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Review

Algal Biosensors for Detection of Potentially Toxic Pollutants and Validation by Advanced Methods: A Brief Review

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Abstract: The presence of potentially toxic pollutants, such as pesticides and metal ions, even at low concentrations, can significantly impact aquatic environmental health. This pollution is a globally widespread problem and requires fast and reliable analysis, especially for in-situ identification/quantification. Atomic absorption spectrometry and plasma-based spectrometry techniques have been considered the most analytical tools used to monitor potentially toxic metal ions in aquatic media and other related matrices. The dynamics of global climate change and its correlation with pollution, especially from anthropogenic sources, have encouraged the development of other faster analytical tools for monitoring these pollutants. A noteworthy alternative for determining potentially toxic pollutants is using algae-based biosensors, resulting in a cost reduction and simplification of environmental analysis, enabling a more reliable comprehension of the role of humans in climate change. These biosensors, which may not have the highest sensitivity in quantification, have demonstrated remarkable potential in the identification of potentially toxic pollutants and several field applications. Biosensors can be an excellent biotechnology solution for monitoring global environmental changes. Thus, this review highlights the main advances in developing and comparing algae-based biosensors and other analytical possibilities for the identification of potentially toxic pollutants and their possible applications in environmental analysis.

Keywords: algae-based biosensors; metal ions determination; environmental analysis; aquatic environmental health; herbicide determination

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1. Introduction

Potentially toxic pollutants can bioaccumulate in the environment from both natural and human sources. Human activities, such as mining, smelting, mineral refining, and industrial production, are mainly responsible for most pollution in the natural environment. Industrial effluents may result in several risks to aquatic and human health contaminating water resources through runoff or intentional release of wastewater [1]. Mercury, lead, cadmium, chromium, and arsenic have no biological function and can cause mental, genetic, and morphological abnormalities in humans [2]. Furthermore, these toxic elements are persistent in the environment, and harmful to humans, animals, and plants even at low concentrations (Hg: 0.003 mg L^{-1}; Pb: 6 mg L^{-1}; Cd: 10 mg L^{-1}; As and Cr: 0.05 mg L^{-1}) [2]. Many herbicides, such as simazine, exhibit similar environmental persistence and toxicity, posing a significant risk to aquatic ecosystems by inhibiting photosynthetic activity [3,4].

Furthermore, the widespread use of these chemicals can lead to groundwater contamination, further complicating the challenge of maintaining safe water quality standards [5,6]. The bioaccumulation of potentially toxic elements can cause health problems, such as intoxication, cancer, and mental disorders [4].

Chemical analyses of water and sediments have been conducted to monitor toxic elements in aquatic environments [7,8]. The analytical techniques most frequently used to identify and quantify the presence of elements in aquatic environments are Flame Atomic Absorption Spectrometry (F AAS), Graphite Furnace Atomic Absorption Spectrometry (GF AAS), Flame Atomic Emission Spectrometry (F AES), Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES), Microwave Induced Plasma Optical Emission Spectrometry (MIP OES), and Inductively Coupled Plasma Mass Spectrometry (ICP–MS) [8,9]. Although those analytical techniques have been reported for metal ion determination in water and sediment samples, with high accuracy and precision, they can be considered expensive, requiring trained personnel, and laboratory settings [5,10,11]. In this sense, research into innovative and economical alternatives for the specific and rapid detection of these pollutants is crucial.

In this context, biosensors have emerged as a modern analytical strategy for identifying and quantifying pollutants in aquatic environments. Unlike physicochemical analyzers, which quantify toxic substances, biosensors can measure toxicity. They offer several advantages, including lower analysis costs and faster response than spectrometric techniques [12,13]. Biosensors work as devices connected to biological recognition elements, which are associated with a transducer that converts a stimulus into a measurable unit of energy. Whole algae, cyanobacteria—or algae components—can be utilized as biological recognition elements in biosensors [12].

