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Wastewater effects on *Phaeodactylum tricornutum* (Bohlin): Setting up a classification system

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ABSTRACT

Phytoplankton is highly productive in marine coastal ecosystems that generally present high levels of anthropogenic pressures. Microalgae presents very high surface-to-volume ratio and may respond promptly to contaminants showing either an increase in growth or inhibition effects. Thus phytoplankton organisms provide information on the potential impacts of contaminants on the supported marine-coastal food webs. The diatom *Phaeodactylum tricornutum* Bohlin is commonly used in toxicity testing according to the ISO 10253:2006 standardised protocol, but a comprehensive inventory of tested substances has not been compiled yet. The aim of this study is to establish a wastewater effect score based on *P. tricornutum* exposed to domestic, municipal and industrial wastewaters from activated sludge sequencing batch reactor and ultra-filtration membrane biological reactors. Wastewater samples produced stimulation and inhibition effects identified by biostimulation unit (BU_{50}) and toxicity unit (TU_{50}) both at 50% effect, respectively. Within the stimulation scenario, toxicity was low if $0 < BU_{50} \leq 0.31$, medium if $0.31 < BU_{50} \leq 1.05$, high if $1.05 < BU_{50} \leq 1.64$, and very high if $BU_{50} > 1.64$. Within the inhibition scenario, toxicity was low if $0 < TU_{50} \leq 0.07$, medium if $0.07 < TU_{50} \leq 2.67$, high if $2.67 < TU_{50} \leq 5.86$, and very high if $TU_{50} > 5.86$. Results evidenced that nitrogen and phosphorus concentrations were not correlated to ecotoxicological values, probably due to the presence of undetected micronutrients, confirming the importance of toxicity-based hazard assessment.

1. Introduction

One of the objectives of the Water Framework Directive (WFD) (60/2000/EC) is to integrate national and international policies and actions in order to achieve a good environmental (i.e. chemical and ecological) status of European water bodies (freshwater, and transitional and coastal waters within 1 mile) by 2015; local assessment strategies and interventions are strictly linked to this (Andersen et al., 2010). The treatment and monitoring of wastewater point sources has become increasingly important, particularly to prevent excess discharge of toxic substances and nutrients (e.g. nitrogen and phosphorus) in the aquatic systems that can affect the structure and functioning of ecological communities. The Nitrates Directive (91/676/EEC) regulates water quality across Europe by preventing discharge of nitrates from non-point sources and promoting the use of good farming practices. The threshold for the identification of polluted water or at risk of pollution was set at 50 mg $N\text{-}NO_3^-$ L^{-1} for freshwater bodies, estuaries, and coastal and marine waters. Also, the European Marine Strategy Framework Directive (56/2008/EC) required Member States to achieve good environmental status (GES) in marine waters to prevent eutrophication.

According to the European Inventory of Existing Commercial Chemical Substances (2013), point sources can contain more than 100,000 substances and their relative by-products. Often the information about the identity of compounds present in wastewater is limited as well as their possible biological effects such as toxicity, genotoxicity or estrogenic potential (Newman and Unger, 2003). The same occurs for nutrients concentration, which vary in accordance with the density of human activities (e.g. in the catchment basin or along the coast), locations and route of discharge. Nutrient enrichment can lead to nuisance algal blooms, under appropriate temperature and light conditions (Newman and Unger, 2003). The control of discharges has been traditionally carried out using methods that provide non-specific responses and through the measurement of global parameters (dissolved organic content, chemical oxygen demand) providing limited information on biological effects (Farré and Barceló, 2003). The traditional approach,

