Lifting the veil: Impact of contaminants on coastal phytoplankton

Sjollema, S.B.

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Chapter 1

General introduction
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Coastal and estuarine waters are covered by a ‘veil’ of contaminants which might affect the organisms present in these waters, thereby forming a potential threat to the productive food web. Exposure of coastal microalgal communities to contaminants potentially results in shifts in species composition thereby modifying the exploitation by herbivores and filterfeeding animals, ultimately affecting the carrying capacity of coastal and estuarine ecosystems. Nevertheless, not all contaminants are harmful for microalgae and currently, neither the chemical composition of the coastal waters, nor the effects of chemical contaminants on microalgae are known. The aim of the present thesis was therefore to lift this veil by determining hazardous contaminants for microalgae living in contaminated coastal and estuarine waters, and to determine the hazard and risk of these contaminants under laboratory as well as field conditions.

Coastal ecosystems

Coastal waters are among the most productive ecosystems on the planet in which the primary production is mostly generated by microalgae and mainly driven by the input of light and nutrients (Kaiser et al., 2011). Light, or more specifically Photosynthetic Active Radiation (PAR 400-700nm), is essential for the photosynthetic process by microalgae in which the light energy is used to

Fig. 1.1. World primary productivity in oceans indicated via the concentration of chlorophyll $a$ in seawater. The highest primary production, represented by the warm colors, can be observed close to continents. Source: oceancolor.gsfc.nasa.gov.
convert carbon dioxide and water into organic compounds and oxygen. As chlorophyll *a* is the main photosynthetic pigment present in all microalgal species, this parameter is frequently used as a proxy for microalgal biomass (Kaiser *et al*., 2011). Satellite images show that the highest chlorophyll *a* concentrations are indeed found near the continents (Fig. 1.1). The high primary production in the coastal and estuarine waters is caused by the high input of nutrients from the continents through natural weathering from rocks and soil or from coastal upwelling of deeper nutrient rich waters. In addition, large scale use of (artificial) fertilizers and sewage discharges result in a strongly increased human induced flux of nutrients into the coastal and estuarine waters (Correll *et al*., 1992; Nixon, 1995; Zaytsev *et al*., 2003). Although nutrients are essential for primary production, the man-made input of nutrients has a downside via the formation of harmful algal blooms (HABs). These HABs are found to increase on a global scale and have several adverse effects on the coastal ecosystems, for example: 1) shifts in microalgal community composition via changes in competitive advantage and/or production of allelochemicals which can inhibit the growth of co-occurring microalgal species (Graneli *et al*., 2008); 2) local oxygen depletion of the water; 3) production of natural algal toxins which are harmful for aquatic life as well as humans, or 4) damage to fish or invertebrates by clogging or damaging of their gills (Hallegraeff, 1993). In addition, the increased anthropogenic input of nutrients causes a shift in the nutrient stoichiometry in the receiving waters, favoring certain algal species over others (Justić *et al*., 1995). Previous studies have indeed demonstrated that changes in the microalgal community composition in the Dutch coastal zone can be linked to changes in nutrients over time (Philippart *et al*., 2007; Lie *et al*., 2011; Prins *et al*., 2012). Changes in the microalgal community composition eventually leads to changes in the nutritional value (Brown, 2002), thereby affecting the food quality for higher trophic levels.

Due to the obvious correlation between nutrient composition and changes in microalgal community composition, observed changes in communities are mainly ascribed to changes in nutrients. However, concurrently with the increase in eutrophication, our coastal waters have become polluted with an almost endless number of other anthropogenic contaminants (e.g. Tolosa *et al*., 1996; Monirith *et al*., 2003; Laane *et al*., 2012). Yet, it is currently unknown if
the presence of this cocktail of contaminants also affects microalgal communities.

**Chemical contaminants in coastal and estuarine waters**

Currently, about 40% of the world’s human population live within 100 kilometres of the coast (United Nations), resulting in a high population density close to the coastal sea. As the human population in these areas is expected to further increase in the coming decades (United Nations, 2004), the effects of anthropogenic pressure on the coastal and estuarine ecosystem are of major concern. Our coastal waters are polluted through the input from large rivers, by intense shipping and via atmospheric input and thus receive a mixture of many diffuse contaminants together forming a 'grey veil'. This cocktail consist of a wide range of contaminant like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), metals, pesticides, pharmaceuticals and potentially natural toxins produced by HAB species. The exact chemical composition of this cocktail does not only depend on local sources, but is also constantly changing due to the regulation of existing compounds and the introduction of new products. A well-known example is the ban on tributyltin (TBT) which had a widespread use as an effective biocide in antifouling paints on ship hulls. However, several studies demonstrated the adverse effect of TBT on growth, development, and reproduction of several marine species (Antizar-Ladislao, 2008), resulting in a global ban of this product in 2008 (Thomas, 2001). As a result, alternative antifouling compounds were developed (Nehring, 2001) and at this moment increased concentrations of for example the alternative compound irgarol are found in the Dutch coastal waters (Laane et al., 2012).

