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Methodological advances and future directions of microalgal bioassays for evaluation of potential toxicity in environmental samples: A review

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ABSTRACT

Microalgal bioassays are widely applied to evaluate the potential toxicity of various persistent toxic substances in environmental samples due to multiple advantages, including high sensitivity, short test duration, and cost-effectiveness. Microalgal bioassay is gradually developing in method, and the scope of application to environmental samples is also expanding. Here, we reviewed the published literature on microalgal bioassays for environmental assessments, focusing on types of samples, sample preparation methods, and endpoints, and highlighted key scientific advancements. Bibliographic analysis was performed with the keywords 'microalgae' and 'toxicity' or 'bioassay', and 'microalgal toxicity'; 89 research articles were selected and reviewed. Traditionally, most studies implementing microalgal bioassays focused on water samples (44%) with passive samplers (38%). Studies using the direct exposure method (41%) of injecting microalgae into sampled water mainly evaluated toxic effects by growth inhibition (63%). Recently, various automated sampling techniques, in situ bioanalytical methods with multiple endpoints, and targeted and non-targeted chemical analyses have been applied. More research is needed to identify causative toxicants affecting microalgae and to quantify the cause-effect relationships. This study provides the first comprehensive overview of recent advances in microalgal bioassays performed with environmental samples, suggesting future research directions based on current understanding and limitations.

1. Introduction

Coastal environments are threatened by increased levels of toxic substances, including pesticides, pharmaceuticals, and industrial chemicals, originating from anthropogenic activities (Escher et al., 2008a; Lee et al., 2020). Accurately assessing the degree of contamination of persistent toxic substances (PTSs) in environmental samples and their potential toxicological effects is very important for the management of healthy coastal ecosystems. PTSs in seawater and sediments can adversely affect coastal pelagic and benthic organisms and accumulate in the body due to their low biodegradability. There are a very large number of PTSs in environmental samples; thus, there is a limit to evaluating adverse effects on organisms only by target chemical analysis (Brack, 2003; Hong et al., 2016a). Mixture toxic effects (e.g., synergistic or antagonistic effects) between (un)known toxic chemicals in environmental samples may occur, which raises ecotoxicological concerns. In addition, the bioavailability of PTSs and specific mechanisms of biological effects remain poorly understood in target chemical analysis. Thus, in order to accurately evaluate and predict the potential risks posed by PTSs in environmental samples, both chemical-based assessment and effect-based assessment should be performed together (Khim and Hong, 2014).

(Eco)toxicological tests are defined as exposing different trophic levels of organisms (i.e., microalgae, zooplankton, and fish) to environmental mixtures of chemicals to assess biological responses. Aquatic organisms could be directly exposed to PTSs, among which the toxic effects of microalgae are of serious concern (Torres et al., 2008; O'Neill et al., 2019). Since microalgae play an important role as primary producers in the marine ecosystem, the toxic effects of microalgae may disrupt the ecosystem balance. Adverse effects on microalgae could negatively impact higher trophic-level organisms, such as zooplankton and fish (Nestler et al., 2012). Microalgae respond immediately to toxic substances in samples, with their rapid growth rate, high surface-to-volume ratio facilitating short response times, and strong sensitivity (Aruoja, 2011; Bauer et al., 2012; Nestler et al., 2012). Thus, microalgae are considered a useful and crucial indicator to evaluate the

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deterioration of environmental quality. Microalgal bioassays have been used to evaluate the toxic potential of single compounds in many studies, and studies evaluating the effects of complex mixtures are limited. Thus, an improved understanding of the ecotoxicological effects of microalgae caused by PTSs in environmental samples is needed.

Microalgal bioassays have been used for toxicity screening of chemicals for the past 40 years; more recently (since 2002) have been applied to assessing potential toxicity in environmental samples (Fig. 1a). This delay may be due to the difficulty of implementing microalgal bioassays in methodological and analytical aspects associated with the complexity of environmental samples. We collected case studies of applying microalgal bioassays to evaluate the potential toxicity of PTSs in environmental samples and conducted an in-depth review. A particular focus has been set on methodological advances, including test species (Section 2), sample types and sample preparation (Section 3), and toxicological endpoints of microalgal bioassays (Section 4). Studies performed with a combination of chemical analysis and microalgal bioassay are discussed in Section 5. This review provides the development, current status, and knowledge gap of environmental assessment using microalgal bioassays and suggests directions for future research.

2. Literature collection and keyword analysis

Peer-reviewed articles on microalgal bioassays for assessing adverse effects of environmental toxicants were searched with keywords 'microalgae' and 'toxicity' or 'bioassay', and 'microalgal toxicity' using Scopus (https://www.scopus.com/). Scopus offers an extensive collection of scholarly materials from various publishers, regularly updated to provide the latest information and presented in a user-friendly manner (Al-Jabi, 2017; Falagas et al., 2008). A total of 485 articles were found from 1980 to 2021 (Fig. 1a). All of these papers were reviewed in detail one by one. Among them, 89 papers related to environmental assessment using microalgal bioassays were selected, and their methodology for microalgal bioassays was reviewed (Fig. 1a). These papers include both the traditional microalgal bioassay methods most used by

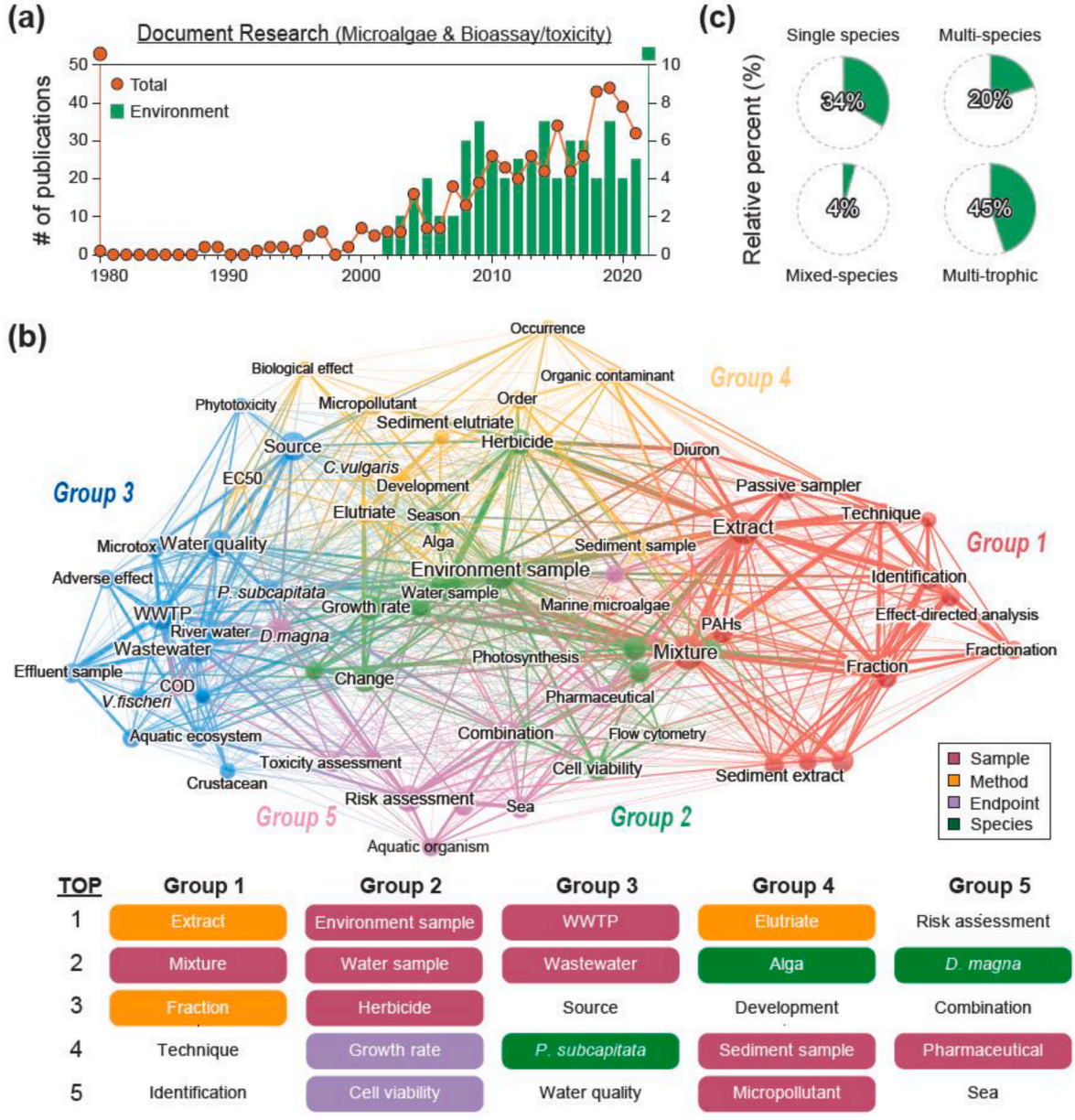

Fig. 1. **(a)** Number of publications on microalgal bioassay over the last 40 years. **(b)** Network analysis of keywords in abstracts and titles from reviewed articles (89 papers). **(c)** Percentage of published methods on species composition used in microalgal bioassays performed for environmental assessments.

researchers in this field and the novel methods recently introduced. The major topics (keywords) and similarities of the reviewed articles were identified through network analysis using VOS viewer software (Van Eck and Waltman, Leiden University, Leiden, The Netherlands; version 1.6.10). This software was developed to provide research trends as concise bibliometric networks graphics by recognizing keywords or frequently mentioned words in titles and abstracts (van Eck and Waltman, 2010). The occurrence of keywords was expressed as a positive correlation with the size of circles (Lee and Khim, 2022) (Fig. 1b). Only keywords that occurred more than four times were used in the final analysis. Out of 3,126 keywords, 72 keywords met the criteria. These keywords were separated into five groups. Based on two standard weighting scores, "Links score" and "Total link strength score" (Stephan et al., 2017), the top five keywords in each group were listed (Fig. 1b).

Most of the top five keywords were related to types of samples (e.g., water, sediment, and wastewater), methods of sample treatment (e.g., extract, elutriate, and fraction), and endpoints (e.g., growth rate and cell viability). *Pseudokirchneriella subcapitata* was the only microalgal species included as a keyword, and the crustacean *Daphnia magna* was also included (Fig. 1b). This result indicated that *P. subcapitata* was the most used microalgal species (36 papers) out of 33 species used in the reviewed articles (Table 1). *P. subcapitata* is also commonly known as *Raphidocelis subcapitata* or *Selenastrum capricornutum*. It is a unicellular alga that has been used as standard species for ecotoxicological tests of freshwater ecosystems by the International Organization for Standardization (8692:2012) guidelines (ISO, 2012) and Organisation for Economic Cooperation and Development (OECD) method 201 (OECD, 2006), due to its high growth rate, sensitivity, and reproducibility (Table 1). The second most used microalga was *Phaeodactylum tricornutum*. *P. tricornutum* is a marine diatom that is used in ecotoxicological tests for coastal ecosystems. This microalgal bioassay is an internationally accepted toxicity test procedure (ISO 10253) (Moreno-Garrido et al., 2007; Zhuravel et al., 2009; ISO (The International Organization for Standardization), 2006) (Table 1). This species is tolerant of wide salinity fluctuations, making it suitable for the evaluation of estuarine environments (Santos et al., 2002).

Microalgal bioassays are traditionally performed using a single standard species (34% of all reviewed papers). In recent years, mixed species have been increasingly used (4%). In other words, various microalgal species are mixed in a single medium and exposed to one sample. This approach reflects natural plankton assemblages with environmental conditions more relevantly (Fig. 1c). The mixed species bioassay is desirable because the natural environment contains diverse populations of microalgae competing for light, nutrients, trace elements, and/or produce substances, which could affect the growth of other species (Sharp et al., 1979; Gross, 2003).

Assessments of multiple species (20%) have increased, whereby the same sample is exposed to various microalgal species separately treated with different mediums (Fig. 1c). Previous studies have shown that the sensitivity to pollutants differs by about one order of magnitude, depending on the microalgal species being tested (Pavlić et al., 2005; Claessens et al., 2013). Cell size is considered a factor that may explain the species-specific toxic effects of various contaminants (Litchman and Klausmeier, 2008). Previous studies have shown that smaller cells have a larger surface-to-volume ratio, which results in higher uptake rates of toxic substances (Ben Othman et al., 2012, 2023; Echeveste et al., 2010). Thus, assessing the state of the environmental health with only one species of toxicity outcome may sometimes not be accurate. Since the toxic response of microalgae depends on the characteristics of pollutants and the sensitivity of test species, it is crucial to select an appropriate bioassay method.

A number of (eco)toxicity studies have assessed the biological effects of different trophic levels with microalgae, accounting for about half (45%) of all reviewed articles. Different trophic-level organisms, including bacteria (decomposer), crustaceans (consumer), and fish (consumer) were frequently used with microalgae (producer) (Fig. 1c). For this reason, *D. magna* was included in the top five keywords in group 5 (Fig. 1b). As a result of a detailed review of the collected literature, the most frequently performed bioassay with microalgal assay was the luminescent bacteria test (LBT). This test is related to the energy metabolism of a bacterium and is used to measure baseline toxicity. The LBT was not included in the top five keywords because the keywords were separated as Microtox (assay name) and *Vibrio fischeri* (species name) (Table 1). In addition, the crustacean *Daphnia* sp. bioassay (DB) and fish embryo test (FET) are widely used together with microalgal bioassays (Hafner et al., 2015; Minguez et al., 2016; Wu et al., 2018). The use of species from different trophic levels in bioassays is important to better understand the potential risk of pollutants in real environments. It is recommended that future studies use a strategy that includes multiple species from different trophic levels inhabiting the same environmental conditions. Overall, the bibliometric network analysis provided a useful starting point for reviewing the published articles regarding the scientific evolution of microalgal bioassays.

