The effects of micropollutants mixtures on marine and brackish microorganisms

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Risk assessment challenges

Micropollutants - substances that occur in surface waters at very low concentrations (micro/ nanograms per liter)

Over 350 000 chemicals and mixtures of chemicals on the market (expected to double by 2030) (Wang et al. 2020)

Over **2000 active pharmaceutical** ingredients are being administered worldwide (OECD, 2019)

Pharmaceuticals are:

- biologically active
- stable (pseudo - persistent)
- present in mixtures

Managing the risks of pharmaceuticals in the environment requires a multi-disciplinary and multi-stakeholder approach

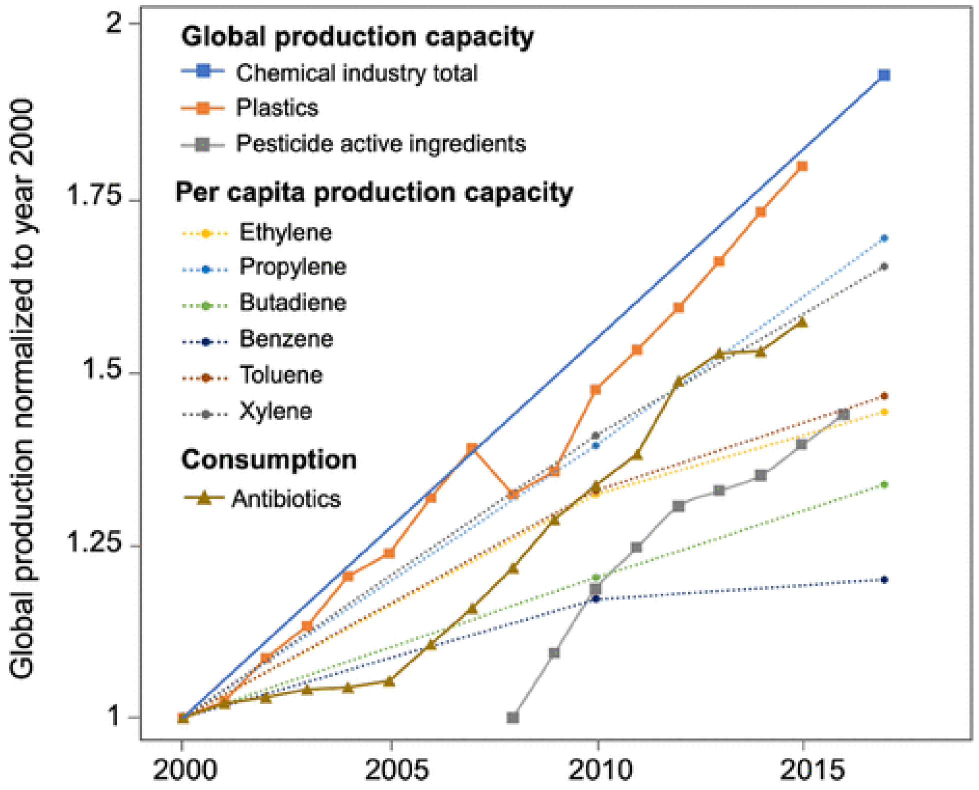


Fig 1. Global production capacity for a number of groups of novel entities (Perrson et al. 2022)

Risk assessment challenges

Complex interaction between chemicals and biological systems

- Cumulative Exposure
- Synergistic or Antagonistic Effects
- Regulatory Considerations

