_{PS\ Mix}) + 0.000995*(\text{turbidity}_{AUC}) - 0.027233 \qquad \text{Eq. (5)}$$

Here, $a_{PS\ Mix}$ represents the total chemical activity measured by the passive sampler, and turbidity_{AUC} represents the area under the curve, indicating the cumulative turbidity over time. Although $a_{PS\ Mix}$ is the strongest predictor in the model, as indicated by the comparison of the two-regression coefficients (where the coefficient for $a_{PS\ Mix}$ is 342 times higher than that for turbidity_{AUC}) (Eq. 3), the uptake of the PAH mixture by *C. tenuicorne* cannot be explained solely by the passive sampler's uptake without considering the effects of turbidity.

When the turbidity$_{AUC}$ values measured in the experiment (ranging from 8.8 to 28.6) were applied to the model, the specific contribution of turbidity to the chemical activity in algae ranged from 0.008 to 0.028. When applying the measured chemical activities from the passive sampler to Eq. 3, we obtained the estimated chemical activity in biota (estimated $a_{Biota\ Mix}$). The passive samplers represented the worst-case exposure scenario for *C. tenuicorne* in the system, as it reflects the highest chemical activity that C. *tenuicorne* can achieve.

Finally, we established a dose-response relationship between the chemical activity of the PAH mixture in *C. tenuircorne* (observed $a_{Biota\ Mix}$) and the resulting photosynthesis inhibition, which reached up to 23% at the highest chemical activity (0.03) (Fig. 9). The "estimated $a_{Biota\ Mix}$" was also fitted to the response curve, and there was insufficient evidence to support a significant difference in either the top of the sigmoidal curve (23%) or the Ea$_{50}$ between two curves ($F_{(2, 22)} = 1.78$ and $p = 0.135$). In paper **IV**, passive samplers were shown to accumulate PAHs in a manner similar to the alga *C. tenuicorne*, representing biota exposure to PAH mixtures. A linear model combining chemical activity measured by passive samplers with measured turbidity predicted mixture's bioaccumulation and the toxicity in *C. tenuicorne*.

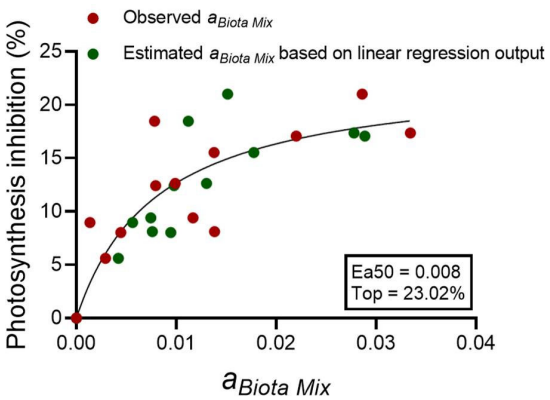

Figure 9: Photosynthesis inhibition (percentage of the control) for $a_{Biota\ Mix}$ measured in *C. tenuicorne* (red) and $a_{Biota\ Mix}$ estimated from the PAH accumulation data in the passive sampler (PE) using a linear regression model (green). Dose-response curves were plotted for both observed $a_{Biota\ Mix}$ and estimated $a_{Biota\ Mix}$ to assess the predictive ability of the estimated $a_{Biota\ Mix}$ in modeling toxicity. (Fig. 5 from **Paper IV**).

As discussed in **Papers I** and **II**, photosynthesis inhibition is also a consequence of thylakoid membranes disruption (Aksmann and Tukaj, 2008; Jajoo, 2017) and reductions in cellular photosynthetic activity can lead to strong inhibition in population growth, as observed for *R. salina* (**Paper I**). While the bioaccumulation estimates from passive samplers are specific to the experiment in **Paper IV**, it remains uncertain whether the relationship between uptake in passive sampler and *C. tenuicorne* can be generalized across a broader range of species and chemical profiles. However, considering sediment resuspension as a factor can help improve assessments of chemical mixtures in biota, especially in field applications where turbidity can vary by several orders of magnitude, and equilibrium is unlikely to be achieved (Dong et al., 2016; Friedman et al., 2009).

5. Conclusions

The approaches presented in this thesis for assessing the ecological impact of chemical activity using PAHs can be extended to other HOC mixtures, contributing to holistic evaluations of environmentally relevant mixtures in aquatic systems. Chemical activity was employed as a key metric to quantify the overall chemical load across multiple compartments, including the exposure medium, biota, passive dosing and sampler polymers. The studied algae species exhibited clear responses to the chemical mixture, with effects observed across multiple levels of biological organization. The effects ranged from physiological disruptions such as photosynthesis inhibition at the cellular level, to reductions in population growth rate and, ultimately, alterations in community structure.

