1. Summary in English

Micropollutants encompass a diverse array of substances, including pharmaceuticals, personal care products, plasticizers, and other emerging chemical groups that are introduced into the environment from anthropogenic sources such as industrial discharges, agricultural runoff, and wastewater treatment plants (Narwal et al. 2023). These compounds are detected in the environment at trace concentrations, typically in the microgram to nanogram per liter. The current state of knowledge regarding the toxicity, environmental fate, and properties of substances classified as emerging contaminants remains incomplete. Their environmental persistence or pseudo-persistence along with their potential for bioaccumulation and biomagnification, presents significant ecological risks to both terrestrial and aquatic ecosystems. Ecotoxicological impacts of micropollutants on aquatic organisms can include sub-lethal effects such as endocrine disruption, reproductive impairments, and behavioral changes, which can have cascading effects on ecosystem biodiversity and services (Sharma et al. 2024). Detection and monitoring of micropollutants are challenging due to their low concentrations and the need for advanced analytical techniques. Additionally, regulatory frameworks and wastewater treatment technologies struggle to effectively manage and mitigate these pollutants (Li et al. 2024).

Ionic liquids (ILs) represent a new group of micropollutants only recently detected in the environment thanks to application of novel analytical techniques including high resolution mass spectrometry non-target screening studies (Neuwald et al. 2021). ILs are salts in a liquid state at or near room temperature, characterized by low volatility, high thermal stability, and tunable physicochemical properties (Maculewicz et al. 2022). The unique properties of ILs arise from the vast array of possible cation-anion combinations, which can be independently selected, allowing for extensive customization of physicochemical properties. Ionic liquids have been increasingly used in various industrial applications, including chemical synthesis, carbon capture and sequestration, catalysis, and electrochemistry. They are considered promising solvents in green chemistry due to their tunable properties, and potential to reduce environmental impact compared to traditional organic solvents (Gonçalves et al 2021). Additionally, their potential for maritime applications has been explored, particularly in antifouling ship coatings, as certain ILs exhibit biocidal properties that inhibit microbial adhesion and biofilm formation (Taylor et al. 2024). Ionic liquids are beginning to play a significant role in the space industry (Cook et al., 2017). Given the steadily increasing frequency of spacecraft launches and associated incidents, their potential environmental impact is becoming increasingly concerning. The market for ILs is projected to grow, driven by

increasing research activities and technological advancements (Zhou et al. 2023). The global ILs market was valued at over USD 2.3 billion in 2023, with an anticipated compound annual growth rate (CAGR) of 13.5% from 2024 to 2032 (Global Market Insights, 2023). However, ILs unique chemical structures, incorporating complex organic cations and anions, can interact with biological membranes and proteins, leading to toxic effects (Gonçalves et al. 2021, Vieira Sanches et al. 2023, Maculewicz et al. 2024). The presence of these compounds in the environment has been documented in a series of recent studies (Neuwald et al., 2020, 2021; Pati and Arnold, 2020), highlighting the need for continued investigation into their ecological impacts.

Pharmaceuticals are recognized as significant group of emerging micropollutants with distinct pathways, environmental behaviors, and ecotoxicological impacts. They are a diverse group of bioactive compounds used in human and veterinary medicine, that enter aquatic environments primarily through wastewater treatment plant effluents, where conventional treatment processes are often inadequate for their complete removal (Samal et al. 2022). Additional sources of pharmaceutical contamination include agricultural runoff from livestock farming, improper disposal of unused medications, leachates from landfills, and direct excretion by humans and animals (aus der Beek et al. 2016). The presence of pharmaceuticals in aquatic ecosystems is concerning due to their biological activity, even at low concentrations. Despite the widespread occurrence of pharmaceuticals in the environment, many questions remain regarding their long-term ecological impacts, the effects of chronic exposure on non-target species, and the interactions within complex mixtures of pharmaceuticals and other micropollutants.

