Chapter 4

Effects on the Photosynthetic Activity of Algae after Exposure to Various Organic and Inorganic Pollutants: Review

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Additional information is available at the end of the chapter

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Abstract

Algal studies remain necessary for risk assessment and their utility in ecotoxicology is the evaluation of lethal and sub-lethal toxic effects of potential toxicants on inhabitants of several ecosystems. Effects on algal photosynthetic apparatus caused by various chemical species have been extensively studied. The present chapter summarizes the published data concerning the toxicity of various organic and inorganic pollutants such as oils, pesticides, antifoulants and metals on photosynthesis of aquatic primary producers. Biochemical mode of action resulting in the disruption of photosynthesis depends on the chemical's nature and the characteristics of the exposed microorganism. Observed differences in response and sensitivity by different species to the same toxicant were attributed to several algal characteristics including photosynthetic capacity, pigment type, cellular lipid and protein content, and cell size. Single species bioassays either for one chemical alone or in mixture have been well reported and tolerance of both marine and freshwater water-column phytoplaktonic species has been examined. Adequate published information on multispecies tests (communities) in laboratory and field studies exists. However, risk assessment on photosynthesis of microbenthic periphyton is inadequate, though it is essential especially for hydrophobic organic molecules. Further studies are required to evaluate the adverse effects of metabolites on aquatic microalgae.

Keywords: aquatic toxicology, microorganisms, chlorophyll, photosynthesis, pollutants

1. Introduction

Aquatic ecosystems receive direct or indirect inputs of a wide diversity and a variety of chemical species among which polychlorinated biphenyls (PCBs), chlorinated dioxins, polycyclic aromatic

hydrocarbons (PAHs), insecticides, herbicides, oils, metals and metalloids, inorganic nonmetallic elements, effluents, surfactants, synthetic detergents, and pharmaceuticals are included. Especially sediments (estuarine, river, and lake) accept the highest loads of all these aforementioned organic and inorganic molecules in both marine and freshwater aquatic environments. As a consequence, several compounds can play the role of toxic agents that inevitably expose inhabitants of these ecosystems which are vulnerable to pollution [1].

Fortunately, over the past few decades an enormous emphasis was placed on the section of aquatic toxicological research. Environmental protection agencies in a number of countries, particularly in Europe, North America, Japan, Southeast Asia, and Australia-New Zealand, in order to deal with wastewater discharges and in addition in their efforts to curb aquatic pollution, have recognized the great value of applying aquatic hazard assessment principles and procedures to effluents and their component chemicals and properties.

Phototrophic microorganisms such as micro- and macroalgae contribute significantly to primary productivity, nutrient cycling, and decomposition in the aquatic ecosystems; therefore, their importance in providing energy that sustains invertebrates and fish of those environmental compartments is very crucial. Microalgal communities form an essential functional group in aquatic habitats not only as key primary producers (important food source for feeders) but as regulators of oxygen levels; even at the water sediment interface, oxygen (O_2) production is highly dependent on the photosynthesis of microphytobenthos. Thus, the effects of toxic substances on algae are important not only for those microorganisms themselves but have subsequent impacts on higher trophic levels of the food chain. Since photosynthesis forms the fundamental basis of the food webs, even sub-lethal effects on primary producers could impact the energy transfer throughout the food chain [2].

As a result, toxicity tests have been developed that assess the effects of toxicants on photosynthetic activity of exposed species. The scientific published data demonstrate that the inhibition of photosynthetic activity is a common effect parameter monitored not only in numerous laboratory toxicity tests with cultured algae but also *in situ* with natural phytoplankton and periphyton communities [3].

The focus of this chapter is to provide a review of studies describing the toxicity of various organic and inorganic contaminants on the photosynthetic apparatus of aquatic microorganisms, such as algae. It describes the biochemical mode of action of each organic and inorganic pollutant concerning the disruption of photosynthesis, discusses the methods that have been employed for its analysis, compares the sensitivities of tested algal species to various toxicants, comments on the ecological relevance of the findings, and declines areas where future research is needed to be conducted.

2. Photosynthesis

Photosynthesis is an energy transformation process that converts light energy into chemical energy and is carried out by phototrophic organisms. Photosynthesis involves a series of

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biochemical and biophysical reactions occurring simultaneously in photosynthetic organisms (plants, algae, and cyanobacteria) that are always starting with the absorption of photons and ending with the incorporation of inorganic carbon into stable organic compounds called carbohydrates, such as sugars. The process of photosynthesis can be divided into two phases: the light reactions and the light independent or dark reactions. The light-dependent reactions of photosynthesis are mediated by four large protein complexes (also referred as supra-molecular complexes), embedded in the thylakoid membrane of the chloroplast: Photosystem I (PSI), Photosystem II (PSII), Cytochrome b_6/f Complex, and adenosine triphosphate (ATP) synthase [4]. In brief, light reactions involve the excitation of electrons of chlorophyll (chl) molecules within the PSII Complex to a higher energy state, which is the excited triple state (*chl^3). This energy is harvested in the formation of several ATP molecules from ADP and inorganic phosphorus. In the PSI Complex, a similar excitation of electrons occurs, with the energy harvested to form reduced nicotinamide adenine dinucleotide phosphate (NADPH) from $NADP^+$. The electron transfer processes involved in the light-dependent reactions of photosynthesis are depicted in **Figure 1**, which is also known as Z-scheme of photosynthesis.

Algae during the light-dependent reactions of photosynthesis that take place in chloroplasts use pigment chl to absorb light, split the molecule of water, and therefore produce oxygen gas, and energy storage compounds of NADPH and ATP. Despite the fact that algae constitute a large, diverse, and polyphyletic group of organisms that exhibit enormous variations in morphology and physiology, the most important common biochemical attribute that unites photosynthetic algal species is their ability to perform photosynthesis.

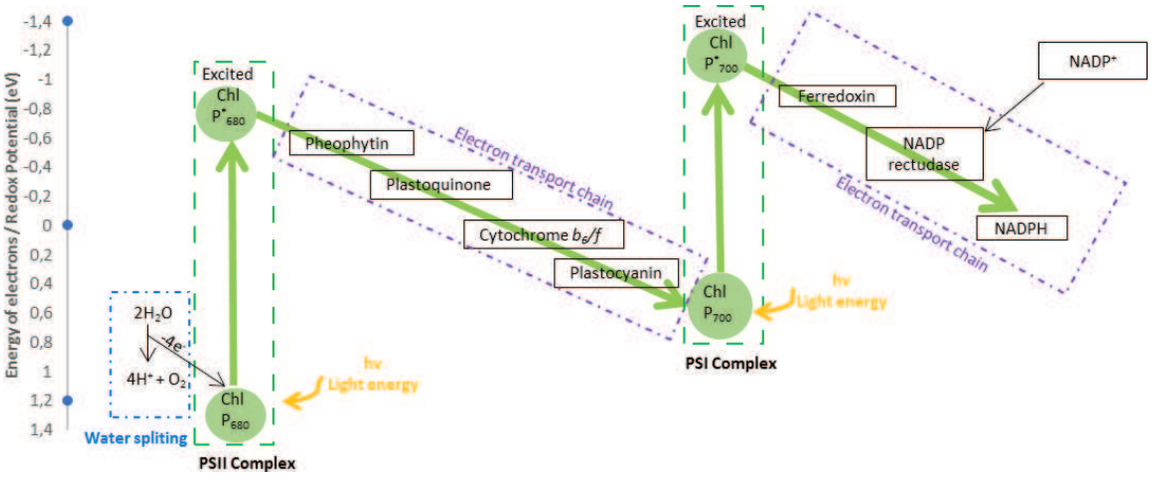

Figure 1. The Z-scheme of electron transfer processes involved in the light-dependent reactions of photosynthesis.

3. Methodologies of algal photosynthesis inhibition tests

Historically since the early 1900s, a variety of toxicity tests using algal species as exposed organisms have been performed for the evaluation of phytotoxic effects of several types of

potential toxicants on aquatic inhabitants (including commercial chemicals, industrial and municipal effluents, and hazardous wastes). In the early 1970s and after taking into account the enormous ecological importance of bioassays, a number of regulatory and standard development agencies such as the Organization for Economic Cooperation and Development (OECD), International Standards Organization (ISO), European Economic Community (EEC), American Public Health Association (APHA), American Society for Testing and Materials (ASTM), and US Environmental Protection Agency (USEPA) developed and standardized phytotoxicity test methods. Current test methods are designed under the assumption that effects can be studied by three general approaches: (I) in a controlled laboratory experiment with limited number of variables, (II) in an experimental model ecosystem (indoor or outdoor simulator), and finally (III) in a natural ecosystem (*in situ*) [5].

Cause and effect relationships of specific chemicals to different types of target species are easily studied by the conduction of single-species laboratory-controlled experiments. The various methodologies of single-species bioassays differ slightly in design, but basically they utilize a uni-algal population of an available, easily cultivated, and sensitive algal test species (based on these criteria several microalgae have been recommended as standard test species, such as *Selenastrum capricornutum*), which is exposed during its log-growth phase to a range of concentrations of the toxicant [6].

The main disadvantage and limitation of single-species bioassays is the fact that they focus on assessing the effects of toxicants on single species and are performed under controlled laboratory conditions which are considerably different from the conditions of a realistic environment. In natural aquatic ecosystems, many complex species interactions and environmental influences and changes that cannot be simulated in laboratory studies continually occur. Other types of laboratory-conducted toxicological studies and beyond the level of single-species test are the multispecies tests and the small ecosystem tests, which are also called laboratory microcosms, and involve small-scale enclosures that contain natural samples (water, sediment, and algae) providing a simple simulation of natural systems. Phytoplankton and periphyton are the flora utilized in most multispecies toxicity tests [7].

