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

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A B S T R A C T
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.

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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 contaminant loads due to riverine inputs, land run-off and shipping systems on the planet (Nichols et al., 2010). Yet, they also suffer from high risk of these 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.
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 EC10 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 Em–Dollard (Groote Gat Noord), located in the Em 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 into 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 (EC10) 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 EC10 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 EC10 values (Eq. (1)).

\[
\text{TU} = \frac{\text{field concentration}}{\text{EC}_{10}}
\]  

(1)

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

\[
\Sigma TU \text{EC}_{10} = TU_{\text{atrazine}} + TU_{\text{diuron}} + TU_{\text{irgarol}} + TU_{\text{isoproturon}} + TU_{\text{terbutryn}} + TU_{\text{terbutylazine}}
\]  

(2)

If \(\Sigma TU \text{EC}_{10} = 1\), 10% effect is expected in the PAM assay. If \(\Sigma TU \text{EC}_{10} \leq 1\), less than 10% effect is expected in the PAM assay. If \(\Sigma TU \text{EC}_{10} > 1\) 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...
CF at EC_{10} level was determined (CF EC\textsubscript{10}). Assuming, as demonstrated by Booij et al. (2014), that the 6 key contaminants are responsible for the observed effect, the ΣTU EC\textsubscript{10} in the PAM assay is calculated with Eq. (3).

\[ \text{ΣTU EC}_{10} \text{ PAM assay} = \frac{1}{\text{CF EC}_{10}} \]  

(3)

The ΣTU data were classified in five classes of toxicity: 1) ΣTU (0.10–0.43); 2) ΣTU (0.10–0.04); 3) ΣTU (0.04–0.02); 4) ΣTU (0.01–0.02); and 5) ΣTU (<0.01). These categories indicate that water has to be concentrated (CF) respectively 1) 2.3–10 times; 2) 10–25 times; 3) 25–50 times; 4) 50–100 times; and 5) >100 times to obtain an EC\textsubscript{10} level in the PAM assay. Class 1 indicates a relatively high contribution to the toxic pressure on ΦPSII, class 5 indicates a relatively low contribution to the toxic pressure.

2.3. Field study

Spot water samples of a sampling campaign conducted in the estuarine Western Scheldt in 2011 (Hansweert) (Booij et al., 2013) were collected for the determination of the total concentrations of atrazine, diuron, isoproturon, irgarol, terbutryn, and terbutylazine with LC-MS/MS. To this end, spot water samples were filtered and extracted with hydrophilic–lipophilic balanced (HLB) solid phase cartridges according to Booij et al. (2013). An aliquot of this spot water sample extract (corresponding to 0.4 L water) was evaporated and dissolved in 100 μL of Milli-Q water. The analytes were separated by an Agilent 1200SL HPLC system (Agilent, Amstelveen, The Netherlands) equipped with a Waters (Etten-Leur, The Netherlands) Symmetry C18 (100 × 2.1 mm, 3.5 μm) column. A flow rate of 0.35 mL/min was used. A gradient started at 90% A (100% Milli-Q water + 0.1% formic acid, v/v) and changed linear to 90% B (100% MeOH + 0.1% formic acid, v/v) in 30 min. Ten μL of each extract was injected. The column was kept at 30 °C. An Agilent G6410A quadrupole mass spectrometer (Agilent, Amstelveen, The Netherlands) equipped with an electrospray ionization source was used for quantification of the contaminants. Multiple reaction monitoring (MRM) was performed in positive mode. The optimized settings for the MRM conditions used for the analysis of the contaminants are shown in Table S2 (supporting information).