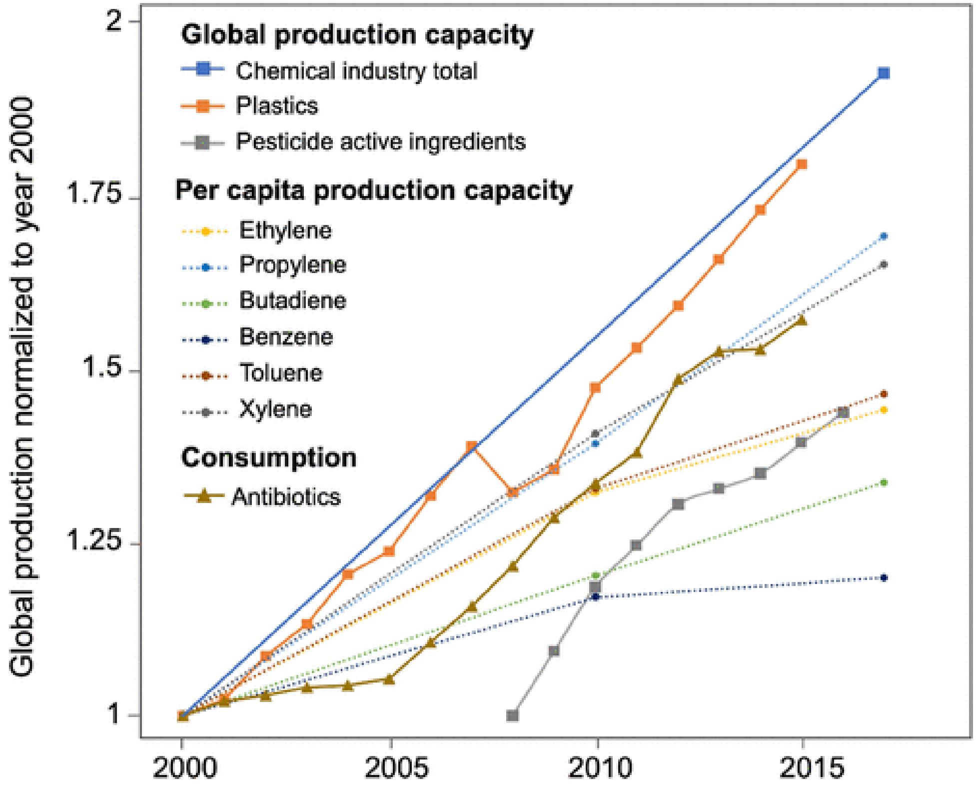


Fig 1. Global production capacity for a number of groups of novel entities (Perrson et al. 2022)

Ionic liquids – potential micropollutants?

Ionic liquids:

- Ionic liquids (ILs) are organic salts that exist as liquids at low temperature (<100^{o}C)
- Advantages: very low vapor pressure, non-flammability, thermal stability, electrochemical stability