There are significant research gaps in understanding the long-term effects of chronic exposure to micropollutant mixtures. Moreover, most research has focused on the effects of individual pollutants. However, organisms and ecosystems are consistently exposed to complex mixtures of multiple contaminants, resulting in unknown and often unpredictable consequences. The cumulative effects of exposure to multiple chemicals can be additive, but interactions within chemical mixtures can also lead to synergistic and antagonistic effects. Additionally marine and coastal waters are considered sinks for micropollutants due to their ability to accumulate and retain pollutants from various sources (Spindola et at. 2022). The presence of micropollutants in marine environments poses significant risks to marine photoautotrophic microorganisms responsible for primary production. This threat is intensified by the potential for long-term, chronic exposure, which can lead to disruptions in photosynthesis, oxidative stress, and changes in species composition.

The foundation of primary production in aquatic ecosystems relies on the photosynthetic activity of autotrophic organisms, including phytoplankton, phytobenthos, and macroalgae, with phytoplankton serving as the predominant contributor (Purina et al. 2018). Among these, diatoms represent one of the most ecologically significant phytoplankton groups, accounting for approximately 20% of global primary productivity (Malviya et al. 2016). Algae are extensively utilized as indicator organisms in ecotoxicological risk assessments due to their rapid response to environmental stressors and high sensitivity. In particular, cyanobacteria exhibit significant susceptibility to xenobiotics, making them a key model group for ecotoxicological evaluations (OECD, 2011). The test organisms selected for this study were chosen due to their status as established model species and their roles in primary production and nutrient cycling in the Baltic Sea—a unique and sensitive brackish ecosystem (Russo et al., 2023). The Baltic Sea is particularly susceptible to xenobiotic pollution because of its limited water exchange, low species diversity, and extensive catchment area. It serves as a heavily trafficked maritime route, with a continuous increase in both the number and size of vessels, particularly oil tankers, over recent decades (Ringbom, 2018). This upward trend is projected to persist in the future. Moreover, the expansion of offshore wind energy production in the Baltic Sea poses potential environmental risks due to the release of chemical contaminants. Increased emissions from construction and vessel traffic may disturb seabed sediments, leading to the mobilization of sediment-bound pollutants and the introduction of chemicals used in infrastructure development (Reckermann et al. 2022). Additionally, the region is under significant anthropogenic pressure from intensive agricultural practices, industrial operations, and dense coastal populations – factors that can potentially affect composition, abundance, and diversity of Baltic microorganisms (Mazur-Marzec et al. 2024).

Considering the documented widespread distribution of pharmaceuticals and the emerging concerns surrounding ILs, it is crucial to assess the impact of mixtures of these compounds within aquatic environments. A comprehensive review of existing literature highlighted significant knowledge gaps in the study of antibiotics, a critical category of emerging pollutants **(Publication 1)** and ILs **(Publication 3).** Key findings revealed a notable lack of data concerning their impact on marine and brackish microorganisms, a predominant focus on acute toxicity assessments rather than long-term chronic effects, and limited investigation into the combined effects of pollutant mixtures. The co-occurrence of these distinct pollutant groups in aquatic ecosystems presents complex challenges, owing to their varied chemical characteristics, mechanisms of action, and the possibility of interactive effects.

The primary objective of this dissertation was to address a knowledge gap in the assessment of environmental risks associated with mixtures of organic micropollutants. Specifically, the study investigated the effects of imidazolium-based ionic liquids (IM1-12Br and IM1-8C$(CN)_3$), the antibiotic oxytetracycline, the anticonvulsant drug carbamazepine metabolite 10,11-epoxy carbamazepine, and the stimulant caffeine on selected microorganisms, including the cyanobacteria *Microcystis aeruginosa*, the green algae *Chlorella vulgaris*, and the diatom *Phaeodactylum tricornutum*. As a result, the following research objectives were established:

i. Assess and compare the sensitivity of various non-target marine/brackish photosynthetic microorganisms to low concentrations of individual pollutants and their mixtures. This evaluation will include determining the effective concentration causing a specified percentage of growth and photosynthetic efficiency parameter (F_v/F_m) inhibition (EC_{50}) in accordance with the OECD 201 Guidelines for Testing of Chemicals (OECD, 2011).

ii. Investigate the underlying mechanisms of action for the individual target compounds and their mixtures by utilizing non-standard biomarkers. This includes assessing their adverse effects on the growth and physiological functioning of marine microorganisms present in the Baltic Sea, through a range of physiological and biochemical endpoints such as superoxide dismutase (SOD) antioxidant enzyme activity, photosynthetic performance (with an emphasis on photosystem II), and photoprotective mechanisms (e.g., the xanthophyll cycle).

iii. Assess the toxicological impact of ionic liquids and their potential to exacerbate toxicity when combined with other organic micropollutants, including representative pharmaceuticals and caffeine—a recognized marker of anthropogenic pollution—utilizing theoretical models of concentration addition (CA) and independent action (IA), alongside model deviation ratio (MDR) calculations to quantify deviations from predicted additive behaviors.

iv. Examine the accuracy of the CA and IA theoretical models in predicting and characterizing the observed binary mixture effects, thereby evaluating their reliability in identifying interactions across diverse combinations of ionic liquids and other organic micropollutants.