Natural field studies or natural aquatic ecosystems tests (pond, stream, lake, or estuary) are defined as those in which both the test system and exposure to the stressor are naturally derived [8]. Field tests are very important and reliable for evaluating and understanding the biological and ecological effects of chemicals under real environmental conditions. Outdoor microcosms or mesocosms are simulated field studies that are composed of either an isolated subsection of the natural aquatic reservoir or a man-made physical model of an aquatic ecosystem, whereas the test systems are manually treated with the test chemical at predetermined test concentrations [8]. In general, the utilization of microcosms and mesocosms for assessing the effects of toxicants can reduce the possibility of an inaccurate estimation of the adverse effects of pollutants on aquatic species belonging to different ecological categories [9].

Photosynthetic activity is considered as a significant effect parameter of a variety of toxicants on algae (physiological and morphological effects). The primary advantage of photosynthesis tests is their short duration, which is usually 2–4 h, but exposure times have also ranged from 30 min to 24 h [7]. Therefore, the inhibitory and stimulatory effects of many organic and inorganic compounds on algal photosynthesis have been determined in laboratory and field studies. According

to an extended published literature, several algal biochemical parameters linked to photosynthesis process such as ATP formation, CO_2 fixation, O_2 evolution, carbon uptake (^{14}C), and chlorophyll content have been adopted as traditional and classical indicators for the evaluation of environmental stresses caused by many classes of various contaminants on photosynthetic algal species [10].

A great progress in the area of algal photosynthesis research has been made during the last decades. Based on the fact that a proportion of the absorbed light energy in PSII photochemistry cannot be used to drive electron transport and is dissipated via non-radiative energy as heat or chlorophyll fluorescence emission associated with the PSII complex [2, 11–15], information about changes in the efficiency of photosynthesis can be acquired by measuring the yield of Chl-*α*-fluorescence [2, 16]. Chl-*α*-fluorescence is a physical signal defined as the radiative energy evolved from de-exciting Chl-*α*-molecules (λ = 690 nm for PSII, λ = 740 nm for PSI) [17] that has been used as a rapid, non-intrusive, and highly sensitive bioindicator of algal stress in response to different chemicals in recent years [2, 18, 19]. Apart from their utility in determining the physiological status of photosynthesizers in the natural environment, Chl-*α*-fluorescence-based methods are applied in ecophysiological and toxicological studies [2]. Among the various fluorescence techniques, pulse amplitude modulation (PAM) fluorometry, introduced by Schreiber et al. [11], has been demonstrated as a rapid, non-invasive, reliable, economically feasible, time-saving, and accurate technique, well suited for investigating changes in photochemical efficiency of aquatic algae, that permits *in vivo* non-destructive determination of changes in the photosynthetic apparatus much earlier than the appearance of visible damage [19]. Several types of PAM are known including the Maxi Imaging-PAM, Diving PAM, and ToxY-PAM fluorometer [2]. Numerous articles provide the efficiency of several Chl-*α*-fluorescence parameters that have been employed in assessing the effects of toxicants or their combinations on microalgae and macroalgae (seaweeds). Detailed definitions of certain Chl-*α*-fluorescence parameters along with their photosynthetic importance are available in the literature [16, 20–22]. The most commonly used Chl-*α*-fluorescence key parameters that are becoming recognized as valid sub-lethal indicators of photosystem stress and have been used to examine the sub-lethal toxicity of toxicants toward a variety of microalgae are maximum quantum yield (F_v/F_m), effective PSII quantum yield ($\Phi_{PSII,}$ or Φ_m or $\Delta F/F_{m'}$), operational PSII quantum yield (Φ'_{PSII} or Φ'_m), proportion of open PSII (*qP*), non-photochemical quenching (*NPQ*), and electron transport rate (*ETR*) [2, 23–26]. Hence, new types of devices of dual-channel PAM Chl fluorometers have been developed, which are specialized in the detection of extremely small differences in photosynthetic activity in algae or thylakoids suspensions. In conjunction with standardized algae cultures or isolated thylakoids, they provide an ultrasensitive bioassay system occupied frequently for the detection of toxic substances in water samples [24, 27]. Furthermore, many studies have directly compared the sensitivity of Chl-*α*-fluorescence end points to traditional indicators of organic and inorganic chemical stress on algae; these surveys include herbicides [26], antifouling agents, organometallic compounds [28], and metals [29].

4. Oils, dispersants, and dispersed oils

Naturally occurring raw or unprocessed crude oil and petroleum products are both included in the term "petroleum." Petroleum is a mixture of hydrocarbons of various molecular weights

(most of which are alkanes, cycloalkanes, and various aromatic hydrocarbons), other organic compounds containing nitrogen, oxygen, and sulfur, and trace amounts of metals such as iron, nickel, copper, and vanadium. Hence, crude oil is a highly toxic compound comprising a mixture of up to 10,000 different types of hydrocarbons, both aliphatic and aromatic, which produce great damage to aquatic ecosystems [30]. On the other hand, processed and refined petroleum products include a large number of fuels, lubricants, and petrochemicals, such as gasoline, kerosene, diesel, paraffin wax, and many others that can cause important environmental contamination if released in ecosystems.

Hydrocarbons in aquatic environments have biogenic, natural geologic, and anthropogenic origins such as oil spills (releases of crude oil from tankers, offshore platforms, drilling rings, as well as spills of refined petroleum products and their by-products, or spills of any oil refuge or waste oil) [31–33]. Adverse effects resulting from spilled oil can be a result of (I) dissolved materials, (II) physical effects due to contact with oil droplets, (III) enhanced uptake of petroleum hydrocarbons through oil/organism interactions, or (IV) a combination of these factors [34]. Besides all the above, the insoluble and mainly the soluble fractions of oil reduce light penetration into the water column affecting phytoplankton photosynthesis process [35].

The ecological effects of accidental oil spills have been the subject of relevant laboratory and field research. Since the decade of 1950s, it has been known that crude and refined oils are phytotoxic [32], whereas the scientific interest concerning the sub-lethal effects of oils and their components on enzyme systems, photosynthesis, respiration, and protein and nucleic acid synthesis of primary producers is steadily increasing nowadays. According to published scientific data, it is demonstrated that toxic effect concentrations for oils and algae vary greatly. As previously reported in a recent review paper, the toxic effect concentrations range is between 0.002 and 10,000 ppm for crude oils and between 0.09 and 50 ppm for refined oils [32].

Based on information presented in the same bibliographic review of Lewis et al. on toxicity of oils, dispersants (mixtures of emulsifiers and solvents that break an oil slick into smaller droplets of oil), and dispersed oils toward algae and aquatic plants, 22 species of freshwater and 63 species of saltwater algae have been exposed to more oils (21) and dispersants (27) than any other type of aquatic plant [32]. This numeric example shows that even though damage may occur from low-level continuous discharges to both freshwater and saltwater environments, however, the environmental effects of large oil spills to marine waters have received the most attention by the public and regulatory and scientific communities resulting in the imbalance of entries in toxicity databases. Some of the available literature data concerning the toxicity of several types of oil or individual hydrocarbons on the photosynthetic apparatus reported for various algae are presented in **Table 1**.

The effects of crude oils and oil components on algae have been widely studied [43, 47–55], and among the different employed response parameters the effects on photosynthetic activity were included [43, 56, 57]. For that purpose, several algal species have been exposed to crude oils, fuel oils, dispersants, and dispersed oils not only in uni-algal cultures grown under laboratory-controlled conditions but also *in situ* as well by short- and long-term studies using microcosms, or mesocosms and mostly in short-term laboratory experiments. Toxicology studies

Test compounds	Test species	Observed stress response	References
Crude oils: South Louisiana, Kuwait, Venezuela, and Alaskan Diesel fuel oils No. 2: Amer. Petrol. Institute, Baton Rouge, Baytown, Montana, New Jersey	*Agmenellum quadriplicatum* *Chlorella autotrophica* *Cylindrotheca* sp.	Fuel oil: lethal at 10 mL $(20\ mL)^{-1}$. Crude oils: not toxic at 30 mL $(20\ mL)^{-1}$. Photosynthesis of *Chlorella autotrophica* was only temporarily depressed by the crude oils at 30 mL $(20\ mL)^{-1}$. Four of the fuel oils inhibited photosynthesis, O_2 output decreasing to zero without recovery (exception: Montana fuel oil).	Batterton et al. (1978) [36]
Crude oils: Atkinson Point, Norman Wells, Pembina, and Venezuela Corexit (unnamed)	*Laminaria saccharina* *Phyllophora truncata*	*In situ* primary production was significantly inhibited by all types and concentrations of oil tested (at 10 ppm). Inhibition generally increased with increasing oil concentration. The crude oil-Corexit mixtures were more toxic than crude oil or Corexit alone.	Hsiao et al. (1978) [37]
Coal liquefaction, shale-oil and petroleum products	*Selenastrum capricornutum* *Microcystis aeruginosa*	Based on ^{14}C assimilation measurements, the coal-liquefaction products inhibited algal photosynthesis at water-soluble fractions concentrations two orders of magnitude lower than the petroleum products; shale-oil products were intermediate in toxicity.	Giddings and Washington (1981) [38]
Crude oil: Tunisian	*Skeletonema costatum*	Toxicity is related to nutrient limitation conditions. 100 mg L^{-1} lethal in P and N limited media, and less severe in the Si-limited media. Chl-α and carbon uptake more sensitive parameters for assessing hydrocarbon toxicity than cell counting.	Karydis (1981) [39]
Crude oil: Ekofish	*Skeletonema costatum* *Phaeodactylum tricornutum* *Chaetoceros ceratosporum*	*S. costatum* and growth rate most sensitive than chlorophyll content per cell and the ratio of in vivo fluorescence to chlorophyll content.	Ostgaard et al. (1984) [40]
BP light diesel BP 1100X BP 1100WD Shell Oil Herder	*Chlorella salina*	Stimulatory effects on photosynthesis by low levels of BP light diesel (0.05%) and the oil dispersant BP 1100X (0.005%), either alone or in mixture. Inhibition of Chl-α content at higher levels of BP light diesel, BP 1100X and at all the tested concentrations of oil dispersants BP 1100WD and Shell Oil Herder.	Chan and Chiu (1985) [41]