Spontaneous physicochemical processes, including chemical partitioning and diffusion to biological membranes, were the dominant mechanisms driving baseline toxicity in phytoplankton populations. However, ecological traits and adaptive mechanisms, such as lipid accumulation and chlorophyll hormesis, contributed to remaining unexplained variability. Understanding how functional and ecological traits influence responses to chemical stress could pave the way for identifying biomarkers and key traits that help assess species sensitivity, as well as the vulnerability of populations and communities under specific environmental conditions. As biological complexity increases from individual species to communities and ecosystems, ecological interactions become increasingly relevant in shaping responses to contamination. For instance, interactions between phytoplankton species and their associated microbiomes can significantly influence how populations respond to pollutants. In controlled phytoplankton monocultures, exposure to a chemical activity of approximately 0.1 led to an average of 50% growth inhibition, whereas population reductions ranging from 33% to 94% were observed in field-collected communities. This finding underscores the ecological complexity that we face when assessing real-world contaminant effects.

The cause – effect relationship between mixture chemical activity and photosynthesis inhibition in *C. tenuicorne* allowed for toxicity estimation based on the bioaccumulation levels derived from passive samplers. In practice, passive samplers captured the mixture chemical activity in the surrounding media, providing a worst-case scenario estimate for bioaccumulation in *C. tenuicorne* under sediment resuspension condition. By integrating turbidity as a parameter to estimate chemical activity in *C. tenuicorne*, this approach more accurately approximates field conditions, where sediment resuspension fluctuates over time.

Expanding our understanding of the relationship between uptake of chemical mixtures in passive samplers and aquatic plants – while accounting for environmental factors such as particle resuspension – can contribute to the development and refinement of monitoring protocols.

Despite significant advances in chemical risk assessment and management over the past decade, the risks posed by chemical mixtures remain systematically overlooked. As a result, ecosystems continue to be exposed to complex combinations of anthropogenic contaminants. As society moves toward improved risk management of chemical mixtures, it is essential to recognize the importance of research in safeguarding both human and environmental health. This thesis contributes to this effort by advancing our understanding of chemical mixtures, investigating the biological responses of phytoplankton and macroalgae – acting as sentinels – for the overall chemical load in the environment.

6. Future Work

Integrating chemical activity measurements with advanced methods such as genomics and proteomics would enable a more comprehensive understanding of molecular initiating events and their cascading effects on ecosystem services provided by primary producers, such as nutrient cycling and carbon sequestration. By linking these molecular processes to broader ecological impacts, we could better assess the real-world consequences of chemical pollution in algae. Many of the adaptive mechanisms to cope with exposure to chemical mixtures could confer advantages during the recovery phase, for example, when contaminant exposure levels decrease. For example, the relationship of chemical activity and additional endpoints, such as impairment in motility relevant for flagellates or the ability of diatoms to maintain membrane integrity (proposed to be a characteristic of their tolerance) remains to be studied. Just as cause-effect relationships have been established at the population level for phytoplankton species, similar relationships can be explored at community level, allowing for the quantification of spontaneous physicochemical processes while accounting for intra- and interspecific interactions. Furthermore, despite the clear effects on phytoplankton and bacterioplankton communities, the impact of chemical mixtures on the association between bacteria and phytoplankton, particularly in the context of contaminant biodegradation, remains an area for future investigation. Lastly, by refining the use of passive samplers in measuring chemical activity under environmentally realistic scenarios and expanding the sampler-biota relationship to other aquatic plants, these samplers could offer additional representation for primary producers in environmental monitoring. This approach would improve our ability to estimate bioaccumulation and toxicity in a more standardized way.

7. Acknowledgements

This project began many years ago, even before I arrived at ACES, when **Anna Sobek** envisioned the research opportunity in this area, and the seeds of this thesis were first planted. Thank you, **Anna**, for allowing me to join you on this scientific adventure through life and for being a supportive supervisor during this journey. From you, I learned not only how to be a scientist but also how to be a better human. The experiences we shared will stay with me wherever I go.

I want to extend my gratitude to my supervisors: **Elin**, **Clare**, and **Sebastian**. What a great team!

Elin – Thank you for opening the doors of Linnaeus University and introducing me to your favorite organism. Your warm welcome every time I visited meant a lot to me. I also want to thank you for always bringing your positivity. I extend my gratitude to all the amazing people at Linnaeus University who helped me during this journey.

Clare – Thank you for your support and for always finding time to help with finding solutions and writing. I really appreciated our lunches together on campus.

Sebastian – Thank you so much for all the support you gave in helping me understand difficult concepts, and for teaching me a lot through your excellent laboratory skills. Thanks for always being there when I had questions and doubts.

During my time at ACES, I had the opportunity to be part of an amazing working environment and a great team. **Zhe Li**, thank you for all your support and our discussions—it's always a pleasure to work with you. **Sabrina** and **Sophie**, thank you for all our discussions about chemical activity. **Betty** and **Hoi**, thank you for your kindness and generosity; it was wonderful working with you. Thank you, **Lisa Duvekot**, for being

ACES gave me an amazing network of friends. Starting with close fellows from ACESo: **Eleni**, **Melanie**, **Joost**, and **Kyra** — thank you for being more than colleagues but also friends, supporting me throughout this journey. To all the professors: **Ian**, **Cindy**, **Malte**, **Jon**, and **Matt**. **Michael**, thanks for all our conversations on a variety of topics. **Merle** and **Martin**, thank you so much for your support. **Stella**, what great conversations we had—thank you for your positivity and courage. **Karin**, you are the best. Thanks to ACES and all my colleagues I met in the corridors and cafeteria for this amazing journey together.