Concentrations of all key contaminants were observed for diuron, isoproturon, irgarol and terbutryn decreased over the last decades. The highest reduction (82%) in the average concentration of atrazine, diuron, isoproturon, irgarol and terbutryn concentrations only (Fig. 1, right panels). In the period 2003–2006 and 2009–2012 atrazine contributed less than 3.3% to the ΣTU. Since the contribution of atrazine to the ΣTU is low at all four locations, the exclusion of atrazine in the years 2007 and 2008 is not assumed to affect the calculated toxic pressure substantially. However, terbutylazine contributes up to 72% in the period 2003–2006 and 2009–2012. Therefore, the exclusion of terbutylazine in 2007 and 2008 is expected to affect the calculated toxic pressure considerably, resulting in an underestimation of the ΣTU in those years. In 2003, the annual average concentrations of the key contaminants were highest in the estuarine Western Scheldt, and amounted to maximum 15 times higher concentrations compared to the other three locations. Average concentrations at Western Scheldt in 2003 were in the range of 0.003 μg/L for irgarol and terbutryn to 0.022 μg/L for diuron (Supplementary information Table S1). Concentrations at the locations North Sea and Wadden Sea were relatively low compared to the estuarine Western Scheldt and Ems-Dollard, and of the same order of magnitude in 2003, ranging from 0.001 μg/L for irgarol, terbutryn, and terbutylazine to 0.015 μg/L for diuron. The annual average concentrations in the estuarine Ems-Dollard in 2003 varied between 0.001 μg/L for irgarol and 0.039 μg/L for isoproturon. In a period of ten years (2003–2012), the irgarol annual average concentrations increased in the Western Scheldt (9%) and Ems-Dollard (31%), but decreased in the North Sea and Wadden Sea below the limit of detection. In the same period, the terbutylazine annual average concentration increased in the Ems-Dollard (75%), Western Scheldt (40%) and Wadden Sea (21%). The annual average concentrations of atrazine, diuron, isoproturon and terbutryn decreased over the last decades. The highest concentration of all key contaminants was observed for diuron (0.37 μg/L) in the Western Scheldt in 2003. Nevertheless, the concentration of this contaminant has decreased by 83–90% at all four locations over a period of 10 years. In 2012, the concentration terbutylazine increased in the Western Scheldt and Ems-Dollard, with peak concentrations higher than those of diuron. Overall, the Western Scheldt demonstrated to be the most polluted location in 2003 compared to the other three locations and was more recently (2012) still the most polluted site, despite the observed reduction. While compound and location specific fluctuations can be observed over a period of 10 years, concentrations of the key contaminants are currently relatively low compared to EC_{10} levels of the contaminants in the PAM assay. Especially in the Ems-Dollard and the Western Scheldt, seasonal variations were observed.

3. Results

3.1. Temporal variations

Temporal variations in the water concentrations of the 6 key contaminants at the four selected locations in the Dutch estuarine and coastal water during the period 2003–2012 are presented in Fig. 1 (left panels). Atrazine and terbutylazine were not monitored in 2007 and 2008. Consequently, the ΣTU in 2007 and 2008 was based on diuron, isoproturon, irgarol and terbutryn concentrations only (Fig. 1, right panels). In the period 2003–2006 and 2009–2012 atrazine contributed less than 3.3% to the ΣTU. Since the contribution of atrazine to the ΣTU is low at all four locations, the exclusion of atrazine in the years 2007 and 2008 is not assumed to affect the calculated toxic pressure substantially. However, terbutylazine contributes up to 72% in the period 2003–2006 and 2009–2012. Therefore, the exclusion of terbutylazine in 2007 and 2008 is expected to affect the calculated toxic pressure considerably, resulting in an underestimation of the ΣTU in those years. In 2003, the annual average concentrations of the key contaminants were highest in the estuarine Western Scheldt, and amounted to maximum 15 times higher concentrations compared to the other three locations. Average concentrations at Western Scheldt in 2003 were in the range of 0.003 μg/L for irgarol and terbutryn to 0.022 μg/L for diuron (Supplementary information Table S1). Concentrations at the locations North Sea and Wadden Sea were relatively low compared to the estuarine Western Scheldt and Ems-Dollard, and of the same order of magnitude in 2003, ranging from 0.001 μg/L for irgarol, terbutryn, and terbutylazine to 0.015 μg/L for diuron. The annual average concentrations in the estuarine Ems-Dollard in 2003 varied between 0.001 μg/L for irgarol and 0.039 μg/L for isoproturon. In a period of ten years (2003–2012), the irgarol annual average concentrations increased in the Western Scheldt (9%) and Ems-Dollard (31%), but decreased in the North Sea and Wadden Sea below the limit of detection. In the same period, the terbutylazine annual average concentration increased in the Ems-Dollard (75%), Western Scheldt (40%) and Wadden Sea (21%). The annual average concentrations of atrazine, diuron, isoproturon and terbutryn decreased over the last decades. The highest concentration of all key contaminants was observed for diuron (0.37 μg/L) in the Western Scheldt in 2003. Nevertheless, the concentration of this contaminant has decreased by 83–90% at all four locations over a period of 10 years. In 2012, the concentration terbutylazine increased in the Western Scheldt and Ems-Dollard, with peak concentrations higher than those of diuron. Overall, the Western Scheldt demonstrated to be the most polluted location in 2003 compared to the other three locations and was more recently (2012) still the most polluted site, despite the observed reduction. While compound and location specific fluctuations can be observed over a period of 10 years, concentrations of the key contaminants are currently relatively low compared to EC_{10} levels of the contaminants in the PAM assay. Especially in the Ems-Dollard and the Western Scheldt, seasonal variations were observed.

