Toxic pressure of chemical stressors on pelagic microalgae in Dutch estuarine and coastal waters
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Summary

There is growing concern about the number and different types of contaminants present in estuarine and coastal waters and their possible effects on pelagic microalgae, and hence primary production. Current regulations for the protection of European water bodies are based on the chemical analysis of a limited set of contaminants. In this thesis, we have identified and confirmed the main contributors to the toxic pressure on the effective photosystem II efficiency (ΦPSII) in microalgae in Dutch estuarine and coastal waters. To broaden the investigation of the potential impact of contaminants in estuarine and coastal water on marine microalgae, effect-directed analysis (EDA) is introduced. EDA is a procedure that combines analytical chemistry with bioassays in order to identify the main contributors and possible new compounds which are responsible for the effects observed. The principle and application of EDA and merits of the Pulse Amplitude Modulation fluorometry bioassay (PAM assay) to assess the toxicity on effective photosystem II efficiency (ΦPSII) in microalgae is discussed. In addition, the benefits of passive samplers and spot water sampling to extract contaminants from estuarine and coastal waters are considered. Lastly, the potential use of metabolomics to assess the toxicity of contaminants on marine algae is evaluated.

In Chapter 2 different extraction methods to extract contaminants from water that elicit an effect on microalgae in the PAM assay are evaluated. Four different commonly used extraction methods - passive sampling with silicone rubber sheets, polar organic integrative samplers (POCIS) and spot water sampling using two different solid phase extraction (SPE) cartridges - were compared to assess the benefits of the sampling strategies and their suitability to determine inhibitors of the photosystem II activity of microalgae in estuarine and coastal waters. The main advantage of SPE is its suitability for quantitative analysis since validated protocols are already available. The joint application of POCIS and silicone rubber sheets was shown to extract a broad range of contaminants with different polarities and enabled to sample episodic events which can be missed with spot water sampling. However, passive sampling still needs further development in so far as quantification of field concentrations is concerned. The combination of these passive samplers was used for further identification of contaminants in estuarine and coastal water affecting photosynthesis in microalgae (Chapter 3).

An enhanced throughput EDA approach was developed to identify the main photosynthesis inhibitors of pelagic microalgae in Dutch estuarine and coastal waters. A novel 96-well plate microfractionation technique was used, resulting in a much larger number of less complicated
fractions than commonly encountered in EDA studies. This technique also helped to improve the identification process. Another innovation was the addition of a keeper to the 96-well plates to increase the recovery of compounds during the evaporation process. Chapter 3 further focusses on the structure elucidation, and the analytical and effect confirmation of the compounds that are identified. Six herbicides, i.e. atrazine, diuron, irgarol, isoproturon, terbutryn, and terbutylazine, were identified and confirmed as the main contributors to the observed effect on the ΦPSII in marine microalgae. Besides terbutylazine, all other herbicides (atrazine, diuron, irgarol, isoproturon and terbutryn) are listed as priority substances by the European Union Water Framework Directive (WFD).

In Chapter 4, the field of environmental metabolomics is explored by using complementary analytical techniques for the identification of the metabolomic pathways affected by diuron on the marine microalgae *D. tertiolecta*. The non-target metabolomic profiling results are linked to the PAM data and suggest that besides the inhibitory effect on ΦPSII (obtained in the PAM assay) also the amino acid metabolism and citric acid cycle in microalgae are affected by diuron. It was found that the use of complementary analytical techniques in non-target metabolomics is necessary to identify a broad range of metabolites, and provides additional information to the PAM assay on the health status of the algae.

The toxic pressure of the six herbicides identified (atrazine, diuron, irgarol, isoproturon, terbutryn, and terbutylazine) in relation to the effects on ΦPSII in marine microalgae is investigated in Chapter 5. The toxic pressure on ΦPSII in microalgae has decreased by 55-82% from 2003 to 2012. The highest toxic pressure in the Dutch estuarine and coastal waters was observed in the Western Scheldt estuary. Diuron and terbutylazine were found to be the most harmful contaminants in Dutch estuarine and coastal waters in terms of toxic pressure on ΦPSII in microalgae. Direct effects of herbicides present in Dutch estuarine and coastal waters on ΦPSII are currently not expected, however the toxic pressure is close to the 10% effect level in the PAM assay. The classical method of compliance checking was compared with toxicity data of the PAM assay. It revealed that with respect to the current legislation marine microalgae are not sufficiently protected. Monitoring of chemical concentrations combined with the PAM assay provided information on the toxic pressure on ΦPSII in microalgae. This information can be used in future monitoring programs for water quality assessment to take mixture effects and unanalyzed chemicals into account.

Generally, EDA can be used in investigative monitoring. In addition, it is needed to identify new compounds if biological effects are above a certain level and effects cannot be explained by quantitative chemical analysis. Currently, metabolomics cannot be used in routine monitoring. A
future challenge in metabolomics is the identification of biomarkers, which can be used as an indicator for exposure to specific contaminants or group of contaminants that affect microalgae. Terbutylazine should be considered as a priority substance in monitoring programmes. In addition, the current environmental quality standards (EQS) set by the European Commission for the herbicides identified as main contributors in the present study should be reconsidered because they do not protect marine microalgae sufficiently.