Thanks to the PhD community we formed to get through the pandemic: **Giulia**, la tua luce continua a ispirarmi. **Cedric**, **Lotte** (and **Ilva)**, **Sonja**, **May Britt**, **Flora**, **Gabriel** —"The Crew." You made this journey special, and all the experiences we shared brought me to another level in life. I couldn't have asked for a better group — I learned so many things from each of you. **Martin**, **Felipe**, **Alyssa**, **Nanna**, **Gaston**, thank you for sharing amazing moments with me.

Primeiramente, agradeço à minha família: **Maria Anastácia**, **José Raimundo** (*in memoriam*), **Edna**, **Thays** e **Leo** pelo apoio e por sempre acreditarem em mim. **Tiago**, obrigado pelo seu suporte incondicional. **Daniel**, **Icaro**, **Carol** e **Bianca (Fuleiros)** pela amizade e apoio. **Silmara**, meu pedaço do Brasil em Estocolmo. A todos da **Bio 2010 (Lavando roupa)**. **Carlos**, dondequiera que estés, gracias por el apoyo durante mi trayectoria académica. **Edvan**, obrigado por ajudar a resgatar a brasilidade e por me proporcionar um novo patamar de relacionamento.

8. References

Aksmann, A., & Tukaj, Z. (2008). Intact anthracene inhibits photosynthesis in algal cells: A fluorescence induction study on Chlamydomonas reinhardtii cw92 strain. *Chemosphere*, *74*(1), 26–32. https://doi.org/10.1016/j.chemosphere.2008.09.064

Allan, I. J., Bæk, K., Haugen, T. O., Hawley, K. L., Høgfeldt, A. S., & Lillicrap, A. D. (2013). In vivo passive sampling of nonpolar contaminants in brown trout (Salmo trutta). *Environmental Science and Technology*, *47*(20), 11660–11667. https://doi.org/10.1021/ES401810R/SUPPL_FILE/ES401810R_SI_001.PDF

Allan, I. J., Miège, C., Jahnke, A., Rojo-Nieto, E., Vorkamp, K., Kech, C., Polesello, S., Perceval, O., Booij, K., Dulio, V., Estoppey, N., Mayer, P., McHugh, B., Munschy, C., Staub, P. F., & Vrana, B. (2025). Passive sampling in support of biota monitoring of hydrophobic substances under the Water Framework Directive. *Journal of Hazardous Materials*, *483*, 136672. https://doi.org/10.1016/J.JHAZMAT.2024.136672

Ben Othman, H., Pick, F. R., Sakka Hlaili, A., & Leboulanger, C. (2023). Effects of polycyclic aromatic hydrocarbons on marine and freshwater microalgae – A review. *Journal of Hazardous Materials*, *441*, 129869. https://doi.org/10.1016/J.JHAZMAT.2022.129869

Birch, H., Hammershøj, R., & Mayer, P. (2018). Determining Biodegradation Kinetics of Hydrocarbons at Low Concentrations: Covering 5 and 9 Orders of Magnitude of K_{ow} and K_{aw}. *Environmental Science & Technology*, *52*(4), 2143–2151. https://doi.org/10.1021/acs.est.7b05624

Bretherton, L., Hillhouse, J., Kamalanathan, M., Finkel, Z. V., Irwin, A. J., & Quigg, A. (2020). Trait-dependent variability of the response of marine phytoplankton to oil and dispersant exposure. *Marine Pollution Bulletin*, *153*, 110906. https://doi.org/10.1016/J.MARPOLBUL.2020.110906

Bruno, E. and Eklund, B., 2003. Two new growth inhibition tests with the filamentous algae Ceramium strictum and C. tenuicorne (Rhodophyta). Environmental Pollution, 125(2), pp.287-293.

Buchan, A., LeCleir, G. R., Gulvik, C. A., & González, J. M. (2014). Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nature Reviews Microbiology 2014 12:10*, *12*(10), 686–698. https://doi.org/10.1038/nrmicro3326

Burgess, R. M., Driscoll, S. B. K., Maynard, M. A., Ozretich, R. J., Mount, D. R., & Reiley, M. C. (2012). *Equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: Procedures for the determination of the freely dissolved interstitial water concentrations of nonionic organics.* efaidnbmnnnibpcajpcglclefindmkaj/https://clu-in.org/conf/tio/porewater1/resources/EPA-ESB-Procedures-Determine-freely-dissolved-organics-2012.pdf

Burritt, D. J., & Mackenzie, S. (2003). Antioxidant Metabolism during Acclimation of Begonia × erythrophylla to High Light Levels. *Annals of Botany*, *91*(7), 783–794. https://doi.org/10.1093/AOB/MCG076

Chen, B., He, R., Yuan, K., Chen, E., Lin, L., Chen, X., Sha, S., Zhong, J., Lin, L., Yang, L., Yang, Y., Wang, X., Zou, S., & Luan, T. (2017). Polycyclic aromatic hydrocarbons (PAHs) enriching antibiotic resistance genes (ARGs) in the soils. *Environmental Pollution*, *220*, 1005–1013. https://doi.org/10.1016/J.ENVPOL.2016.11.047