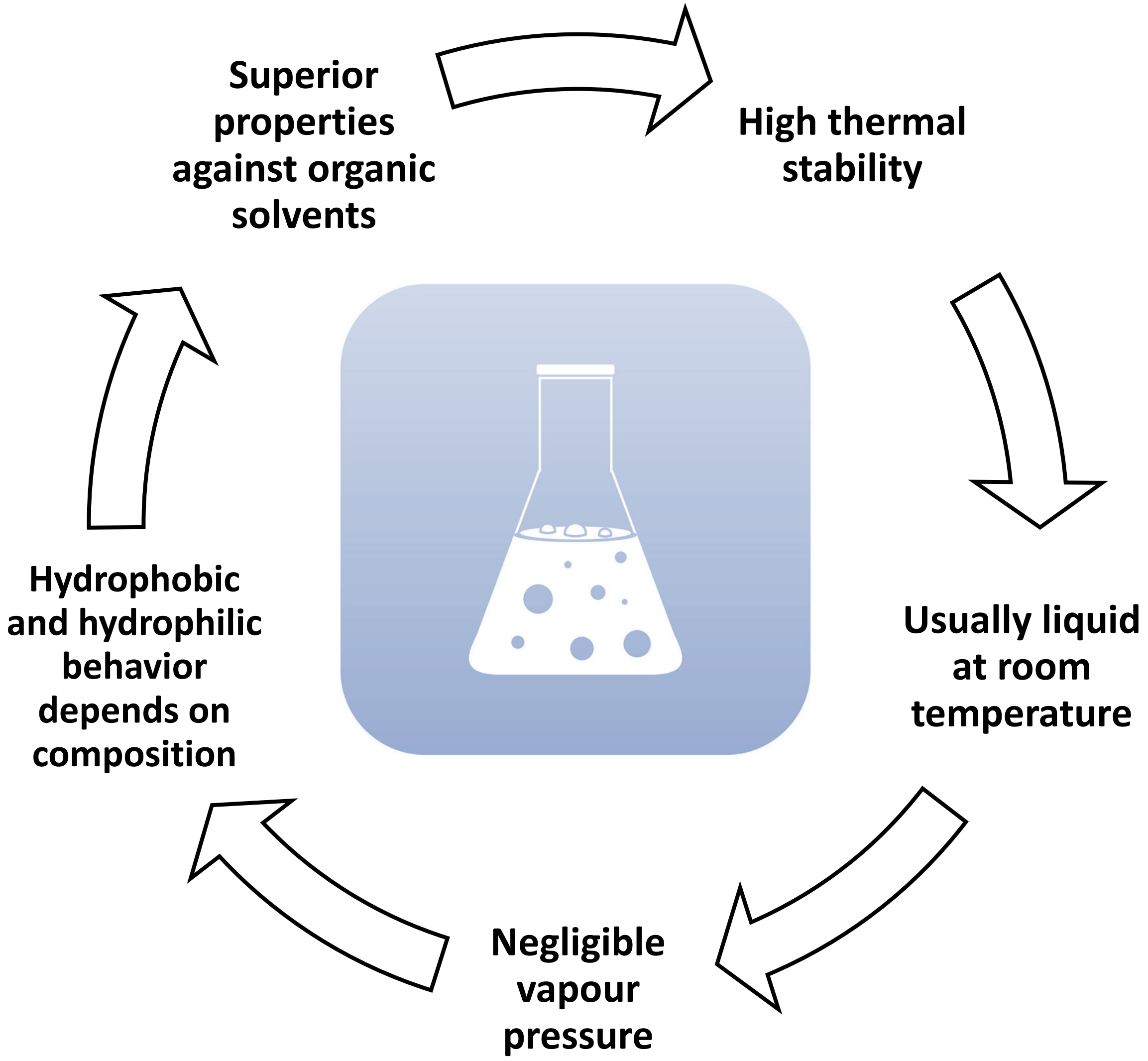
Green solvents ?

Ionic liquids—a threat to health and the environment?

Persistent, mobile, and toxic (PMT) and very persistent and very mobile (vPvM) substances.

Presence in the environment

Detection of a fluorinated ionic liquid with peak concentrations of up to 3.4 µg/L in rivers in Germany (Neuwald et al., 2020)

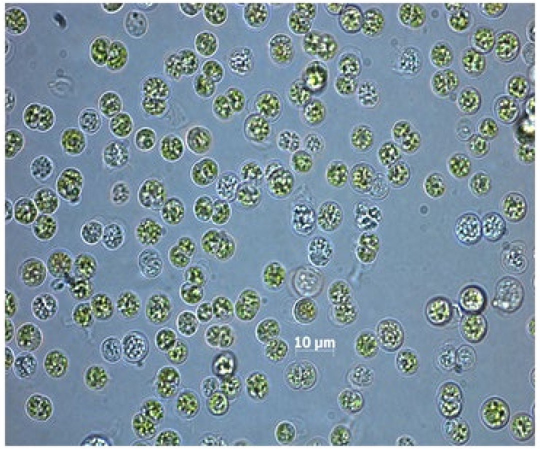


Objectives

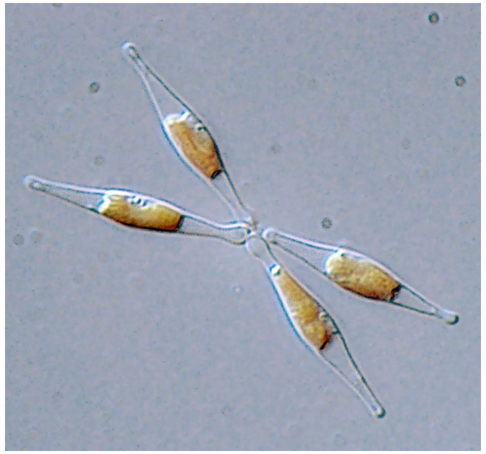
- Assessment of the effects of mixtures of selected compounds - the antibiotic oxytetracycline (OXTC) and the ionic liquid 1-dodecyl-3-methylimidazolium bromide (IM1-12Br), on the growth, pigment content, and photosynthesis efficiency of target marine microorganisms including the luminescent marine bacterium **Aliivibrio fischeri.**