Within Europe, member states are obliged to monitor the 45 priority compounds listed under the European Water Framework Directive (WFD, Box 1). Additionally, under the OSPAR convention there is a list of 42 substances or groups of substances for priority actions (OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic), which partly overlap with the priority compounds of the WFD. Yet, this selection of compounds represent only a small selection of all contaminants present in our coastal waters, missing poorly identified (emerging) compounds with unknown effects. As a consequence, when a good chemical status of surface water, defined as
compliance with Environmental Quality Standards (EQS, Box 1), is based on the selected WFD priority compounds only, it remains uncertain if the ecological status is affected by the presence of all contaminants.

**Box 1. Water Framework Directive and Environmental Quality Standards**

In 2000, the European Water Framework Directive (Directive 2000/60/EC) was adopted with the goal to achieve a good ecological and chemical status of inland, transitional and coastal waters by 2015. In this Directive, a list of 33 priority compounds was incorporated for which Environmental Quality Standards (EQS) were added in 2008 (Directive 2008/105/EC). This list of priority compounds was updated recently and expanded to 45 priority compounds and their respective EQS (Directive 2013/39/EU). Two quality standards, intended to protect marine and freshwater ecosystems, are described for inland, other surface waters and/or biota: 1) annual average concentrations (AA-EQS) for the protection against the occurrence of chronic exposure; 2) maximum allowable concentrations (MAC-EQS) to protect against the occurrence of acute effects. These standards are based on toxicity data and assessment or safety factors are implemented based on the quality and quantity of the available toxicity data (Lepper, 2005). Detailed background information of the derivation of the EQS of individual priority compounds is available in the Environmental Quality Standard Data Sheets (EQSD). It is acknowledged that effects of mixtures of chemical contaminants are not incorporated, and the assessment factors are set to protect against these mixture toxicity effects. The goal of the EQS is to protect the entire ecosystem, including microalgae, against negative effects of chemical contaminants.
Impact of contaminants on microalgae

The toxicity of contaminants to microalgae is a result of a complex set of interacting processes. To quantify toxicity, different types of bioassays are used. A traditional and frequently used method is the determination of effects on microalgal population growth (Walsh, 1972; Casotti et al., 2005; Yang et al., 2008) for which several standardized guidelines are available. Another frequently used endpoint is based on photosynthesis as this is the main metabolic function of microalgae. The photosynthetic efficiency of microalgae can be determined with a rapid, non-destructive technique: Pulse Amplitude Modulation (PAM) fluorometry (Box 2). Ramakrishnan et al. (2010) reviewed numerous toxicity studies on microalgae. Their results suggest that adverse

Box 2. Pulse Amplitude Modulation (PAM) fluorometry

Modification in the photosynthetic process or coupled physiological or biochemical processes will lead to changes in the kinetics and yield of the chlorophyll $a$ fluorescence (Suresh Kumar et al., 2014). Hence, interference of the photosynthetic machinery by chemical contaminants can be determined by measuring chlorophyll $a$ fluorescence. PAM fluorometry, measuring this chlorophyll $a$ fluorescence in vivo, was first described in the 1980s (Schreiber, 1986) and has been used in numerous ecotoxicological studies on algae investigating different types of contaminants (Brack and Frank, 1998; Wiegman et al., 2003; Bengtson Nash et al., 2006; Schmitt-Jansen and Altenburger, 2007; Magnusson et al., 2008; Muller et al., 2008; Baumann et al., 2009). Information on the toxicity of a contaminant can be obtained after several minutes to several hours, depending on the type of contaminant. As herbicides often interfere with the photosynthetic apparatus, this technique has frequently been used to determine herbicide toxicity to microalgae (Juneau et al., 2007; Ralph et al., 2007; Suresh Kumar et al., 2014). As carbon is fixed for growth during photosynthesis, effects on the photosynthetic apparatus will ultimately affect microalgal growth and thereby indirectly affecting energy transfer to higher trophic levels (Ralph et al., 2007; Suresh Kumar et al., 2014).
effects of contaminants on microalgae are compound and species specific. In addition, it has been demonstrated that significant mixture effects, due to the presence of other compounds at levels at or below the individual No Observed Effect Concentrations (NOEC), might occur (Silva et al., 2002; Walter et al., 2002). Also the interaction with environmental factors such as light, temperature and nutrient availability (multi-stress) can affect the toxicity towards microalgae (Guasch et al., 2004; Bengtson Nash and Quayle, 2007; Chalifour and Juneau, 2011). Since these environmental factors and the development of microalgal blooms are subjected to seasonal dynamics (Fig. 1.2 top panels), it can be expected that the toxic pressure of the many different contaminants (Fig. 1.2 bottom panel) also varies over the seasons.

Fig. 1.2. Schematic overview of the factors throughout the year influencing contaminant toxicity to microalgae. The three main factors are: 1) environmental factors (Panel 1), such as light (solid line) and nutrients (dashed line); 2) development of microalgal blooms (Panel 2) presence of contaminants (Panel 3).
In contrast, the majority of ecotoxicological studies are performed using single compounds and single species bioassays under controlled laboratory conditions, thereby avoiding interaction with other compounds and various environmental factors.