Based on an extensive review of the literature, the key hypotheses of the dissertation are as follows:

i. The presence of ionic liquids, pharmaceuticals including transformation products, and the anthropogenic marker—stimulant caffeine— as components of micropollutant mixtures results in different interaction effects such as synergism or antagonism.

ii. Chronic exposure to low concentrations of individual compounds or mixtures of these organic micropollutants adversely affects photosynthetic processes and photoprotective mechanisms, leading to oxidative stress in photoautotrophic microorganisms.

iii. The use of sensitive, non-target biomarkers provides a means to identify the modes of action of target compounds and to detect subtle physiological changes in marine microorganisms prior to the onset of measurable growth inhibition.

iv. Studied species of diatoms, green algae, and cyanobacteria exhibit distinct responses and varying degrees of sensitivity to exposure to selected substances and their mixtures.

The research aimed to assess the toxicological properties of two imidazolium-based cations with varying alkyl chain lengths, specifically $[IM1\text{-}12]^+$ (**Publication 3**) $[IM1\text{-}8]^+$ (**Publication 4 and 5**) along with individual organic micropollutants. The higher affinity of cations with longer side chains for biological membranes aligns with the "side-chain effect," which describes the inverse relationship between EC_{50} values and increasing hydrophobicity. This well-documented phenomenon is evident across a range of biological assays for ionic liquids (Markiewicz et al., 2011). The selection of these specific cations was based on prior studies investigating the partitioning of IL cations into membranes and their bioconcentration potential (Dołżonek et al., 2017; Maculewicz et al., 2023[a]). The findings suggest that ILs may possess a greater potential for bioaccumulation than previously indicated by their logarithmic partition coefficient (log K_{ow}) alone (Dołżonek et al., 2017). A study investigating ILs interactions with human serum albumin (HSA), a representative blood protein, demonstrated that both examined cations exhibited binding affinity; however, $[IM1\text{-}12]^+$ displayed significantly stronger interactions compared to $[IM1\text{-}8]^+$, supporting the hypothesis of differential bioconcentration potential (Kowalska et al., 2023). In vivo research further substantiated these observations, identifying long-chain imidazolium ionic liquids, such as $[IM1\text{-}12]^+$, as meeting the criteria for high bioaccumulation. Conversely, the bioconcentration factor (BCF) for $[IM1\text{-}8]^+$ was determined to be below 100, reflecting a minimal bioaccumulation capacity (Maculewicz et al., 2023[a], 2023[b]).

The selection of representative antibiotic and ILs for interaction studies was guided by a comprehensive review of the literature (**Publication 1 and 3**). Oxytetracycline, a commonly used antibiotic, was chosen as the representative compound for its well-documented presence in aquatic environments. Its effects on growth and chlorophyll *a* fluorescence have been comprehensively investigated in green algae (*C. vulgaris*), cyanobacteria (*M. aeruginosa*) - inhabitants of brackish and coastal waters—and the marine diatom (*P. tricornutum*), which is prevalent in the Baltic Sea basin (**Publication 2**). Additionally, the study explored its interactions with the ionic liquids $[IM1\text{-}12]^{+}[Br]^{-}$ (**Publication 3**) and $[IM1\text{-}8]^{+}[C(CN)3]^{-}$ (**Publication 4**), assessing their combined effects on ecologically relevant microorganisms, including *C. vulgaris*, *M. aeruginosa*, and *P. tricornutum*.