- A better understanding of the mode of action through a evaluation of different endpoints

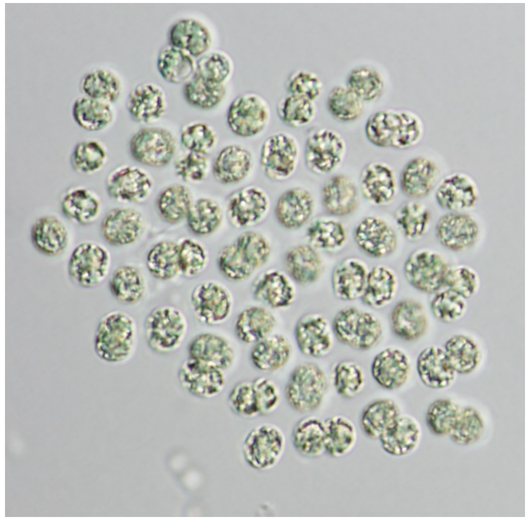
- **The classification and prediction of synergistic, antagonistic, and additive effects** of binary mixtures was carried out using mathematical models, specifically the concentration addition (CA) and independent action (IA) models



Chlorella vulgaris



Phaeodactylum tricornutum



Microcystis aeruginosa

Materials and methods

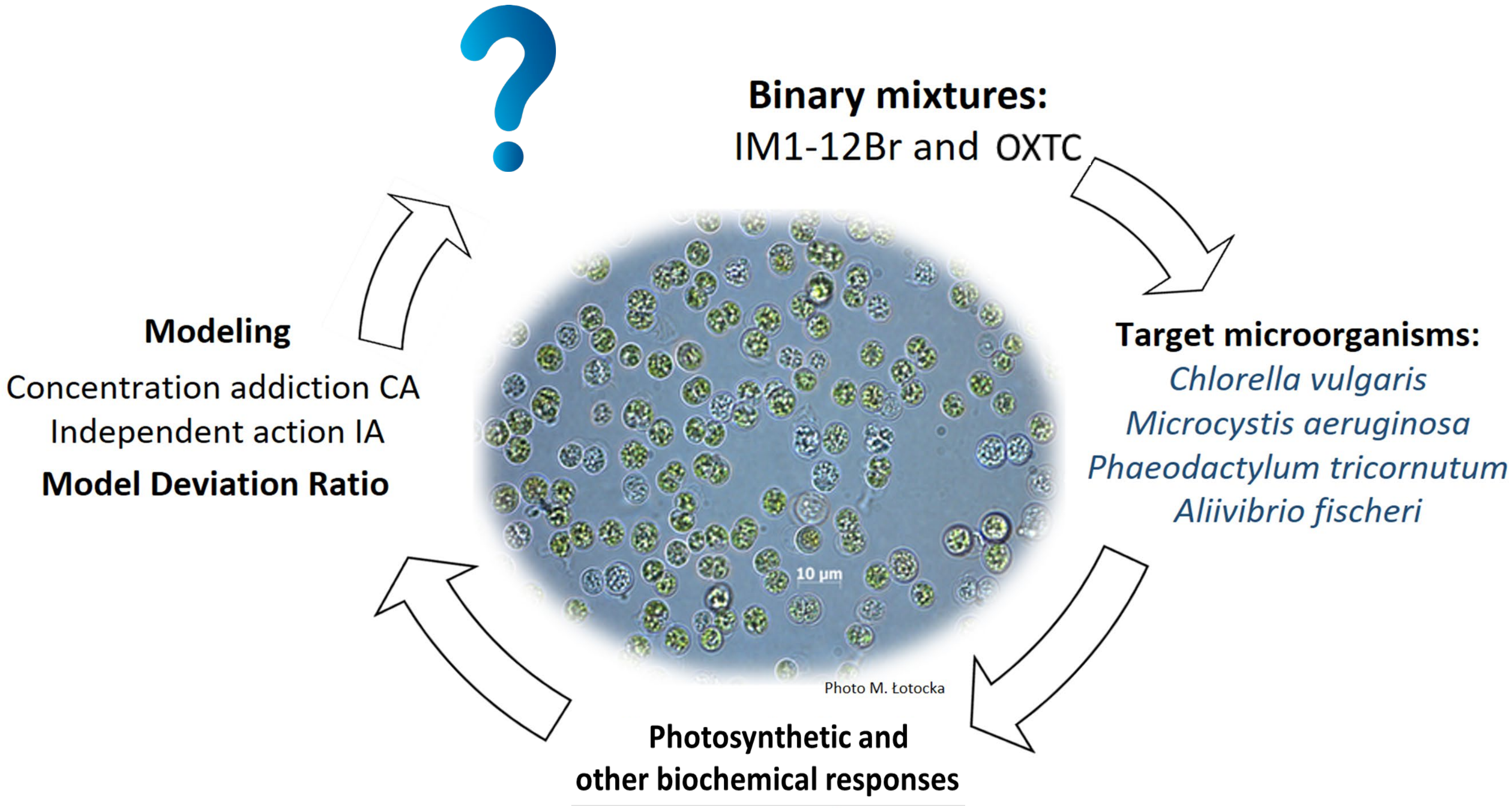


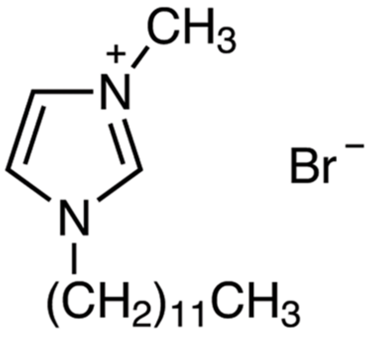
Fig. 2. Conceptual approach of the present study

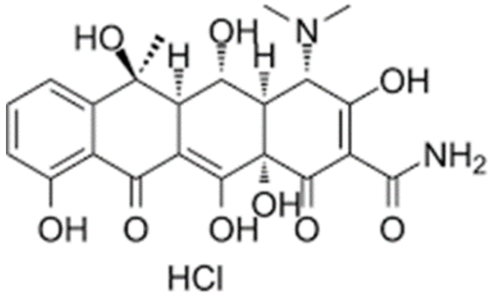
Materials and methods

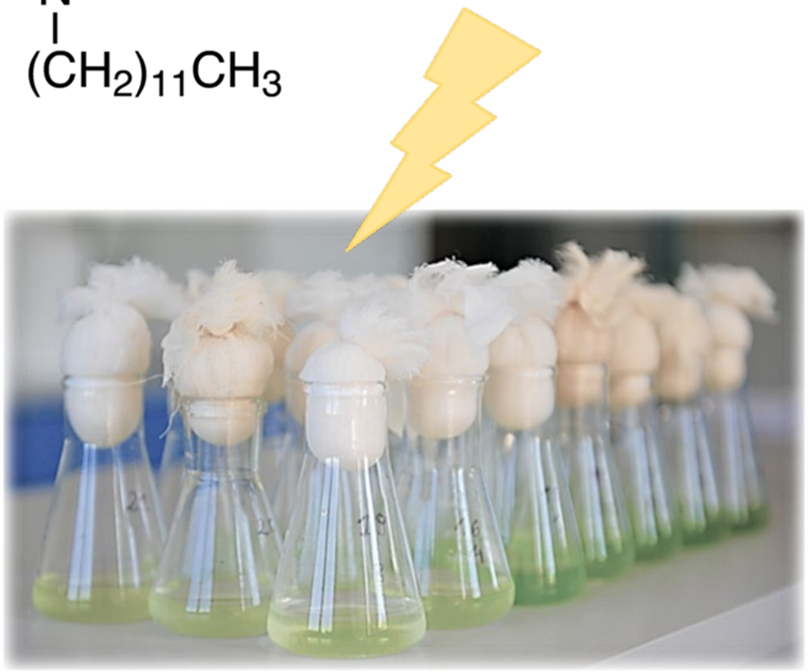
Target ionic liquids 1-dodecyl-3-methylimidazolium bromide (IM1-12Br) and **antibiotic** oxytetracycline (OXTC)