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based on chemical analyses, adopted for assessing the environmental impact of discharges is therefore not appropriate, and an integrated/extended assessment of wastewater effects is essential to ascertain potential hazard (Munawar et al., 1989). Several authors (Burkhard and Durhan, 1991; Fernández et al., 1995; Tothill and Turner, 1996) recommended combining different standard chemical analyses of target compounds for toxicity assays for identifying the main components of toxic effluents and sludge. A toxicity test can be defined as a biological assay performed to measure the effects of a substance or an environmental matrix (leachate, elutriate, contaminated sediment/soil) on a living organism, providing a qualitative or quantitative output (Whadhia and Thomson, 2007). Toxicity provides an integrated measure of the impact of wastewater combining different factors such as pH, compound solubility and bioavailability as well as the potential for mixture effect (Farré and Barceló, 2003; Libralato et al., 2010a). Due to the impossibility of determining the specific toxicity of each component of a complex effluent, the Whole Effluent Toxicity (WET), using aquatic organisms (bioassay), was proposed as a direct suitable method, relatively inexpensive, for the toxicity determination (USEPA, 2004). Bioassays can also monitor pollutants that are not detected by common analytical approaches (Tothill and Turner, 1996). Whadhia and Thomson (2007) pointed out that the use of biological assays is a holistic approach that allows the assessment of toxicity and the total effect of all components, including possible additive, synergistic and antagonistic effects. Living organisms integrate the positive and negative effects of chemicals with which they come into contact with the environmental conditions they are exposed to during the experiment responding to the biologically active components present (Keddy et al., 1995). Wastewater samples can be tested using any level of biological organisation from the molecular level up to the whole organism, population or community (Calow, 1989). To date, the assessment of toxicity of wastewater has been carried out using different types of assays (e.g. *Daphnia magna* 24 h acute test), but gaps still remain about how to use, interpret and integrate their results. Some toxicity tools already exist for assessing (Toxicity Identification Evaluation and Toxicity Reduction Evaluation) (USEPA, 2004) and ranking of the potential ecotoxicity of wastewater samples such as those proposed by Bulich (1982), Calleja et al. (1986), Ross (1993), Costan et al. (1993), Swedish EPA (1997), Tonkes et al. (1999), Vindimian et al. (1999), Sarakinos et al. (2000), Phillips et al. (2001), Persoone et al. (2003) and Libralato et al. (2010b). At the same time, only few authors made explicit reference to phytotoxicity and phytostimulation dichotomy for environmental quality assessment (Sbrilli et al., 2005). Generally, phytostimulation can adversely affect environmental quality similarly to phytotoxicity. Only few saltwater phytoplanktonic biological models are really widespread and standardised for toxicity testing such as the benthic unicellular diatom *Phaeodactylum tricornutum* Bohlin (Muller et al., 2007). This alga is present in transitional, marine-coastal and marine waters (Kim et al., 2004; Rech et al., 2005; Toepel et al., 2005) and its use in ecotoxicology is standardised within ISO (2006). Several authors (PAN Pesticide Database, 2013) considered *P. tricornutum* as a reference biological model for pure inorganic and organic chemicals with a record of 524 tested substances. *P. tricornutum* was also used to assess emerging contaminants such as engineered nanomaterials (Clément et al., 2013; Morelli et al., 2013) and pharmaceuticals (Claessens et al., 2013), surface and water column samples (Okay et al., 1998; Macova et al., 2010; Moreira-Santos et al., 2006a; Shaw et al., 2009), lignin and tannin (Libralato et al., 2011), whole sediment and other sediment related samples (elutriate, pore water, water from the sediment–water interface) (Puddu et al., 1988; Tolun et al., 2001; Mucha et al., 2003; Moreira-Santos et al., 2006b; Moreno-Garrido et al., 2007a, 2007b; Morelli et al., 2009). Various other complex liquid environmental specimens were also evaluated such as domestic, municipal and industrial wastewaters (Clarkson et al., 1998, 1999; Peters et al., 2002; Moser et al., 2004; Okay et al., 2005). The morphology and genome of *P. tricornutum* are also well known thus it has frequently been used as a model diatom in studies of algal physiology and ecology (Bowler et al., 2008), and of heavy metals transportation and transformation in ocean ecosystems (Morelli et al., 2009; Deng et al., 2013).

This paper evaluates the ability of *P. tricornutum* in discriminating the quality of domestic, municipal and industrial wastewater samples in order to develop and calibrate a species-specific effect score integrating Libralato et al. (2010b, 2010c). The ranking of wastewater samples is essential for identifying potential effect-based hazards for receiving water bodies and for undertaking adequate management actions considering both toxicity and biostimulation events.