3. Exposure of environmental samples to microalgae

3.1. Types of environmental samples

Among the environmental media, 44% of microalgal bioassays were conducted to assess the adverse effects of pollutants in water samples (Fig. 2). Water samples included freshwater, brackish water, and coastal seawater. Studies conducted in freshwater regions, including lakes and rivers, accounted for 51% of all studies on water samples, followed by brackish regions (estuaries and inland creeks) (27%), and coastal regions (22%). Toxicity evaluation of freshwater samples is in high demand compared to seawater; thus, the number of standard test species of freshwater microalgae has been developed more than those of marine species (Bandow et al., 2009a, 2009b; Moreno-Garrido et al., 2007). Water samples are widely used in microalgal bioassays because of the convenience of preparing the culture media with the water sample to be tested. The toxic effects of primary producers in water samples are ecologically significant (Booij et al., 2014). Microalgae respond very sensitively to water pollution and can serve as an early pollution warning indicator (Emelogu et al., 2013).

Next, microalgal bioassays were frequently applied to sediment pollution assessment (27%) (Fig. 2). Sediments are considered the final sink of contaminants in freshwater and coastal environments and reflect a longer period of contamination compared to water samples (Hong et al., 2016a, 2016b). Microalgae do not come into direct contact with bottom sediments, but sedimentary pollutants could be eluted into the water column, and resuspended sediments can be exposed to microalgae (Bandow et al., 2009a; Lee et al., 2020). Thus, it is important to evaluate the ecotoxicological effects of sedimentary pollutants. Thirdly, the environmental medium to which the microalgae bioassay was widely applied was effluent (21%). The treated water that has undergone sewage treatment or wastewater treatment is directly discharged to the nearby aquatic environment, and untreated pollutants can affect the aquatic ecosystem (Bellemare et al., 2006; Cai et al., 2020). Microalgal bioassays have been used to assess the potential risk of (un)known pollutants in effluents (Meriç et al., 2005; O'Neill et al., 2019; Tousova et al., 2018; Vermeirssen et al., 2010).

During the last five years, risk assessments of particle samples in the environment have been performed, with examples including vehicle-emitted particles (VEPs) (Pikula et al., 2019, 2021). Although VEPs and fine dust have received increasing attention, there remains a lack of studies on particles in the aquatic environment (Takahashi and Yokoyama, 2016). Certain types of fine atmospheric particles bring pollutants into aquatic ecosystems via wet and dry depositions and surface runoff (Takahashi and Yokoyama, 2016). Recently, studies on the distribution and behavior of fine atmospheric particles, road dust, and microplastics in the environments have been actively conducted (Pikula et al., 2021), and there are concerns about the potential risks to

Table 1
A summary of microalgal species for environmental pollution assessment.

Species	# [a]	Phylum	Class	Habitat			Characteristic		Standard species	# of publication (Multi-trophic [b])			References
				Fresh	Brackish	Marine	Pelagic	Benthic		LBT (26)	DT (18)	FET (9)	
Raphidocelis subcapitata [c] *(Pseudokirchneriella subcapitata)*	36	Chlorophyta	Chlorophyceae	V			V		V	16	14	5	1–36
Phaeodactylum tricornutum [d]	14	Bacillariophyta	Bacillariophyceae			V	V		V				34, 37–50
Chlorella vulgaris	10	Chlorophyta	Chlorophyceae	V			V			3	1	1	8, 14, 15, 32, 40, 51–55
Dunaliella tertiolecta [c]	8	Chlorophyta	Chlorophyceae			V	V				1		49, 56–62
Isochrysis galbana	6	Haptophyta	Prymnesiophyceae			V	V			4		1	44, 48, 49, 63–65
Scenedesmus vacuolatus	4	Chlorophyta	Chlorodendrophyceae	V				V					66–69
Desmodesmus subspicatus [c]	2	Chlorophyta	Chlorophyceae	V			V			1	1	1	34, 70
Diacronema lutheri	2	Haptophyta	Pavlovophyceae			V	V	V				1	71, 72
Microchloropsis gaditana *(Nannochloropsis gaditana)*	2	Ochrophyta	Eustigmatophyceae			V	V						63, 73
Navicula sp.	2	Bacillariophyta	Bacillariophyceae	V	V	V		V					39, 74, 75
Skeletonema costatum [d]	2	Bacillariophyta	Bacillariophyceae			V		V	V				41, 76
Tetraselmis chuii	2	Chlorophyta	Chlorodendrophyceae			V	V						63, 64
Tetradesmus obliquus *(Scenedesmus obliquus)*	2	Chlorophyta	Chlorophyceae	V			V	V		1			77, 78
Tetraselmis suecica	2	Chlorophyta	Chlorodendrophyceae			V	V						79, 80
Attheya ussuriensis	1	Bacillariophyta	Bacillariophyceae			V		V					81
Chaetoceros muelleri	1	Bacillariophyta	Bacillariophyceae			V	V						81
Chlorella kessleri *(Parachlorella kessleri)*	1	Chlorophyta	Chlorophyceae	V			V						82
Cylindrotheca closterium	1	Bacillariophyta	Bacillariophyceae	V		V		V					83
Entomoneis sp.	1	Ochrophyta	Bacillariophyceae					V					84
Euglena gracilis	1	Euglenozoa	Euglenoidea	V			V						85
Heterosigma akashiwo	1	Ochrophyta	Bacillariophyceae			V	V						86
Lepocinclis acus	1	Euglenozoa	Euglenoidea	V			V						87
Microcystis aeruginosa	1	Cyanobacteria	Cyanophyceae	V		V		V		1	1		88
Monoraphidium arcuatum *(Ankistrodesmus arcuatus)*	1	Chlorophyta	Chlorophyceae	V			V						73
Nephroselmis pyriformis	1	Chlorophyta	Nephroselmidophyceae	V				V					74
Nitzschia closterium	1	Ochrophyta	Bacillariophyceae	V				V					39
Pediastrum duplex	1	Chlorophyta	Chlorophyceae	V			V						73
Porphyridium purpureum	1	Rhodophyta	Porphyridiophyceae	V	V	V	V						86
Scenedesmus acutus	1	Chlorophyta	Chlorophyceae	V			V						87
Skeletonema marinoi	1	Ochrophyta	Bacillariophyceae			V	V						23
Stichococcus bacillaris	1	Chlorophyta	Trebouxiophyceae	V	V	V	V						89
Thalassiosira pseudonana	1	Ochrophyta	Bacillariophyceae	V		V	V						63
Thalassiosira weissflogii	1	Ochrophyta	Bacillariophyceae	V		V	V						41

The following is a list of all references cited in this paper; [1]Latif and Licek, 2004; [2]Moreira-Santos et al., 2004; [3]Emmanuel et al., 2005; [4]Hernando et al., 2005; [5]Escher et al., 2008a; [6]Escher et al., 2008b; [7]Källqvist et al., 2008; [8]Marques et al., 2008; [9]Mendonça et al., 2009; [10]Wang et al., 2009; [11]Köck et al., 2010; [12]Pérez et al., 2010; [13]Vermeirssen et al., 2010; [14]Marques et al., 2011; [15]Moreira-Santos et al., 2011; [16]Pignata et al., 2012; [17]Tang et al., 2013; [18]De Liguoro et al., 2014;[19] Patroncini et al., 2014; [20]Jia et al., 2015; [21]de Castro-Català et al., 2016; [22]Mano and Okamoto, 2016; [23]Minguez et al., 2016; [24]Biruk et al., 2017; [25]Mano et al., 2017; [26]Neale et al., 2017; [27]Tousova et al., 2017; [28]Alvarenga et al., 2018; [29]Lors et al., 2018; [30]Tousova et al., 2018; [31]Wu et al., 2018; [32]Gosset et al., 2019; [33]O'Neill et al., 2019; [34]Riegraf et al., 2019; [35]Berrebaan et al., 2020; [36]Rodrigues et al., 2021; [37]Santos et al., 2002; [38]Moreira et al., 2006; [39]Moreno-Garrido et al., 2007; [40]Muller et al., 2008; [41]Morelli et al., 2009; [42]Zhuravel et al., 2009; [43]Markina and Aizdaicher, 2011; [44]Carballeira et al., 2012; [45]Zhuravel et al., 2012; [46]Everaert et al., 2016; [47]Moeris et al., 2019; [48]Lee et al., 2020; [49]An et al., 2021; [50]Moeris et al., 2021; [51]Bellemare et al., 2006; [52]Leusch et al., 2014; [53]Ma et al., 2016; [54]Ajitha et al., 2019; [55]Pei et al., 2020; [56]Meriç et al., 2005; [57]De Nicola et al., 2007; [58]Morales-Caselles et al., 2008; [59]Prato et al., 2012; [60]Booij et al., 2013; [61]Booij et al., 2014; [62]Booij et al., 2015; [63]Garrido-Pérez et al., 2003; [64]Maranho et al., 2015; [65]Carballeira et al., 2018; [66]Grote et al., 2005; [67]Bandow et al., 2009a; [68]Bandow et al., 2009b; [69]Schwab et al., 2009; [70]Hafner et al., 2015; [71]Emelogu et al., 2013; [72]Emelogu et al., 2014; [73]Stone et al., 2021; [74]Magnusson et al., 2013; [75]Olano et al., 2019; [76]Mariño-Balsa et al., 2003; [77]Yu et al., 2007; [78]Cai et al., 2020; [79]Giacco et al., 2011; [80]Mustajärvi et al., 2017; [81]Pikula et al., 2021; [82]Takahashi and Yokoyama, 2016; [83]Araújo et al., 2010; [84]Adams and Stauber, 2004; [85]Li et al., 2014; [86]Pikula et al., 2019; [87]Bauer et al., 2012; [88]Kwok et al., 2010; [89]Kvíderová and Elster, 2013.

[a] #: Number of publications for test microalgal species.

[b] LBT: Luminescent bacteria test, DT: *Daphnia* bioassay, FET: Fish embryo test. Numbers in parentheses indicate the number of publications.

[c] Standard test species for OECD (2006) and ISO (2012).

[d] Standard test species for ISO (2006).

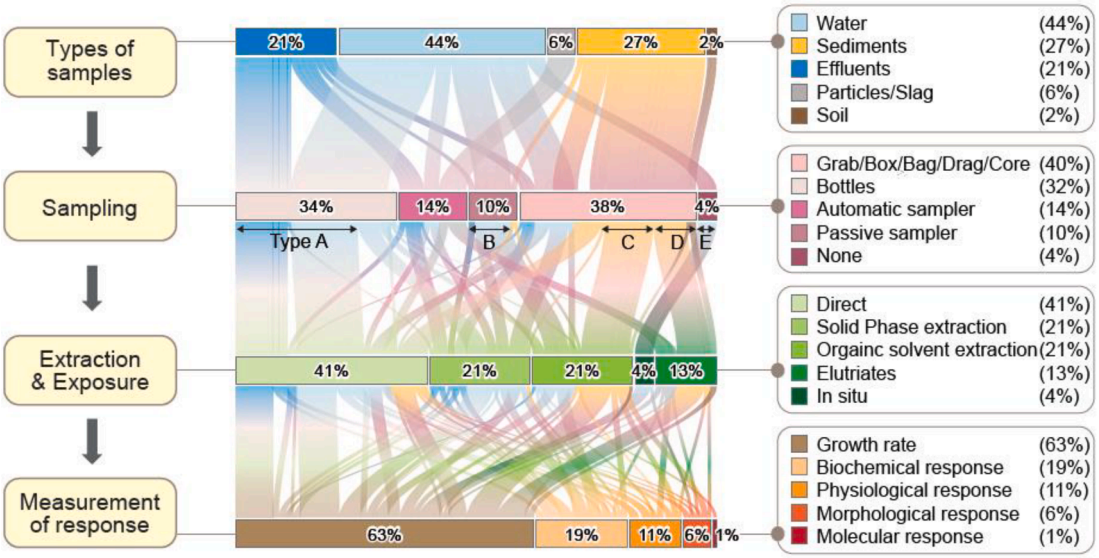

Fig. 2. Sankey diagram of microalgal bioassay methods with the relative percentage of publications.

the aquatic ecosystem. Although microalgae seem to be suitable test species to evaluate their potential risk, standardization of sampling, sample preparation, and exposure methods should be performed first.

3.2. Sample preparation and exposure

In terms of the methodology of microalgal bioassays, it was grouped into five types according to sampling, pretreatment methods, and microalgal exposure methods. Details descriptions of types A to E are shown in Table S1 of the Supplementary Material. The first and most popular type 'A' is the traditional method used for samples in liquid forms, such as freshwater and effluent samples. This method involves directly exposing the sample for testing to the culture medium to measure dose–effect relationships. According to toxicity identification evaluation (TIE) procedures for freshwater samples developed by US Environmental Protection Agency (USEPA), serial dilution with five concentrations and three replicates per concentration of samples is recommended for initial toxicity tests (USEPA, 1996). Along with the type 'A' method, even samples with different types of sampling methods with no pretreatment (termed direct method) accounted for 41% of all reviewed papers (Fig. 2). The advantage of this approach might reflect the potential risks of interactions that occur in complex mixtures in the environment (Marques et al., 2011). However, this method has a limitation in that it is difficult to identify the causative microalgal toxic substances in complex mixtures.

Direct exposure of sediment samples is referred to as a whole-sediment toxicity test, with this approach being less frequently adopted for microalgal bioassays (Adams and Stauber, 2004; Moreno-Garrido et al., 2007; Araújo et al., 2010; Giacco et al., 2011; Pei et al., 2020). This is because the presence of sediment particles makes it difficult to distinguish and count microalgal cells, and the photosynthesis of algae is blocked (Adams and Stauber, 2004). To compensate for this, Zhang et al. (2012) developed a whole-sediment toxicity test using immobilized microalgae. However, the biomass of immobilized microalgae in the control group only increased five to six times after 72-h incubation and did not reach the minimum 16-fold increase recommended by the OECD guideline (OECD, 2006). During the last five years, a whole-sediment toxicity test was developed using optimized sediment immobilization and the free microalgae *Chlorella vulgaris*. This test could overcome the current limitations of immobilized microalgal beads (Pei et al., 2020).