Algae is a heterogeneous group inhabiting various planet regions: oceans, rivers, lakes, soils, and organism surfaces [14,15]. There are two main types of algae: macroalgae, also known as seaweeds, inhabiting coastal zones and encompass varieties (green, brown, and red algae); and microalgae, found in both benthic and coastal habitats, distributed throughout the oceans as phytoplankton [16]. Furthermore, algae are a promising alternative for carbon sequestration due to photosynthesis [15].

Microalgae possess physiological and biochemical characteristics that make them promising candidates for application in biosensors aimed at monitoring heavy metals and pesticides in contaminated environments [17–19]. These unicellular organisms are able to detect and respond to toxic contaminants through well-established detoxification mechanisms, which include initial adsorption at the cell wall, followed by intracellular accumulation and activation of specific antioxidant systems. In response to heavy metals, species such as *Chlorella vulgaris* and *Scenedesmus obliquus* perform passive adsorption on the cell surface, utilizing functional groups such as carboxyl and hydroxyl to retain metal ions. Metals may also be chelated by metallothioneins and phytochelatins, which protect the cell by sequestering these ions in cellular compartments such as vacuoles [19]. Additionally, microalgae activate antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), which neutralize reactive oxygen species (ROS) generated by oxidative stress, thereby preserving cellular viability [17].

For pesticides, microalgae like *Scenedesmus obliquus* promote compound decomposition into less toxic by-products, such as phthalic acid, via the action of enzymes like glutathione S-transferase (GST). This process is further supported by antioxidants, including glutathione (GSH) and the ascorbate cycle, which mitigate the oxidative stress induced by the pesticide, demonstrating the ability of these microalgae to act as effective biosensors for environmental monitoring of both organic and inorganic pollutants [18]. Algae can be used as biosensors for chemical elements, providing an accurate analysis of the potential risk to human and environmental well-being [20–22]. Thus, algae-based biosensors for the detection of potentially toxic metal ions are an interesting topic aiming at environmental and agroecological contamination problems. This review article is focused on using algae or their components for application in biosensors for the detection of potentially toxic

elements (Figure 1). This review highlights the latest advances in biosensors derived from algae and their components for potentially toxic pollutant detection *in situ* or *ex situ*.

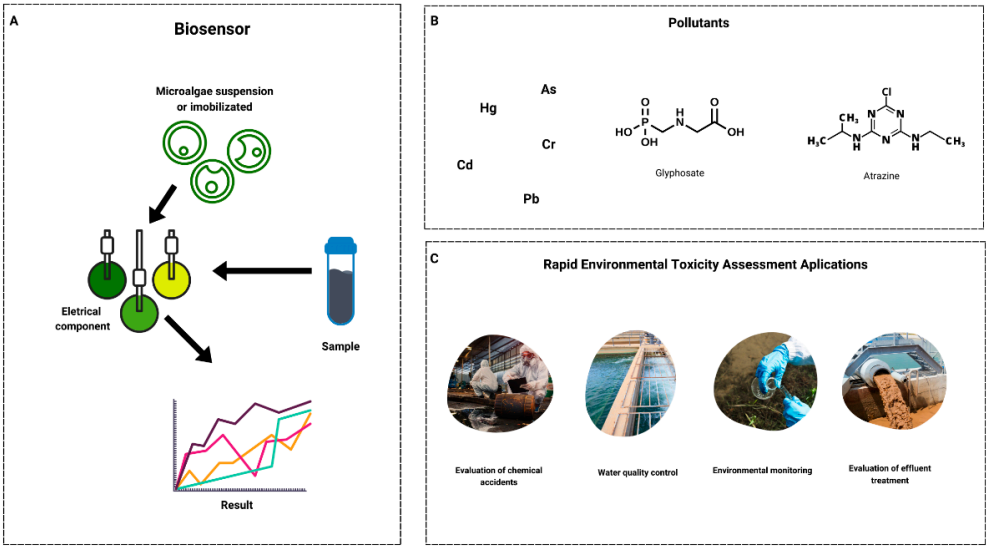

Figure 1. Biosensors based on algae and their applications. (**A**) Biosensor components. (**B**) Toxic elements. (**C**) Locals for in situ use biosensors based on algae.