- Incubation under controlled light conditions and constant temperature (duration of the experiments: 11 days)
- The chlorophyll *a* fluorescence analysis (AquaPen Ap-100C)
- Pigment content
  Spectrofluorometric phycobilin analysis (Sobiechowska-Sasim et al. 2014)
- Statistical analysis (one- way ANOVA and Dunnett's multiple comparisons post-hoc test)

$$MDR = \frac{Expectation}{Observation} = \frac{Expected\ value\ of EC_{X_{Mix}}}{Observed\ value\ of\ EC_{X_{Mix}}}$$







Results

Model Deviation Ratio results

Table 1

MDR values calculated based on the F_v/F_m parameter representing photosynthetic activity changes in *C. vulgaris, M. aeruginosa, P. tricornutum* exposed to a mixture of S1 – IM1-12Br, S2-OXTC for both CA and IA models.

synergism antagonism underestimation overestimation

C .vulgaris						
	CA			**IA**		
	S1C1	S1C2	S1C3	S1C1	S1C2	S1C3
S2C1	1.025	0.896	0.718	0.895	0.855	0.364
S2C2	1.009	0.575	0.061	0.828	0.842	0.266
S2C3	1.211	0.431	0.140	0.859	0.719	0.306
M. aeruginosa						
	S1C1	S1C2	S1C3	S1C1	S1C2	S1C3
S2C1	0.995	1.034	0.737	0.787	0.734	0.585
S2C2	1.061	1.050	1.071	0.257	0.350	0.322
S2C3	1.549	1.671	1.431	0.122	0.167	0.133
P. tricornutum						
	S1C1	S1C2	S1C3	S1C1	S1C2	S1C3
S2C1	1.237	0.769	2.155	0.831	0.780	0.173
S2C2	0.968	1.213	1.332	0.725	0.481	0.070
S2C3	0.467	1.040	1.053	0.220	0.076	0.043

Results

Model Deviation Ratio results

Table 2

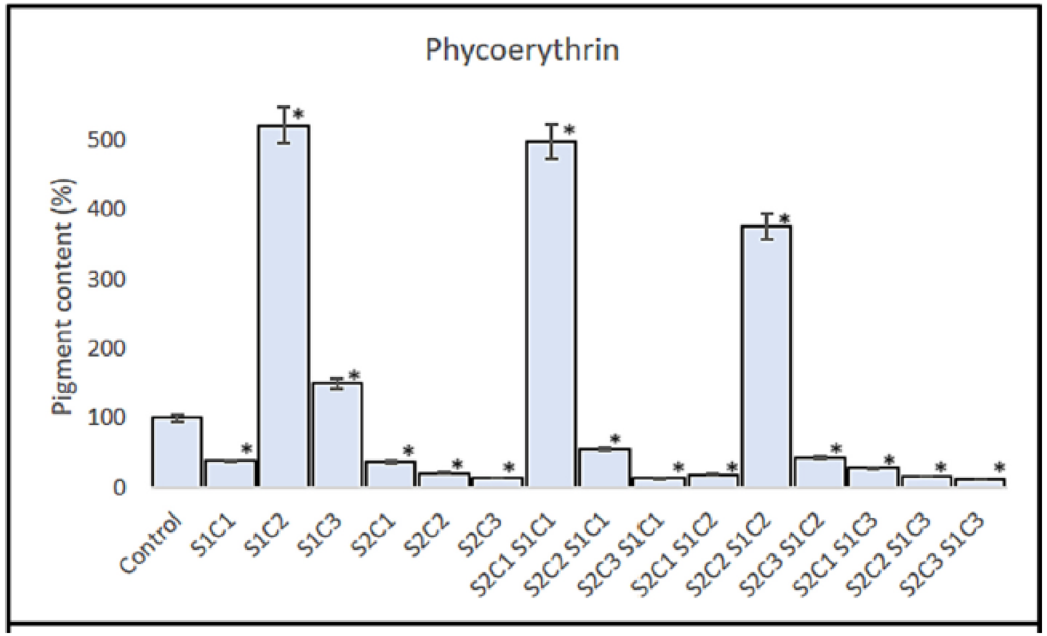
MDR value calculated based on bioluminescence inhibition of *A. fischeri* exposed to a mixture of S_1 – IM1-12Br, S_2-OXTC for both CA and IA models.