A primary metabolite of carbamazepine (carbamazepine-10, 11-epoxide), a widely prescribed anticonvulsant, was also included in mixture toxicity assessments (**Publication 4**). Carbamazepine and its metabolites are consistently detected in aquatic environments including Baltic Sea, with the parent compound characterized by significant environmental persistence, including an estimated half-life of 3.5 years in seawater. This persistence, coupled with its high detection rates, establishes carbamazepine and its metabolites as a reliable indicators of anthropogenic pollution (Brezina et al., 2017). The available research on the ecotoxicological effects of carbamazepine-10,11-epoxide in microorganisms remains limited. To address this knowledge gap, the present research systematically evaluated the impact of carbamazepine-10,11-epoxide on *M. aeruginosa* and *P. tricornutum* (**Publication 4**)

Caffeine, another ubiquitous micropollutant, was also investigated due to its extensive global consumption and environmental presence (**Publication 5**) (Korekar et al., 2020; Szymczycha et al. 2020). Residual caffeine in coastal and marine ecosystems subjects aquatic organisms to chronic exposure, causing neurotoxicity, oxidative stress, and developmental and reproductive impairments (Rizzi et al., 2020). Its persistence and adverse effects have led some researchers to advocate for its classification as a hazardous compound and an emerging pollutant (Vieira et al., 2022). Furthermore, caffeine serves as an anthropogenic pollution marker, reflecting human activity and wastewater discharge in aquatic ecosystems. Previous studies indicate that caffeine's effects on marine photosynthetic microorganisms vary depending on concentration and species sensitivity, with low environmental concentrations potentially exerting minimal or even stimulatory effects (Hutárová et al., 2023; de Sousa et al., 2021). The present research focuses on environmentally relevant caffeine concentrations, assessing both its individual effects and interactions within pollutant mixtures. By addressing critical knowledge gaps, it provides new insights into caffeine’s role as an emerging pollutant.

The following analytical methods were applied to evaluate the physiological responses of microorganisms during the 11-day chronic exposure experiments:

i. **Chlorophyll *a* Fluorescence Analysis:** The O-J-I-P transient was analyzed using the AquaPen AP110-C fluorometer (**Publications 2, 3,4 and 5**),

ii. **Pigment Concentration Quantification:** Pigment concentrations were determined via Agilent HP1200 chromatographic system (Perlan Technologies, USA) equipped with fluorescence and diode array absorbance detectors following the method established by Stoń-Egiert and Kosakowska (2005) (**Publications 4 and 5**),

iii. **Phycobiliprotein Analysis:** Analysis of phycobiliproteins was conducted using the Cary Eclipse spectrofluorometer (Agilent Technologies) as outlined by Sobiechowska-Sasim et al. (2014) (**Publication 3, 4 and 5**)

iv. **Total Superoxide Dismutase (T-SOD) Enzyme Activity:** T-SOD activity was quantified using commercially available kits (Elabscience Biotechnology Inc., China) based on the WST-1 method (**Publications 4 and 5**)

v. **Protein Content Measurement:** Soluble protein content was evaluated using the Bradford method (Bradford, 1976), following a modified trichloroacetic acid (TCA) precipitation method, as outlined by Peterson (1977, 1983) and Clayton et al. (1988). This analysis was performed using a Thermo Scientific™ Varioskan microplate reader **(Publications 4 and 5)**

vi. **Optical Density Analysis of microbial cultures:** Optical density measurements were conducted using the HITACHI U-2800 UV-VIS spectrophotometer at 680 nm and 750 nm (**Publications 2, 3**, **4** and **5**)

vii. **Ionic Liquid Concentration Determination**: The nominal versus measured concentrations of IM1-12Br and IM1-8C$(CN)_3$ were quantified using ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS), following the protocol by Maculewicz et al. (2023[a], 2023[b]) (**Publications 3** and **4**).

viii. **Carbamazepine-10, 11-epoxide and caffeine concentration determination**: Analysis of the nominal versus measured concentration of carbamazepine-10, 11-epoxide and caffeine using LC-MS analysis following extraction on BAKERBOND Speedisk (H2O-Philic DVB) (JT Baker, Heidelberg, Germany) (**Publications 4** and **5**),

ix. **Mixture Toxicity Modeling:** Mixture toxicity effects of target compounds were evaluated using theoretical models Concentration Addition (CA) and Independent

Action (IA), followed by Model Deviation Ratio (MDR) (Wieczerzak et al. 2018) (**Publications 3, 4** and **5**)