Except for type 'A', B to D types of approaches include sample pretreatments, such as extraction, concentration, and clean-up. The processes of "extraction and exposure" were interpreted together in Fig. 2, because they were closely related and connected. The second type 'B' method for liquid samples includes an approach that uses passive samplers for water sampling as an alternative to grab sampling, followed by extraction. Passive sampling is conducted using silicone rubber sheets (Booij et al., 2013, 2014, 2015; Emelogu et al., 2013, 2014), polar organic integrative samplers (POCIS) (Muller et al., 2008; Vermeirssen et al., 2010; Booij et al., 2013, 2014), and chemcatcher passive samplers, which use Empore™ styrenedivinylbenzene (SDB)-RPS disks (Riegraf et al., 2019) and Speedisk™ (Moeris et al., 2019). Passive sampling is not limited to constant water quality and allows the concentration of trace and contaminant mixtures over extended periods because it can concentrate large volumes of water (Booij et al., 2013). Exposing species of concern to this method provides a valuable tool to improve the relevance of test results for predicting real effects (Moeris et al., 2021). However, passive samplers have some limitations; for instance, environmental conditions affect analyte uptake, and difficulties exist with the in situ calibrations of sampling rates (Emelogu et al., 2013). For the extraction process, solid phase extraction with C18 or HLB cartridges and extraction of organic solvents with acetone, n-hexane, methanol, or acetonitrile, were mainly used. This approach is used to extract organic pollutants from water (Booij et al., 2013). Compounds with different polarities and chemical properties in the environment and the components of extracted compounds tend to vary depending on the method of extraction. For example, the backbone of Oasis HLB sorbent consists of non-polar moieties (benzyl groups, aliphatic chains) and polar groups (pyrrolidone) that adsorb both polar and non-polar compounds from aqueous media, while the resin ENV + and octadecylsilane (C18) were used for extracting mid-polar to non-polar compounds.

Types 'C' and 'D' methods were classified based on the method of extracting the sediment and soil samples collected using grab, box core, and drag sampling. Extraction using organic solvents corresponds to type 'C' (Grote et al., 2005; Källqvist et al., 2008; Bandow et al., 2009a, 2009b; Schwab et al., 2009; Hafner et al., 2015; Maranho et al., 2015; Biruk et al., 2017; Lee et al., 2020; An et al., 2021). The use of elutriation corresponds to type 'D' (Mariño-Balsa et al., 2003; Marques et al., 2008, 2011; Morales-Caselles et al., 2008; Morelli et al., 2009; Wang et al., 2009; Giacco et al., 2011; Prato et al., 2011; Maranho et al., 2015; Biruk et al., 2017; Alvarenga et al., 2018). Various organic solvents are used, including hexane, dichloromethane, and acetone, along with mixed solvents in Soxhlet extraction systems and/or accelerated solvent extraction (ASE). Type 'C' could not be used to determine the

bioaccessibility and/or bioavailability of organic chemicals in environmental media; thus, this approach is limited in assessing the ecotoxicological effects in the real environment. To overcome this issue, methods for extraction that assess the rapidly desorbed fraction were developed to improve estimates of bioaccessibility, such as TENAX (60–80 mesh, Alltech International) (Schwab et al., 2009). The type 'D' approach improves estimates of bioaccessibility and assesses the potential of contaminants being transferred from sediment to the adjacent water column (Maranho et al., 2015). It is very important to select an appropriate extraction method based on the physicochemical properties of the anticipated contaminants.

Type 'E' represents 'in situ' bioassays performed without sampling or laboratory manipulation (Santos et al., 2002). Fewer studies have used in situ microalgal bioassays compared to laboratory assays (only 5% of reviewed articles) (Santos et al., 2002; Moreira-Santos et al., 2004, 2011; Moreira et al., 2006; Bauer et al., 2012). The limited number of in situ microalgal bioassays is probably due to the inherent difficulties of exposing algal cells to changing environmental conditions (Santos et al., 2002). Traditionally, for in situ bioassays of microalgae, cultured or natural microalgae were exposed in the form of confinement in dialysis bags/chambers; however, approaches to immobilize algal cells in an alginate gel matrix were subsequently developed (Munawar and Munawar, 1987). These bioassays can be easily exposed to a variety of natural environmental conditions for final recovery without risking removal by water movement, predation, sedimentation, or biological contamination (Twist et al., 1997). In situ assays provide more realistic environmental conditions than laboratory assays using natural fluctuations, such as ultraviolet light, suspended solids, high flow, and multiple stressor interactions. Furthermore, artifacts associated with the sampling and pretreatment process are minimized (Crane et al., 2007; Moreira-Santos et al., 2011). However, in situ assays has disadvantages in that it is difficult to establish the relationship between exposure and effect, as well as influencing variables, such as organism transportation and caging and environmental conditions (Culp et al., 2000). To more accurately evaluate ecotoxicological effects, in situ bioassays should ideally be used as part of integrated toxicity assessments, in combination

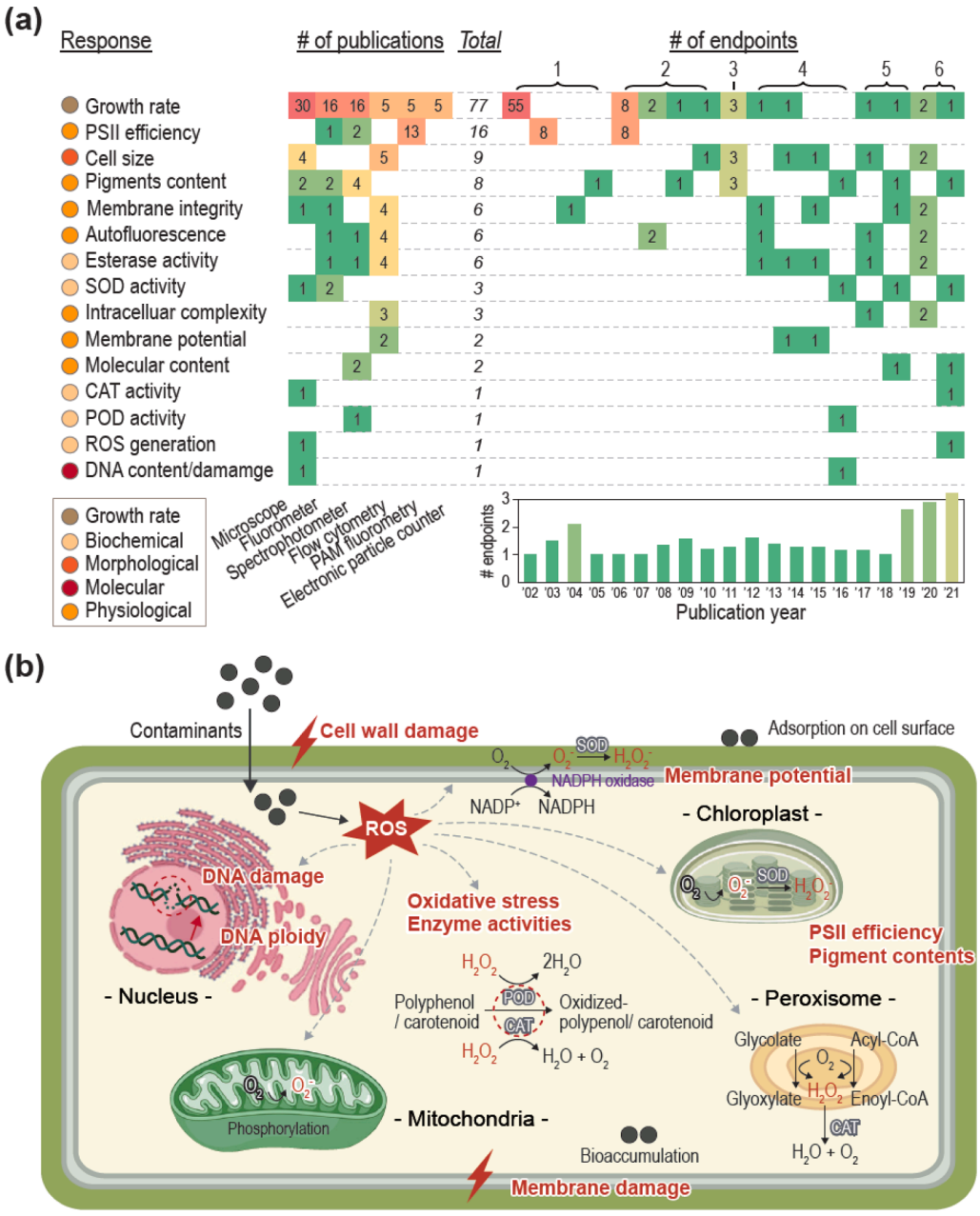

Fig. 3. (a) Number of publications on microalgal bioassays with respect to endpoints and measurement instruments. The number of endpoints refers to the number of endpoints evaluated simultaneously in each study. **(b)** Schematic of the environmental effects of predicted contaminants on microalgal species with several endpoints, including total pigment content, photosynthetic efficiency, reactive oxygen species (ROS) production, and antioxidant enzymatic activity (i.e., superoxide dismutase [SOD], peroxidase [POD] and catalase [CAT]). Created with BioRender.com.

with other parameters, such as physicochemical parameters, concentrations of chemicals, and laboratory assays.

4. Advances in microalgal response evaluation

4.1. Growth inhibition

Growth inhibition is the only standardized endpoint in the international guidelines for toxicity testing methods with freshwater and marine organisms (Emelogu et al., 2014) (Fig. 2, Fig. 3a). Although growth inhibition is a physiological response, it was isolated in this study because it is the endpoint used in most of the reviewed articles (63% of articles; $n = 78$). Microalgal growth inhibitions are sensitive indicators against environmental stress, and 55 studies used growth inhibition as the sole endpoint (Fig. 3a). Out of 24 papers that measured multiple endpoints, only two did not measure growth inhibition (Li et al., 2014; Pikula et al., 2019) (Fig. 3a). In general, growth inhibition is calculated by measuring the difference in cell growth rate between the control group, which is not exposed to the sample, and the experimental group, which is exposed to the sample. One case study used a method to count the number of cells with chlorophyll *a* (Chl *a*) autofluorescence (live cells) and exclude dead cells (Pikula et al., 2019).

An optical microscope has been most commonly used to count cells to measure cell growth rate since 1958 (Di Caprio, 2020). Recently, various types of microscopes, including fluorescence (Mustajärvi et al., 2017; Cai et al., 2020), electron, and confocal (Ajitha et al., 2019) microscopes have been developed and used to measure cell abundances and cell viability (Cerezo et al., 2017). Errors may occur in the process of performing sampling and cell counting if the researcher is not sufficiently skilled (Di Caprio, 2020). This error range is known to decrease as the number of counted cells increases; thus, it is recommended that at least 100 cells are counted (Guillard and Sieracki, 2005). To compensate for this limitation, several studies have used an electronic particle counter to count cells (Grote et al., 2005; Meriç et al., 2005; Everaert et al., 2016; Moeris et al., 2019, 2021). In addition to directly counting cells, cell density is also indirectly measured using Chl *a* concentration, a photosynthetic pigment in microalgae (Lichtenthaler, 1987). Chl *a* content is measured using fluorometers, spectrophotometers, and Pulse Amplitude Modulation (PAM) fluorometry. This approach is widely used, as it is fast and simple. However, when exposed to environmental pollutants or harmful substances, even if cells do not die, Chl *a* concentration decreases; thus, caution is needed when using it as a substitute for cell counts (Zhuravel et al., 2009; Markina and Aizdaicher, 2011).

4.2. Biochemical responses

Aside from growth inhibition, other endpoints were used in the previous studies in the order of biochemical (19%), physiological (11%), morphological (6%), and molecular responses (1%) (Fig. 3a). Biochemical responses included the generation of reactive oxygen species (ROS) and enzymatic activities, such as esterase, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Fig. 3a). Esterase activity is reported to be a sensitive and rapid response to assess microalgae metabolic activity (Jochem, 2000). Fluorescein diacetate (FDA) staining was used in all six studies that measured esterase activity (Adams and Stauber, 2004; Gosset et al., 2019; Pikula et al., 2019, 2021; Lee et al., 2020; An et al., 2021). FDA is a non-fluorescent lipophilic dye that diffuses across the cell membrane to the cell and is then degraded to fluorescing fluorescein by esterase. Due to the high polarity of fluorescein, it accumulates inside the cell (Seoane et al., 2014). Thus, esterase activity is a sensitive endpoint of sublethal toxicity of microalgae. It is measured based on the fluorescent intensity signal emitted from cells, with esterase activity and fluorescent intensity being positively correlated (Yu et al., 2007; Pikula et al., 2019).

ROS include hydrogen peroxide (H_2O_2) and superoxide (O_2^-), which are generated in the metabolic process of cells from mitochondria, chloroplasts, peroxisome, and cell membranes (Ugya et al., 2020) (Fig. 3b). ROS are produced in healthy cells, with low concentrations of ROS contributing to signaling pathways (Rezayian et al., 2019). However, large amounts of ROS, generated by the toxic effects of heavy metals and organic contaminants, cause various metabolic dysfunctions, such as peroxidation when they target the lipids and proteins of the algal cell membrane, and damage to DNA (Esperanza et al., 2015; Rezayian et al., 2019). Chen and Jiang (2011) showed that when the alga *Dunaliella salina* was exposed to adverse environmental conditions, the first site attacked by ROS was the cell membrane. Peroxidation of lipid membranes destroys the structure and function of cell membranes (Kramárová et al., 2012). Furthermore, the activity of antioxidant enzymes (such as glutathione, SOD, CAT, and POD) that neutralize the toxicity of ROS is further inhibited, leading to antioxidants in the cell becoming imbalanced (Ajitha et al., 2019). Thus, changes to these enzymes can be used as sensitive and functional biomarkers of exposure and adaptive responses to contaminants (Li et al., 2014). Ajitha et al. (2019) measured ROS production and antioxidant enzymatic activity (including CAT and SOD) in *Chlorella vulgaris* to assess the toxicity of metals found in discharged effluent from the electroplating industry. Although the study did not determine which enzyme activities occur rapidly, SOD was selected as a prominent enzymatic biomarker of oxidative stress.