2. Possibilities for Metal Ions and Pesticides Determination: Advantages and Limitation

Classical analytical methods for detecting potentially toxic metal ions, such as gravimetric and volumetric assays, were widely used in the mid-20th century. Gravimetric analysis, which measures the mass of an isolated substance in an insoluble form, and volumetric analysis, which determines the concentration of the substance based on the volume of a reagent solution, were fundamental to analytical chemistry [20–25]. However, these methods have limitations, including low sensitivity, detection limits above 10 mg L^{-1}, and the need for significant quantities of reagents, making them less suitable for modern environmental analysis [26,27].

Recent advancements in analytical technologies have addressed the limitations of classical methods for the identification and quantification of potentially toxic metals, yielding reliable results characterized by precision, accuracy, repeatability, and reproducibility. Atomic spectrometry techniques, including atomic absorption spectrometry (AAS) and plasma-based methods, play a crucial role in metal determination [7]. The choice of analytical technique depends on factors such as the number of elements to be determined, sample form (solid or solution), sensitivity, linear range, interferences, and the cost and maintenance of the instrumentation [8,28]. AAS employs various atomizers, such as flame or graphite furnace, which differ in sensitivity and operational efficiency; the graphite furnace offers higher sensitivity with lower detection limits (in μg L^{-1}) compared to the flame atomizer (in mg L^{-1}), albeit at a higher operational cost [7].

Plasma-based analytical techniques, such as inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS), have gained prominence due to their high sensitivity and multielemental capabilities, enabling the determination of concentrations ranging from μg L^{-1} to mg L^{-1} [9,29]. Despite their advantages, these techniques are often hindered by high acquisition and operational costs due to substantial argon gas consumption [29]. Given the ongoing exploration of new analytical methods, biosensors have emerged as a promising alternative, offering significant benefits in application, cost, versatility, and robustness by detecting signals produced from the interaction between analytes and biological components, which correlates with metal concentrations [5,30].

The detection of pesticides in environmental ecosystems can be achieved through various analytical techniques, including gas chromatography (GC) and liquid chromatography (LC). GC is predominantly employed for volatile and non-polar compounds, utilizing detectors such as electron capture detectors (ECD), flame photometric detectors (FPD), and mass spectrometry (MS) for enhanced sensitivity [31,32]. High-performance liquid chromatography (HPLC), on the other hand, is particularly suitable for high-polarity pesticides and can be paired with classical detectors such as ultraviolet (UV) and fluorescence, as well as MS, which offers exceptional sensitivity without the need for derivatization [33].

In addition, capillary electrophoresis (CE) is an effective analytical technique that requires minimal reagents and sample volumes, providing high separation efficiency. Despite its limitations in sensitivity due to the small capillary diameter, CE is often coupled with sensitive detection methods, including MS (Sánchez-Hernández et al., 2014) [34], and integrated with pre-concentration techniques. Recent studies have demonstrated the application of innovative approaches, such as electrokinetic supercharging (EKS) and biomimetic immunoassays (BI-CE), for efficient pesticide detection, offering more accessible alternatives to conventional methods [32,35,36].

Recent developments in plasma-based techniques, such as Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), have significantly improved detection capabilities. These methods allow multielement analysis with detection limits down to μg L^{-1} or less but also have high operational costs [37–42]. Challenges of these advanced techniques include their susceptibility to spectral and non-spectral interferences and the need for extensive sample preparation [42–45]. In response to these challenges, there is increasing interest in the development of alternative methods, such as algal biosensors. These biosensors provide a sustainable and cost-effective solution for in situ metal ion and pesticide detection by leveraging biological interactions for sensitive and robust environmental monitoring [3,6,46].