The first objective focused on **accessing and comparing the sensitivity of selected photosynthetic microorganisms to individual target analytes and their binary mixtures including determination of the effective concentration (EC_{50}) based on growth and photosynthetic efficiency inhibition.** This approach provided a quantitative basis for evaluating the differential responses of target species highlighting the variability in susceptibility among taxa within the Baltic Sea ecosystems (**Publications 2, 3, 4** and **5**). According to the regulatory framework established by the European Chemicals Agency (ECHA, 2008), toxicity classifications are defined based on EC_{50} concentration thresholds: highly toxic (EC_{50} = 0.1–1 mg/L), marginally toxic (1–10 mg/L), moderately toxic (10–100 mg/L), and non-toxic (100–1000 mg/L). Among the tested microorganisms, *M. aeruginosa* showed the highest sensitivity to both ionic liquids, with IM1-12Br exhibiting the lowest $EC_{50\ IM1\text{-}12Br}$ value (0.03 mg/l) compared to $EC_{50\ IM1\text{-}18C(CN)_3}$ (0.10 mg/l), indicating its high toxicity. In contrast, *P. tricornutum* and *C. vulgaris* were less sensitive, with EC_{50} values of 8.00 mg/L, and 0.37 mg/l for IM1-12Br, respectively. *M. aeruginosa* and *P. tricornutum* exhibited moderate sensitivity to oxytetracycline, while *C. vulgaris* showed a comparable EC_{50} of 1.00 mg/l. Caffeine and carbamazepine-10,11-epoxide did not inhibit growth or photosynthetic efficiency at concentrations up to 100 mg/l in both *M. aeruginosa* and *P. tricornutum*. The results underscore the differential sensitivity among the tested microorganisms, with the cyanobacterium *M. aeruginosa* demonstrating heightened susceptibility to the tested ionic liquids, while the diatom *P. tricornutum* exhibited a comparatively higher tolerance. These findings align with previously reported patterns in the literature, emphasizing the varying toxicological responses of distinct taxonomic groups to chemical stressors (Guo et al. 2016).

The second objective focused on **elucidating the underlying mechanisms of action of the target compounds and their mixtures through the application of non-standard biomarkers.** This approach encompassed the evaluation of physiological and biochemical endpoints, including chlorophyll *a* fluorescence analysis, quantification of pigment content (including phycobiliproteins in cyanobacterium), and assessment of T-SOD enzyme activity (**Publications 2, 3, 4** and **5**). The growth, photosynthetic processes, and pigment ratios in all target microorganisms were significantly affected following exposure to individual pollutants, as well as their mixtures. The use of nonstandard biomarkers facilitated the identification of stress response mechanisms, even in the absence of observable changes in growth or biomass

inhibition. This approach enabled the detection of photoprotective mechanisms activated in response to low concentrations of target compounds including caffeine. The differential responses of *M. aeruginosa*, *C. vulgaris*, and *P. tricornutum* underscore the importance of organism-specific factors such as physiological traits and stress tolerance mechanisms, in determining sensitivity to contaminants. Among the tested microorganisms, *M. aeruginosa* exhibited the highest sensitivity to both ionic liquids, with significant inhibition of the F_v/F_m (the maximum efficiency of photosystem II) photochemistry parameter observed even at low concentrations (**Publications 3** and **4**). Conversely, *C. vulgaris* displayed a biphasic response to IM1-12Br, with low concentrations stimulating photosynthetic efficiency, followed by inhibition at higher concentrations, indicative of a hormetic effect where mild stress levels elicit adaptive responses (**Publication 3**). *P. tricornutum* was the least sensitive, with F_v/F_m values declining at elevated concentrations but to a lesser extent than in *M. aeruginosa* (**Publications 3** and **4**).The binary mixtures of IM1-8C$(CN)_3$ with carbamazepine-10,11-epoxide or oxytetracycline resulted in synergistic toxicity for *M. aeruginosa*, significantly reducing F_v/F_m values (**Publication 4**). The presence of caffeine in mixtures, however, had a less pronounced effect, with only high-concentration combinations showing moderate inhibition (**Publication 5**). *C. vulgaris* demonstrated pronounced changes in ABS/RC (absorbed energy flux per reaction center) and DI_0/RC (dissipation of photochemical energy per reaction center) in response to high concentrations of IM1-12Br, oxytetracycline and their mixtures (**Publications 2 and 3**). All organisms exhibited increased DI_0/RC values in response to ionic liquids and their mixtures (**Publications 3, 4** and **5**). This reflects a common stress response mechanism, where excess absorbed energy is dissipated as heat or fluorescence to prevent photodamage under unfavorable conditions. Specifically, environmentally relevant caffeine concentration (10 μg/l) significantly increased the DI_0/RC parameter, indicating heightened energy dissipation as a stress response in cyanobacterium and diatom (**Publication 5**). Additionally, a sharp increase in ABS/RC was observed in all microorganisms exposed to high concentrations of ionic liquids and binary mixtures. This response likely represents a compensatory mechanism to optimize light capture and maintain photosynthetic function under stress **(Publications 3, 4 and 5**).