Previous studies have measured SOD activity as an endpoint indicative of lipid peroxidation in microalgal bioassays (Li et al., 2014; Ajitha et al., 2019; Cai et al., 2020). SOD acts as a catalyst for converting superoxide to hydrogen peroxide and oxygen at relatively low toxicities, with CAT and POD being removed by accelerating the conversion of hydrogen peroxide to water and molecular oxygen (Fig. 3b). Thus, among the various enzymes involved in removing ROS, SOD is an early indicator of oxidative stress as it is increased by environmental contaminants. Among the various endpoints of *Scenedesmus obliquus* (including SOD activity), Cai et al. (2020) showed that the general order of sensitivity of toxicity endpoints is SOD activity > Chl *a* synthesis > algal growth. It seems to appear in this order because the membrane of chloroplasts is destroyed by peroxidation, which inhibits chlorophyll synthesis and reduces photosynthesis (Bährs and Steinberg, 2012). In addition, glutathione which is involved in ROS reduction, and malondialdehyde as a product of lipid peroxidation, are two typical biochemical markers of oxidative stress of microalgae. Studies evaluating exposure to single compounds, such as PAHs, have been previously conducted (Lei et al., 2006; Wang and Zheng, 2008), but none have used this approach for environmental risk assessment.

4.3. Physiological response

Endpoints for physiological responses include photosystem II (PSII) efficiency, Chl *a* content, autofluorescence (Chl *a* fluorescence), intracellular complexity, membrane potential, and molecular (protein) content. Photosynthesis is a key function of microalgae, and Chl *a* is the primary pigment in algal cells for collecting solar energy for photosynthesis (Van Baalen and O'Donnell, 1978). Because Chl *a* reduction is the result of oxidative stress (Clijsters and Van Assche, 1985; di Toppi et al., 2004), Chl *a* content represents a typical indicator of algal toxicity. Various metals interfere with overall physiological processes and have notable effects on Chl *a* and *b* (Clijsters and Van Assche, 1985). Zhuravel et al. (2009) recorded significant negative correlations between the concentrations of organic pollutants and Chl *a* content in the microalga *P. tricornutum*.

The efficiency of PSII is measured by changes to fluorescence yield induced by PSII inhibitors and is directly related to the growth rate and biomass of microalgae (Magnusson et al., 2008). PSII inhibitors, such as herbicides (Escher et al., 2008a) and insecticides (Hamers et al., 2000), act by inhibiting energy transfer by competing with plastoquinone at the quinone-B binding site of the D1 protein in the PSII reaction center

(Oettmeier, 1992). Consequently, some of the light energy absorbed in PSII cannot be used to drive electron transport and is dissipated as heat or chlorophyll fluorescence (Schreiber, 1986; Genty et al., 1989). Out of the 16 reviewed papers that detected inhibition of PSII, thirteen used the Imaging-PAM assay (Escher et al., 2008a, 2008b; Muller et al., 2008; Vermeirssen et al., 2010; Booij et al., 2013, 2014, 2015; Magnusson et al., 2013; Tang et al., 2013; Leusch et al., 2014; Jia et al., 2015; Neale et al., 2017; Riegraf et al., 2019), and the other three used other approaches (Garrido-Pérez et al., 2003; Bellemare et al., 2006; Kvíderová and Elster, 2013). The PAM assay is a sensitive and rapid high-throughput screening method used to measure the inhibition of photosynthesis in various microalgal species, including coral reef and seagrass, but mainly microalgae (Juneau et al., 2002; Ralph et al., 2007). Changes to PAM fluorescence signaling reflect the physiological status of species; thus, this method provides a consistent indicator of various environmental stressors. For example, Booij et al. (2014) exhibited that herbicides (e.g., irgarol, diuron, terbuthylazine, etc.) induced a negative effect on the PSII efficiency of *D. tertiolecta*.

Two other endpoints of physiological responses related to the viability of microalgae include the integrity and potential of membranes. Both endpoints are measured by staining with dye. Membrane integrity (or permeability) is measured using SYTOX Green (Gosset et al., 2019), SYTOX Blue (Lee et al., 2020; An et al., 2021), propidium iodide (PI) (Pikula et al., 2019; Cai et al., 2020; Lee et al., 2020; An et al., 2021), or TO-PRO-1 iodide (Mustajärvi et al., 2017) dyes. These dyes can only diffuse through the damaged (no longer intact) membranes of living cells and are incorporated in nucleic acids (Pikula et al., 2019). Since the dye increases the fluorescence intensity of cells 20–30 times, a higher fluorescence intensity reflects the inhibition of cell viability (Suzuki et al., 1997; Gorokhova et al., 2012). Two studies measured the membrane potential of microalgal cells using a lipophilic, positively charged fluorescent dye 3,3′-dihexyloxacarbocyanine iodide (DIOC6(3)) (Pikula et al., 2019; Pikula et al., 2021). This dye binds to membranes (mitochondria and endoplasmic reticulum) and other hydrophobic negatively charged cellular components (Sabnis et al., 1997). After staining, the condition of microalgal cells can be observed using a fluorescence microscope (Cai et al., 2020), a fluorometer (Gosset et al., 2019), or a flow cytometry.

Flow cytometry is a high-throughput technique that allows automated single-cell counting and simultaneous multiparametric analysis of different cellular properties. Examples include the auto-fluorescence of cell size and pigments (Marie et al., 2000), DNA content and cell cycle (Marie et al., 1997), and metabolic changes in cells based on appropriate staining (Shapiro, 2005). Staining with two or more fluorescent dyes facilitates the measurement of multiple cellular characteristics simultaneously. Five out of nine studies that measured four or more endpoints simultaneously used flow cytometry (Adams and Stauber, 2004; Pikula et al., 2019, 2021; Lee et al., 2020; An et al., 2021). One of the main drawbacks is given by aggregation of microalgae and cell damage during the pretreatment (Di Caprio, 2020).

4.4. Other responses

DNA damage as a reaction at the molecular level represents another endpoint. There is limited information on the environmental contaminants that cause genotoxicity or DNA damage of microalgae. Li et al. (2014) assessed genotoxicity by measuring fragmentation during the alkaline unwinding of DNA by comet assays. The comet assay has been used in several studies to measure DNA damage in two microalgal species, such as *Chlamydomonas reinhadtii* (Erbes et al., 1997) and *Euglena gracilis* (Watanabe and Suzuki, 2002; Aoyama et al., 2003).

The morphological response of microalgae has also been used as an endpoint. The cell size of microalgae can be estimated differently depending on the strain, culture conditions, and nutrient status, even in the same species (Ben Othman et al., 2023). Thus, rather than absolute values, the effect of contamination is evaluated by comparing the size of samples before and after exposure (Lee et al., 2020; An et al., 2021). Cell size is measured by both flow cytometry and microscopy (Morelli et al., 2009; Bauer et al., 2012; Zhuravel et al., 2012). de Filippis and Pallaghy (1994) showed that the volume of microalgae continuously exposed to high concentrations of metals increased because the export of certain photo-assimilation products to the medium declined. They also showed that the cell size enlarged after metal exposure because the growth-induced volume increase of the microalgal cells was not accompanied by cell division. In the case of normal healthy cells, increased cell size is evaluated as an insensitive endpoint because size is controlled by cell division and/or apoptosis (Savage-Dunn, 2008).

4.5. Multiple endpoints

Traditionally, microalgal bioassays have been performed on environmental samples by evaluating growth inhibition as a single endpoint. The average number of endpoints used in the previous studies conducted from 2002 to 2018 was approximately 1.3, with this number only being higher in 2004 (2.1 endpoints) because Adams and Stauber (2004) assessed five endpoints using flow cytometry (Fig. 3a). This average number of endpoints increased after 2019 to over three endpoints in 2021. Recent advances in various fluorescent viability probe staining technologies that detect changes to the metabolism of microalgal cells using flow cytometry or fluorescence microscopy are opening up opportunities for the simultaneous evaluation of the functional capacity of cells (Gorokhova et al., 2012). When microalgae are exposed to environmental pollutants, such as metals and organic toxic substances, oxidative stress responses and inhibition of photosynthesis generally appear. It is very important to measure the cell viability and growth rate of microalgae together and to interpret the responses in an integrated manner. If cell viability is not considered, false positive and false negative responses (overestimation/underestimation) might occur when assessing the risk of environmental samples (Lee et al., 2020). Thus, multiple endpoints (at least two, ideally) are required to prevent mischaracterizing the potential microalgal toxicity. Out of the 89 articles reviewed here, only 11 mentioned assessing cell viability, and none reported the criteria used to determine the acceptability of bioassay data regarding cell viability. It is difficult to establish a standard criterion because each species has different characteristics. In future studies, it is necessary to establish a standard for cell viability representing cell death.

Several studies analyzing the microalgal toxicity of a single compound (organic contaminants or metals) use multi-endpoints (Koppel et al., 2017; Seoane et al., 2017; Esperanza et al., 2019). For example, several endpoints, including growth inhibition, Chl *a* fluorescence, membrane integrity, esterase activity, cellular metabolic activity, oxidative stress, and cytoplasmic membrane- and mitochondrial membrane-potential, were measured for *Chlamydomonas reinhardtii* after exposure to benzophenone-3 and benzophenone-4 (Esperanza et al., 2019). Growth rate, vital population and metabolic activity (esterase activity), and intracellular pH decreased significantly, whereas cytoplasmic membrane potential and ROS levels increased significantly in cultures exposed to benzophenone-3 and benzophenone-4 (Esperanza et al., 2019). Koppel et al. (2017) showed that five metals (Cu, Pb, Zn, Cd, and Ni) affected the autofluorescence, cell complexity, size, and lipid concentrations of *Cryothecomonas armigera*, but did not alter cell membrane permeability. These results demonstrate the importance of measuring multiple endpoints because the toxic effects of chemical mixtures on microalgae in the environments do not appear in just one aspect but appear simultaneously as complex effects that are organically linked in cells. In addition to multiple endpoints, multiple species should be measured because the responses induced by contaminants have species-specific and toxicity endpoint-specific differences (Nam et al., 2018; Lee et al., 2020).

Future research should focus more on molecular responses, such as genomics, transcriptomics, and proteomics. For instance, Nagarajan

et al. (2022) showed that differentially expressed genes and proteins exhibited coordinated cellular responses by altering gene expression. However, more studies are needed to understand the relationship between genes and proteins. For example, the omics approach for molecular response assessments is expected to provide valuable insights into algal physiology and metabolism during exposure to environmental samples. Studies are also required that reflect various environmental factors (e.g., ultraviolet [UV] radiation, light and nutrient availability, infections) and physiological processes (e.g., collapsing blooms and other mass-death events) that exist under natural conditions.

5. Major environmental toxic substances in microalgal responses

Out of the reviewed articles, 88% conducted chemical analyses to quantify potential toxicants in environmental samples (Fig. 4). Environmental toxicants included organohalogens, metals, biocides, pharmaceuticals, phenols, polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs). Metal concentrations were analyzed using inductively coupled plasma atomic/optical emission spectrometry (ICP-AES/OES) (Araújo et al., 2010; Takahashi and Yokoyama, 2016; Ajitha et al., 2019) or -mass spectrometry (ICP-MS) (Araújo et al., 2010; de Castro-Català et al., 2016; Berrebaan et al., 2020; Rodrigues et al., 2021). PAHs, PCBs, pesticides, and other chemicals were analyzed using gas chromatography (GC) and liquid chromatography (LC) with mass spectrometry (MS) or electron capture detector (ECD) (Booij et al., 2013; Emelogu et al., 2013; Mano and Okamoto, 2016).

To link observed microalgal toxic effects and concentrations of toxicants, principal component analysis (PCA) was performed by about half of the reviewed papers. Predictions of targeted compounds toxicity were assessed using a concentration–response model, and it was assumed that the compounds had the same mode of action and provided a conservative estimate of mixture toxicity (Adams and Stauber, 2004; Muller et al., 2008; Schwab et al., 2009; Stone et al., 2021). However, this assumption is unlikely to be accurate, as chemicals in mixtures might have antagonistic or synergistic effects (Hong et al., 2016b). In addition, interactions of compounds with other abiotic and biotic factors, including nutrients, UV, and predators, can also lead to unpredictable effects (Ben Othman et al., 2023). The establishment of causal links between chemical concentrations and the toxic effects of environmental samples remains a major challenge in ecotoxicology.

The classical analytical instruments (e.g., low-resolution mass spectrometry) might not be able to identify biologically active substances, and conversely, the identification of the compounds responsible for biological activity using bioassays is very challenging. A significant advance to microalgal bioassays introduced in 2005 involved applying effect-directed analysis (EDA) (Grote et al., 2005; Bandow et al., 2009a, 2009b; Schwab et al., 2009; Booij et al., 2014; Tousova et al., 2017, 2018; Riegraf et al., 2019; Lee et al., 2020; An et al., 2021). EDA includes toxicity testing, fractionations, and instrumental analyses. As such, it has been widely applied to identify key toxicant(s) in various environmental samples (Brack, 2003). Many studies using EDA have successfully identified various microalgal toxicants, such as triclosan (Bandow et al., 2009b), n-phenyl-2-naphthylamine (Grote et al., 2005; Schwab et al., 2009), diuron (Riegraf et al., 2019), azithromycin, and biocides (Tousova et al., 2018). Only 12% of studies confirmed toxicity by exposing target chemicals to microalgae. In addition, it is not easy to identify the microalgal toxicants of untargeted/unknown substances, even when EDA is applied.