synergism | antagonism | underestimation | overestimation

	CA				IA		
	S1C1	S1C2	S1C3		S1C1	S1C2	S1C3
S2C1	0.745	0.476	0.398		1.016	0.921	0.933
S2C2	0.733	0.433	0.279		1.178	0.908	0.708
S2C3	0.622	0.217	0.190		0.982	0.844	0.692

Results

Pigment content



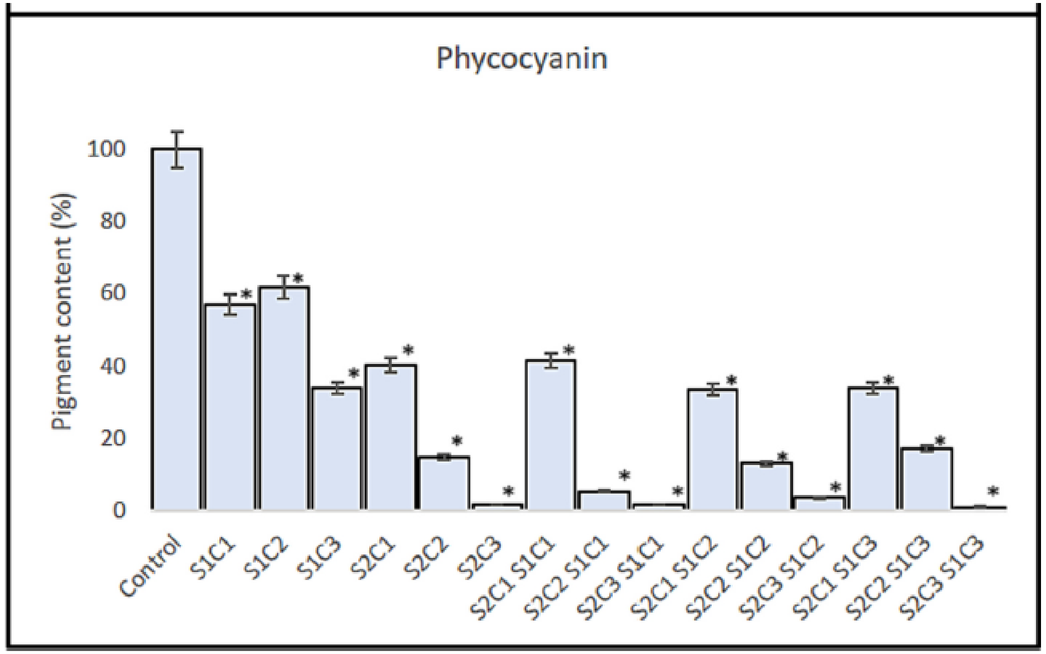


Fig. 3. The phycoerythrin and phycocyanin content in *M. aeruginosa* presented as % of the control on the 11th day of the experiment (S1- IM1–12Br, S2- OXTC, C1–0.01 mg/L, C2–0.02 mg/L, C3–0.03 mg/L of IM1–12Br, C1–0.4 mg/L, C2–0.8 mg/L, C3–1.2 mg/L of OXTC) (* result statistically significant).

Summary

General Conclusions

- The results indicate the presence of **synergistic interactions** between binary mixtures of a representative imidazolium based ionic liquid, IM1-12Br, and oxytetracycline.
- The combined effect of both compounds affects the photosynthetic machinery of PSI and PSII in target microorganisms and leads to changes in pigment content in a model cyanobacterium.
- Bioluminescence measurements of the marine bacterium *A. fischeri* allowed the identification of synergistic interactions.
- Both Concentration Addiction (CA) and Independent Action (IA) mathematical models accurately predict direct mixture results.
- This study is the first to report on the effects of ionic liquids and other organic micropollutants, providing valuable information on the toxicity of mixtures of these compounds.

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