Concentrations from the monitoring study and predicted TU are compared to annual average concentration environmental quality standards (AA-EQS) and maximum allowable concentration environmental quality standards (MAC-EQS) set by the European Commission (Directive 2013/39/EU, 2013). For terbutylazine, the EQS values were not available because this compound was not included in the WFD. Therefore, the maximum allowable risk concentration was used for compliance checking (RIVM, 2014).
panels), compound and location specific fluctuations can be observed over a period of 10 years. Generally, diuron showed the highest contribution to the ΣTU, especially in the Western Scheldt, although contribution of other key contaminants can be observed at the other locations (Fig. 1). In 2012, the highest contributor fluctuated between diuron, isoproturon, terbutryn and terbutylazine.

![Fig. 1. Temporal variation in water concentrations and sum of the toxic units (ΣTU) at four locations in the Dutch estuarine and coastal waters. Atrazine and terbutylazine were not determined in 2007 and 2008 and not included in the ΣTU EC10 in 2007 and 2008. Note that the y-axes have different scales.](image-url)
3.2. Spatial variations

A clear spatial variation in the maximum $\Sigma TU$ at various locations in the Dutch estuarine and coastal waters in 2012 is demonstrated in Fig. 2. The toxic pressure of the key contaminants, expressed as maximum $\Sigma TU$, is highest in the estuarine waters of the Western Scheldt and Ems-Dollard and lowest in the coastal areas and off shore locations. The average, minimum and maximum $\Sigma TU$ for 2 fresh, 15 estuarine and 13 coastal water locations in 2012 are presented in Table S1 (supporting information). The average $\Sigma TU$ in estuarine water varies much more compared to coastal waters. Especially, the Western Scheldt (Hansweert and Terneuzen boei 20) showed a high toxic pressure, with a maximum $\Sigma TU$ of respectively 0.43 and 0.32 (Table S1).

The spatial variation in the contribution of individual contaminants to the maximum TU for various locations in the Dutch estuarine and coastal waters in 2012 is shown in Fig. 3. The maps of diuron, isoproturon, terbutryn, and terbutylazine show several orange and red dots (class 1 or 2) (Fig. 3) indicating that these herbicides are the main contributors to the $\Sigma TU$ in 2012. The contribution of atrazine was marginal, corresponding to class 5. The map of irgarol only shows one location (Lake Veere), which is situated in a brackish lake, in class 2, other locations where irgarol was monitored were in class 4 or 5.

The current Dutch monitoring programme is not consistent in the monitoring of the key contaminants, especially for the fresh water systems. Irgarol was not monitored at any of the fresh water locations. Therefore, a proper comparison between the toxic pressure on $\Phi PSII$ in fresh water and estuarine/coastal water was impossible for the key contaminants.

For example, the river Meuse (Eijsden) at which only 5 key contaminants (atrazine, diuron, isoproturon, terbutryn, and terbutylazine) were monitored, showed a maximum $\Sigma TU$ of 0.21 (Table S1). In the river Rhine (Lobith) only diuron, isoproturon, and terbutylazine were monitored and showed a maximum $\Sigma TU$ of 0.65 (Table S1). The two main rivers (Meuse and Rhine) entering the Netherlands from Belgium and Germany showed a toxic pressure in class 1, which is in the same range as the Western Scheldt and the Ems Dollard. In the river Rhine (Lobith), the toxic pressure on $\Phi PSII$ is comparable to that of the Western Scheldt, where 6 key contaminants were monitored. It is possible that atrazine, isoproturon and irgarol were also present in river water as a consequence of agricultural activities and shipping.
along the river Rhine and Meuse. Therefore, the values of the toxic pressure on ΦPSII presented here for the rivers Rhine (Lobith) and Meuse (Eijsden) may be an underestimation of the actual toxic pressure.