With the development of analytical instruments, EDA was combined with full-scan screening analysis (FSA) to detect unknown and potentially toxic substances in environmental samples holistically (Grote et al., 2005; Bandow et al., 2009b; Schwab et al., 2009; Booij et al., 2014; Tousova et al., 2018; Lee et al., 2020; An et al., 2021) (Fig. 4). FSA is a powerful tool that is used to identify unknown toxicants in environmental samples based on accurate molecular mass using high-resolution mass spectrometry (HRMS), such as time-of-flight mass spectrometry (TOFMS).

Quantitative structure–activity relationship (QSAR) modeling can be an alternative tool when toxicological confirmation cannot be performed due to the unavailability of authentic standards (OECD, 1994; US EPA, 2012). ECOSAR is a program developed by the US EPA to predict toxicity to fish, invertebrates (Daphnia), and green algae (Nabholz et al., 1993). The use of this approach was presented in four reviewed articles (Ma et al., 2016; Tousova et al., 2017, 2018; An et al., 2021). Unfortunately, because ECOSAR only targets freshwater microalgae, its use for microalgal bioassays in seawater is limited (Reuschenbach et al., 2008). To date, no study has reconfirmed the toxicity of newly derived microalgal toxicants, nor has it evaluated the contribution of new chemical substances. Future studies should focus on evaluating the contribution of toxic causative substances using a mass balance approach. Of course, mass balance cannot be easily calculated as a simple mechanism-based in vitro assay; instead, it is necessary to give each endpoint a different weighting or to elucidate the mechanisms of toxic action of individual compounds on aquatic organisms.

In the present review, we analyzed methodological advances of microalgal bioassays for environmental risk assessments over the last 20 years (total 89 studies reviewed). Recently, environmental assessment studies using microalgae bioassays have considered multiple species and

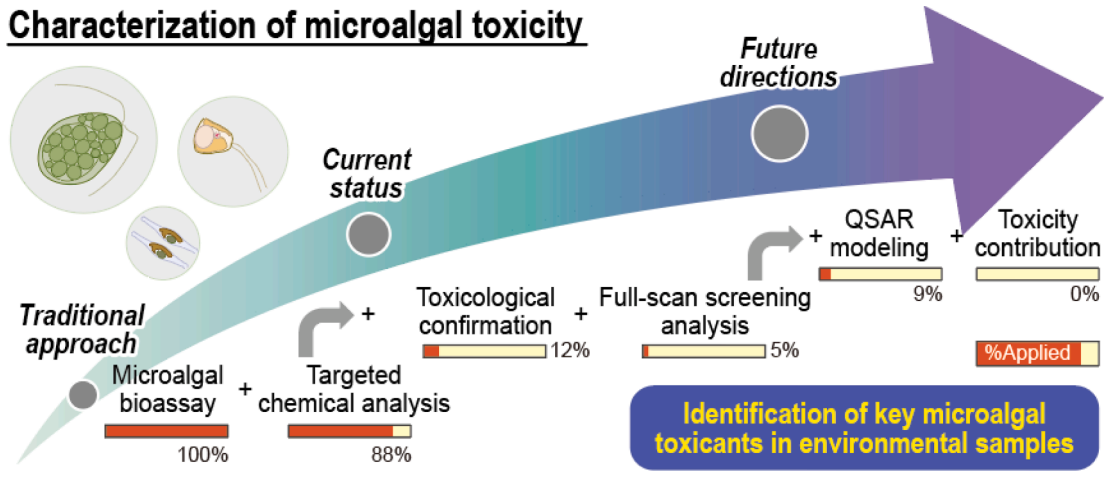

Fig. 4. A frame of the microalgal bioassay development showing the methodological flow with the evolution of chemical analysis towards identification of major algal toxicant(s) in environmental samples.

endpoints, which provide additional information on the potential adverse effects and toxicity mechanisms of various toxic substances in the environment. Flow cytometry and DNA analysis are two of the most powerful and promising techniques for the measurements of cell density and cell viability. In the future, these multiple endpoints microalgal bioassays will be useful for more accurate ecological risk assessment and selection of priority toxic substances. More research is needed to identify microalgal toxicants in the environment combined with EDA and FSA.

CRediT authorship contribution statement

Junghyun Lee: Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Seongjin Hong:** Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing. **Seong-Ah An:** Formal analysis. **Jong Seong Khim:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107869.

References

Adams, M.S., Stauber, J.L., 2004. Development of a whole-sediment toxicity test using a benthic marine microalga. Environ. Toxicol. Chem. 23, 1957–1968. https://doi.org/10.1897/03-232.

Ajitha, V., Sreevidya, C.P., Kim, J.H., Bright Singh, I.S., Mohandas, A., Lee, J.-S., Puthumana, J., 2019. Effect of metals of treated electroplating industrial effluents on antioxidant defense system in the microalga *Chlorella vulgaris*. Aqua. Toxicol. 217, 105317 https://doi.org/10.1016/j.aquatox.2019.105317.

Al-Jabi, S.W., 2017. Global research trends in West Nile virus from 1943 to 2016: a bibliometric analysis. Global. Health 13, 55. https://doi.org/10.1186/s12992-017-0284-y.

Alvarenga, P., Ferreira, C., Mourinha, C., Palma, P., de Varennes, A., 2018. Chemical and ecotoxicological effects of the use of drinking-water treatment residuals for the remediation of soils degraded by mining activities. Ecotox. Environ. Safe. 161, 281–289. https://doi.org/10.1016/j.ecoenv.2018.05.094.

An, S.-A., Hong, S., Lee, J., Cha, J., Lee, S., Moon, H.-B., Giesy, J.P., Khim, J.S., 2021. Identification of potential toxicants in sediments from an industrialized area in Pohang, South Korea: Application of a cell viability assay of microalgae using flow cytometry. J. Hazard. Mater. 405, 124230 https://doi.org/10.1016/j.jhazmat.2020.124230.

Aoyama, K., Iwahori, K., Miyata, N., 2003. Application of *Euglena gracilis* cells to comet assay: Evaluation of DNA damage and repair. Mutat. Res.-Gen. Tox. En. 538, 155–162. https://doi.org/10.1016/s1383-5718(03)00113-x.

Araújo, C.V.M., Tornero, V., Lubián, L.M., Blasco, J., Bergeijk, S.A.V., Cañavate, P., Cid, Á., Franco, D., Prado, R., Bartual, A., López, M.G., Ribeiro, R., Moreira-Santos, M., Torreblanca, A., Jurado, B., Moreno-Garrido, I., 2010. Ring test for whole-sediment toxicity assay with -a- benthic marine diatom. Sci. Total Environ. 408, 822–828. https://doi.org/10.1016/j.scitotenv.2009.10.018.

Aruoja, V., 2011. Algae *Pseudokircheriella subcapitata* in environmental hazard evaluation of chemicals and synthetic nanoparticles. Eesti Maaülikool, http://hdl.handle.net/10492/154.

Bährs, H., Steinberg, C.E.W., 2012. Impact of two different humic substances on selected coccal green algae and cyanobacteria—changes in growth and photosynthetic performance. Environ. Sci. Pollut. R. 19, 335–346. https://doi.org/10.1007/s11356-011-0564-7.

Bandow, N., Altenburger, R., Lübcke-von Varel, U., Paschke, A., Streck, G., Brack, W., 2009a. Partitioning-based dosing: An approach to include bioavailability in the effect-directed analysis of contaminated sediment samples. Environ. Sci. Technol. 43, 3891–3896. https://doi.org/10.1021/es803453h.

Bandow, N., Altenburger, R., Streck, G., Brack, W., 2009b. Effect-directed analysis of contaminated sediments with partition-based dosing using green algae cell multiplication inhibition. Environ. Sci. Technol. 43, 7343–7349. https://doi.org/10.1021/es901351z.

Bauer, D.E., Conforti, V., Ruiz, L., Gómez, N., 2012. An in situ test to explore the responses of *Scenedesmus acutus* and *Lepocinclis acus* as indicators of the changes in water quality in lowland streams. Ecotox. Environ. Safe. 77, 71–78. https://doi.org/10.1016/j.ecoenv.2011.10.021.

Bellemare, F., Rouette, M.-E., Lorrain, L., Perron, É., Boucher, N., 2006. Combined use of photosynthetic enzyme complexes and microalgal photosynthetic systems for rapid screening of wastewater toxicity. Environ. Toxicol. 21, 445–449. https://doi.org/10.1002/tox.20205.

Ben Othman, H., Leboulanger, C., Le Floc'h, E., Hadj Mabrouk, H., Sakka Hlaili, A., 2012. Toxicity of benz(a)anthracene and fluoranthene to marine phytoplankton in culture: Does cell size really matter? J. Hazard. Mater., 243, 204–211, 10.1016/j.jhazmat.2012.10.020.

Ben Othman, H., Pick, F.R., Sakka Hlaili, A., Leboulanger, C., 2023. Effects of polycyclic aromatic hydrocarbons on marine and freshwater microalgae – A review. J. Hazard. Mater. 441, 129869 https://doi.org/10.1016/j.jhazmat.2022.129869.

Berrebaan, I., Montassir, L., El Alami, M., Saadallah, M., Bessi, H., 2020. The Integration of bioanalytical approaches in the assessment of municipal wastewater treatment plant performances. J. Water Environ. Technol. 18, 383–397. https://doi.org/10.2965/jwet.18-092.

Biruk, L.N., Moretton, J., Abrizio, A., Weigandt, C., Etcheverry, J., Filippetto, J., Magdaleno, A., 2017. Toxicity and genotoxicity assessment in sediments from the Matanza-Riachuelo river basin (Argentina) under the influence of heavy metals and organic contaminants. Ecotox. Environ. Safe. 135, 302–311. https://doi.org/10.1016/j.ecoenv.2016.09.024.

Booij, P., Sjollema, S.B., Leonards, P.E.G., de Voogt, P., Stroomberg, G.J., Vethaak, A.D., Lamoree, M.H., 2013. Extraction tools for identification of chemical contaminants in estuarine and coastal waters to determine toxic pressure on primary producers. Chemosphere 93, 107–114. https://doi.org/10.1016/j.chemosphere.2013.04.095.

Booij, P., Vethaak, A.D., Leonards, P.E.G., Sjollema, S.B., Kool, J., de Voogt, P., Lamoree, M.H., 2014. Identification of photosynthesis inhibitors of pelagic marine algae using 96-well plate microfractionation for enhanced throughput in effect-directed analysis. Environ. Sci. Technol. 48, 8003–8011. https://doi.org/10.1016/j.seares.2015.05.001.

Booij, P., Sjollema, S.B., van der Geest, H.G., Leonards, P.E.G., Lamoree, M.H., de Voogt, W.P., Admiraal, W., Laane, R.W.P.M., Vethaak, D., 2015. Toxic pressure of herbicides on microalgae in Dutch estuarine and coastal waters. J. Sea Res. 102, 48–56. https://doi.org/10.1021/es405428t.

Brack, W., 2003. Effect-directed analysis: a promising tool for the identification of organic toxicants in complex mixtures? Anal. Bioanal. Chem. 377, 397–407. https://doi.org/10.1007/s00216-003-2139-z.

Cai, H., Liang, J., Ning, X.-A., Lai, X., Li, Y., 2020. Algal toxicity induced by effluents from textile-dyeing wastewater treatment plants. J. Environ. Sci. 91, 199–208. https://doi.org/10.1016/j.jes.2020.01.004.

Carballeira, C., De Orte, M.R., Viana, I.G., Carballeira, A., 2012. Implementation of a minimal set of biological tests to assess the ecotoxic effects of effluents from land-based marine fish farms. Ecotox. Environ. Safe. 78, 148–161. https://doi.org/10.1016/j.ecoenv.2011.11.022.

Carballeira, C., Cebro, A., Villares, R., Carballeira, A., 2018. Assessing changes in the toxicity of effluents from intensive marine fish farms over time by using a battery of bioassays. Environ. Sci. Pollut. Res. 25, 12739–12748. https://doi.org/10.1007/s11356-018-1403-x.

Cerezo, M.I., Linden, M., Agustí, S., 2017. Flow cytometry detection of planktonic cells with polycyclic aromatic hydrocarbons sorbed to cell surfaces. Mar. Pollut. Bull. 118 (1–2), 64–70. https://doi.org/10.1016/j.marpolbul.2017.02.006.

Chen, H., Jiang, J.-G., 2011. Toxic effects of chemical pesticides (trichlorfon and dimehypo) on *Dunaliella salina*. Chemosphere 84, 664–670. https://doi.org/10.1016/j.chemosphere.2011.03.032.

Claessens, M., Vanhaecke, L., Wille, K., Janssen, C.R., 2013. Emerging contaminants in Belgian marine waters: Single toxicant and mixture risks of pharmaceuticals. Mar. Pollut. Bull. 71, 41–50. https://doi.org/10.1016/j.marpolbul.2013.03.039.

Clijsters, H., Van Assche, F., 1985. Inhibition of photosynthesis by heavy metals. Photosynth. Res. 7, 31–40. https://doi.org/10.1007/BF00032920.

Crane, M., Burton, G.A., Culp, J.M., Greenberg, M.S., Munkittrick, K.R., Ribeiro, R., Salazar, M.H., St-Jean, S.D., 2007. Review of aquatic in situ approaches for stressor and effect diagnosis. Integr. Environ. Assess. Manag. 3, 234–245. https://doi.org/10.1897/ieam_2006-027.1.