3. Algae-Based Biosensors

3.1. Pollutants Capture Properties of Algae

Algae, as photosynthetic organisms found in various aquatic habitats, are essential for the health and biodiversity of aquatic ecosystems [47]. They act as primary producers in food chains, provide nourishment for invertebrates and fish, and contribute to nutrient recycling and oxygen production [48]. In addition, algae help maintain the stability of coastal environments by filtering pollutants and preventing coastal erosion [49]. Due to their rich composition of vitamins, proteins, lipids, pigments, polysaccharides, and bioactive compounds, algae are utilized in various industries such as food, cosmetics, and pharmaceuticals [50,51].

Algae have only been used in developing biosensors in the last two decades [14]. In this sense, the use of algae as biosensors has been reported as a low-cost and eco-friendly option for environmental monitoring [52–55]. Their cell walls rich in polysaccharides, proteins, and lipids (Figure 2) give them a high capacity for adsorbing contaminant molecules, such as metal ions and pesticides, through functional groups that offer negatively charged sites: carboxyl, hydroxyl, amino, sulfate, and carboxylate; while physiological processes, such as active transport, contribute to the internalization of these substances [56–58]. The carboxyl group is recognized as a key factor in adsorption, with carboxylic moieties able to bind metal ions, such as Cd^{2+}, through two distinct mechanisms: either by replacing the H^+ in the carboxyl group (type-I) or, after prolonged exposure, forming carboxylates (type-II) [59]. This adsorption process follows a biphasic mechanism. In the initial, rapid phase, metal ions are adsorbed by various components of the microalgal cell wall and its functional groups [60]. This non-metabolic phase is influenced by several factors, including pH, temperature, metal concentration, biosorbent dosage, and contact time, with the microalgal strain, contact time, and pH being the most critical parameters [61], while temperature primarily affects the kinetics rather than the capacity [62].

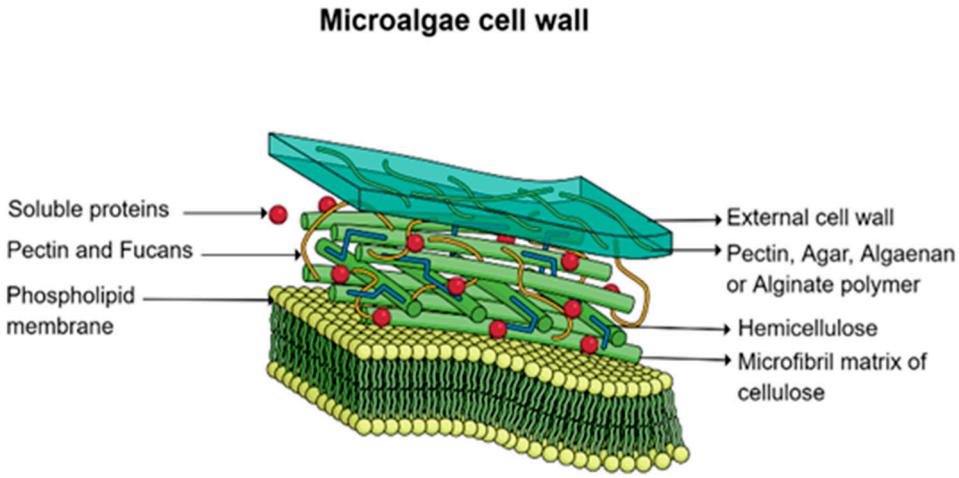

Figure 2. Microalgae cell wall and its components (adapted [63]).

Additionally, the nature of the metal ion also affects the process; cationic metal uptake is enhanced at higher pH, while anionic ion removal is more efficient at lower pH [64,65]. In the second, metabolic phase, metal ions are absorbed and accumulated within the cells through active transport and binding to proteins like phytochelatins or metallothioneins, making this phase slower [17,18,65]. Unlike the first phase, the second phase is dependent on factors such as temperature and the metabolic state, as active metal transport requires energy [19]. This affinity for metal ions has led to the development of algae-based biosensors, such as whole-cell biosensors constructed utilizing the microalga *Chlorella vulgaris* as the recognition component, with its fluorescence response employed as the measurement parameter to detect the presence of titanium dioxide (TiO_2) and silver (Ag) nanoparticles in water. This biosensor demonstrated significant sensitivity, with a detection limit of 1×10^{-3} mg L^{-1} [66–71].