3.3. Field study

The chemical concentrations observed in samples collected in the field study (Western Scheldt) and transformed into ΣTU and the ΣTU determined in the PAM assay are shown in Fig. 4. The ΣTU calculated based on chemical data from the field study correlated well with the ΣTU determined in the field study extracts with the PAM assay. We can conclude that the effect of the PAM assay at this field location could be predicted from chemical measurements. The ΣTU calculated from chemical concentrations of key contaminants determined in the MWTL monitoring programme is higher, however, still in the same order of magnitude. The seasonal variations observed in the field study correspond to those observed in the MWTL samples.

4. Discussion

Diuron and ieharol are used as antifouling agents to prevent the attachment of living organisms to the submerged surfaces of ships (Lamoree et al., 2002) and are known to have a negative impact on the marine microalgae (Buma et al., 2009; Knauert et al., 2008;
leading to significant seasonal peak concentrations in coastal waters (Carafa et al., 2007). In the present study, indeed, a clear seasonal trend in concentrations of these herbicides was observed in the Western Scheldt (Figs. 1). Additionally, in an estuary, fresh water is mixed with sea water due to dispersion processes induced by tidal action. Consequently, contaminant concentrations are expected to vary with the residence time of the river water (Steen et al., 2001). Since samples are taken at low tides, fluctuations in concentration are expected to be related to the fluxes of river water. The present study demonstrated a clear temporal and spatial variation in the total toxic pressure of the key contaminants to microalgae.

In this study, we found that diuron contributed 57 ± 2% (n = 405) to the overall toxic effect calculated for the Western Scheldt, North Sea, Wadden Sea and Ems-Dollard in the period 2003–2012. Although the toxicity of diuron has been well described for individual microagal species (Gatidou and Thomaidis, 2007; Magnusson et al., 2008) as well as for microagal communities (Dorigo et al., 2007; Pesce et al., 2010), it has to be noted that this herbicide is not the most toxic compound of the five key contaminants under investigation, as terbutryn has the lowest EC10. In 2012, the maximum toxic pressure was highest in the Western Scheldt (ΣTU = 0.43). This was probably mainly due to the high contribution of terbutylazine entering the Netherlands from the river Scheldt and the Ghent (Belgium)–Terneuzen (Western Scheldt) Canal.

During the past ten years, the individual concentrations of atrazine, diuron, isoproturon, and terbutryn at the four selected locations were all at least 5 times below the maximum allowable concentration (MAC-EQS) according to the environmental quality standards set by the European Commission (European Commission, Directive, 2013/39/EU) (supporting information Table S4).

The irgarol concentration observed in the Western Scheldt in 2008 was equal to the MAC-EQS in the Western Scheldt (0.016 μg/L) in 2008. However, the annual average concentration of irgarol has been above the allowable annual average concentrations (AA-EQS) (European Commission, Directive 2013/39/EU) at the Western Scheldt during every year in the period 2003–2012 (supporting information Table S3). In the Wadden Sea, North Sea and Ems-Dollard irgarol concentrations were less than or close to the AA-EQS of irgarol. None of the irgarol concentrations at the 4 locations reached an EC10 level of 0.6 μg/L. The concentration of diuron exceeded the AA-EQS (0.2 μg/L) in the Western Scheldt with a concentration of 0.22 μg/L only in 2003. The other four key contaminants (atrazine, isoproturon, terbutryn and terbutylazine) neither exceeded the AA-EQS, nor reached the EC10. In 2012, the concentration terbutylazine exceeded AA-EQS in the river Meuse in Belgium (Lierde), close to the Dutch border (RIWA, 2012). A recent study showed that the Dutch estuarine and coastal waters are still in moderate ecological status according to WFD yardsticks (Solheim et al., 2012). This is confirmed by the present study, as environmental quality standards set by the European Union of the key contaminants, mainly irgarol, are exceeded. Where in the past ten years, irgarol frequently exceeded the AA-EQS, failing to achieve good quality status of the water body, the present study demonstrated that for microalgae, diuron and terbutylazine are compounds of higher concern.