The analysis of pigment concentrations revealed that both *M. aeruginosa* and *P. tricornutum* experienced a significant decline in chlorophyll *a* levels when exposed to the highest concentrations of ionic liquids, with the extent of inhibition proportional to the compound concentration. Both organisms demonstrated changes in stress-associated pigments, such as β-carotene and xanthophyll derivatives (e.g., violaxanthin, diadinoxanthin, and diatoxanthin), with relative increases observed at lower ionic liquid concentrations, indicative

of adaptive responses to mitigate oxidative stress (**Publication 4 and 5**). Furthermore, chlorophyllide *a*, a degradation product of chlorophyll *a*, increased under elevated ionic liquid concentrations and binary mixtures, suggesting enhanced chlorophyll degradation. Similarly, even low oxytetracycline concentrations had a significant impact on *C. vulgaris* and *P. tricornutum*, leading to a reduction in phaeopigment and chlorophyll *a* content per cell, with a clear dose-dependent decline observed (**Publication 2**).

In *M. aeruginosa*, phycobiliproteins (e.g., phycoerythrin and phycocyanin) were differentially affected, with an increase in phycoerythrin levels and a decrease or absence of phycocyanin under stress conditions (**Publications 3, 4 and 5**). In *P. tricornutum*, stress responses were marked by alterations in xanthophyll cycle pigments and light-harvesting chlorophyll derivatives, with the absence of violaxanthin at higher stress levels indicating impaired photoprotective mechanisms (**Publications 4 and 5**).

Total superoxide dismutase (T-SOD) activity, a critical oxidative stress marker, was significantly influenced by IM1-8C$(CN)_3$ ionic liquid and their binary mixtures with oxytetracycline, carbamazepine-10,11-epoxide, and caffeine (**Publication 4** and **5**). Responses were species-specific, concentration-dependent, and varied based on analyte combinations. *M. aeruginosa* exhibited heightened oxidative stress under combined exposure to ionic liquid and its mixtures, with increased T-SOD activity at elevated concentrations. In contrast, *P. tricornutum* showed a stronger oxidative stress response to ionic liquid alone compared to their mixtures, highlighting the pronounced toxicity of ionic liquid relative to binary combinations (**Publication 4**). Both organisms displayed substantial oxidative stress in response to mixtures of ionic liquid and caffeine, with synergistic effects observed at higher concentrations (**Publication 5**).

The third objective involved **assessment of the toxicological impact of ionic liquids and their potential to exacerbate toxicity when combined with other organic micropollutants utilizing theoretical models of concentration addition (CA) and independent action (IA), alongside model deviation ratio (MDR) calculations to quantify deviations from predicted additive behaviors.** Simultaneously, the fourth objective focused on **examining the accuracy of the CA and IA theoretical models in predicting and characterizing the observed binary mixture effects, thereby evaluating their reliability in identifying interactions across diverse combinations of ionic liquids and organic micropollutants.** The study revealed complex interaction patterns between pollutants, characterized by concentration-dependent synergistic and antagonistic effects, with notable variations in predictions between the CA and IA models (**Publications 3, 4,** and **5**). *C. vulgaris*