Culp, J.M., Lowell, R.B., Cash, K.J., 2000. Integrating mesocosm experiments with field and laboratory studies to generate weight-of-evidence risk assessments for large rivers. Environ. Toxicol. Chem. 19, 1167–1173. https://doi.org/10.1002/etc.5620190450.

de Castro-Català, N., Kuzmanovic, M., Roig, N., Sierra, J., Ginebreda, A., Barceló, D., Pérez, S., Petrovic, M., Picó, Y., Schuhmacher, M., Muñoz, I., 2016. Ecotoxicity of sediments in rivers: Invertebrate community, toxicity bioassays and the toxic unit

approach as complementary assessment tools. Sci. Total Environ. 540, 297–306. https://doi.org/10.1016/j.scitotenv.2015.06.071.
de Filippis, L.F., Pallaghy, C.K., 1994. Heavy Metals: Sources and Biological Effects. In: Rai, L.C., Gaur, J.P., Soeder, C.J. (Eds.), Advances in Limnology Series: Algae and Water Pollution, E. Scheizerbartsche Press, Stuttgart, pp. 31–77.
De Liguoro, M., Bona, M.D., Gallina, G., Capolongo, F., Gallocchio, F., Binato, G., Di Leva, V., 2014. A monitoring of chemical contaminants in waters used for field irrigation and livestock watering in the Veneto region (Italy), using bioassays as a screening tool. Environ. Sci. Pollut. Res. 21, 3546–3557. https://doi.org/10.1007/s11356-013-2357-7.
De Nicola, E., Meriç, S., Della Rocca, C., Gallo, M., Iaccarino, M., Manini, P., Petruzzelli, D., Belgiorno, V., Cheggour, M., Di Gennaro, A., Moukrim, A., Tünay, O., Pagano, G., 2007. Wastewater toxicity of Tannin- Versus chromium-based leather tanneries in Marrakesh. Morocco. Arch. Environ. Contam. Toxicol. 53, 321–328. https://doi.org/10.1007/s00244-006-0181-5.
Di Caprio, F., 2020. Methods to quantify biological contaminants in microalgae cultures. Algal Res. 49, 101943 https://doi.org/10.1016/j.algal.2020.101943.
di Toppi, L.S., Musetti, R., Marabottini, R., Corradi, M.G., Vattuone, Z., Favali, M.A., Badiani, M., 2004. Responses of *Xanthoria parietina* thalli to environmentally relevant concentrations of hexavalent chromium. Funct. Plant Biol. 31, 329–338. https://doi.org/10.1071/FP03171.
Echeveste, P., Agustí, S., Dachs, J., 2010. Cell size dependent toxicity thresholds of polycyclic aromatic hydrocarbons to natural and cultured phytoplankton populations. Environ. Pollut. 158 (1), 299–307. https://doi.org/10.1016/j.envpol.2009.07.006.
Emelogu, E.S., Pollard, P., Dymond, P., Robinson, C.D., Webster, L., McKenzie, C., Dobson, J., Bresnan, E., Moffat, C.F., 2013. Occurrence and potential combined toxicity of dissolved organic contaminants in the Forth estuary and Firth of Forth, Scotland assessed using passive samplers and an algal toxicity test. Sci. Total Environ. 461–462, 230–239. https://doi.org/10.1016/j.scitotenv.2013.05.011.
Emelogu, E.S., Seiler, T.-B., Pollard, P., Robinson, C.D., Webster, L., McKenzie, C., Heger, S., Hollert, H., Bresnan, E., Best, J., Moffat, C.F., 2014. Evaluations of combined zebrafish (*Danio rerio*) embryo and marine phytoplankton (*Diacronema lutheri*) toxicity of dissolved organic contaminants in the Ythan catchment, Scotland, UK. Environ. Sci. Pollut. Res. Int. 21, 5537–5546. https://doi.org/10.1007/s11356-013-2488-x.
Emmanuel, E., Perrodin, Y., Keck, G., Blanchard, J.-M., Vermande, P., 2005. Ecotoxicological risk assessment of hospital wastewater: a proposed framework for raw effluents discharging into urban sewer network. J. Hazard. Mater. 117 (1), 1–11. https://doi.org/10.1016/j.jhazmat.2004.08.032.
Erbes, M., Weßler, A., Obst, U., Wild, A., 1997. Detection of primary DNA damage in *Chlamydomonas reinhardtii* by means of modified microgel electrophoresis. Environ. Mol. Mutagen. 30, 448–458. https://doi.org/10.1002/(sici)1098-2280(1997)30:4<448::aid-em10>3.0.co;2-d.
Escher, B.I., Bramaz, N., Mueller, J.F., Quayle, P., Rutishauser, S., Vermeirssen, E.L., 2008a. Toxic equivalent concentrations (TEQs) for baseline toxicity and specific modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples. J. Environ. Monitor. 10, 612–621. https://doi.org/10.1039/B800949J.
Escher, B.I., Bramaz, N., Quayle, P., Rutishauser, S., Vermeirssen, E.L.M., 2008b. Monitoring of the ecotoxicological hazard potential by polar organic micropollutants in sewage treatment plants and surface waters using a mode-of-action based test battery. J. Environ. Monitor. 10, 622–631. https://doi.org/10.1039/B800951A.
Esperanza, M., Cid, Á., Herrero, C., Rioboo, C., 2015. Acute effects of a prooxidant herbicide on the microalga *Chlamydomonas reinhardtii*: Screening cytotoxicity and genotoxicity endpoints. Aqua. Toxicol. 165, 210–221. https://doi.org/10.1016/j.aquatox.2015.06.004.
Esperanza, M., Seoane, M., Rioboo, C., Herrero, C., Cid, Á., 2019. Differential toxicity of the UV-filters BP-3 and BP-4 in *Chlamydomonas reinhardtii*: A flow cytometric approach. Sci. Total Environ. 669, 412–420. https://doi.org/10.1016/j.scitotenv.2019.03.116.
Everaert, G., De Laender, F., Claessens, M., Baert, J., Monteyne, E., Roose, P., Goethals, P. L.M., Janssen, C.R., 2016. Realistic environmental mixtures of hydrophobic compounds do not alter growth of a marine diatom. Mar. Pollut. Bull. 102, 58–64. https://doi.org/10.1016/j.marpolbul.2015.11.058.
Falagas, M.E., Pitsouni, E.I., Malietzis, G.A., Pappas, G., 2008. Comparison of PubMed, Scopus, Web of Science, and Google Scholar: strengths and weaknesses. FASEB J. 22, 338–342. https://doi.org/10.1096/fj.07-9492LSF.
Garrido-Pérez, M.C., Acevedo-Merino, A., Nebot-Sanz, E., Sales-Márquez, D., 2003. A set of marine microalgae bioassays for the evaluation of biological water quality in enclosure areas in south of Spain. Water Sci. Technol. 47, 85–92. https://doi.org/10.2166/wst.2003.0499.
Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990, 87–92. https://doi.org/10.1016/S0304-4165(89)80016-9.
Giacco, E., Mariottini, G.L., Pane, L., 2011. Ecotoxicological evaluation of marine sediments using free and immobilized phytoplanktonic algae. J. Biol. Res. 84 https://doi.org/10.4081/jbr.2011.4486.
Gorokhova, E., Mattsson, L., Sundström, A.M., 2012. A comparison of TO-PRO-1 iodide and 5-CFDA-AM staining methods for assessing viability of planktonic algae with epifluorescence microscopy. J. Microbiol. Meth. 89, 216–221. https://doi.org/10.1016/j.mimet.2012.03.005.
Gosset, A., Durrieu, C., Barbe, P., Bazin, C., Bayard, R., 2019. Microalgal whole-cell biomarkers as sensitive tools for fast toxicity and pollution monitoring of urban wet weather discharges. Chemosphere 217, 522–533. https://doi.org/10.1016/j.chemosphere.2018.11.033.
Gross, E.M., 2003. Allelopathy of Aquatic Autotrophs. Crit. Rev. Plant Sci. 22, 313–339. https://doi.org/10.1080/713610859.
Grote, M., Brack, W., Walter, H.A., Altenburger, R., 2005. Confirmation of cause-effect relationships using effect-directed analysis for complex environmental samples. Environ. Toxicol. Chem. 24, 1420–1427. https://doi.org/10.1897/04-278R.1.
Guillard, R.R.L., Sieracki, M.S., 2005. Counting cells in cultures with the light microscope. In: Andersen, R.A. (Ed.), Algal culturing techniques. Elsevier, Burlington, MA, pp. 239–252.
Hafner, C., Gartiser, S., Garcia-Käufer, M., Schiwy, S., Hercher, C., Meyer, W., Achten, C., Larsson, M., Engwall, M., Keiter, S., Hollert, H., 2015. Investigations on sediment toxicity of German rivers applying a standardized bioassay battery. Environ. Sci. Pollut. R. 22, 16358–16370. https://doi.org/10.1007/s11356-015-4482-y.
Hamers, T., Molin, K.R.J., Koeman, J.H., Murk, A.J., 2000. A small-volume bioassay for quantification of the esterase inhibiting potency of mixtures of organophosphate and carbamate insecticides in rainwater: Development and optimization. Toxicol. Sci. 58, 60–67. https://doi.org/10.1093/toxsci/58.1.60.
Hernando, M.D., Fernández-Alba, A.R., Tauler, R., Barceló, D., 2005. Toxicity assays applied to wastewater treatment. Talanta 65, 358–366. https://doi.org/10.1016/j.talanta.2004.07.012.
Hong, S., Giesy, J.P., Lee, J.-S., Lee, J.-H., Khim, J.S., 2016a. Effect-directed analysis: Current status and future challenges. Ocean Sci. J. 51, 413–433. https://doi.org/10.1007/s12601-016-0038-4.
Hong, S., Yim, U.H., Ha, S.Y., Shim, W.J., Jeon, S., Lee, S., Kim, C., Choi, K., Jung, J., Giesy, J.P., Khim, J.S., 2016b. Bioaccessibility of AhR-active PAHs in sediments contaminated by the Hebei Spirit oil spill: Application of Tenax extraction in effect-directed analysis. Chemosphere 144, 706–712. https://doi.org/10.1016/j.chemosphere.2015.09.043.
ISO (The International Organization for Standardization), 2006, Water quality — Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water. ISO Water Quality, 14442.
ISO (The International Organization of Standardization), 2012. Water quality — Fresh water algal growth inhibition test with unicellular green algae. ISO Water Quality, 8692, Geneva, Switzerland.
Jia, A., Escher, B.I., Leusch, F.D.L., Tang, J.Y.M., Prochazka, E., Dong, B., Snyder, E.M., Snyder, S.A., 2015. In vitro bioassays to evaluate complex chemical mixtures in recycled water. Water Res. 80, 1–11. https://doi.org/10.1016/j.watres.2015.05.020.
Jochem, F.J., 2000. Probing the physiological state of phytoplankton at the single-cell level. Sci. Mar. 64, 183–195. https://doi.org/10.3989/scimar.2000.64n2183.
Juneau, P., El Berdey, A., Popovic, R., 2002. PAM fluorometry in the determination of the sensitivity of *Chlorella vulgaris*, *Selenastrum capricornutum*, and *Chlamydomonas reinhardtii* to copper. Arch. Environ. Con. Tox. 42, 155–164. https://doi.org/10.1007/s00244-001-0027-0.
Källqvist, T., Milačič, R., Smital, T., Thomas, K.V., Vranes, S., Tollefsen, K.-E., 2008. Chronic toxicity of the Sava River (SE Europe) sediments and river water to the algae *Pseudokirchneriella subcapitata*. Water Res. 42, 2146–2156. https://doi.org/10.1016/j.watres.2007.11.026.
Källqvist, T., Milačič, R., Smital, T., Thomas, K.V., Vranes, S., Tollefsen, K.-E., 2008. Chronic toxicity of the Sava River (SE Europe) sediments and river water to the algae *Pseudokirchneriella subcapitata*. Water Res 42, 2146–2156. https://doi.org/10.1016/j.watres.2007.11.026.
Khim, J.S., Hong, S., 2014. Assessment of trace pollutants in Korean coastal sediments using the triad approach: A review. Sci. Total Environ. 470–471, 1450–1462. https://doi.org/10.1016/j.scitotenv.2013.07.052.
Köck, M., Farré, M., Martínez, E., Gajda-Schrantz, K., Ginebreda, A., Navarro, A., Alda, M.L.d., Barceló, D., 2010. Integrated ecotoxicological and chemical approach for the assessment of pesticide pollution in the Ebro River delta (Spain). J. Hydrol. 383, 73–82. https://doi.org/10.1016/j.jhydrol.2009.12.029.
Koppel, D.J., Gissi, F., Adams, M.S., King, C.K., Jolley, D.F., 2017. Chronic toxicity of five metals to the polar marine microalga *Cryothecomonas armigera*–Application of a new bioassay. Environ. Pollut. 228, 211–221. https://doi.org/10.1016/j.envpol.2017.05.034.
Kramárová, Z., Fargašová, A., Molnárová, M., Bujdoš, M., 2012. Arsenic and selenium interactive effect on alga *Desmodesmus quadricauda*. Ecotox. Environ. Safe. 86, 1–6. https://doi.org/10.1016/j.ecoenv.2012.08.028.
Kvíderová, J., Elster, J., 2013. Standardized algal growth potential and/or algal primary production rates of maritime antarctic stream waters (King George Island, South Shetlands). Polar Res. 32, 11191. https://doi.org/10.3402/polar.v32i0.11191.
Kwok, C.K., Yang, S.M., Mak, N.K., Wong, C.K.C., Liang, Y., Leung, S.Y., Young, L., Wong, M.H., 2010. Ecotoxicological study on sediments of Mai Po marshes, Hong Kong using organisms and biomarkers. Ecotox. Environ. Safe. 73, 541–549. https://doi.org/10.1016/j.ecoenv.2010.01.012.
Latif, M., Licek, E., 2004. Toxicity assessment of wastewaters, river waters, and sediments in Austria using cost-effective microbiotests. Environ. Toxicol. 19, 302–309. https://doi.org/10.1002/tox.20027.
Lee, J., Hong, S., Kim, T., Lee, C., An, S.-A., Kwon, B.-O., Lee, S., Moon, H.-B., Giesy, J.P., Khim, J.S., 2020. Multiple bioassays and targeted and nontargeted analyses to characterize potential toxicological effects associated with sediments of Masan Bay: Focusing on AhR-mediated potency. Environ. Sci. Technol. 54, 4443–4454. https://doi.org/10.1021/acs.est.9b07390.
Lee, J., Khim, J.S., 2022. Revisited a sediment quality triad approach in the Korean coastal waters: Past research, current status, and future directions. Environ. Pollut. 292, 118262 https://doi.org/10.1016/j.envpol.2021.118262.
Lei, A., Hu, Z., Wong, Y., Tam, N.F., 2006. Antioxidant responses of microalgal species to pyrene. J. Appl. Phycol. 18, 67–78. https://doi.org/10.1007/s10811-005-9016-4.