Cohen, M. K., West, A. S., Cosper, E. M., & Wurster, C. F. (1991). Mechanisms Of Resistance to Polychlorinated Biphenyls (PCB) in Two Species of Marine Diatoms. *Journal of the Marine Biological Association of the United Kingdom*, *71*(2), 247–263. https://doi.org/10.1017/S0025315400051596

European Comission (2019). European Commission: Commission Proposal for a Regulation... - Google Scholar. https://scholar.google.com/scholar_lookup?title=Commission proposal for a regulation%3A European climate Law&publication_year=2020&author=European Commission

Croxton, A. N., Wikfors, G. H., & Schulterbrandt-Gragg, R. D. (2015). The use of flow cytometric applications to measure the effects of PAHs on growth, membrane integrity, and relative lipid content of the benthic

diatom, Nitzschia brevirostris. *Marine Pollution Bulletin*, *91*(1), 160–165. https://doi.org/10.1016/J.MARPOLBUL.2014.12.010

Di Toro, D. M. (1985). A particle interaction model of reversible organic chemical sorption. *Chemosphere*, *14*(10), 1503–1538. https://doi.org/10.1016/0045-6535(85)90008-6

Dong, J., Xia, X., Wang, M., Xie, H., Wen, J., & Bao, Y. (2016). Effect of recurrent sediment resuspension-deposition events on bioavailability of polycyclic aromatic hydrocarbons in aquatic environments. *Journal of Hydrology*, *540*, 934–946. https://doi.org/10.1016/J.JHYDROL.2016.07.009

Duarte, C. M., Gattuso, J. P., Hancke, K., Gundersen, H., Filbee-Dexter, K., Pedersen, M. F., Middelburg, J. J., Burrows, M. T., Krumhansl, K. A., Wernberg, T., Moore, P., Pessarrodona, A., Ørberg, S. B., Pinto, I. S., Assis, J., Queirós, A. M., Smale, D. A., Bekkby, T., Serrão, E. A., & Krause-Jensen, D. (2022). Global estimates of the extent and production of macroalgal forests. *Global Ecology and Biogeography*, *31*(7), 1422–1439. https://doi.org/10.1111/GEB.13515

Echeveste, P., Agustí, S., & Dachs, J. (2011). Cell size dependence of additive versus synergetic effects of UV radiation and PAHs on oceanic phytoplankton. Environmental Pollution, 159(5), 1307–1316. https://doi.org/10.1016/J.ENVPOL.2011.01.023

Echeveste, P., Dachs, J., Berrojalbiz, N., & Agustí, S. (2010). Decrease in the abundance and viability of oceanic phytoplankton due to trace levels of complex mixtures of organic pollutants. *Chemosphere*, *81*(2), 161–168. https://doi.org/10.1016/j.chemosphere.2010.06.072

Engraff, M., Solere, C., Smith, K. E. C., Mayer, P., & Dahllöf, I. (2011). Aquatic toxicity of PAHs and PAH mixtures at saturation to benthic amphipods: Linking toxic effects to chemical activity. *Aquatic Toxicology*, *102*(3–4), 142–149. https://doi.org/10.1016/J.AQUATOX.2011.01.009

Escher, B. I., Eggen, R. I. L., Schreiber, U., Schreiber, Z., Vye, E., Wisner, B., & Schwarzenbach, R. P. (2002). Baseline toxicity (narcosis) of organic chemicals determined by in vitro membrane potential measurements in energy-transducing membranes. *Environmental Science and Technology*, *36*(9), 1971–1979. https://doi.org/10.1021/ES015844C

Escher, B. I., Quayle, P., Muller, R., Schreiber, U., & Mueller, J. F. (2006). Passive sampling of herbicides combined with effect analysis in algae using a novel high-throughput phytotoxicity assay (Maxi-Imaging-PAM). *Journal of Environmental Monitoring*, *8*(4), 456–464. https://doi.org/10.1039/B517512G

Fenner, K., & Scheringer, M. (2021). The Need for Chemical Simplification As a Logical Consequence of Ever-Increasing Chemical Pollution. In *Environmental Science and Technology* (Vol. 55, Issue 21, pp. 14470–14472). https://doi.org/10.1021/acs.est.1c04903

Fields, M. W., Hise, A., Lohman, E. J., Bell, T., Gardner, R. D., Corredor, L., Moll, K., Peyton, B. M., Characklis, G. W., & Gerlach, R. (2014). Sources and resources: Importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. *Applied Microbiology and Biotechnology*, *98*(11), 4805–4816. https://doi.org/10.1007/S00253-014-5694-7/FIGURES/3

Friedman, C. L., Burgess, R. M., Perron, M. M., Cantwell, M. G., Ho, K. T., & Lohmann, R. (2009). Comparing polychaete and polyethylene uptake to assess sediment resuspension effects on PCB bioavailability. *Environmental Science and Technology*, *43*(8), 2865–2870. https://doi.org/10.1021/ES803695N/SUPPL_FILE/ES803695N_SI_001.PDF

Gasol, J. M. & Morán, X. A. G. (eds Terry J. McGenity, Kenneth N. Timmis, & B. N. (2016). Single-Cell and Single-Molecule Methods. In *Hydrocarbon and Lipid Microbiology Protocols. Biochemical methods* (pp. 159–187). Springer.