Test compounds	Test species	Observed stress response	References
Crude oils: Ekofisk and Stratjford	*Skeletonema costatum* *Thalassiosira pseudonana* *Phaeodactylum tricornutum*	Reduced photosynthetic capacity. Highest sensitivity: *S. costatum*. Similar results by lab batch and *in situ* dialysis culture.	Hegseth and Ostgaard (1985) [42]
Crude oil: Norman Wells Corexit 9550	St. Laurence Estuary phytoplankton (*in situ* dosing)	Chl-α reduced at oil exposure concentration of 1–2 mg L^{-1}; No observed affection in marine community composition.	Siron et al. (1993) [43]
Diesel fuel oil No. 2: American Petroleum Institute	*Selenastrum capricornutum*	In terms of Chl-α content: 3d EC_{50} = 0.015 g L^{-1}; 5d EC_{50} = 0.014 g L^{-1}; 7d EC_{50} = 0.0156 g L^{-1}.	El-Dib et al. (1997) [44]
Chrysene (water soluble PAH)	Microcosms	Photosynthetic activity and chlorophyll-α concentration decreased after 24–72 h.	González et al. (2009) [35]
Oil samples from the tanker *Prestige* spill	*Dunaliella tertiolecta*	Significant inhibition of photosynthesis (based on F_v/F_m, ETR_{max}, and photosynthetic efficiency α-values) after only 1 h of oil exposure with clear concentration dependency. After 3 d, photosynthesis remained inhibited although cell survival was only slightly effected.	Carrera-Martinez et al. (2010) [30]
Eight groups of crude oil	Marine phytoplankton community	High concentrations of oil (≥2.28 mg L^{-1}) of decreased Chl-α content.	Huang et al. (2010) [45]
Accidental oil spill in Mumbai Harbor	Natural periphyton	*In situ*: significant decrease in phytoplankton population, inhibition of photosynthesis associated with degradation of pigments (increase in phaeophytin).	Jaiswar et al. (2013) [46]

Note: *Pseudokirchneriella subcapitata*, known as *Selenastrum capricornutum*.

Table 1. Examples of oils and hydrocarbons toxicity on the photosynthetic apparatus reported for various algae. Reports in chronological order.

conducted with photosynthetic aquatic communities usually indicate a shift of species composition and abundance after an oil spill due to the replacement of sensitive species by resistant ones (observations of short-term studies) [58]. Long-term studies in most cases reported cascades of late, indirect impacts on coastal communities due to chronic exposures to environment-sequestered petroleum products that delayed ecosystem recovery for years after an oil spill [59, 60]. Results of phytoplankton community studies are quite variable depending on characteristics of the oil, characteristics of the exposed algal species, influence of dispersants, type of ecosystem affected, dynamics of water masses, and numerous other variables [60, 61]. Therefore, the ecological impact following an oil spill depends on the volume spilled, oil type, geographical location of the spill, the characteristics of the receiving water, and its biota (e.g., sensitivity of organisms), and duration of contact with oil [62].

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Short-term laboratory experiments, using laboratory-tolerant taxa and model experimental designs, have also been performed in order to evaluate more specifically the effects of different petroleum products on algal photosynthesis. Toxicity data obtained from laboratory assays indicate that toxic effects depend on the phytoplanktonic species, the group of oils involved, and the physical characteristics of the water, such as concentrations of dissolved organic compounds, temperature, salinity currents, redox potential, and nutrient loading [60].

In general, responses of microscopic photosynthesizers to oil are diverse [63]. In some case studies, growth rate has been shown as a more sensitive end point parameter than photosynthetic activity [40], whereas in others Chl-α-content and carbon uptake were more sensitive parameters for assessing hydrocarbon toxicity than cell counting [39]. In our knowledge, the dominant effect observed on photosynthetic activity after exposure to petroleum hydrocarbons is inhibition, while stimulation effects at low exposure levels of the toxicants have been also reported [37, 41, 64].

These findings are in accordance with the observations that microalgae have the capability to grow in the crude oil-contaminated environments, such as in the case of the rapid adaptation of mesophile species to crude oil of the Arroyo Minero River (Argentina) [30]. Hence, microalgae are able to survive in adverse environments as a result of physiological acclimation due to the modification of gene expression [30]. However, when values of environmental stress exceed physiological limits, survival depends exclusively on adaptive evolution, which is supported by the occurrence of mutations that confer resistance [30].

5. Pesticides

Pesticides are phytotoxins that are widely used all over the world in agriculture to kill unwanted vegetation. Pesticides are defined as substances or mixtures of substances intended for controlling, preventing, destroying, repelling, or attracting any biological organism deemed to be a pest. Insecticides, herbicides, defoliants, desiccants, fungicides, nematicides, avicides, and rodenticides are some of the many categories of pesticides. Many members of these compounds are very selective and are applied against certain target species, whereas many others are completely nonselective and thus effective to almost every species of plants acting as wide-spectrum molecules.

Paradoxically, these substances do not always remain in agricultural soils where they are applied for crop protection and fruit tree treatment, but sometimes they find their way into aquatic systems through leaching, surface runoff, spray-drift, soil erosion, and volatilization. Estimates indicate that the average agricultural herbicide loss is around 1% of the applied volume [27, 65]. In addition, millions of pounds of active pesticide ingredients are applied in coastal watersheds each year and that way pesticides may affect marine inhabitants via spills, runoff, and drift [66]. As a consequence, aquatic reservoirs receive direct and indirect pesticide inputs, inevitably exposing microorganisms to pesticides.

Pesticides have been classified by scientists according to their mechanisms of action. Photosynthetic inhibitors include many chemical groups of herbicides that disrupt photosynthesis pathways by four basic mechanisms that are summarized in **Figure 2**.

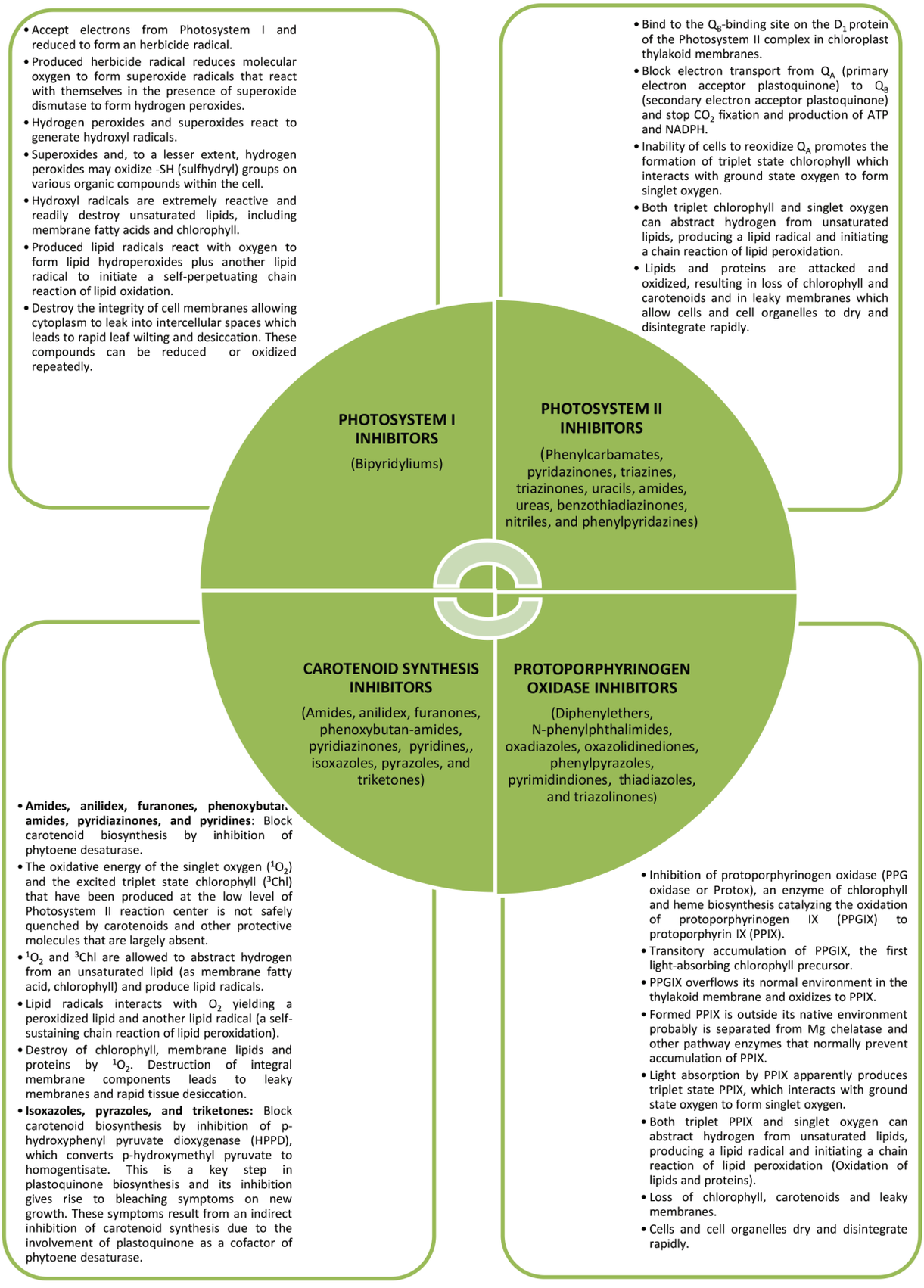

Figure 2. Photosynthetic inhibitors and their mechanism of action.