2. Material and methods

2.1. Wastewater samples

A total of 93 samples were collected from three small-scale decentralised wastewater treatment plants (WWTPs), located in Venice (Italy) historical centre (Libralato et al., 2009c, 2012). Samples included domestic ($n = 25$), municipal ($n = 59$) and industrial ($n = 9$) wastewaters. WWTP technologies included an Activated Sludge Sequencing Batch Reactor (AS-SBR) and two Ultra-Filtration Membrane Biological Reactors (UF-MBR1 and UF-MBR-2). Details of the WWTPs (AS-SBR, UF-MBR1 and UF-MBR2) can be found in Libralato et al. (2010c). Samples were labelled in accordance to their origin (A = domestic, B = municipal and C = industrial). Few hours after their manual collection, samples were adjusted to the salinity of the receiving water body (34‰) (Libralato et al., 2009a). During sampling, dissolved oxygen (DO), pH and temperature were recorded, and samples were stored in completely filled polyethylene terephthalate containers. Some aliquots were used for physico-chemical investigations and stored at $4 \pm 1\,^{\circ}C$ for a maximum of 36 h, while the remaining samples were used for ecotoxicological analyses and stored at $-18\,^{\circ}C \pm 1\,^{\circ}C$ (OSPAR, 2000, 2005, 2007; Libralato et al., 2009b).

2.2. Physico-chemical analyses

The pH was measured via pHmeter HI 9025 Microcomputer (HANNA Instrument, Beverly, MA, USA), the salinity was checked with a refractometer (Atago, Japan) and the DO by a WTW multiparametric device (Nova Analytics, Weilheim, Germany). The determination of ionic species, chloride (Cl^-), nitrite ($N\text{-}NO_2^-$), nitrate ($N\text{-}NO_3^-$), ammonia ($N\text{-}NH_4^+$), phosphate ($P\text{-}PO_4^{3-}$) and sulphate ($S\text{-}SO_4^{2-}$) was performed using an Ion Chromatograph (IC) system (column Metrohm Metrosep A Supp 5150 – 4 mm, Metrohm 761 Compact IC, Switzerland) according to APHA (1998) methods. Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN), total phosphorus (P_{tot}), raw wastewater Suspended Solids (SS) were analysed according to APHA (1998) methods. The determination of metal and metallic elements such as aluminium (Al), arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), total chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), molybdenum (Sb), selenium (Se), vanadium (V) and zinc (Zn) was carried out according to USEPA (1992) and APHA (1998) methods using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Spectroflame Compact E, Spectro Analytical Instruments, Kleve, Germany). An ICP-OES multi-element standard solution (Merck 10580) was used for calibration and Quality Assurance/Quality Control (QA/QC) procedures. Only UF-MBR2 samples were checked for metal and metallic elements.

2.3. Toxicity test

Toxicity tests with *P. tricornutum* were carried out according to the ISO 10253 method (ISO, 2006) determining the growth inhibition (or biostimulation) of microalgae exposed to increasing dilutions of samples. The algal culture was kept at $20 \pm 2\,^{\circ}C$ and 6000–10,000 lux, obtaining a cellular density of more than 10^6 cells mL^{-1}. The initial algal density in the test was obtained by diluting the algal culture and ranged between 2×10^3 cells mL^{-1} and 10^4 cells mL^{-1}. *P. tricornutum* was exposed to increasing concentrations of samples for 72 ± 2 h at $20 \pm 1\,^{\circ}C$ and 6000–10,000 lux. Negative and positive ($K_2Cr_2O_7$, BDH Prolabo, CAS# 7778-50-9) controls were included in each experiment. Cellular density was evaluated by optical microscopy in bright field (40×) using a Bürker counting chamber. Each experiment included at least a negative control and 6 sample dilutions (5, 10, 30, 50, 70 and 90% wastewater/volume (w/v)) in triplicate. Wastewater samples were diluted with ISO (2006) artificial seawater.

2.4. Data analysis

Toxicity and stimulation effect data normalised on negative controls (Abbott's formula) were determined as percentages of inhibition/stimulation effect inducing a 50% growth inhibition (inhibition concentration, IC_{50}) or 50% growth stimulation (biostimulation concentration, BC_{50}) in the observed population. Data were expressed as percentage of effect at the highest w/v (%) after normalisation to the average effect in the negative controls. Negative values of PE indicated stimulation, whereas positive ones showed toxicity. Negative and positive controls were compared to ISO (2006) threshold for acceptability. Whenever possible, IC_{50} values were transformed in toxicity units ($TU_{50} = 100/IC_{50}$). Negative effect data indicating growth stimulation were treated similarly determining the biostimulation units ($BU_{50} = 100/BC_{50}$). If IC_{50} or BC_{50} could not be determined, TU_{50} or BU_{50} were determined according to TU (or $BU)_{50} = 50/$(percentage of effect (PE)) where PE < 50%.