Gerofke, A., Kömp, P., & McLachlan, M. S. (2005). Bioconcentration of persistent organic pollutants in four species of marine phytoplankton. *Environmental Toxicology and Chemistry*, *24*(11), 2908–2917. https://doi.org/10.1897/04-566R.1

Gobas, F. A. P. C., Mayer, P., Parkerton, T. F., Burgess, R. M., van de Meent, D., & Gouin, T. (2018). A chemical activity approach to exposure and risk assessment of chemicals. *Environmental Toxicology and Chemistry*, *37*(5), 1235–1251. https://doi.org/10.1002/etc.4091

Goni-Urriza, M., Moussard, H., Lafabrie, C., Carre, C., Bouvy, M., Sakka Hlaili, A., & Pringault, O. (2018). Consequences of contamination on the interactions between phytoplankton and bacterioplankton. *Chemosphere*, *195*, 212–222. https://doi.org/10.1016/J.CHEMOSPHERE.2017.12.053

Guillard, R. R. L. (1975). Culture of phytoplankton for feeding marine invertebrates. *In Culture of Marine Invertebrate Animals: Proceedings—1st Conference on Culture of Marine Invertebrate Animals Greenport*, 29–60.

Häder, D. P., & Gao, K. (2015). Interactions of anthropogenic stress factors on marine phytoplankton. *Frontiers in Environmental Science*, *3*(MAR), 120191. https://doi.org/10.3389/FENVS.2015.00014/BIBTEX

Häder, D. P., Villafañe, V. E., & Helbling, E. W. (2014). Productivity of aquatic primary producers under global climate change. *Photochemical & Photobiological Sciences*, *13*(10), 1370–1392. https://doi.org/10.1039/C3PP50418B

Hajeb, P., Zhu, L., Bossi, R., & Vorkamp, K. (2022). Sample preparation techniques for suspect and non-target screening of emerging contaminants. *Chemosphere*, *287*, 132306. https://doi.org/10.1016/J.CHEMOSPHERE.2021.132306

Hermens, J., Canton, H., Janssen, P., & De Jong, R. (1984). Quantitative structure-activity relationships and toxicity studies of mixtures of chemicals with anaesthetic potency: Acute lethal and sublethal toxicity to Daphnia magna. *Aquatic Toxicology*, *5*(2), 143–154. https://doi.org/10.1016/0166-445X(84)90005-5

Jajoo, A. (2017). Effects of environmental pollutants polycyclic aromatic hydrocarbons (PAH) on photosynthetic processes. *Photosynthesis: Structures, Mechanisms, and Applications*, 249–259. https://doi.org/10.1007/978-3-319-48873-8_11/COVER

Jonker, M. T. O., Van Der Heijden, S. A., Kotte, M., & Smedes, F. (2015). Quantifying the effects of temperature and salinity on partitioning of hydrophobic organic chemicals to silicone rubber passive samplers. *Environmental Science and Technology*, *49*(11), 6791–6799. https://doi.org/10.1021/ACS.EST.5B00286

Joyce, A. S., Portis, L. M., Parks, A. N., & Burgess, R. M. (2016). Evaluating the Relationship between Equilibrium Passive Sampler Uptake and Aquatic Organism Bioaccumulation. *Environmental Science and Technology*, *50*(21), 11437–11451. https://doi.org/10.1021/ACS.EST.6B03273/ASSET/IMAGES/ES-2016-03273E_M010.GIF

Karickhoff, S. W. (1981). Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. *Chemosphere*, *10*(8), 833–846. https://doi.org/10.1016/0045-6535(81)90083-7

Kirso, U., & Irha, N. (1998). Role of Algae in Fate of Carcinogenic Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. *Ecotoxicology and Environmental Safety*, *41*(1), 83–89. https://doi.org/10.1006/EESA.1998.1671

Konat, J., & Kowalewska, G. (2001). Polychlorinated biphenyls (PCBs) in sediments of the southern Baltic Sea — trends and fate. *Science of The Total Environment*, *280*(1–3), 1–15. https://doi.org/10.1016/S0048-9697(01)00785-9

Kreutzer, A., Faetsch, S., Heise, S., Hollert, H., & Witt, G. (2022). Passive dosing: Assessing the toxicity of individual PAHs and recreated mixtures to the microalgae Raphidocelis subcapitata. *Aquatic Toxicology*, *249*, 106220. https://doi.org/10.1016/j.aquatox.2022.106220

Landrigan, P. J., Stegeman, J. J., Fleming, L. E., Allemand, D., Anderson, D. M., Backer, L. C., Brucker-Davis, F., Chevalier, N., Corra, L., Czerucka, D., Bottein, M. Y. D., Demeneix, B., Depledge, M., Deheyn, D. D., Dorman, C. J., Fénichel, P., Fisher, S., Gaill, F., Galgani, F., … Rampal, P. (2020). Human Health and Ocean Pollution. *Annals of Global Health*, *86*(1), 151. https://doi.org/10.5334/AOGH.2831