Leusch, F.D.L., Khan, S.J., Gagnon, M.M., Quayle, P., Trinh, T., Coleman, H., Rawson, C., Chapman, H.F., Blair, P., Nice, H., Reitsema, T., 2014. Assessment of wastewater and recycled water quality: A comparison of lines of evidence from in vitro, in vivo and chemical analyses. Water Res. 50, 420–431. https://doi.org/10.1016/j.watres.2013.10.056.

Li, M., Gao, X., Wu, B., Qian, X., Giesy, J.P., Cui, Y., 2014. Microalga *Euglena* as a bioindicator for testing genotoxic potentials of organic pollutants in Taihu Lake, China. Ecotoxicology 23, 633–640. https://doi.org/10.1007/s10646-014-1214-x.

Lichtenthaler, H.K., 1987. [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In: Packer, L., Douce, R. (Eds.), Methods in Enzymology. Academic Press, Washington, pp. 350–382. https://doi.org/10.1016/0076-6879(87)48036-1.

Litchman, E., Klausmeier, C.A., 2008. Trait-based community ecology of phytoplankton. Ann. Rev. Ecol., Evolut., System. 39, 615–639.

Lors, C., Ponge, J.-F., Damidot, D., 2018. Environmental hazard assessment by the ecoscore system to discriminate PAH-polluted soils. Environ. Sci. Pollut. Res. 25, 26747–26756. https://doi.org/10.1007/s11356-017-9906-4.

Ma, X.Y., Wang, X.C., Wang, D., Ngo, H.H., Zhang, Q., Wang, Y., Dai, D., 2016. Function of a landscape lake in the reduction of biotoxicity related to trace organic chemicals from reclaimed water. J. Hazard. Mater. 318, 663–670. https://doi.org/10.1016/j.jhazmat.2016.07.050.

Magnusson, M., Heimann, K., Negri, A.P., 2008. Comparative effects of herbicides on photosynthesis and growth of tropical estuarine microalgae. Mar. Pollut. Bull. 56, 1545–1552. https://doi.org/10.1016/j.marpolbul.2008.05.023.

Magnusson, M., Heimann, K., Ridd, M., Negri, A.P., 2013. Pesticide contamination and phytotoxicity of sediment interstitial water to tropical benthic microalgae. Water Res. 47, 5211–5221. https://doi.org/10.1016/j.watres.2013.06.003.

Mano, H., Okamoto, S., 2016. Preliminary ecological risk assessment of 10 PPCPs and their contributions to the toxicity of concentrated surface water on an algal species in the middle basin of Tama River. J. Water Environ. Technol. 14, 423–436. https://doi.org/10.2965/jwet.15-045.

Mano, H., Takeda, F., Kitamura, T., Okamoto, S., Suzuki, Y., Park, C.-B., Yasui, N., Kobayashi, K., Tanaka, Y., Yamashita, N., Minamiyama, M., 2017. Water quality comparison of secondary effluent and reclaimed water to ambient river water of southern Okinawa Island via biological evaluation. Environ. Monit. Assess. 189, 442. https://doi.org/10.1007/s10661-017-6160-7.

Maranho, L., Garrido-Pérez, M., Baena-Nogueras, R., Lara-Martín, P., Antón-Martín, R., DelValls, T., Martín-Díaz, M.J.E., 2015. Are WWTPs effluents responsible for acute toxicity? Seasonal variations of sediment quality at the Bay of Cádiz (SW, Spain). Ecotoxicology 24 (2), 368–380. https://doi.org/10.1007/s10646-014-1385-5.

Marie, D., Partensky, F., Jacquet, S., Vaulot, D., 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl. Environ. Microb. 63 (1), 186–193. https://doi.org/10.1128/aem.63.1.186-193.1997.

Marie, D., Simon, N., Guillou, L., Partensky, F., Vaulot, D., 2000. Flow cytometry analysis of marine picoplankton. In: Diamond, R.A., De Maggio, S. (Eds.), Living color: Protocols in flow cytometry and cell sorting. Springer, Berlin, pp. 421–454.

Mariño-Balsa, J.C., Pérez, P., Estévez-Blanco, P., Saco-Álvarez, L., Fernández, E., Beiras, R., 2003. Assessment of the toxicity of sediment and seawater polluted by the prestige fuel spill using bioassays with clams (*Venerupis pullastra*, *Tappes decussatus* and *Venerupis rhomboideus*) and the microalga *Skeletonema costatum*. Cienc. Mar. 29, 115–122. https://doi.org/10.7773/cm.v29i1.128.

Markina, Z.V., Aizdaicher, N.A., 2011. *Phaeodactylum tricornutum* Bohlin bioassay of water quality of Amur Bay (the Sea of Japan). Contemp. Probl. Ecol. 4, 74–79. https://doi.org/10.1134/S1995425511010127.

Marques, C.R., Abrantes, N., de Figueiredo, D.R., Pereira, M.J., Gonçalves, F., 2008. Are *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* affected by environmental samples from a rice field? Water Air Soil Poll. 189, 49–59. https://doi.org/10.1007/s11270-007-9554-2.

Marques, C.R., Pereira, R., Gonçalves, F., 2011. Toxicity evaluation of natural samples from the vicinity of rice fields using two trophic levels. Environ. Monit. Assess. 180, 521–536. https://doi.org/10.1007/s10661-010-1803-y.

Mendonça, E., Picado, A., Paixão, S.M., Silva, L., Cunha, M.A., Leitão, S., Moura, I., Cortez, C., Brito, F., 2009. Ecotoxicity tests in the environmental analysis of wastewater treatment plants: Case study in Portugal. J. Hazard. Mater. 163, 665–670. https://doi.org/10.1016/j.jhazmat.2008.07.012.

Meriç, S., Nicola, E.D., Iaccarino, M., Gallo, M., Gennaro, A.D., Morrone, G., Warnau, M., Belgiorno, V., Pagano, G., 2005. Toxicity of leather tanning wastewater effluents in sea urchin early development and in marine microalgae. Chemosphere 61, 208–217. https://doi.org/10.1016/j.chemosphere.2005.02.037.

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H., Halm-Lemeille, M.P., 2016. Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. Environ. Sci. Pollut. Res. Int. 23 (6), 4992–5001. https://doi.org/10.1007/s11356-014-3662-5.

Moeris, S., Vanryckeghem, F., Demeestere, K., Huysman, S., Vanhaecke, L., De Schamphelaere, K.A.C., 2019. Growth stimulation effects of environmentally realistic contaminant mixtures on a marine diatom. Environ. Toxicol. Chem. 38, 1313–1322. https://doi.org/10.1002/etc.4431.

Moeris, S., Vanryckeghem, F., Demeestere, K., De Schamphelaere, K.A.C., 2021. A margin of safety approach for the assessment of environmentally realistic chemical mixtures in the marine environment based on combined passive sampling and ecotoxicity testing. Sci. Total Environ. 765, 142748 https://doi.org/10.1016/j.scitotenv.2020.142748.

Morales-Caselles, C., Rico, A., Abbondanzi, F., Campisi, T., Iacondini, A., Riba, I., DelValls, A., 2008. Assessing sediment quality in Spanish ports using a green alga bioassay. Cienc. Mar. 34, 329–337. https://doi.org/10.7773/cm.v34i3.1398.

Moreira, S.M., Guilhermino, L., Ribeiro, R.J.E.T., Journal, C.A.I., 2006. An in situ assay with the microalga *Phaeodactylum tricornutum* for sediment-overlying water toxicity evaluations in estuaries. Environ. Toxicol. Chem. 25, 2272–2279. https://doi.org/10.1897/05-266r.1.

Moreira-Santos, M., Soares, A.M.V.M., Ribeiro, R., 2004. An in situ bioassay for freshwater environments with the microalga *Pseudokirchneriella subcapitata*. Ecotox. Environ. Safe. 59, 164–173. https://doi.org/10.1016/j.ecoenv.2003.07.004.

Moreira-Santos, M., Antunes, S.C., Gonçalves, F., Soares, A.M.V.M., Ribeiro, R., 2011. An in situ toxicity assay with the local phytoplankton community. Environ. Toxicol. Chem. 30, 196–205. https://doi.org/10.1002/etc.371.

Morelli, E., Marangi, M.L., Fantozzi, L., 2009. A phytochelatin-based bioassay in marine diatoms useful for the assessment of bioavailability of heavy metals released by polluted sediments. Environ. Int. 35, 532–538. https://doi.org/10.1016/j.envint.2008.09.012.

Moreno-Garrido, I., Lubián, L.M., Jiménez, B., Soares, A.M.V.M., Blasco, J., 2007. Estuarine sediment toxicity tests on diatoms: Sensitivity comparison for three species. Estuar. Coast. Shelf S. 71, 278–286. https://doi.org/10.1016/j.ecss.2006.08.003.

Muller, R., Schreiber, U., Escher, B.I., Quayle, P., Bengtson Nash, S.M., Mueller, J.F., 2008. Rapid exposure assessment of PSII herbicides in surface water using a novel chlorophyll a fluorescence imaging assay. Sci. Total Environ. 401, 51–59. https://doi.org/10.1016/j.scitotenv.2008.02.062.

Munawar, M., Munawar, I.F., 1987. Phytoplankton bioassays for evaluating toxicity of in situ sediment contaminants. Hydrobiologia 149, 87–105. https://doi.org/10.1007/BF00048650.

Mustajärvi, L., Eriksson-Wiklund, A.-K., Gorokhova, E., Jahnke, A., Sobek, A., 2017. Transferring mixtures of chemicals from sediment to a bioassay using silicone-based passive sampling and dosing. Environ. Sci.-Proc. Imp. 19, 1404–1413. https://doi.org/10.1039/C7EM00228A.

Nabholz, J., Clements, R., Zeeman, M., Osborn, K., Wedge, R., 1993. Validation of structure activity relationships used by the USEPA's office of pollution prevention and toxics for the environmental hazard assessment of industrial chemicals. Environmental Toxicology and Risk Assessment: Second Volume. ASTM International.

Nagarajan, D., Lee, D.-J., Varjani, S., Lam, S.S., Allakhverdiev, S.I., Chang, J.-S., 2022. Microalgae-based wastewater treatment – Microalgae-bacteria consortia, multi-omics approaches and algal stress response. Sci. Total Environ. 845, 157110 https://doi.org/10.1016/j.scitotenv.2022.157110.

Nam, S.-H., Kwak, J.I., An, Y.-J., 2018. Assessing applicability of the paper-disc method used in combination with flow cytometry to evaluate algal toxicity. Environ. Pollut. 234, 979–987. https://doi.org/10.1016/j.envpol.2017.12.010.

Neale, P.A., Munz, N.A., Aït-Aïssa, S., Altenburger, R., Brion, F., Busch, W., Escher, B.I., Hilscherová, K., Kienle, C., Novák, J., Seiler, T.-B., Shao, Y., Stamm, C., Hollender, J., 2017. Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams. Sci. Total Environ. 576, 785–795. https://doi.org/10.1016/j.scitotenv.2016.10.141.

Nestler, H., Groh, K.J., Schönenberger, R., Behra, R., Schirmer, K., Eggen, R.I., Suter, M.-J.-F., 2012. Multiple-endpoint assay provides a detailed mechanistic view of responses to herbicide exposure in *Chlamydomonas reinhardtii*. Aquat. toxicol. 110, 214–224. https://doi.org/10.1016/j.aquatox.2012.01.014.

OECD (Organization for Economic Cooperation and Development), 1994. US EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships (QSARS). OECD Environment Monographs No. 88. OECD Publishing, Paris, France.

OECD, 2006. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, France.

Oettmeier, W., 1992. Herbicides of photosystem II. Barber J (Ed.). The photosystems: structure, function and molecular biology, Vol II. Elsevier, Amsterdam, pp. 349–408.

Olano, H., Martigani, F., Somma, A., Aubriot, L., 2019. Wastewater discharge with phytoplankton may favor cyanobacterial development in the main drinking water supply river in Uruguay. Environ. Monit. Assess. 191, 146. https://doi.org/10.1007/s10661-019-7288-4.

O'Neill, E.A., Rowan, N.J., Fogarty, A.M., 2019. Novel use of the alga *Pseudokirchneriella subcapitata*, as an early-warning indicator to identify climate change ambiguity in aquatic environments using freshwater finfish farming as a case study. Sci. Total Environ. 692, 209–218. https://doi.org/10.1016/j.scitotenv.2019.07.243.

Patroncini, D., Veronesi, F., Rawson, D., 2014. Evaluation of river water quality: A case study of the Lea Navigation (NE London). Water Air Soil Poll 225, 2090. https://doi.org/10.1007/s11270-014-2090-y.

Pavlić, Ž., Vidaković-Cifrek, Ž., Puntarić, D., 2005. Toxicity of surfactants to green microalgae *Pseudokirchneriella subcapitata* and *Scenedesmus subspicatus* and to marine diatoms *Phaeodactylum tricornutum* and *Skeletonema costatum*. Chemosphere 61, 1061–1068. https://doi.org/10.1016/j.chemosphere.2005.03.051.

