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Toxic pressure of herbicides on microalgae in Dutch estuarine and coastal waters

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ABSTRACT

For several decades now, there has been an increase in the sources and types of chemicals in estuarine and coastal waters as a consequence of anthropogenic activities. This has led to considerable concern about the effects of these chemicals on the marine food chain. The fact is that estuarine and coastal waters are the most productive ecosystems with high primary production by microalgae. The toxic pressure of specific phytotoxic chemicals now poses a major threat to these ecosystems.

In a previous study, six herbicides (atrazine, diuron, irgarol, isoproturon, terbutryn and terbutylazine) were identified as the main contaminants affecting photosynthesis in marine microalgae. The purpose of this study is to investigate the toxic pressure of these herbicides in the Dutch estuarine and coastal waters in relation to the effective photosystem II efficiency (ΦPSII) in microalgae. Temporal and spatial variations in the concentrations of these herbicides were analyzed based on monitoring data. Additionally, a field study was carried out in which chemical analysis of water was performed and also a toxicity assessment using the Pulse Amplitude Modulation (PAM) fluorometry assay that measures ΦPSII. The toxic pressure on ΦPSII in microalgae has decreased with 55–82% from 2003 to 2012, with the Western Scheldt estuary showing the highest toxic pressure. By combining toxicity data from the PAM assay with chemical analysis of herbicide concentrations, we have identified diuron and terbutylazine as the main contributors to the toxic pressure on microalgae. Although direct effects are not expected, the toxic pressure is close to the 10% effect level in the PAM assay. A compliance check with the current environmental legislation of the European Union revealed that the quality standards are not sufficient to protect marine microalgae.

1. Introduction

Estuarine and coastal waters are among the most productive ecosystems on the planet (Nichols et al., 2010). Yet, they also suffer from high contaminant loads due to riverine inputs, land run-off and shipping activities (Hylland and Vethaak, 2011). Moreover, it is expected that the number of chemicals in estuarine and coastal waters will further increase in the coming decades due to the growth of the world population, the coinciding industrial and agricultural activities and an increase of domestic wastewater volumes (Laane et al., 2012). Due to their geographical location downstream of four main rivers (Scheldt, Meuse, Rhine and Ems), the Dutch estuarine and coastal waters are polluted with a wide variety of contaminants, including PAHs, xeno-estrogenic compounds and pesticides (De Voogt and Laane, 2009; Lamoree et al., 2002; Vethaak et al., 2005). Posthuma and Vijver (2007) demonstrated that such a complex mixture of contaminants in upstream freshwater systems might cause ecological effects on aquatic ecosystems. When these contaminants ultimately end up in estuarine and coastal waters, ecological effects can be expected.

Estuarine and coastal waters also receive a high nutrient input from the land, resulting in a higher primary production by microalgae compared to off-shore locations. As microalgae form the basis of the marine food chain, exposure of microalgae to the complex mixture of contaminants in these productive estuarine and coastal waters is, therefore, a major concern.

Recently, we have performed an effect-directed analysis (EDA) study to identify the key contaminants that affect microalgae in Dutch estuarine and coastal waters (Booij et al., 2014). The toxicity of the extracts on the effective photosystem II efficiency (ΦPSII) of these algae was determined by exposing the marine microalgae *Dunaliella tertiolecta* to passive sampler extracts of estuarine and coastal waters

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using a Pulse Amplitude Modulation (PAM) fluorometry bioassay. We identified and confirmed six herbicides (atrazine, diuron, irgarol, isoproturon, terbutryn and terbutylazine) as the main contributors to the ΦPSII inhibitory effects on *D. tertiolecta* (Booij et al., 2014). Another study indicated that only a few pesticides (maneb, fentin-acetate, lambda-cyhalothrin, deltamethrin, chlorpyrifos, isoproturon, and monolinuron) of the hundreds of pesticides present in the Dutch aquatic environment are responsible for possible toxicity to aquatic organisms (De Zwart, 2005). Studies to investigate the relative importance of individual pesticides to the overall toxic pressure have been performed for freshwater locations in the rivers Rhine, Meuse and Scheldt. These studies focused on various aquatic organisms, such as fish, algae and daphnia (Harbers et al., 2006; Henning-De Jong et al., 2008). The ecological risk of contaminants to microalgae communities can be severely underestimated when based on the limited amount of data available from databases, which are primarily derived by the conventional bioassay protocols (Chen et al., 2009). With the PAM assay, all compounds in an extract that contribute to the effect are included, whereas with traditional monitoring, only a limited set of contaminants is determined. Moreover, current monitoring programmes based on the Oslo–Paris convention (OSPAR) and the Water Framework Directive (WFD) depend on chemical concentrations, while bioassays to assess the environmental risk of chemicals on e.g. marine microalgae are not included. The WFD is a European Union directive which commits European Union member states to achieve good qualitative and quantitative status of all water bodies, including marine waters up to one nautical mile from shore, by 2015.

Concentrations of individual contaminants are expected to show temporal and spatial variation mainly due to (seasonal) variations in their use and due to (hydrological) processes during transport to coastal areas. It is therefore essential to analyze the variation in concentrations of these contaminants over longer periods of time and at various locations. Concentrations of individual contaminants are not necessarily indicative for a poor water quality as this will also depend on the toxicity and composition of these contaminants. Consequently, a combination of field concentrations and toxicity data is essential to quantify the actual toxic pressure of the herbicides on marine microalgae.