In this point, it must be mentioned that even though the majority of the pesticides is designed to and produced in the market with the assumption that they directly affect only one primary molecular site of action in the target organism; however, many of these compounds can cause a cascade of secondary and tertiary effects as well. For example, it has been found that most photosynthetic inhibitors also can affect plant respiration at higher doses [67]. Oxidative stress can also occur as a secondary effect of PSII inhibitors [68].

Furthermore, many non-photosynthetic inhibitors have been found to have an effect on photosynthetic process of various algal species. The herbicide flazasulfuron, a member of the chemical group of sulfonylureas, which are known to cause inhibition of amino acid synthesis, belongs to that case; bioassays conducted with the freshwater algae *Scenedesmus obliquus* revealed reduction in chlorophyll content at exposure concentration of 10 $\mu g\ L^{-1}$, while the increase of pigment content was reduced with the lowest tested level of exposure (0.1 $\mu g\ L^{-1}$) [69]. Moreover, studies of pesticide effects on algae showed that some pesticides can inhibit photosynthesis process with two independent mechanisms. For example, it has been reported that fluometuron, a substituted phenylurea compound, not only inhibited the production of Chl pigment in the unicellular algae *Chlorella pyrenoidosa* and *Euglena gracilis* but also blocked the biosynthesis of carotene via a process known as bleaching [70].

A broad base of toxicity data involving ecotoxicology of several classes of herbicides toward non-target microorganisms is available. Numerous reports have elaborated the impacts of various herbicides to algal photosynthetic activity. However, due to limited extent only few of them are selected to be presented herein this chapter. Therefore, only some of the available data in the literature are summarized in **Table 2** so as to depict the wide range among exposed algal species and among the employed photosynthesis parameters.

Algal species vary considerably in sensitivity to herbicides stress, and several factors may contribute to species-specific sensitivity including pigment type and photosynthetic capacity, cellular lipid and protein content, and cell size [71]. For instance, tolerance to atrazine has been linked to cell size in microalgae [71], whereas increased atrazine sensitivity to cell biovolume was observed, with smaller species being more sensitive to the herbicide [72]. What is more, algal subcellular responses to herbicides have been found to be also species dependent. In general, chlorophytes are considered to be more sensitive than bacillariophytes when comparing herbicide toxicity across phyla [73]. It has been well established that environmental parameters (light exposure, nutrient concentrations, etc.) interfere in the responses of algal communities to pesticides [74, 75]. As reported in reference [74], diatoms were more sensitive to atrazine during light exposure, suggesting that in the context of light, the response of algae depends on the season of study and on the site where samples are taken [76]. Light history has previously been implicated in periphytic (attached) microalgae, with shade-adapted (generally diatom-dominated) communities less susceptible than sun-adapted (chlorophyte-dominated) communities [74].

Additionally, in some species, results of algal bioassays may vary significantly based on the end point selected. As reported in a published comparative study of four estuarine microalgal species, a planktonic chlorophyte (*Dunaliella tertiolecta*), a benthic chlorophyte (*Ankistrodesmus* sp.), a cryptophyte (*Storeatula major*), and a dinoflagellate (*Amphidinium*

Pesticide (Chemical class)	Test species	Exposure conditions, observed stress response and findings	References
Glyphosate (Organophosphate)	Periphytic algal communities from 6 small forest ponds	Short-term carbon assimilation. Exposure range: 0.89–1800 mg L^{-1}. Photosynthetic activity decreased with increasing herbicide concentration in most ponds. Range of EC_{50} values: 8.9–89 mg L^{-1}.	Goldsborough et al. (1998) [77]
Flazasulfuron (Sulfonylurea)	*Scenedesmus obliquus*	24 or 48 h at 0.1–1000 μg L^{-1} (Chl-*α* and -*b*, carotenoids content): Reduction in chls content at 10 μg L^{-1}, while the increase of pigment content was reduced with the lowest tested level of exposure (0.1 μg L^{-1}). Among the three pigments studied Chl-*α* was the more sensitive biomarker.	Couderchet and Vernet (2003) [69]
Atrazine (Triazine)	*Dunaliella tertiolecta* *Ankistrodesmus* sp. *Storeatula major* *Amphidinium operculatum*	Nominal concentrations of atrazine tested: 0, 12.5, 25, 50, 100, and 200 μg L^{-1}. Atrazine significantly decreased cell density, productivity rate, biomass, and biovolume in all the algal populations tested at atrazine concentrations ≥12.5 μg L^{-1}. Based on photosynthetic carbon assimilation: *D. tertiolecta*: EC_{50} = 66.81 μg L^{-1}; *Ankistrodesmus* sp.: EC_{50} = 37.07 μg L^{-1}; *Storeatula major*: EC_{50} = 22.17 μg L^{-1}; *A. operculatum:* EC_{50} = 33.07 μg L^{-1}; Based on photosynthetic pigments content: *D. tertiolecta*: EC_{50} = 65.00 μg L^{-1}; *Ankistrodesmus* sp.: EC_{50} = 11.87 μg L^{-1}; *Storeatula major*: EC_{50} = 45.81 μg L^{-1}; *A. operculatum:* EC_{50} = 146.71 μg L^{-1}.	DeLorenzo et al. (2004) [71]
Cypermethrin (Pyrethroid)	*Scenedesmus obliquus*	96 h at 50–250 mg L^{-1} (Chl-*α* and -*b*, carotenoids content): Decreased contents of chls and carotenoids. Carotenoids production more sensitive than the ratio of Chl-*α*/Chl-*b*.	Li et al. (2005) [79]
Atrazine, simazine, hexazinone (Triazine) and diuron (Urea)	*Phaeodactylum tricornutum*	Based on PSII quantum yield: Atrazine: IC_{10} = 4.4 μL L^{-1}; Simazine: IC_{10} = 29.0 μL L^{-1}; Hexazinone: IC_{10} = 2.7 μL L^{-1}; Diuron: IC_{10} = 0.74 μL L^{-1}	Bengtson Nash et al. (2005) [27]
40 herbicides from 18 chemical classes and 9 modes of action	*Raphidocelis subcapitata*	EC_{50} with respect to the photosynthetic processes ranged from 0.0007 to 4.2286 mg L^{-1}. Descending order of the average acute toxicity was photosynthetic process>cell division>lipid synthesis, acetyl-coenzyme A carboxylase>acetolactate synthase> 5-enolpyruvyl-shikimate-3-phosphate-syntha-se, glutamine synthase, hormone synthesis>protoporphyrinogen oxidase.	Ma et al. (2006) [88]

Pesticide (Chemical class)	Test species	Exposure conditions, observed stress response and findings	References
Atrazine (Triazine), diuron, isoproturon (Ureas), paraquat dichloride (Bipyridinium)	*Selenastrum capricornutum*	Based on: $\Phi_{m'}$, $\Phi'_{m'}$, *NPQ* (1.5 h), $F_{684'}$ F_{735} (30 min: Atrazine at concentrations 1.0–500 μg L^{-1} range or IC_{50} = 71.7–205.2 μL L^{-1}; Diuron at concentrations 0.2–100 μg L^{-1} range or IC_{50} = 7–12.3 μL L^{-1}; Isoproturon at concentrations 0.4–3.875 μg L^{-1} range or IC_{50} = 38.7–59.7μL L^{-1}; Paraquat dichloride at concentrations 2.0–1000 μg L^{-1} range of IC_{50} = 65.5–104.7 μL L^{-1}.	Fai et al. (2007) [10]
Diuron, hexazinone and atrazine (triazine/ triazinone)	*Navicula* sp. *Nephroselmis pyriformis*	The relationships between $\Phi_{PSII'}$ growth rate, and biomass increase were consistent ($r^2 \geq 0.90$) and linear (1:1). Order of toxicity (EC_{50} range) was diuron (16–33 nM) > hexazinone (25–110 nM) > atrazine (130–620 nm) for both algal species.	Magnusson et al. (2008) [26]
Mixture of diuron (phenylurea) and tebuconazole (triazole)	Natural periphyton in two series of two lotic outdoor mesocosms	The effects of pulsed acute exposures to pesticides on periphyton depended on whether the communities had previously been exposed to the same stressors or not.	Tlili et al. (2011) [89]

Note: *Pseudokirchneriella subcapitata*, known as *Selenastrum capricornutum*.

Table 2. Examples of pesticides toxicity on the photosynthetic apparatus reported for various algae. Reports in chronological order.

operculatum), which were exposed to atrazine, significant differences in sensitivity were observed depending on the test end point used. Chlorophyll-*α* was a significantly more sensitive test end point for *Ankistrodesmus* sp., biovolume was a significantly more sensitive test end point for *A. operculatum*, and phototrophic carbon assimilation was a significantly more sensitive test end point for *S. major* and *A. operculatum* [71]. In the same survey, it is suggested that species with greater Chl-*α* per cell are expected to be less sensitive to PSII inhibitors, because Chl-*α* is directly related to the amount of PSII in the cell, which is the primary biochemical target of such insecticides, and hence the more photosynthetic targets available, the more pesticide would be required to block it [71].

