Lifting the Veil. Impact of Contaminants on Coastal Phytoplankton
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Coastal waters are among the most productive ecosystems on the planet. Yet, they also suffer from a high contaminant load due to input from rivers and shipping, resulting in a 'grey veil' of contaminants. Exposure of microalgal communities to these contaminants potentially results in shifts in microalgal community composition. As microalgae form the basis of the marine food webs, those shifts can ultimately affect the carrying capacity of coastal and estuarine ecosystems. However, neither the composition, nor the potential harmful effects of the 'grey veil' of contaminants on marine microalgae have been identified. This thesis aimed to 'lift the veil' by identifying contaminants hazardous to microalgae and to determine the hazard and risk of these contaminants. To this purpose the following objectives were set:

I. To develop a tool for the identification of phytotoxic contaminants in coastal and estuarine waters.
II. To determine the hazard and risk of key contaminants under laboratory and environmental relevant conditions in single and multi-species experiments.
III. To investigate the suitability of current environmental quality standards to protect marine microalgae.

The study was focused on the Dutch coastal and estuarine waters as they are known to be polluted due to their geographical location downstream of European rivers.

To identify the phytotoxic contaminants in the water and to determine their toxic effects under different conditions, photosynthesis served as a toxic endpoint. A bioassay based on Pulse Amplitude Modulation (PAM) fluorometry was optimized to serve as a high throughput bioassay, requiring low test volumes. Moreover, the bioassay was capable of assessing toxicity of single compounds as well as complex field samples. Since test conditions within a bioassay are known to influence toxicity results, the effects of the main test factors on the test outcome of a PAM bioassay were discussed (***Chapter 2***). This provided background information for the interpretation of toxicity tests using PAM fluorometry. These technical aspects were taken into account in the development of the acute (4.5h) PAM bioassay used in the rest of the study.

The PAM bioassay was used in an Effect-Directed Analysis (EDA) to identify the main contributors to the phytotoxic pressure in the Dutch coastal and estuarine waters. This EDA was carried out by combining reversed-phase ultra performance liquid chromatography fractionation of extracts from passive samplers, followed by effect assessment using the PAM bioassay with the marine microalgae *Dunaliella tertiolecta* (***Chapter 3***). Next, chemical analysis of biologically active fractions was performed using high-resolution mass spectrometry. It was demonstrated that, of the numerous unknown contaminants forming the 'grey veil' in the Dutch coastal and estuarine waters, the herbicides atrazine, diuron, irgarol, isoproturon, terbutryn, and terbutylazine are the main contributors to the observed effects on the photosynthetic response of *D. tertiolecta*.

To gain insight in the hazard and risk of these herbicides, the toxicity of a selection of the herbicides (atrazine, diuron, irgarol, isoproturon) was determined for three marine microalgal species (*Thalassiosira pseudonana* , *Phaeodactylum tricornutum* and *D. tertiolecta*) (***Chapter 4***). Results obtained in this acute 4.5h PAM bioassay demonstrated a clear herbicide and species specific toxicity. Due to the this species specific toxicity, herbicides potentially contribute to changes in species composition of phytoplankton communities. Moreover, the toxicity of these four herbicides was enhanced when present in a mixture. Additionally it was found that the risk of these herbicides at current field concentration is low, although monitoring data revealed that concentrations occasionally reach potential effect levels. Moreover, we demonstrated that the current legislation (MAC-EQS) does not protect the microalgae sufficiently against isoproturon and to a greater extent against diuron exposure.

The above described results are all based on laboratory experiments. However, environmental factors in the field may interact with the algae and/or the herbicide toxicity, thereby forming a multi-stress factor for microalgae. In the present study light proved to modify the herbicide toxicity in the field. As irradiance is seasonal variable, the toxicity of two selected herbicides to microalgae was determined under different simulated seasonal light conditions (***Chapter 5***). Toxicity of diuron and irgarol to *D. tertiolecta* was determined under irradiance conditions mimicking spring and autumn using PAM fluorometry. We demonstrated that the toxicity of both herbicides individually as well as in a mixture was higher under simulated spring light conditions compared to autumn conditions. Due to this seasonal specific response, it was concluded that toxicity determined under standard light conditions may overestimate or underestimate the toxic effect of these herbicides. Consequently, this seasonal variability creates a margin of uncertainty of the actual risk of the herbicides in the field.

Finally, the effect of one of the herbicides (diuron) was determined in a field experiment with a natural marine phytoplankton community (***Chapter 6***). The community was exposed to a maximum field concentrations (MFC) as well as to the level of the environmental quality standard for acute toxicity (MAC-EQS). Experiments were performed outdoor and effects on the community composition were determined with microscopy and flow cytometry, while effects on photosynthesis were determined by PAM fluorometry. With this experiment we showed that MCF of diuron has no effect on community composition nor on photosynthetic efficiency. In contrast, there is a significant effect on both endpoints of the MAC-EQS. It is concluded that the current EU legislation is not sufficiently protecting marine phytoplankton communities against the risk of diuron exposure. Consequently, this acute quality standard should be adjusted to obtain a safe quality standard.

Concluding, this study lifted the ‘veil’ of contaminants, demonstrating that of the numerous contaminants currently present in the Dutch coastal zone, six herbicides (atrazine, diuron, irgarol, isoproturon, terbutylazine, terbutryn) could be identified as the main contributors to the toxic pressure on phytoplankton in these waters. Although the concentrations of these six herbicides are currently low compared to their observed toxicity to phytoplankton, higher concentrations which reached effect levels have been observed in the past. This suggests that the presence of herbicides might have contributed to previously observed changes in phytoplankton community composition. Nevertheless, mixture toxicity and multi-stress effects will modify the toxicity of the herbicides in the field, thereby creating a ‘margin of uncertainty’ of the ultimate toxic pressure. This margin of uncertainty should also be taken into account in environmental quality standard setting. Finally, the current monitoring system will benefit from an effect-based approach as demonstrate in the present study for phytoplankton. By combining the presented PAM bioassay with other miniaturized bioassays for organisms at higher trophic levels, the main contributors to the toxic pressure for many biological components can be identified. Such an integrated ecotoxicological and chemical monitoring of coastal waters will support the protection of the vital function of coastal ecosystems.