Landrum, P. F., Lotufo, G. R., Gossiaux, D. C., Gedeon, M. L., & Lee, J. H. (2003). Bioaccumulation and critical body residue of PAHs in the amphipod, Diporeia spp.: additional evidence to support toxicity additivity for PAH mixtures. *Chemosphere*, *51*(6), 481–489. https://doi.org/10.1016/S0045-6535(02)00863-9

Lewis, G. N. (1901). The Law of Physico-Chemical Change. *Proceedings of the American Academy of Arts and Sciences*, *37*(3), 49. https://doi.org/10.2307/20021635

Lewis, G. N. (1907). Outlines of a New System of Thermodynamic Chemistry. *Proceedings of the American Academy of Arts and Sciences*, *43*(7), 259. https://doi.org/10.2307/20022322

Litchman, E., & Klausmeier, C. A. (2008). Trait-Based Community Ecology of Phytoplankton. *Https://Doi.Org/10.1146/Annurev.Ecolsys.39.110707.173549*, *39*, 615–639. https://doi.org/10.1146/ANNUREV.ECOLSYS.39.110707.173549

Lohmann, R., Jurado, E., Pilson, M. E. Q., & Dachs, J. (2006). Oceanic deep water formation as a sink of persistent organic pollutants. *Geophysical Research Letters*, *33*(12), 12607. https://doi.org/10.1029/2006GL025953

Lydy, M. J., Landrum, P. F., Oen, A. M., Allinson, M., Smedes, F., Harwood, A. D., Li, H., Maruya, K. A., & Liu, J. (2014). Passive sampling methods for contaminated sediments: State of the science for organic contaminants. *Integrated Environmental Assessment and Management*, *10*(2), 167–178. https://doi.org/10.1002/IEAM.1503

Martin, O., Scholze, M., Ermler, S., McPhie, J., Bopp, S. K., Kienzler, A., Parissis, N., & Kortenkamp, A. (2021). Ten years of research on synergisms and antagonisms in chemical mixtures: A systematic review and quantitative reappraisal of mixture studies. *Environment International*, *146*, 106206. https://doi.org/10.1016/J.ENVINT.2020.106206

Mayer, P., & Holmstrup, M. (2008). Passive dosing of soil invertebrates with polycyclic aromatic hydrocarbons: Limited chemical activity explains toxicity cutoff. *Environmental Science and Technology*, *42*(19), 7516–7521. https://doi.org/10.1021/ES801689Y

Ma, Y., Halsall, C. J., Crosse, J. D., Graf, C., Cai, M., He, J., Gao, G., & Jones, K. (2015). Persistent organic pollutants in ocean sediments from the North Pacific to the Arctic Ocean. *Journal of Geophysical Research: Oceans*, *120*(4), 2723–2735. https://doi.org/10.1002/2014JC010651

Moore, S. A., & Harriss, R. C. (1974). Differential sensitivity to PCB by phytoplankton. *Marine Pollution Bulletin*, *5*(11), 174–176. https://doi.org/10.1016/0025-326X(74)90132-5

Morales, M., Aflalo, C., & Bernard, O. (2021). Microalgal lipids: A review of lipids potential and quantification for 95 phytoplankton species. *Biomass and Bioenergy*, *150*. https://doi.org/10.1016/j.biombioe.2021.106108

Mukherjee, S., Juottonen, H., Siivonen, P., Lloret Quesada, C., Tuomi, P., Pulkkinen, P., & Yrjälä, K. (2014). Spatial patterns of microbial diversity and activity in an aged creosote-contaminated site. *The ISME Journal*, *8*(10), 2131–2142. https://doi.org/10.1038/ISMEJ.2014.151

Neff, J. M., Stout, S. A., & Gunster, D. G. (2005). Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: Identifying sources and ecological hazard. *Integrated Environmental Assessment and Management*, *1*(1), 22–33. https://doi.org/10.1897/IEAM_2004A-016.1

Niehus, N. C., Floeter, C., Hollert, H., & Witt, G. (2018). Miniaturised Marine Algae Test with Polycyclic Aromatic Hydrocarbons − Comparing Equilibrium Passive Dosing and Nominal Spiking. *Aquatic Toxicology*, *198*, 190–197. https://doi.org/10.1016/j.aquatox.2018.03.002

Nordberg, K., Björk, G., Abrahamsson, K., Josefsson, S., & Lundin, L. (2024). Historic distribution of Polycyclic Aromatic Compounds (PAC) in a Skagerrak fjord, Swedish west coast as reflected in a high-resolution sediment record and compared to the Environmental Quality Standards (EQS). *Marine Pollution Bulletin*, *199*, 116014. https://doi.org/10.1016/J.MARPOLBUL.2023.116014

Persson, L., Carney Almroth, B. M., Collins, C. D., Cornell, S., de Wit, C. A., Diamond, M. L., Fantke, P., Hassellöv, M., MacLeod, M., Ryberg, M. W., Søgaard Jørgensen, P., Villarrubia-Gómez, P., Wang, Z., & Hauschild, M. Z. (2022). Outside the Safe Operating Space of the Planetary Boundary for Novel Entities. *Environmental Science and Technology*, *56*(3), 1510–1521. https://doi.org/10.1021/ACS.EST.1C04158