A dose-dependent inhibition of photosynthetic activity of algae has been reported in cases of single species [10, 27, 71] and as well as in periphytic algae exposures to a range of insecticides concentrations [77, 78].

According to the bibliographic data, available pigments content has often been used as a classic biomarker of exposure to pesticides in plants including algae and phytoplankton [69, 79, 80]. In other cases of published ecotoxicology studies evaluating the inhibition of photosynthesis by PSII inhibitors, Chl-*α*-fluorescence parameters were selected instead as test end points, emphasizing the precision and time-saving virtues of the technique [10, 24, 81]. For example, the inhibition of effective quantum yield (Φ_{PSII} or $\Delta F/F_{m'}$) has been used by many authors in

order to examine the sub-lethal toxicity of herbicides toward a variety of microalgae, with some being sensitive to diuron at environmentally relevant concentrations [24, 25, 27]. Similar sensitivities were measured using ^{14}C uptake in benthic microalgae in temperate waters [82].

Taking into account the possible interactions between substances in combination, many mixture ecotoxicological experiments were performed using binary or ternary combinations of herbicides [83, 84]. Furthermore, a large body of literature data is available concerning the prediction of the joint effect of mixtures of pesticides based on their individual impacts and specific modes of action [85, 86]. Concentration addition (CA) and independent action (IA) model are the most commonly used models to predict mixture effects for similar- and dissimilar-acting compounds, respectively. Both theories assume enhanced effects with an increasing number of compounds and non-interaction between substances. Therefore, a deviation from the prediction indicates antagonism (weaker effects than predicted) or synergism (stronger effects) [87].

Pesticides are probably the most well-studied chemical group within ecotoxicological mixtures studies. This is not only due to the use of chemical mixtures in pesticide formulations and tank mixtures and the resulting co-occurrence in agricultural areas, but just as much because of the in-depth knowledge of their physiological mode of action [87]. These facts make them ideal candidates for testing mixture models based on the chemical mode of action and understanding the physiological mechanisms behind possible interactions [85, 90]. Mixture toxicity studies focused on single species [85, 86], natural communities in laboratory experiments [3, 82, 91], or outdoor microcosms and mesocosms [83, 92–94] data. Many reviews and critical analysis have shown that synergistic interactions within pesticide mixtures and realistic low-dose chemical mixtures in species are a rather rare phenomenon, constituting very low percentages of the tested mixture combinations and often occurs at high concentrations [87, 95–101]. According to the results of a comprehensive systematic review in which cocktail effects and synergistic interactions of chemicals in mixtures were predicted, synergy phenomena occurred only in 7% of the 194 binary pesticide mixtures included in the data compilation on frequency [101] (the database of Belden et al. [98] provided data on 207 pesticide mixtures of which 194 were binary and another 13 consisted of more than two pesticides). Results of the same study showed that PSII herbicides did not induce synergy in any of the 33 mixtures performed on algae in the pesticide database [101].

6. Antifouling biocides

Antifouling biocides are chemical substances that deter the microorganisms responsible for biofouling. Biofouling or biological fouling is the accumulation of microorganisms, plants, algae, or animals on wetted surfaces; hence, it can occur almost anywhere where water is present (marine vessels, swimming pools, drinking water and liquid lines for cooling electronics, medical devices and membranes, etc.). Biofouling takes place on surfaces after the formation of a biofilm that creates a surface onto which successively larger microorganisms can attach. Specifically designed antifouling materials and coatings/paints have the ability to remove or prevent biofouling by any number of organisms on such surfaces.

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Antifouling biocides are introduced to antifouling paints in order to improve their efficacy against photosynthetic organisms [2]. The biocides often target the microorganisms which create the initial biofilm, typically bacteria. Other biocides are toxic to larger organisms in biofouling, such as the fungi and algae. Many different booster biocides have been currently added to antifouling paints including tributyltin (TBT), 2-methylthio-4-tetr-butylamino-6-cyclopropylamino-s-triazine (Irgarol 1051), 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (Sea-Nine 211), diuron, cuprous oxide, chlorothalonil, zinc pyrithione, dichlofluanid, 2,3,3,6-tetrachloro-4methylsulfonyl (TCMS), pyridine, 2-(thiocyanomethylthio) benzothiazole (TCMTB), and zineb [102].

One of the most commonly used biocides, and anti-fouling agents, is TBT. It is toxic to both microorganisms and larger aquatic organisms [103]. The mechanism of action of the TBT in algae is based on its interference with energy metabolism in chloroplasts and mitochondria, but it is also shown that TBT interacts with proteins and membranes and binds to or interacts with any protein containing free sulfhydryl groups [3, 104]. Bioassays conducted with the marine algae *Tetraselmis suecica* revealed that in chronic exposure to TBT, at higher concentrations (0.5–1 μg mL^{-1}) growth rate, chlorophyll pigments, carbohydrate, and protein contents were reduced [105]. Different responses have been described among three species of marine microalga *T. tetrathele*, *Nannochloropsis oculata,* and *Dunaliella* sp., which were exposed to three concentrations of TBT (0.1, 0.5, and 1μg L^{-1}). For *T. tetrathele*, exposure to TBT resulted in an increase of chlorophyll contents, even up to 210 and 225% at highest concentration of TBT (1μg L^{-1}) for chlorophyll α and b, respectively. However, acquired results for the other two algal species, *N. oculata* and *Dunaliella* sp., showed that stimulation effects occurred only at the lowest concentration tested (0.1 μg L^{-1}), as chlorophyll contents decreased at higher exposure levels, whereas *N. oculata* was the most sensitive microalga [106]. Similar results had been published in a previous study by Sidharthan et al. in which photosynthetic pigment content of the marine eustigmatophyte *N. oculata* was significantly affected, especially at elevated TBT concentrations. The same authors found that Chl-α content decreased more than 50% at TBT concentrations above 0.50 nM level, whereas at high concentration of 4 nM both the pigments were completely leached. Comparatively, carotenoid content was less inhibited by TBT toxicity (r = 0:917; $P < 0.05$) [107]. Reduction (60%) in the net photosynthetic activity of *Ruppia maritina* (seagrass) in TBT-spiked and impacted sediments was measured [108]. In a microcosm approach survey that was designed to study the combined effects of TBT from antifouling paints and ultraviolet-B radiation (UVBR: 280–320 nm), on a natural planktonic assemblage (<150 μm) isolated from the St. Lawrence Estuary (eastern Canada), it was demonstrated that phytoplankton cells were affected in their physiological functions, such as their photosynthetic efficiency. According to the obtained experimental data, the reduction in the maximum quantum yield (F_v/F_m) values were due to damage of PSII reaction centers and inhibition of ATP synthesis. Moreover, results clearly showed that the combination of TBT and UVBR stresses has synergistic effects affecting the first trophic levels of the marine food web [28]. Finally, the inhibition of photosynthesis of periphyton community has been observed after exposure to TBT (EC_{50} = 0.02 mg L^{-1}) [3].

Irgarol 1051 is a triazine herbicide that has been described as an inhibitor of algal photosynthesis. More specifically, it belongs in PSII inhibitors, as it results in oxidative stress, including photooxidation of chlorophyll [109], and inhibition of the photosynthetic electron transport in

chloroplasts by binding to the D_1 protein [110]. Irgarol 1051 was introduced after the restrictions on using TBT in antifouling paints (as a replacement) [111] and has found its application as an algicide in antifouling paints for boats and vessels. Irgarol is the most hydrophobic compound of the family of the triazines due to the presence of both tert-butyl group and the cyclopropyl group [102]. It is mainly used in combination with copper [3] and is the most frequently detected antifouling biocide worldwide [102]. Even though Irgarol 1051 is a relatively new compound, several papers have been published in the last years dealing with its ecotoxicological behavior toward non-target microorganisms. For example, in algal symbionts isolated from *M. mirabilis, D. strigosa*, and *F. fragum* 40–50% reduction of net ^{14}C incorporation has been demonstrated after their 6-h exposure to 10 mg L^{-1} of Irgarol 1051 [112]. Inhibition of the algal photosynthetic activity of several algal species including *D. tertiolecta, Synechococcus* sp., *E. huxleyi, Fucus vesiculosus, Enteromorpha intestinalis*, *Ulva intestinalis,* and seagrass *Z. marina* by Irgarol 1051 has been summarized [113]. In addition, the destruction of periphyton photosynthesis process after exposure to the same biocide has been demonstrated (EC_{50} = 0.82 nM) [114]. According to the available data, Irgarol 1051 has the potential to affect the F_v/F_m of phytoplankton even at very low (0.03 μg L^{-1}) environmentally relevant concentrations [115]. This conclusion is in accordance with the assumption that Irgarol 1051 concentration up to 0.23 mg L^{-1} negatively impacted the photosynthetic activity of the green alga *U. intestinalis* [116]. The effect of Irgarol on the values of several Chl-*α*-fluorescences parameters for numerous freshwater and marine algal species has been reported including the following data: according to F_v/F_m values: EC_{50} = 0.33 mg L^{-1} for *T. weissflogii*; EC_{50} = 0.60 mg L^{-1} for *E. huxleyi*; EC_{50} = 0.23 mg L^{-1} for *Tetraselmis* sp.; EC_{50} = 0.11 mg L^{-1} for *F. japonica* [117], reduction of F_v/F_m values in the presence of high concentrations for *Potamogeton pectinatus* [118]; whereas according to Φ_{PSII} or $\Delta F/F_{m'}$ values: 72 h EC_{50} = 0.327 mg L^{-1} for *T. weissflogii*; 72 h EC_{50} = 0.604 mg L^{-1} for *Emiliania huxleyi*; 72 h EC_{50} = 0.230 mg L^{-1} for *Tetraselmis* sp.; 72 h EC_{50} = 0.110 mg L^{-1} for *Fibrocapsa japonica* [119]; 72 h EC_{50} = 0.17 mg L^{-1} for *H. banksii* [120]; and 72 h EC_{50} = 2500 ng L^{-1} for *E. intestinalis* [121].