Our study aims to quantify variations in toxic pressure of the identified herbicides for marine microalgae at several locations in the Dutch estuarine and coastal waters of the past 10 years. To this end, we first analyzed the variations in the concentrations of atrazine, diuron, irgarol, isoproturon, terbutryn, and terbutylazine (hereafter referred to as key contaminants) that are reported in the Dutch MWTL monitoring programme (Rijkswaterstaat, 2014). Secondly, we determined the toxic pressure on ΦPSII in microalgae of the contaminants by comparing their actual concentrations to individual effect levels (based on PAM bioassay responses) and expressing the effect as the sum of the individual toxic units (ΣTU). Additionally, the concentrations and toxicity of the key contaminants were evaluated in relation to the current environmental quality standards (EQS). Finally, data from the MWTL monitoring programme were compared to our own results obtained from a field study performed in 2011. The first objective of our study is to determine temporal and spatial trends in concentrations of the key contaminants to provide information on the toxic pressure on marine microalgae in Dutch estuarine and coastal waters. The second objective is to compare current legislation of the key contaminants with EC_{10} levels in the PAM assay to evaluate the suitability of the PAM assay in monitoring programmes as an indicator for toxic pressure on microalgae.

2. Materials and methods

2.1. Monitoring data

Data on total concentrations were obtained from results of the Dutch MWTL monitoring programme of the Dutch Ministry of Infrastructure and the Environment for collecting physical, chemical, biological and morphological measurement data for surface waters in the Netherlands (Ministry of Infrastructure and the Environment, 2014). Samples for monitoring were collected in a bucket during identical tidal conditions to minimize the effect of dilution of fresh water with sea water. The limits of detection in this monitoring programme varied and were dependent on the sampling location and date of sampling. Locations with contaminant concentrations reported as "< reporting threshold" were considered as "not present". If one or more contaminant(s) were not determined at a specific date and location, they were not included in the calculations and consequently toxic pressure was based on the remaining contaminants determined.

In our study, four locations, representing different water bodies, were selected to analyze the temporal variation in concentrations and toxic pressure for the period 2003–2012. Both seasonal as well as long term trends were investigated. The locations include the Ems-Dollard (*Groote Gat Noord*), located in the Ems estuary in the north-eastern part of the Netherlands; Western Scheldt (*Hansweert*), an estuary located in the south-western part of the Netherlands which connects the main harbor of Antwerp (Belgium) to the North Sea; Wadden Sea (*Marsdiep*), located in the most north-western part of the Netherlands; and North Sea (*Noordwijk*), located 2 km off shore of the village of Noordwijk.

In addition, 15 estuarine and 13 coastal water locations were selected from the MWTL monitoring programme to describe spatial differences in the concentrations and the toxic pressure of the key contaminants in the most recent year for which data was available (2012). More information on the locations (e.g. geographical location, type of water body and number of sampling points in 2012) is provided in the Supplementary Information Table S1.

2.2. Toxic pressure

To gain insight in the toxic pressure of the key contaminants on marine microalgae at the reported field concentrations, these concentrations were compared to the effect levels towards microalgae obtained in a laboratory bioassay. To this end, 10% effect levels (EC_{10}) were derived for the key contaminants from previously obtained dose–response curves based on a PAM fluorometry bioassay (Booij et al., 2014; Sjollema et al., 2014). The EC_{10} values, based on the effective photosystem II efficiency (ΦPSII) of the marine microalgae *D. tertiolecta* after 4.5 h of exposure, were 5.8, 0.3, 0.6, 1.4, 0.1 and 0.4 μg/L for atrazine, diuron, irgarol, isoproturon, terbutryn, and terbutylazine, respectively. *D. tertiolecta* was used as a model species, since they are commonly used in ecotoxicity tests on pesticides and have a European distribution. The Toxic Units (TU) of the contaminants were calculated by dividing concentrations of the individual contaminants in the field by their respective EC_{10} values (Eq. (1)).

$$TU = \frac{\text{field concentration}}{EC_{10}} \quad (1)$$

The TU of the mixture of contaminants is expressed as $\Sigma TU\ EC_{10}$, representing the toxic pressure of the contaminants on microalgal photosynthesis (Eq. (2)).

$$\Sigma TU\ EC_{10} = TU_{atrazine} + TU_{diuron} + TU_{irgarol} + TU_{isoproturon} + TU_{terbutryn} + TU_{terbutylazine} \quad (2)$$

If $\Sigma TU\ EC_{10} = 1$, 10% effect is expected in the PAM assay. If $\Sigma TU\ EC_{10}$ is <1, less than 10% effect is expected in the PAM assay, if $\Sigma TU\ EC_{10}$ is >1 more than 10% effect is expected in the PAM assay.

In addition to the toxic unit approach, dose–response curves of the spot water samples were used to calculate the concentration factor (CF) of the extracts by plotting on the x-axis CF versus the response in the PAM assay on the y-axis. From these plots (data not shown) the