The hypothesis test was verified using Analysis of Variance (ANOVA) and Tukey's test to check any difference among the groups. When ANOVA revealed significant differences among treatments, post hoc tests were carried on with Dunnett's method testing the pairwise difference between each treatment and the control. Parametric methods were considered for experimental points' estimation. All results are presented as means with the relative standard error using for all statistical analysis the default 5% rejection level. Statistical analyses were performed using Microsoft® Excel 2013/XLSTAT©-Pro (Version 7.2, 2003, Addinsoft, Inc., Brooklyn, NY, USA).

Univariate and multivariate (principal component analysis) analyses were considered to assess physico-chemical and ecotoxicological data in order to check for potential correlations.

3. Results and discussion

3.1. Effects of wastewater specimen

Within 93-wastewater specimen, only 17 samples showed the ability to inhibit algae growth and only one sample (C06) showed no toxicity at all (Supplementary Materials S1 and S2). The remaining 75 samples displayed a stimulation effect ranging between −2 and −102%; this can be explained considering that algae growth does not reach a maximum value presenting the potential for an unlimited carrying capacity of the system. BU_{50} ranged between 0.06 and 2.04 varying two orders of magnitude (S1 and S2). The algae growth inhibition ranged between 3 and 100%, while TU_{50} varied two orders of magnitude between 0.06 and 5.88. Amongst domestic, municipal and industrial wastewater samples presenting algae stimulation, the average effect was of 74% (A, $n = 25$), 40% (B, $n = 47$) and 36% (C, $n = 3$), respectively. Concerning domestic, municipal and industrial wastewater samples showing algae inhibition, the average effect was of 66% (A, $n = 2$), 43% (B, $n = 10$) and 67% (C, $n = 5$), in that order. The most toxic samples belonged to domestic and municipal wastewaters not to the industrial ones (S1 and S2). However, all industrial wastewater samples, except for C06, presented from medium to high levels of algae growth inhibition suggesting that industrial wastewater always represents a potential toxicity-based hazard for the receiving water body. The range of toxicities presented by domestic, municipal and industrial wastewater samples allowed the definition and calibration of *P. tricornutum* effect score for wastewater classification.

3.2. Physical and chemical data

The univariate and multivariate statistical analyses showed no significant correlations between physico-chemical and ecotoxicological data ($p > 0.05$) (Supplementary Materials S3 and S4).

Table 1
Wastewater effect score for BU_{50} ($n = 75$) and TU_{50} ($n = 18$, including the no effect sample C06) from *P. tricornutum*; all data must be assessed after negative control normalisation.

Endpoint	Toxicity Score	Effect	Score
Algae growth stimulation	$BU_{50} > 1.64$	very high	4
	$1.05 < BU_{50} \leq 1.64$	high	3
	$0.31 < BU_{50} \leq 1.05$	medium	2
	$0 < BU_{50} \leq 0.31$	low	1
	BU_{50} or $TU_{50} = 0$	absent	0
Algae growth inhibition	$0 < TU_{50} \leq 0.07$	low	1
	$0.07 < TU_{50} \leq 2.67$	medium	2
	$2.67 < TU_{50} \leq 5.86$	high	3
	$TU_{50} > 5.86$	very high	4

Table 2
Results from the application of *P. tricornutum* effect score; data were plotted in S5; A = domestic, B = municipal and C = industrial wastewater samples – the integer number indicated the progression in sample collection.

Endpoint	Samples	BU_{50} values
Algae growth stimulation	A17	2.04
	A27	2.04
	A31	1.92
	A30	1.90
	A08	1.76
	B59	1.72
	A32	1.70
	A05	1.64
	B36	1.64
	A28	1.60
	B62	1.60
	A01	1.58
	A04	1.56
	B51	1.56
	A26	1.54
	A20	1.50
	B05	1.50
	A09	1.46
	A03	1.44
	A24	1.44
	A11	1.38
	A12	1.38
	A21	1.38
	A19	1.34
	A22	1.34
	B11	1.34
	B56	1.32
	C01	1.32
	B06	1.32
	B33	1.32
	A02	1.26
	A18	1.26
	B24	1.24
	B07	1.18
	B29	1.16
	A14	1.10
	B04	1.10
	B45	1.06
	B48	1.04
	B30	1.04
	B39	0.98
	B27	0.98
	B32	0.96
	B09	0.86
	B13	0.84
	B08	0.82
	B35	0.82
	A13	0.78