Using the PAM assay, we also experimentally demonstrated in our previous study that the current environmental legislation (MAC-EQS) does not protect algae sufficiently against diuron and isoproturon (Sjollema et al., 2014). The maximum ΣTU EC10 was above 1 in the Western Scheldt in 2003 (Table S6), indicating a predicted effect in the PAM assay above EC10 level. This effect was mainly caused by the high contribution of diuron. For the three other locations, the calculated TU EC10 (Tables S5 and S6) were below 1, indicating that the TU of individual contaminants are below the EC10 level in the PAM assay.

For diuron, the TU EC10 of the MAC-EQS is 5.4, indicating that at the MAC-EQS level (1.8 μg/L) an effect is expected on the PSII in microalgae. Summation of the TU EC10 of individual contaminants at their respective AA-EQS levels would result in a value of 1.4, indicating that if all key contaminants are present at AA-EQS concentrations, a mixture effect above EC10 level is predicted. Summation of the TU EC10 of individual contaminants at their MAC-EQS levels would result in a value of 7.4, indicating that the predicted effect of such a mixture on PSII in microalgae is far above the EC10 level in the PAM assay. Based on these predictions, we can conclude that the current environmental legislation protects algae insufficiently.

Herbicides affecting microalgal photosynthesis, as the ones investigated in the present study, are a worldwide problem. Diuron, atrazine and metolachlor exceeded Australian and New Zealand water quality guideline trigger values, which aim to protect 95% of the species, at eight freshwater sites (Smith et al., 2012). Diuron also contributed...
most significantly to the toxic pressure at various locations across the Great Barrier Reef due to both its concentration and relative potency as a PSII inhibitor (Kennedy et al., 2012). In Swiss surface waters, herbicides and insecticides dominated the risk, exceeding critical concentrations (Moshct et al., 2014). A large scale study was recently performed, based on data derived from 4000 locations in Europe, indicating that chemical pollution in European rivers is still a large-scale environmental problem, despite the legislation over the past decades (Malaj et al., 2014). Malaj et al. (2014) showed that herbicides accounted for most of the exceeded actual risk threshold related to algae.

Currently, water monitoring is based on measuring and reporting chemical concentrations, as requested by the European Union. Our study demonstrated that combining monitoring data with toxicity data provides essential information on the effects of potential harmful contaminants in the field.

Bioanalytical tools such as the PAM assay have not been used for regulatory purposes in the water quality field, basically because there are no effect-based water quality guidelines or trigger values available yet (Escher et al., 2013). By using bioassays like the PAM assay, mixture toxicity effects can be tested. Although the PAM assay measures the effect on microalgal photosynthesis, a good correlation of the PAM response with the effect on algal growth has been described for several herbicides (Buma et al., 2009; Fai et al., 2007; Magnusson et al., 2008), indicating that the PAM assay is an ecologically relevant bioassay.

Recently, an EU-report was published to discuss potential effect-based tools (e.g. biomarker, bioassays) that could be used in the context of the different monitoring programmes (surveillance, operational and investigative) linking the chemical and ecological status assessment of aquatic environments (European Union, 2014). In the context of the WFD, the use of effect-based tools can be foreseen for the elaboration and implementation of monitoring programmes. It is important for water managers to know the identity of the contaminants causing the adverse effect, so that these contaminants can be monitored and traced back to their sources. If water is chemically monitored, bioassays performed on the same sample (or at least taken from the same location and at the same time) could be complementary tools to cover additional substances that are not analyzed chemically (Durand et al., 2009). The present study demonstrates that the implementation of the PAM assay as an additional effect-based tool can improve the monitoring programme to assess the water quality and provide additional information on the ecological status of microalgae.

5. Conclusion

By combining the classical method of compliance checking with toxicity data, we were able to provide valuable information on the toxic pressure of six contaminants (all herbicides) in the field affecting microalgal photosynthesis. The toxic pressure of herbicides on microalgae in the Dutch estuarine and coastal waters appears to be low in recent years, with a highest maximum of 0.43 (based on EC50) in the Western Scheldt Estuary in 2012. Although hundreds of compounds are present in the estuarine and coastal waters, only six herbicides did seem to affect 0PSII in microalgae. Diuron and terbutylazine are currently the highest contributors to the toxic pressure. To determine the effects of potential harmful contaminants in the future, chemical monitoring should be combined with bioassay testing. The PAM assay has shown to provide a useful additional tool and early warning system for assessing the toxic pressure on microalgae in estuarine and coastal waters due to mixtures of contaminants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.seares.2015.05.001.

References