showed synergistic effects at higher IL and oxytetracycline concentrations, which were accurately predicted by both models (**Publication 3**). For mixtures of IM1-12Br and oxytetracycline with *M. aeruginosa*, the IA model effectively captured pronounced synergistic interactions. Additionally, both models successfully predicted interactions between these compounds and their effects on *P. tricornutum* (**Publication 3**). In the case of *M. aeruginosa* and *P. tricornutum*, the CA model identified concentration-dependent interactions between IMI-8C$(CN)_3$ and oxytetracycline, demonstrating antagonistic effects at higher concentrations and synergistic effects at lower concentrations. These findings suggest overlapping modes of action where compounds either mitigate or amplify each other's toxicity based on their relative concentrations. Conversely, the IA model consistently overestimated toxicity, especially at higher oxytetracycline concentrations and in antagonistic interactions of carbamazepine-10,11-epoxide, failing to align with observed antagonistic effects. Similarly, for photosynthetic activity, the CA model revealed synergistic effects at high ionic liquid concentrations and antagonistic effects at its lower levels, highlighting the complex, concentration-dependent nature of these interactions, which the IA model was less effective at capturing (**Publication 4**). Lastly, the evaluation of caffeine and IMI-8C$(CN)_3$ mixtures demonstrated that caffeine significantly mitigates the ionic liquid's toxic effects, as shown by the CA model. Predominantly antagonistic interactions were identified by both models, particularly at higher caffeine concentrations, with the CA model providing more accurate predictions. The IA model often overestimated toxicity at medium ionic liquid concentrations, reflecting its limitations in addressing interactions between compounds with differing mechanisms of action (**Publication 5**).

Based on the findings of this study, all hypotheses were verified, confirming the predicted impacts of micropollutants on photosynthetic processes and biomarker responses. The following **conclusions** were drawn:

i. The toxicological effects of ionic liquids and their binary mixtures with organic micropollutants, including oxytetracycline, caffeine and carbamazepine-10,11-epoxide, are highly species-specific and concentration-dependent. *M. aeruginosa* exhibited the highest sensitivity, with pronounced inhibition of photosynthetic efficiency (e.g., F_v/F_m), growth, and pigment production.

ii. Non-standard biomarkers, such as chlorophyll *a* fluorescence kinetics, pigment composition alterations, and T-SOD activity, proved essential in detecting sub-lethal stress responses that were not captured by conventional growth inhibition test. Parameters like DI_0/RC (dissipated energy per reaction center) and pigment

composition changes highlighted the activation of photoprotective mechanisms in target microorganisms in response to ILs and their mixtures with organic micropollutants.

iii. Variations in responses among representative species of diatoms, green algae, and cyanobacteria are anticipated to result in community-level shifts. These detrimental effects could alter algal population structures and functions, with potential cascading impacts on the broader marine ecosystem.

iv. Complex, concentration-dependent interactions between ILs and organic micropollutants were identified. Synergistic and antagonistic effects were observed, influenced by the specific pollutants and their relative concentrations.

v. The Concentration Addition (CA) model provided accurate predictions of binary mixture toxicity across various combinations. The Independent Action (IA) model consistently overestimated toxicity and failed to account for nuanced interactions.

vi. The combined effects of micropollutants on non-target organisms emphasize the necessity of evaluating pollutant interactions within ecologically relevant, real-world contexts, particularly in under-researched marine and brackish environments such as the Baltic Sea. Additionally, the findings underscore the critical importance of conducting chronic exposure assessments to elucidate the cumulative and long-term impacts of these compounds on aquatic ecosystems.

Based on the findings of this study, future research should prioritize the further exploration and validation of non-standard biomarkers, such as chlorophyll fluorescence kinetics and pigment composition changes, as sensitive indicators for detecting early sub-lethal stress responses in microalgae. Given the importance of cumulative and long-term exposure effects, future investigations should focus on chronic toxicity assessments of micropollutants. Prolonged exposure experiments will help reveal delayed physiological effects, adaptive responses, and potential recovery mechanisms in microalgae and other primary producers. Since the study demonstrated complex, concentration-dependent interactions between micropollutants, future research should prioritize mixture toxicity studies over individual compound assessments. Advanced modeling approaches should be integrated to enhance the characterization of mixture behavior and reactivity, addressing the limitations of CA and IA models. This will facilitate a more comprehensive assessment of their environmental impact and associated risks. While laboratory studies provide valuable insights, studies should extend to field-based assessments to validate laboratory findings

under real-world environmental conditions. Studying algal community shifts, trophic interactions, and ecosystem-level impacts in marine and brackish water environments, such as the Baltic Sea, will be crucial for understanding long-term ecological consequences.