Ptacnik, R., Solimini, A. G., Andersen, T., Tamminen, T., Brettum, P., Lepistö, L., Willén, E., & Rekolainen, S. (2008). Diversity predicts stability and resource use efficiency in natural phytoplankton communities. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(13), 5134–5138. https://doi.org/10.1073/PNAS.0708328105/SUPPL_FILE/0708328105SI.PDF

Quero, G. M., Cassin, D., Botter, M., Perini, L., & Luna, G. M. (2015). Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). *Frontiers in Microbiology*, *6*(OCT), 154095. https://doi.org/10.3389/FMICB.2015.01053/BIBTEX

Reichenberg, F., & Mayer, P. (2006). Two complementary sides of bioavailability: Accessibility and chemical activity of organic contaminants in sediments and soils. *Environmental Toxicology and Chemistry*, *25*(5), 1239–1245. https://doi.org/10.1897/05-458R.1

Ren, G., Ren, W., Teng, Y., & Li, Z. (2015). Evident bacterial community changes but only slight degradation when polluted with pyrene in a red soil. *Frontiers in Microbiology*, *6*(JAN), 119776. https://doi.org/10.3389/FMICB.2015.00022/ABSTRACT

Ribbenstedt, A., Mustajärvi, L., Breitholtz, M., Gorokhova, E., Mayer, P., & Sobek, A. (2017). Passive dosing of triclosan in multigeneration tests with copepods – stable exposure concentrations and effects at the low µg/L range. *Environmental Toxicology and Chemistry*, *36*(5), 1254–1260. https://doi.org/10.1002/ETC.3649

Rojo-Nieto, E., Smith, K. E. C., Perales, J. A., & Mayer, P. (2012). Recreating the seawater mixture composition of HOCs in toxicity tests with Artemia franciscana by passive dosing. *Aquatic Toxicology*, *120–121*, 27–34. https://doi.org/10.1016/j.aquatox.2012.04.006

Rubach, M. N., Ashauer, R., Buchwalter, D. B., De Lange, H. J., Hamer, M., Preuss, T. G., Töpke, K., & Maund, S. J. (2011). Framework for traits-based assessment in ecotoxicology. *Integrated Environmental Assessment and Management*, *7*(2), 172–186. https://doi.org/10.1002/IEAM.105

Schäfer, R. B., Jackson, M., Juvigny-Khenafou, N., Osakpolor, S. E., Posthuma, L., Schneeweiss, A., Spaak, J., & Vinebrooke, R. (2023). Chemical Mixtures and Multiple Stressors: Same but Different? *Environmental Toxicology and Chemistry*, *42*(9), 1915–1936. https://doi.org/10.1002/ETC.5629

Schmidt, S. N., Holmstrup, M., Smith, K. E. C., & Mayer, P. (2013). Passive dosing of polycyclic aromatic hydrocarbon (PAH) mixtures to terrestrial springtails: Linking mixture toxicity to chemical activities, equilibrium lipid concentrations, and toxic units. *Environmental Science and Technology*, *47*(13), 7020–7027. https://doi.org/10.1021/ES3047813/SUPPL_FILE/ES3047813_SI_001.PDF

Schmidt, S. N., & Mayer, P. (2015). Linking algal growth inhibition to chemical activity: Baseline toxicity required 1% of saturation. *Chemosphere*, *120*, 305–308. https://doi.org/10.1016/j.chemosphere.2014.07.006

Schuhmann, H., Lim, D. K. Y., & Schenk, P. M. (2012). Perspectives on metabolic engineering for increased lipid contents in microalgae. *Biofuels*, *3*(1), 71–86. https://doi.org/10.4155/BFS.11.147

Schuijt, L. M., Peng, F. J., van den Berg, S. J. P., Dingemans, M. M. L., & Van den Brink, P. J. (2021). (Eco)toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. *Science of The Total Environment*, *795*, 148776. https://doi.org/10.1016/J.SCITOTENV.2021.148776

Schwarzenbach, R. P., Gschwend, P. M., & Imboden, D. M. (2016). *Environmental organic chemistry* (third edit). John Wiley & Sons.

Smith, K. E. C., Dom, N., Blust, R., & Mayer, P. (2010). Controlling and maintaining exposure of hydrophobic organic compounds in aquatic toxicity tests by passive dosing. *Aquatic Toxicology*, *98*(1), 15–24. https://doi.org/10.1016/j.aquatox.2010.01.007

Smith, K. E. C., Schmidt, S. N., Dom, N., Blust, R., Holmstrup, M., & Mayer, P. (2013). Baseline toxic mixtures of non-toxic chemicals: "solubility addition" increases exposure for solid hydrophobic chemicals. *Environmental Science and Technology*, *47*(4), 2026–2033. https://doi.org/10.1021/ES3040472

Sobek, A., Gustafsson, Ö., Hajdu, S., & Larsson, U. (2004). Particle-Water Partitioning of PCBs in the Photic Zone: A 25-Month Study in the Open Baltic Sea. *Environmental Science and Technology*, *38*(5), 1375–1382. https://doi.org/10.1021/ES034447U