The other most commonly detected biocide in areas of high boating activity is diuron (phenylurea herbicide) [102]. The toxic effects of diuron on the photosynthetic apparatus of different algal species have been examined by many authors [10, 24–27, 89, 93, 115, 117] and among other ecotoxicological data the values of IC_{10} = 0.74 μL L^{-1} (based on PSII quantum yield) for *Phaeodactylum tricornutum* [27] and IC_{50} = 7 μL L^{-1} (based on Φ_m, 1.5 h) for *S. capricornutum* [10] are included. Natural periphyton studies have reported an induced increase in Chl-*α* content after long-term (29 days) exposure to low concentrations (1μg L^{-1}) of diuron [122]. This observation is in agreement with other previous studies of Tlili et al., who found that periphyton chronically exposed to 1 μg L^{-1} of diuron showed higher Chl-*α* pigments and carbon incorporation rates than control periphyton from day 21 to day 32 of their microcosm experiment [123]. That was confirmed in a more recent survey conducted in two series of two lotic outdoor mesocosms exposed to mixture of diuron and tebuconazole (triazole fungicide) which revealed induced tolerance to diuron, and therefore it was indicated that the effects of pulsed acute exposures to pesticides on periphyton depended on whether the communities had previously been exposed to the same stressors or not [89].

It has become well known that the antifouling biocide Sea-Nine 211 has an impact as an inhibitor of PSII electron transport [2, 113]. In addition, like other, more water-soluble representatives from the so-called Kathon group of biocides, Sea-Nine 211 quickly penetrates cell membranes and inhibits specific enzymes in the cell by reacting with intracellular thiols [3, 124]. Sea-Nine also seems to be able to affect more than one thiol group by generating a cascade of intracellular radicals [3]. Based on F_v/F_m measurements of natural phytoplankton communities, the toxicity of few biocides has been ranked as follows: Irgarol 1051 > zinc pyrithione>Sea-Nine 211>diuron. Thereby, it is suggested that Sea-Nine is more toxic than diuron, but less toxic than Irgarol [115]. In another survey, the toxicity of the antifoulants Sea-Nine, Irgarol, and TBT has been determined individually and in mixtures in two tests with microalgae and the effects on periphyton community photosynthesis and reproduction of the unicellular green algae *S. vacuolatus* have been investigated. The tested antifoulants have been found to be highly toxic in both tests. Observed mixture toxicities were compared with predictions derived from two concepts: independent action (IA) and concentration addition (CA), and IA failed to provide accurate predictions of the observed mixture toxicities. Mixture effects at high concentrations were slightly overestimated and effects at low concentrations were slightly underestimated [3].

Synergistic interactions have been foreseen not only between irgarol and diuron but between irgarol and chlorothalonil or 2-(thiocyanomethylthio)benzothiazole (TCMTB) as well. The synergies between irgarol and the two general fungicides, chlorothalonil and TCMTB, could be similar to the mechanism proposed for the PSII/metal interactions, as both fungicides create reactive oxygen species (ROS) and additionally chlorothalonil conjugates with glutathione, an important ROS scavenger [101].

7. Heavy metals and metalloids

In general, heavy metals are defined as metals with relatively high densities, atomic weights, or atomic numbers. On the basis of density, the term "heavy metal" is used for the elements that possess a density value greater than 4.5–5 g cm^{-3}, such as silver (Ag), arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn), while metalloid is the definition of a chemical element that has properties intermediate between metals and non-metals, such as germanium(Ge), antimony (Sb), selenium (Se), tellurium (Te), polonium (Po), technetium (Tc), and astatine (At) [125].

Several metals are essential for living beings at very low concentrations, but at higher doses most of them are toxic for organisms belonging to different levels of the food chain [126]. Based on that criterion, metals are separated into the three following classes:

- **The essentials (class A)**: calcium (Ca), magnesium (Mg), Mn, potassium (K), sodium (Na), and strontium (Sr) (including macroelements which are metals that are required for algal growth, metabolism, and physiology (e.g., K and Mg) and microelements, which are metals that are required in trace amounts for certain biological processes and therefore must be obtained from the external environment).

- **The non-essentials (class B)**: Cd, Cu, Hg, and Ag.
- **The borderline class**: Zn, Pb, iron (Fe), chromium (Cr), cobalt (Co), Ni, As, vanadium (V), and tin (Sn) [127].

With regard to Ecotoxicology and Environmental Science, the term "heavy metals" is used to refer to metals that have caused environmental problems and includes chemical elements from the non-essentials and the borderline classes.

A steadily growing interest in the investigations on heavy metals is recorded and a large number of scientific surveys focused on the speciation of metals, their toxicity, accumulation, biomagnification, bioindication, migration, removal, phytoremediation, and biomonitoring have been conducted during the last decades. Cd, Hg, Zn, Cu, Ni, Cr, Pb, Co, V, titanium (Ti), Fe, Mn, Ag, and Sn are the metals that have been studied more extensively, whereas Hg, Cd, and Pb are some of the elements that have received the most scientific attention, possibly due to their highly toxic properties and their effectiveness on the environment and the living organisms [128].

Heavy metals can be naturally produced in aquatic system by the slow leaching from soil to water, usually at low levels [129]. Several other large natural inputs of heavy metals into water ecosystems are from the erosion or rocks, wind-blowing dusts, volcanic activity, and forest fires [128]. In addition, several anthropogenic activities such as energy production technologies, industrial effluents, and wastes (from coal mines, thermal power plants, metallurgy, plating, chemical plant, curry and paper-making industries, and other allied industries) alter the physicochemical characteristics of water bodies and elevate the heavy metals concentration according to the nature of effluent being discharged [130, 131]. Therefore, aquatic ecosystems receive inputs of different source containing a variety of metal ions (M^{x+}) that are directly or indirectly discharged into them.

Aquatic plants assimilate easily heavy metals, which are strongly phytotoxic and pose a threat to freshwater and marine life. Moreover, it has been well established that, depending on its bioaccumulation characteristics, a heavy metal can disperse through the various trophic levels of an ecosystem and its concentration levels are magnified [129]. Metals are not accessible to plants in their elemental forms (valence state of 0). On the contrary, they are available only in solution; hence, only metal ions play a role in biological systems [132]. The toxicity of metals and their compounds, however, largely depends on their bioavailability, that is, the mechanisms of uptake through cell membranes, intracellular distribution, and binding to cellular macromolecules [133]. In other words, the bioavailability of the metal, which depends on both biological factors and on the physicochemical properties of metallic forms (elements, their ions, and their compounds), is one of the key parameters in the assessment of the potential toxicity of metallic elements and their compounds toward organisms [125]. Metal availability is strongly dependent on environmental components, such as pH, redox and organic content, and soluble and bio-available metals. Hence, metals in the environment can be divided into two classes: (I) bio-available (soluble, non-sorbed, and mobile) and (II) non-bio-available (precipitated, complexed, sorbed, and no mobile).

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Heavy metals enter algal cells by means of either active transport or endocytosis through chelating proteins and affect various physiological and biochemical processes of the algae. The mechanisms by which metals exert their toxicity on algae are very diverse and depend on the algal species, the nature and concentration of the metal, and the environmental conditions accompanying heavy metal stress [134]. Generally, their toxicity toward algal cells primarily results from (I) direct binding to the sulfhydryl groups (−SH) in functional proteins which disrupts their structure and function, and thus renders them inactive; (II) displacement of essential cations from specific binding sites that lead to a collapse of function; and (III) generation of reactive oxygen species, which consequently damages the macromolecules [126, 135].

At the sub-lethal level, heavy metals can interact with the vital process of photosynthesis. Interference of heavy metals with the photosynthesis of algae is a subject of intensive research that has been well documented. Almost all heavy metals are known to cause a negative impact on nearly all the components of the photosynthetic apparatus of primary producers [2, 132]. Direct effects of heavy metals on light and dark reactions and indirect effects resulting in the decrease of the photosynthetic pigment (including chlorophyll and carotenoid) content, as well as changes in stomata function, have been reported in the literature [132, 136]. Additionally, ions of heavy metal can damage the chloroplast membrane structure, disturb the light-harvesting and oxygen-evolving complexes, inhibit the photosystems and constituents of the photosynthetic electron transport chain, and also block the reductive pentose phosphate cycle [132, 137]. Moreover, toxic metals cause the inhibition of enzyme activities that are important in photosynthetic pathway. For example, it was found that Cd^{2+}, Zn^{2+}, and Hg^{2+} inhibited the NADP-oxidoreductase in *Euglena*, thereby significantly lowering the cell supply of NADPH [138], whereas Cu^{2+} was shown to inhibit plasma membrane H^{+}-ATPase activity in *Nitella flexis* [139]. Several enzymes involved in the Calvin cycle are also inhibited, especially Rubisco (bisphosphate carboxylase oxygenase) and PEPcarboxylase [132, 136]. Reaction of heavy metals with the enzyme-SH groups in proteins, substitution of essential ions, enhancement of photoinhibition and oxidative stress, impediment of plastocyanin function, change in lipid metabolisms, and disturbances in the uptake of essential microelements are other phenomena revealed due to heavy metal exposure [140, 141]. For instance, Cu^{2+} and Zn^{2+} substituted the Mg^{2+} in Chl molecules bound predominantly in the light-harvesting complex II of Chlorophyta, thereby impeding the PSII reaction centers, such as in the green alga *S. quadricauda* [141].