Endpoint	Samples	BU_{50} or TU_{50} values
Algae growth stimulation	A29	0.76
	B61	0.76
	C08	0.74
	B10	0.72
	B43	0.66
	B31	0.66
	B41	0.64
	B34	0.62
	B01	0.54
	B44	0.54
	B02	0.52
	B40	0.48
	B17	0.44
	B15	0.38
	B03	0.38
	B19	0.36
	B21	0.36
	B20	0.34
	B60	0.32
	B23	0.32
	B22	0.30
	B38	0.20
	B28	0.18
	C04	0.12
	B26	0.10
	B12	0.06
	B18	0.04
No effect	C06	0.00
Algae growth inhibition	B49	0.06
	B42	0.08
	C05	0.14
	B25	0.34
	B16	0.44
	A10	1.64
	C03	1.86
	C02	1.92
	C09	2.67
	C07	2.75
	B52	2.96
	B50	3.36
	A33	3.70
	B47	5.75
	B53	5.81
	B46	5.85
	B54	5.88

Stimulation effects were not directly related to nitrogen ($N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$) and phosphorus ($P\text{-}PO_4^{3-}$ and total P) concentrations that were present in excess almost in all wastewater samples. Thus nitrogen and phosphorous did not represent a limiting factor for the system carrying capacity. It is likely that undetected stimulating or additive agents were present in wastewater samples like as micronutrients such as silica, calcium, magnesium, potassium, iron, manganese, sulphur, and cobalt potentially stimulating nitrogen fixation (Buesseler et al., 2004). For example, nutrients required in trace amounts to support algal

growth for biofuel production rarely limits algal growth when wastewater is used (Knud-Hansen et al., 1998).

3.3. P. tricornutum *effect score for wastewater classification*

A 5-class effect score was defined ranking stimulation and toxicity data separately, but in a symmetrical way. The system of effect scores was centred on the "no effect" rank as displayed in Table 1 based on a simplification of the method reported in Libralato et al. (2010b) for species-specific scores. Preliminarily, all stimulation/toxicity data were normalised to the average effect on negative controls before their assessment. Then, due to the precautionary principle, it was stated that only when BU_{50} or TU_{50} were equal to zero the sample was deemed as presenting "no effect". Low, medium, high and very high toxicity classes were organised to classify all effects (Table 2 and S5). This approach avoided the use of the minimum significance distance (MSD) for each test-matrix pair easing the preliminary phases of data elaboration. Three levels of effect were determined considering multiples of the 90th-percentile of effects' distribution accounting for stimulation and toxicity separately. In order to reduce to a minimum the expert judgement and considering some previous similar approaches (Burton, 2002; Libralato et al., 2010b), the choice was addressed to the 10th- and 50th-percentile values of effect data providing information on the probability of effects rarely or likely to occur (Leotsinidis and Sazakli, 2008). The 10th-, 50th- and 90th-percentile of stimulation effect data ($n = 75$) corresponded to 0.31, 1.05 and 1.64 BU_{50}, respectively, while the 10th-, 50th- and 90th-percentile of toxicity effect values ($n = 18$, including the "no effect" sample) were 0.07, 2.67 and 5.86 TU_{50}, respectively. Finally, the effect score was summarised in Table 1. An integer score number from 0 (no effect) to 4 (very high effect) and a colour accompanied each class to summarise and represent the observed effect in a simple visual way.

For the stimulation scenario, if $0 < BU_{50} \leq 0.31$ effect is low (1 green), if $0.31 < BU_{50} \leq 1.05$ effect is medium (2, yellow), if $1.05 < BU_{50} \leq 1.64$ effect is high (3, orange), and if $BU_{50} > 1.64$ effect is very high (4, red). When $BU_{50} < 1$, the wastewater sample present a stimulation effect that is < 50% ($BU_{50} = 50/PE$); values of $BU_{50} \geq 1$ ($BU_{50} = 100/BC_{50}$) can be deemed as the dilution factor required to obtain a 50% algae growth stimulation in the treatment. Thus, high and very high stimulation rates occur when it is required to dilute the sample at least 1.05 times to obtain a 50% algae growth stimulation compared to the negative control.