Spaan, K. M., Seilitz, F., Plassmann, M. M., de Wit, C. A., & Benskin, J. P. (2023). Pharmaceuticals Account for a Significant Proportion of the Extractable Organic Fluorine in Municipal Wastewater Treatment Plant Sludge. *Environmental Science and Technology Letters*, *10*(4), 328–336. https://doi.org/10.1021/ACS.ESTLETT.3C00108/ASSET/IMAGES/LARGE/EZ3C00108_0002.JPEG

Swackhamer, D. L., & Skoglund, R. S. (1993). Bioaccumulation of PCBs by algae: Kinetics versus equilibrium. *Environmental Toxicology and Chemistry*, *12*(5), 831–838. https://doi.org/10.1002/ETC.5620120506

Szabo, G., Prosser, S. L., & Bulman, R. A. (1990). Determination of the adsorption coefficient (KOC) of some aromatics for soil by RP-HPLC on two immobilized humic acid phases. *Chemosphere*, *21*(6), 777–788. https://doi.org/10.1016/0045-6535(90)90265-U

Tauler, M., Vila, J., Nieto, J. M., & Grifoll, M. (2016). Key high molecular weight PAH-degrading bacteria in a soil consortium enriched using a sand-in-liquid microcosm system. *Applied Microbiology and Biotechnology*, *100*(7), 3321–3336. https://doi.org/10.1007/S00253-015-7195-8/TABLES/4

Thompson, P. L., MacLennan, M. M., & Vinebrooke, R. D. (2018). An improved null model for assessing the net effects of multiple stressors on communities. *Global Change Biology*, *24*(1), 517–525. https://doi.org/10.1111/GCB.13852

Tijani, J. O., Fatoba, O. O., Babajide, O. O., & Petrik, L. F. (2015). Pharmaceuticals, endocrine disruptors, personal care products, nanomaterials and perfluorinated pollutants: a review. *Environmental Chemistry Letters 2015 14:1*, *14*(1), 27–49. https://doi.org/10.1007/S10311-015-0537-Z

Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, S., Gehlen, M., Aumont, O., Bittner, L., Dugdale, R., Finkel, Z., Iudicone, D., Jahn, O., Guidi, L., Lasbleiz, M., Leblanc, K., Levy, M., & Pondaven, P. (2017). Influence of diatom diversity on the ocean biological carbon pump. *Nature Geoscience 2017 11:1*, *11*(1), 27–37. https://doi.org/10.1038/s41561-017-0028-x

Van Straalen, N. (1994). Biodiversity of ecotoxicological responses in animals. *Neth J Zool*, *441/442*, 112–129.

Van Wezel, A. P., & Opperhuizen, A. (1995). Narcosis due to environmental pollutants in aquatic organisms: Residue-based toxicity, mechanisms, and membrane burdens. *Critical Reviews in Toxicology*, *25*(3), 255–279. https://doi.org/10.3109/10408449509089890

Vermeirssen, E. L. M., Hollender, J., Bramaz, N., Van Der Voet, J., & Escher, B. I. (2010). Linking toxicity in algal and bacterial assays with chemical analysis in passive samplers deployed in 21 treated sewage effluents. *Environmental Toxicology and Chemistry*, *29*(11), 2575–2582. https://doi.org/10.1002/ETC.311

Vrana, B., Allan, I. J., Greenwood, R., Mills, G. A., Dominiak, E., Svensson, K., Knutsson, J., & Morrison, G. (2005). Passive sampling techniques for monitoring pollutants in water. *TrAC Trends in Analytical Chemistry*, *24*(10), 845–868. https://doi.org/10.1016/J.TRAC.2005.06.006

Wang, Z., Walker, G. W., Muir, D. C. G., & Nagatani-Yoshida, K. (2020). Toward a Global Understanding of Chemical Pollution: A First Comprehensive Analysis of National and Regional Chemical Inventories. *Environmental Science and Technology*, *54*(5), 2575–2584. https://doi.org/10.1021/ACS.EST.9B06379

Warne, M. S. J., & Hawker, D. W. (1995). The Number of Components in a Mixture Determines Whether Synergistic and Antagonistic or Additive Toxicity Predominate: The Funnel Hypothesis. *Ecotoxicology and Environmental Safety*, *31*(1), 23–28. https://doi.org/10.1006/EESA.1995.1039

Winding, A., Modrzyński, J. J., Christensen, J. H., Brandt, K. K., & Mayer, P. (2019). Soil bacteria and protists show different sensitivity to polycyclic aromatic hydrocarbons at controlled chemical activity. *FEMS Microbiology Letters*, *366*(17), 214. https://doi.org/10.1093/FEMSLE/FNZ214

Zheng, G. xia, Li, Y. jie, Qi, L. lin, Liu, X. ming, Wang, H., Yu, S. ping, & Wang, Y. hua. (2014). Marine phytoplankton motility sensor integrated into a microfluidic chip for high-throughput pollutant toxicity assessment. *Marine Pollution Bulletin*, *84*(1–2), 147–154. https://doi.org/10.1016/J.MARPOLBUL.2014.05.019

Zokm, G. M. El, Ismail, M. M., & Okbah, M. A. E. (2022). Seaweed as bioindicators of organic micropollutants polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs). *Environmental Science and Pollution Research*, *29*(23), 34738–34748. https://doi.org/10.1007/S11356-022-18634-Z/TABLES/2