Finally, many heavy metals have been reported to influence the photosynthetic activity of algae through bleaching process. The observed bleaching effects have been connected with the tendency of toxic metals to generate ROS, such as singlet oxygen ($^1O_2$) and the hydroxyl radical (*OH), which can attack thylakoid lipids and initiate oxidation biochemical reactions that destroy membranes and damage structural pigment-protein complexes. For example, the toxicity of Cr^{6+} compounds has been traced to the reactive intermediates (formation of *OH radicals from H_2O_2 via a Fenton reaction) generated during the reduction of Cr by living cells [142]. As observed in the case of *Chlamydomonas reinhardtii* [134], this toxic metal tends to generate ROS, which can attack thylakoid lipids (mainly unsaturated fatty acids). This

initiates peroxyl-radical chain reactions, destroying membranes and damaging indirectly structural pigment-protein complexes located in chloroplast membranes [2].

According to the numerous reported data on the photosynthesis inhibition by metals, three main experimental approaches can be distinguished: (I) results obtained from experiments with isolated chloroplasts or enzymes, to which heavy metals were supplied in the assay medium, (II) data acquired from experiments performed on excised leaves, exposed to a solution of the heavy metal, and (III) comparative laboratory experiments conducted on intact higher plants or algae, grown in a control medium and on a substrate enriched with heavy metals [140]. A summary of selected references on the toxicity of metals toward the photosynthetic apparatus for various microalgae is presented in **Table 3**.

Mercury is considered as the most toxic element among those having"no known physiological function" in algae. Based on results of ecotoxicological studies, Hg is recognized globally as an important pollutant and a serious threat to ecosystems. Hg and its compounds are persistent, bioaccumulative, and toxic. Inorganic Hg is the most common form of Hg released in the aquatic environment by industries [133]. Organic forms of Hg, such as methylmercury, revealed to have much stronger inhibitory effect than the inorganic mercury chloride on photosynthetic process [143]. Hg is able to alter the photosynthetic machinery including the chloroplastic PSI reaction center, subunit PSII, the oxygen-evolving protein, and the chloroplastic ATP synthase β-subunit [133, 144]. High levels of Hg in the form of Hg^{2+} have strong phytotoxic effects and when present in toxic concentrations can induce visible injuries and physiological disorders in plant cells triggering the production of ROS leading to cellular disruption [133].

Metallic form	Test species	Observed stress response	References
Cu^{2+}, Zn^{2+}	*Scenedesmus quadricauda* *Antithamnion plumula* *Ectocarpus siliculosus*	Under low irradiance heavy metal substitution of Mg in chl molecules bound predominantly in PSII of Chlorophyta; Under high irradiance the chls were inaccessible to substitution and the damage occurred in the PSII reaction center instead.	Kupper et al. (2002) [141]
Cu^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, Cr^{6+}	*Scenedesmus obliquus*	Inhibition of PSII photochemistry. Among the fluorescence parameters measured (after 12 h: F_o, F_v/F_m, *qN*, *qP* and after 1 h: F_m, $F_v/2$, and F_o/F_m) the highest sensitivity to all the five test metals had F_v/F_m.	Mallick and Mohn (2003) [29]
Co^{2+}	*Monoraphidium minutum* *Nitzschia perminuta*	Pigment content and photosynthetic O_2 evolution: increased at low levels and inhibited in high levels. Photosynthetic electron transport in *M. minutum* was more sensitive to Co^{2+} than in *N. perminuta*.	El-Sheekh et al. (2003) [156]

Metallic form	Test species	Observed stress response	References
CH_3Hg, Hg^{2+}	*Chlamydomonas reinhardtii*	$CH_3Hg \geq 1$ μM: Damaged the electron transfer chain at several sites; donor side of PSII, electron transfer from Q_A to Q_B, electron transfer between photosystems. Reduction of F_v/F_m, $\Delta F/F_m'$ and qN values. Hg^{2+} ($HgCl_2$) ≤ 5 μM did not affect F_v/F_m and $\Delta F/F_m'$ ratios.	Kukarskikh et al. (2003) [143]
Cd^{2+}, Cu^{2+}, Zn^2	*Dunaliella tertiolecta* *Promocentrum minimum* *Synechococcus* sp. *Thalassiosira weissflogii*	Comparable sensitivities of F_v/F_m and the cell-specific growth rate in quantifying the toxic effects of metals. *Synechococcus* sp. was the most sensitive species among the four algal species tested.	Miao et al. (2005) [145]
Ag^{1+}	*Chlamydomonas reinhardtii* *Pseudokirchneriella subcapitata*	Influence on proteins and enzymes for *C. reinhardtii* and on photosynthetic apparatus of *P. subcapitata.*	Hiriat-Baer et al. (2006) [157]
Cr^{6+}	*Chlamydomonas reinhardtii*	Complete pheophinitization of the chls and modification of the carotenoids.	Rodríguez et al. (2007) [134]
Cr^{6+}	*Eudorina unicocca* *Chlorella kessleri*	In *E. unicocca*: complete pheophinitization of the chls and modification of the carotenoids. In *C. kessleri*: no effect on the photosynthetic machinery even at higher levels of Cr^{6+}.	Juarez et al. (2008) [158]
Silver nano-particles (AgNP), Ag^{1+}	*Chlamydomonas reinhardtii*	Inhibition of photosynthesis by both AgNP and Ag^+. Based on total Ag concentration: Ag^{1+} ($AgNO_3$) displayed higher toxicity than AgNP. Based on Ag^{1+} concentration: AgNP displayed higher toxicity than Ag^{1+} ($AgNO_3$).	Navarro et al. (2008) [159]
Cu^{2+}, Cr^{6+}	*Euglena gracilis* (MAT and UTEX 753)	In the applied light conditions occurred, mainly damages to the PSII reaction center. Dark reactions were less sensitive.	Rocchetta et al. (2009) [150]
Cu^{2+}, Cr^{6+}, Zn^{2+}, Cd^{2+} Pb^{2+}	*Chlorella vulgaris*	Different effects on chl fluorescence for different metals: Cu and Cr had an inhibiting effect and Zn and Cd had a promoting effect.	Ou-Yang et al. (2012) [154]
Cd^{2+}	*Micrasterias denticulata*	Inhibition of PSII activity. Reduction of O_2 production. Structural damage of the chloroplast. Disturbance of Ca homeostasis by displacing Ca.	Andosch et al. (2012) [160]

Metallic form	Test species	Observed stress response	References
Cd^{2+}, Cu^{2+}, Zn^{2+}	*Planothidium lanceolatum* (Brébisson)	Significant effect on F_v/F_m at concentrations of Cd^{2+} ≥0.1, Zn^{2+} ≥0.2, and Cu^{2+} ≥0.4 mg L^{-1}.	Sbihi et al. (2012) [155]
Cd^{2+}, Cr^{6+}, Cu^{2+}, Zn^{2+}	*Pseudokirchneriella subcapitata*	Modification of mitochondrial membrane. Reduction of photosynthetic activity.	Machado et al. (2015) [161]
Hg^{2+}	*Gracilaria salicornia* *Sargassum* sp. *Ulva reticulata*	Reduction of F_v/F_m and Chl-α content.	Bakar et al. (2015) [133]
Cu^{2+}, Pb^{2+}	*Gracilaria edulis* *Gracilaria manilaensis* *Gracilaria salicornia*	Reduction of the algal F_v/F_m in both metals. Cu^{2+} induced the synthesis of chl-a in *G. edulis* and *G. salicornia* but inhibited chl-α synthesis in *G. manilaensis*. Pb^{2+} induced the production of Chl-α in all tested algae.	Bakar et al. (2015) [162]
Pb^{2+}	*Anabaena* sp.	Reduction of pigment content (Chl-α and car) and photosynthetic efficiency (F_v/F_m) of PSII.	Deep et al. (2016) [163]

Note: *Pseudokirchneriella subcapitata*, known as *Selenastrum capricornutum*.

Table 3. Examples of metals toxicity on the photosynthetic apparatus reported for various algae. Reports in chronological order.

Copper is unquestionably an essential element in various metabolic processes of algae, such as amine oxidase and cytochrome c oxidase system, prosthetic group of the chloroplastic antioxidant enzyme Cu/Zn superoxide dismutase, and regulator of PSII-mediated electron transport. However, Cu is still considered as one of the most toxic heavy metal ions to algae and is a potent inhibitor of photosynthesis [2]. Many studies have examined ecotoxicological effects of Cu on photosynthetic activity of plants and phytoplankton [145]. From an evaluation of the literature, Cu can affect photosynthetic electron transport on the reducing side of PSI at the level of the ferredoxin [146], alter the PSII on the oxidizing side by inhibiting the electron transport at P680 (the primary donor of PSII) or by inactivating some PSII reaction centers [147]. Cu may also impair the PSII electron transport on its reducing side by affecting the rate of oxidoreduction [148]. The inhibitory effect of copper on the photosynthetic apparatus of several species of algae has been examined, including *E. gracilis* [149, 150], *S. quadricauda* [141], *S. obliquus* [151], *S. incrassatulus* [152], *C. pyrenoidosa* [153], *C. vulgaris* [154], *Planothidium lanceolatum* and *Isochrysis galbana* [155], *D. tertiolecta, Promocentrum minimum, Synechococcus* sp., and *Thalassiosira weissflogii* [145].