Pei, Z.-T., Xu, R.-R., Liu, H.-Y., Wang, W.-Q., Zhang, M., Zhang, L.-L., Zhang, J., Wang, W.-Q., Yu, R., Sun, L.-W., 2020. Development and application of a novel whole sediment toxicity test using immobilized sediment and *Chlorella vulgaris*. Ecotox. Environ. Safe. 189, 109979 https://doi.org/10.1016/j.ecoenv.2019.109979.

Pérez, J.R., Loureiro, S., Menezes, S., Palma, P., Fernandes, R.M., Barbosa, I.R., Soares, A.M.V.M., 2010. Assessment of water quality in the Alqueva Reservoir (Portugal) using bioassays. Environ. Sci. Pollut. Res. 17, 688–702. https://doi.org/10.1007/s11356-009-0174-9.

Pignata, C., Fea, E., Rovere, R., Degan, R., Lorenzi, E., de Ceglia, M., Schilirò, T., Gilli, G., 2012. Chlorination in a wastewater treatment plant: acute toxicity effects of the effluent and of the recipient water body. Environ. Monit. Assess. 184, 2091–2103. https://doi.org/10.1007/s10661-011-2102-y.

Pikula, K.S., Chernyshev, V.V., Zakharenko, A.M., Chaika, V.V., Waissi, G., Hai, L.H., Hien, T.T., Tsatsakis, A.M., Golokhvast, K.S., 2019. Toxicity assessment of particulate matter emitted from different types of vehicles on marine microalgae. Environ. Res. 179, 108785 https://doi.org/10.1016/j.envres.2019.108785.

Pikula, K., Tretyakova, M., Zakharenko, A., Johari, S.A., Ugay, S., Chernyshev, V., Chaika, V., Kalenik, T., Golokhvast, K., 2021. Environmental risk assessment of vehicle exhaust particles on aquatic organisms of different trophic levels. Toxics 9, 261. https://doi.org/10.3390/toxics9100261.

Prato, E., Parlapiano, I., Biandolino, F., Assessment, 2011. Evaluation of a bioassays battery for ecotoxicological screening of marine sediments from Ionian Sea (Mediterranea Sea, Southern Italy). Environ. Monit. Assess. 184, 5225–5238, 10.1007/s10661-011-2335-9.

Prato, E., Parlapiano, I., Biandolino, F., 2012. Evaluation of a bioassays battery for ecotoxicological screening of marine sediments from Ionian Sea (Mediterranea Sea, Southern Italy). Environ. Monit. Assess. 184, 5225–5238. https://doi.org/10.1007/s10661-011-2335-9.

Ralph, P.J., Smith, R.A., Macinnis-Ng, C.M.O., Seery, C.R., 2007. Use of fluorescence-based ecotoxicological bioassays in monitoring toxicants and pollution in aquatic systems: Review. Toxicol. Environ. Chem. 89, 589–607. https://doi.org/10.1080/02772240701561593.

Reuschenbach, P., Silvani, M., Dammann, M., Warnecke, D., Knacker, T., 2008. ECOSAR model performance with a large test set of industrial chemicals. Chemosphere 71, 1986–1995. https://doi.org/10.1016/j.chemosphere.2007.12.006.

Rezayian, M., Niknam, V., Ebrahimzadeh, H., 2019. Oxidative damage and antioxidative system in algae. Toxicol. Rep. 6, 1309–1313. https://doi.org/10.1016/j.toxrep.2019.10.001.

Riegraf, C., Reifferscheid, G., Becker, B., Belkin, S., Hollert, H., Feiler, U., Buchinger, S., 2019. Detection and quantification of photosystem II Inhibitors using the freshwater alga *Desmodesmus subspicatus* in combination with high-performance thin-layer chromatography. Environ. Sci. Technol. 53, 13458–13467. https://doi.org/10.1021/acs.est.9b04634.

Rodrigues, S., Pinto, I., Formigo, N., Antunes, S.C., 2021. Microalgae growth inhibition-based reservoirs water quality assessment to identify ecotoxicological risks. Water 13, 2605. https://doi.org/10.1021/acs.est.9b04634.

Sabnis, R.W., Deligeorgiev, T.G., Jachak, M.N., Dalvi, T.S., 1997. DiOC6(3): A useful dye for staining the endoplasmic reticulum. Biotech. Histochem. 72, 253–258. https://doi.org/10.3109/10520299709082249.

Santos, M.M.D., Moreno-Garrido, I., Gonçalves, F., Soares, A., Ribeiro, R., 2002. An in situ bioassay for estuarine environments using the microalga *Phaeodactylum tricornutum*. Environ. Toxicol. 21, 567–574. https://doi.org/10.1002/etc.5620210315.

Savage-Dunn, C., 2008. Cell size: A matter of life or death? Curr. Bio. 18, R738–R740. https://doi.org/10.1016/j.cub.2008.07.058.

Schreiber, U., 1986. Detection of rapid induction kinetics with a new type of high-frequency modulated chlorophyll fluorometer. Photosynth. Res. 9, 261–272. https://doi.org/10.1007/BF00029749.

Schwab, K., Altenburger, R., Lübcke-von Varel, U., Streck, G., Brack, W., 2009. Effect-directed analysis of sediment-associated algal toxicants at selected hot spots in the River Elbe basin with a special focus on bioaccessibility. Environ. Toxicol. Chem. 28, 1506–1517. https://doi.org/10.1897/08-340.1.

Seoane, M., Rioboo, C., Herrero, C., Cid, Á., 2014. Toxicity induced by three antibiotics commonly used in aquaculture on the marine microalga *Tetraselmis suecica* (Kylin) Butch. Mar. Environ. Res. 101, 1–7. https://doi.org/10.1016/j.marenvres.2014.07.011.

Seoane, M., Esperanza, M., Rioboo, C., Herrero, C., Cid, Á., 2017. Flow cytometric assay to assess short-term effects of personal care products on the marine microalga *Tetraselmis suecica*. Chemosphere 171, 339–347. https://doi.org/10.1016/j.chemosphere.2016.12.097.

Shapiro, H.M., 2005. How flow cytometers work, Practical flow cytometry. John Wiley & Sons 101–223.

Sharp, J.H., Underhill, P.A., Hughes, D.J., 1979. Interaction (allelopathy) between marine diatoms: *Thalassiosira pseudonana* and *Phaeodactylum triconutum*. J. Phycol. 15, 353–362. https://doi.org/10.1111/j.1529-8817.1979.tb00705.x.

Stephan, P., Veugelers, R., Wang, J., 2017. Reviewers are blinkered by bibliometrics. Nature 544 (7651), 411–412.

Stone, S., Adams, M.S., Stauber, J.L., Jolley, D.F., Warne, M.S.J., 2021. Toxicity of herbicide mixtures to tropical freshwater microalgae using a multispecies test. Environ. Toxicol. Chem. 40, 473–486. https://doi.org/10.1002/etc.4932.

Suzuki, T., Fujikura, K., Higashiyama, T., Takata, K., 1997. DNA Staining for fluorescence and laser confocal microscopy. J. Histochem. Cytochem. 45, 49–53. https://doi.org/10.1177/002215549704500107.

Takahashi, T., Yokoyama, S., 2016. Bioassay of components eluted from electric arc furnace steel slag using microalgae *Chlorella*. ISIJ Int. 56, 1497–1505. https://doi.org/10.2355/isijinternational.ISIJINT-2015-539.

Tang, J.Y.M., Aryal, R., Deletic, A., Gernjak, W., Glenn, E., McCarthy, D., Escher, B.I., 2013. Toxicity characterization of urban stormwater with bioanalytical tools. Water Res. 47, 5594–5606. https://doi.org/10.1016/j.watres.2013.06.037.

Torres, M.A., Barros, M.P., Campos, S.C.G., Pinto, E., Rajamani, S., Sayre, R.T., Colepicolo, P., 2008. Biochemical biomarkers in algae and marine pollution: A review. Ecotox. Environ. Safe. 71, 1–15. https://doi.org/10.1016/j.ecoenv.2008.05.009.

Tousova, Z., Oswald, P., Slobodnik, J., Blaha, L., Muz, M., Hu, M., Brack, W., Krauss, M., Di Paolo, C., Tarcai, Z., Seiler, T.-B., Hollert, H., Koprivica, S., Ahel, M., Schollée, J. E., Hollender, J., Suter, M.J.F., Hidasi, A.O., Schirmer, K., Sonavane, M., Ait-Aissa, S., Creusot, N., Brion, F., Froment, J., Almeida, A.C., Thomas, K., Tollefsen, K. E., Tufi, S., Ouyang, X., Leonards, P., Lamoree, M., Torrens, V.O., Kolkman, A., Schriks, M., Spirhanzlova, P., Tindall, A., Schulze, T., 2017. European demonstration program on the effect-based and chemical identification and monitoring of organic pollutants in European surface waters. Sci. Total Environ. 601–602, 1849–1868. https://doi.org/10.1016/j.scitotenv.2017.06.032.

Tousova, Z., Froment, J., Oswald, P., Slobodník, J., Hilscherova, K., Thomas, K.V., Tollefsen, K.E., Reid, M., Langford, K., Blaha, L., 2018. Identification of algal growth inhibitors in treated waste water using effect-directed analysis based on non-target screening techniques. J. Hazard. Mater. 358, 494–502. https://doi.org/10.1016/j.jhazmat.2018.05.031.

Twist, H., Edwards, A.C., Codd, G.A., 1997. A novel in-situ biomonitor using alginate immobilised algae (*Scenedesmus subspicatus*) for the assessment of eutrophication in flowing surface waters. Water Res. 31, 2066–2072. https://doi.org/10.1016/S0043-1354(97)00035-3.

U.S. Environmental Protection Agency (EPA), 1996. Guidelines for Carcinogen Risk Assessment. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA, 2012. Ecological Structure Activity Relationships (ECOSAR) v.1.11, U.S. Environmental Protection Agency, Washington D.C.

Ugya, A.Y., Imam, T.S., Li, A., Ma, J., Hua, X., 2020. Antioxidant response mechanism of freshwater microalgae species to reactive oxygen species production: A mini review. Chem. Ecol. 36, 174–193. https://doi.org/10.1080/02757540.2019.1688308.

Van Baalen, C., O'Donnell, R., 1978. Isolation of a nickel-dependent blue-green alga. Microbiology 105, 351–353. https://doi.org/10.1099/00221287-105-2-351.

van Eck, N.J., Waltman, L., 2010. Software survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics 84, 523–538. https://doi.org/10.1007/s11192-009-0146-3.

Vermeirssen, E.L.M., Hollender, J., Bramaz, N., van der Voet, J., Escher, B.I., 2010. Linking toxicity in algal and bacterial assays with chemical analysis in passive samplers deployed in 21 treated sewage effluents. Environ. Toxicol. Chem. 29, 2575–2582. https://doi.org/10.1002/etc.311.

Wang, F., Leung, A.O.W., Wu, S.C., Yang, M.S., Wong, M.H., 2009. Chemical and ecotoxicological analyses of sediments and elutriates of contaminated rivers due to e-waste recycling activities using a diverse battery of bioassays. Environ. Pollut. 157, 2082–2090. https://doi.org/10.1016/j.envpol.2009.02.015.

Wang, L., Zheng, B., 2008. Toxic effects of fluoranthene and copper on the marine diatom *Phaeodactylum tricornutum*. J. Environ. Sci. 20 (11), 1363–1372. https://doi.org/10.1016/S1001-0742(08)62234-2.

Watanabe, M., Suzuki, T., 2002. Involvement of reactive oxygen stress in cadmium-induced cellular damage in *Euglena gracilis*. Comp. Biochem. Phys. C 131, 491–500. https://doi.org/10.1016/s1532-0456(02)00036-4.

Wu, C., Li, Y., Zhou, Y., Li, Z., Zhang, S., Liu, H., 2018. Upgrading the Chinese biggest petrochemical wastewater treatment plant: Technologies research and full scale application. Sci. Total Environ. 633 (15), 189–197. https://doi.org/10.1016/j.scitotenv.2018.03.164.

Yu, Y., Kong, F., Wang, M., Qian, L., Shi, X., 2007. Determination of short-term copper toxicity in a multispecies microalgal population using flow cytometry. Ecotox. Environ. Safe. 66, 49–56. https://doi.org/10.1016/j.ecoenv.2005.10.014.

Zhang, L.J., Ying, G.G., Chen, F., Zhao, J.L., Wang, L., Fang, Y.X., 2012. Development and application of whole-sediment toxicity test using immobilized freshwater microalgae *Pseudokirchneriella subcapitata*. Environ. Toxicol. Chem. 31 (2), 377–386. https://doi.org/10.1002/etc.734.

Zhuravel, E., Markina, Z., Aizdaicher, N., 2009. Growth and physiological state of the microalgae *Phaeodactylum tricornutum* Bohlin (Bacillariophyta) in the water taken from Peter the Great Bay. Ocean Sci. J. 44, 173–179. https://doi.org/10.1007/s12601-009-0015-2.

Zhuravel, E.V., Markina, Z.V., Aizdaicher, N.A., 2012. Influence of water pollution on the growth and pigment concentration of the microalgae *Phaeodactylum tricornutum* Bohlin (Bacillariophyta). Environ. Monit. Assess. 184, 2909–2919. https://doi.org/10.1007/s10661-011-2159-7.

<Supplementary Material>

Review

Methodological advances and future directions of microalgal bioassays for evaluation of potential toxicity in environmental samples: A review

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Supplementary Table

Table S1. Detailed description of the 5 types according to sampling, pretreatment methods, and microalgal exposure methods.

Type	Procedure of microalgal bioassay			
	Sample type	**Sampling**	**Extraction**	**Exposure**
A	Water or Effluents	Bottles	**None**	Direct
B	Water or Effluents	Passive sampler	Solid phase extraction or organic solvent extraction	In-direct
C	Sediments or Soil	Grab/Box/Bag/etc.	Organic solvent extraction	In-direct
D	Sediments or Soil	Grab/Box/Bag/etc.	Elutriate	In-direct
E	**None**	**None**	**None**	In situ