For the toxicity scenario, if $0 < TU_{50} \leq 0.07$ effect is low (1 green), if $0.07 < TU_{50} \leq 2.67$ effect is medium (2, yellow), if $2.67 < TU_{50} \leq 5.86$ effect is high (3, orange), and if $TU_{50} > 5.86$ effect is very high (4, red).

If $TU_{50} < 1$, the wastewater sample presents a toxicity effect that is <50% ($TU_{50} = 50/PE$); values of $TU_{50} \geq 1$ ($TU_{50} = 100/EC_{50}$) can be deemed as the dilution factor required to obtain a 50% algae growth inhibition in the treatment. Thus, high and very high toxicity rates occur when it is required to dilute the sample at least 2.67 times to obtain a 50% algae growth toxicity compared to the negative control.

The assessment of wastewater samples via the effect score (Table 2 and S5) showed 7, 30, 31 and 7 samples with low, medium, high and very high effects, respectively, amongst the 75-specimen inducing algae growth stimulation. Thus 91% of samples presenting a stimulation effect belonged to a rank from medium to very high. The 17 samples showing algae growth inhibition presented 1, 8, 7 and 1 specimen with low, medium, high and very high effects, accordingly. In this case, 94% of samples presenting a toxicity effect belonged to a rank from medium to very high. Only one sample, C06, presented no effect at all compared to the negative control. In general, the main part of wastewater samples (90%) showed from medium to very high stimulation or toxicity effects.

The same results can be discussed in light of Tonkes et al. (1999) and Persoone et al. (2003) toxicity scores for wastewater sample classification, as reported in Libralato et al. (2010b).

The output of Tonkes et al. (1999) classification system identified 3 samples not acutely toxic (i.e. >100%, w/v) and 15 moderately acutely toxic (i.e. 10–100%, w/v). The application of Persoone et al. (2003) ranking identified 5 samples presenting no acute toxicity, 1 sample with slight acute toxicity and 12 samples with acute toxicity. Both scoring systems deemed as not classifiable 75 samples due to their stimulation effect. Excess in algae growth stimulation seems not to be considered by Tonkes et al. (1999) and Persoone et al. (2003) as a clear undesirable phenomenon; this is not the case as shown by algal blooms associated to eutrophication phenomena.

Actually, the suggested scoring system does not have the intent to provide a classification of a wastewater sample by an index of trophic state; in fact the latter is generally focused on nutrients (nitrogen and phosphorus), chlorophyll concentration, on site algal biomass quantification or species diversity distribution, and considering that a complex interplay among factors that influence the trophic state can occur (Dodds et al., 1998; Dodds, 2007). The aim of the suggested scoring system is to classify wastewater samples by the integrated approach offered by toxicity tests mainly focusing on Whole Effluent Assessment (WEA) (OSPAR, 2005), thus including not only toxicity (growth inhibition), but also stimulation effects. The amount of stimulation effect detected at the laboratory scale, and not on a field basis observation, could be an alternative measure on how wastewater influences the trophic state of the receiving water body. This could be interesting for retroactive and predictive risk assessment and for risk management as well. Within the present case study, for example, the analysis of N and P concentrations and effect data did not evidence any significant correlation suggesting that effects can be difficult to forecast just on the basis of traditional parameters. This result highlights the importance of a more integrated and holistic approach in wastewater sample hazard characterisation and assessment that should include not only contamination-based data, but also toxicity-based issues.

4. Conclusions

Marine algal bioassays are viable methods to assess a variety of environmental pollutants as well as complex environmental matrices such as wastewaters. Toxicity tests with *P. tricornutum* showed to be useful not only to detect growth inhibition effects, but also wastewaters inducing biostimulation phenomena. Effect data have been assessed providing effect scores specific for toxicity and stimulation based on their percentile distribution of effect. The *P. tricornutum* toxicity score is an open source tool that showed to improve the ability of discriminating wastewater samples compared to other traditional classification methods, potentially increasing the ability to manage the quality of the receiving water bodies and supporting the (near-)zero-emission approach as well as non-potable water reuse.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2015.06.014

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