Cadmium is a heavy metal that occurs naturally in ores along with zinc, lead, and copper. Its compounds are used as stabilizers in PVC products, color pigment, several alloys, and in rechargeable nickel-cadmium batteries. Cd forms complexes with various organic particles and thereby triggers a wide range of reactions that collectively put the aquatic ecosystems

to risk [2]. Due to its high toxicity at low concentration, Cd is considered as an important contaminant of natural waters [164]. Research regarding the adverse effects of Cd on microorganisms demonstrated that Cd^{2+}, via a variation of mechanisms, affected several biochemical algal processes. References include the displacement of $Zn^{2+}$and Ca^{2+} co-factors from undefined protein targets or directly binding amino acid residues, including cysteine, glutamate, aspartate, and histidine [165]; the inhibition of chlorophyll formation and the reduction of both chlorophyll content and Chl a/b ratio through disturbances in the electron transport chain in both PSI and PSII; and the reduction of Rubisco and enhancement of lipoxygenase activity [2, 145].

Chromium is a transition element that comprises the seventh most abundant metal in the earth's crust, whereas trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) ions are its two most common and stable oxidation states in the environment. Whereas Cr^{3+} is considered a micronutrient, essential for the proper function of living organisms, Cr^{6+} instead can display numerous toxic effects on biological systems. Cr^{6+} is usually associated with oxygen to form chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions that can easily go through cell membranes as an alternative substrate for the sulfate transport system and exhibit strong oxidative potential [166]. Therefore, Cr^{6+} is associated with several intracellular and ultra-structural modifications, among which the inhibition of photosynthesis is included. As observed in the cases of the algal species *Chlamydomonas* [134], *C. pyrenoidosa* [167], *Eudorina unicocca*, *C. Kessleri* [168], *E. gracilis* [150], *S. obliquus* [169], and *Monoraphidium convolutum* [170], Cr^{6+} caused an enhanced destruction of the reaction centers and a reduction in measured Chl-α-fluorescence parameters such as Φ_{PSII}, F_v/F_m, Φ'_{PSII}, *ETR*, and *qP* [2].

Zinc is an essential element for the activity of several enzymatic systems of organisms. Stimulatory effects on algal photosynthesis at low exposure concentrations of Zn^{2+} have been observed. For example, *C. vulgaris* after 96 h of exposure at treatment concentration of 5 μmol L^{-1} showed that the proportion of the maximum quantum yield of PSII promoted by Zn was approximately 10% [154]. However, when the external concentration of Zn^{2+} is beyond a limited value, it causes harmful effects; hence, its concentration in the cells must be controlled. Zn deficiency in *E. gracilis* has been shown to affect growth, morphology, cell cycle, and mitosis. These observations are best explained by a role for zinc in gene regulation, through zinc-dependent enzymes [149]. Significant effect on F_v/F_m ratio of *P. lanceolatum* (Brébisson) at a concentration level of 0.2 mg L^{-1} of Zn^{2+} was observed, while the sensitivity of the same algal species toward all tested heavy metals was diminishing in the order: $Cd^{2+} > Zn^{2} > Cu^{2+}$ [155].

The toxicity of ionic silver to a variety of aquatic organisms, such as algae, has been studied and shown to be significant, whereas from an evaluation of the literature, Ag+ displayed toxicity to aquatic photosynthetic microorganisms in the nanomolar (nM) concentration range [157, 159]. The toxicity of other forms of silver, such as silver nanoparticles (AgNP) ranged in size from 10 to 200 nm, has been examined as well and according to fluorometry values AgNPs were found to influence the photosynthesis of *C. reinhardtii* as well as ionic silver (Ag^{+}) [159].

At this point, it must be mentioned that due to the fact that aquatic ecosystems act as reservoirs of several mixtures of metals, it is essential to evaluate the combined or cumulative effect

of metals or metal mixtures on photosynthesis. Therefore, toxicological studies dealing with heavy metal pollution in aquatic organisms must take into account the interactions among metals that may influence uptake, accumulation, and toxicity [2, 128]. For instance, it has been reported that interactions between Cu^{2+} and Mg^{2+} may have special significance regarding phytoplankton growth [2]. In another survey assessing the effect of Cu^{2+}, Cr^{6+}, and Ni^{2+} on growth, photosynthesis, and chlorophyll, a synthesis of *C. pyrenoidosa,* it was demonstrated that various bimetallic combinations of those metals interacted synergistically [171]. Combined effects of Cu^{2+} and Cd^{2+} on the growth and photosynthesis-related gene transcription of *C. vulgaris* have been also investigated [154].

In a more realistic approach, metals could also occur along with other contaminants in mixtures. In that respect, synergistic interactions have been predicted between pesticides that act as PSII inhibitors (and are included in the database of Belden et al. [98]) and the metals Cd, Cu, and Zn [101]. A proposed synergistic mechanism between metals and PSII inhibitors in autotrophs could be that metals might prevent the repair of not only damaged PSII complexes, which are constantly repaired during photosynthesis, but also the damage caused by the reactive oxygen species (ROS) created by the PSII inhibition and the metals themselves, by interacting with enzymes responsible for the repair [101].

Finally, metal bioassays must take into account the synthetic organometallic compounds or the ones formed under environmental conditions. These organometallic substances, especially of Hg, Pb, and Sn, might have completely different toxicological properties and can be more toxic to aquatic organisms because of their high bioaccumulation, as is the cases of methyl mercury compounds (methylation process is thought to be bacterially mediated) [128, 143] and tributyltin chloride [3, 105].

However, it must be underlined that several metal-tolerant algal strains, which have been adapted to environments contaminated with toxic metals (such as Cu and Cd), have been isolated and identified and a variety of tolerance mechanisms have been described [172]. Metallothioneins (MTs) consist one of the most important cellular defense mechanisms against metal stress that regulate the toxicity of various metals and trace elements. MT is a family of cysteine-rich and low-molecular-weight proteins localized to the membrane of the Golgi apparatus, which have the ability to bind several metals through the thiol clusters of their cysteine residues [173]. Some algal MTs are gene products, while others are secondary metabolites [172]. According to relevant studies, these molecules chelate toxic trace metals, for example, Cd, thereby reducing the concentration of cytotoxic, free-metal ions. Furthermore, some MTs are believed to be involved in zinc and copper homoeostasis [172]. The removal of heavy metals from polluted waters by the use of algae (e.g., *C. pyrenoidosa* and *Scenedesmus* sp.) is called phycoremediation and is an expanding technology with several advantages over physical remediation methods [174].

8. Conclusions and trends

One of the common and main goals of environmental science and ecotoxicology is the environmental sustainability that concerns the natural aquatic ecosystems and how they endure

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and remain diverse and productive. Taking into account that photosynthetic microorganisms are the main primary producers and consist of the basis of the food chains, a large number of toxicity tests have been conducted in order to assess the effects of a variety of environmental pollutants on algal photosynthetic activity. According to the available vast information, several bioassays have been performed with a great variety of standard test species of both freshwater and saltwater algae, though various "non-standard" algal species have been used on occasion. In our knowledge, in most cases freshwater microalgae were used more frequently in laboratory toxicity tests than any other types of aquatic plant, except in the case of oil spills where more data for marine algae are available. Moreover, literature data showed that the most commonly used microalgae in marine toxicity tests are green algae and diatoms. The observed differences in response and sensitivity by various microalgal and macroalgal species to the same toxicant can be several orders of magnitude for toxicants such as crude oils, oil products, pesticides, antifouling biocides, and metals. Evidenced heterogeneous sensitivity of different algal species to the same pollutant is attributed to several characteristics of the exposed alga such as photosynthetic capacity and pigment type, cellular lipid and protein content, and cell size.

Algae have been suggested and used as potential bioindicators of aquatic pollution [1, 175]. Damage of their photosynthetic apparatus is a very sensitive response to xenobiotics that could point to an important biomarker [79]. Carried out studies confirmed that inhibition of photosynthesis is one basic reflex of the toxic effects of several organic and inorganic pollutants on microalgae which in many cases is a more sensitive end point than inhibition of growth [39]. Therefore, we can conclude that measuring the photosynthetic activity is a good screening method for detecting a variety of possible stress situations [132].

Loadings of several anthropogenic pollutants are usually nearly and chronically synchronous with discharges, leading to marked changes in exposure levels of inhabitants of aquatic reservoirs. Depending on the nature, concentration, frequency, and duration of toxicants exposure, their impacts on biological communities can prove highly variable [89]. Until nowadays, many experimental studies of aquatic communities of microorganisms have been done using water-column phytoplanktonic species, but only a few have attempted to assess the effect of environmentally realistic pollution exposure scenarios on microbenthic periphyton [89, 122, 123, 176]. The distribution characteristics of chemical toxicants between water phase and sediment are of major importance in the evaluation of their fate and ecotoxicological effects into environmental compartments, especially for organic hydrophobic pollutants. Therefore, more vivid studies need to be performed in the future on the bioavailability of organic pollutants and the possible link between pollutant dynamics in the adsorbed phase (bottom sediment periphyton matrices) and their impacts on microbenthic photosynthetic algae.

Last but not least, there is still not much known about the possible toxic effects of transformation and degradation products of several synthetic organic compounds on aquatic microalgae. This lack of data makes the toxicity assessment of formed organic molecules metabolites essential, because these molecules may be more toxic than the parent ones; hence, further studies are required to evaluate the adverse effects of these produced chemical species on algal photosynthetic activity.

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