As a consequence, the environmental relevance of most available toxicity data on the effects of contaminants on microalgae can be disputed. Nevertheless, Echeveste et al. (2010) demonstrated in a field study in the NE Atlantic Ocean that a mixture of organic contaminants at low concentrations affected marine microalgae. Therefore, it can also be expected that the ‘grey veil’ of contaminants in the coastal and estuarine waters might have an adverse effect on microalgal community composition. However, the current lack of knowledge on the composition of chemical contaminants in the Dutch coastal waters, combined with the variability resulting from mixture effects and interaction with multiple environmental factors obscures the risk of these contaminants for microalgal communities.

**Aim and objectives**

The aim of this thesis is to identify the most important phytotoxic contaminants in the Dutch coastal and estuarine waters, and to determine their hazard and risk in the field. Additionally, the level of protection by the current legislation will be determined. To meet this aim, the following three objectives have been 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 the identified contaminants under laboratory and environmentally relevant conditions in single and multi-species experiments.

III. To investigate the suitability of current environmental quality standards to protect marine microalgae.
Study area

The Dutch coastal and estuarine waters belong to the best studied coastal areas based on long term monitoring series of both biological as well as chemical parameters. Moreover, these waters are an interesting study area to determine effect of chemical contamination as they are a pollution hotspot due to the geographical location downstream of large European rivers (Scheldt, Rhine, Meuse, Ems, Fig. 1.3A). Contaminants, including the priority compounds as set by the WFD, in the Dutch surface waters are monitored in freshwater, estuarine and coastal locations as part of a large monitoring program (‘Monitoring Waterstaatkundige Toestand des Lands Milieumeetnet Rijkswateren’, MWTL) of the Dutch Ministry of Infrastructure and Environment. Trends and compliance with the EQS are determined and as depicted in fig. 1.3B, these standard are not always met, resulting in a chemical status ‘bad’ (red areas).

![Fig. 1.3. Main European rivers (Scheldt, Meuse, Rhine and Ems) (3A) and chemical water quality (3B) in The Netherlands. Red: areas with a chemical status ‘bad’. Source:www.rijksoverheid.nl (A); KRW Waterbeheerders 2009.](image)

Toxic effects of chemical contaminants on aquatic organisms have been investigated for several Dutch fresh water systems (Hendriks et al., 1994; Posthuma and Vijver, 2007; Durand et al., 2009) and the contaminants present in these waters may ultimately end up in the coastal and estuarine waters.
Additionally, due to the use of anti-fouling paints on ship hulls, contaminants may also originate from the sea. Over the years, a wide variety of contaminants has been described in the Dutch coastal and estuarine waters (Lamoree et al., 2002; Vethaak et al., 2005; de Voogt and Laane, 2009; Laane et al., 2013) which may potentially affect microalgae in these waters. Although the primary production of the shallow tidal flats in the Wadden Sea is dominated by microphytobenthos, phytoplankton are the main contributors to the primary production in the North Sea (Reid et al., 1990). Hence, this study focused on the effects of contaminants on marine phytoplankton.

**Outline of this thesis**

To determine the phytotoxic compounds in coastal and estuarine waters, a tool for quick identification is required. Although PAM fluorometry is a frequently used technique to assess contaminant toxicity to microalgae, a standardized test protocol is currently lacking. As test conditions are known to influence test results, we discuss the effects of the main factors on the test outcome (Chapter 2). This information is taken into account in all PAM bioassays presented in this thesis, starting with the implementation in an effect-directed analysis (EDA). With this tool, in which a PAM bioassay is combined with chemical analysis, we identify and confirm the contaminants in the Dutch coastal and estuarine waters affecting microalgal photosynthesis (Chapter 3). To determine the hazard of a selection of the key contaminants, the (mixture) toxicity to marine microalgal species is determined using the PAM bioassay (Chapter 4). The actual risk in the field and level of protection by the current legislation is determined by additional toxicity testing in which microalgae are exposed to the maximum field concentration and the maximum allowable concentration (MAC-EQS) of the individual contaminants respectively (Chapter 4). All these toxicity experiments are performed under standardized laboratory conditions, thereby excluding potential environmental multi-stress factors. Since light (PAR) is essential for microalgal photosynthesis, solar radiation might also affect microalgae directly or modify the toxicity of the contaminants in the water, converting it into a multi-stress factor in algal toxicity tests. As irradiance is known to be seasonally variable, the toxicity of selected contaminants to microalgae is determined under different simulated light conditions (Chapter 5). To determine the actual toxicity in the field, effects of one of the identified contaminants is determined for a natural phytoplankton
community under field conditions (Chapter 6). To this purpose, phytoplankton communities are exposed to the maximum field concentration as well as to the MAC-EQS for 72h. Effects on the community composition are determined with microscopy and flowcytometry, while effects on photosynthesis are determined by PAM fluorometry. In the synthesis (Chapter 7), I discuss the identified phytotoxic contaminants in more detail and evaluate the ecological consequences of their presence in coastal and estuarine waters for phytoplankton. Finally, I discuss the effectivity of the current legislation to protect microalgae as well as the implication of the developed approach for monitoring purposes.