Similarly for pesticides, hydroxyl, carboxyl, and amine groups have been identified as the predominant active surface groups in the adsorption of 2,4-dichlorophenoxyacetic acid (2,4-D) by *Gracilaria verrucosa* [67]. The cell wall of microalgae is composed of a fibrillar matrix of carbohydrates, intercellular spaces, and sulfated polysaccharides, which facilitate the adsorption of organic contaminants from water [68,69]. Pesticide adsorption depends on two aspects: the growth of the microalgae, and the chemical structure of the pesticide and organism-related factors (microalgae) [70]. Therefore, several studies use microalgae for bioremediation and the development of sensors for pesticides. Moro et al. (2018) used the inhibition of microalgae fluorescence to identify pesticides in seawater. In this study, fluorescence parameters of several species of microalgae were analyzed in the presence of three common marine pesticides that act as photosynthesis inhibitors. The three pollutants were detected in a 10-min interval, at concentrations ranging from ng L^{-1} to µg L^{-1}. The different species of microalgae demonstrated slightly different sensitivities to pesticides, with *Chlorella mirabilis* being the most sensitive [71].

3.2. Features of Algae-Based Biosensor

Algae-based biosensors were initially employed in the 1980s and 1990s, involving the cultivation of algae that underwent alterations in their cellular growth and photosynthetic activity in the presence of pollutants [12,14]. Biosensors are currently applied in a wide range of areas such as industrial process control, food control, and environmental monitoring [5,6]. Biosensors are considered highly promising tools for the detection of potentially toxic pollutants due to their specificity, low cost, portability, real-time monitoring capability, fast response time, ease of handling, compactness, sensitivity, user-friendliness, and reliability [72,73]. Furthermore, biosensors have a fast response time, are portable, and can be used for in situ applications, as is their ability to assess the biotoxicity and bioavailable of pollutants in living beings. Biosensing technology represents a synergistic

combination of nanotechnology, biotechnology, and microelectronics. These analytical devices rely primarily on a biological sensing component capable of recognizing the target analyte with the signal transduced by an appropriate transduction element [72–75]. The construction of an effective biosensor involves several steps: selection of the bioreceptor, characterization and adaptation to be used as a sensor, selection of a transducer, system assembly, signal amplification, and verification of the real efficiency of the biosensor. A bioreceptor or biological recognition element is a biological entity that specifically reacts with the molecule to be analyzed and produces a measurable signal. Meanwhile, the transducer is the device that converts the signal from the bioreceptor into electrical energy. Biosensors can utilize different analytes, biological receptors, and transducers. The careful selection of each biosensor component is crucial, as it directly affects the sensitivity and specificity of detection; therefore, optimization is necessary to adjust the biosensor to the specific pollutant and environmental conditions [12,14,74].

Conceptually, all biomolecules and molecular assemblies have the ability to recognize target analytes and then become a bio-receiver. The first elements to be used in biorecognition for biosensors were elements of a living system [75]. Biorecognition elements mainly include whole cells, enzymes, molecularly imprinted polymers, and others, such as aptamers and antibodies, with greater application in the healthcare field [74]. Bacteria [76], yeasts [77], cyanobacteria [78], and microalgae [66] have been utilized as bioreceptors in these devices' fabrication. The principle of using bio-recognition elements in algae-based biosensors for the detection of potentially toxic pollutants is based on the ability of these elements to selectively and specifically bind to the target compound [79]. When the algae binds to the toxic compound, it triggers a signal detected and quantified by the biosensor. This signal is typically a change in fluorescence, conductivity, or other physical or chemical property that can be measured to determine the concentration of the pesticides, chemicals, toxic compounds, and metal ions in the sample [80–83].

Among the various biosensors, those based on algae have shown a good perspective on potentially toxic pollutant detection. These biosensors are adaptable to adverse conditions, reproducible with low nutrient requirements, and more stable than enzyme-based biosensors. Microalgae biosensors utilize intact microalgal cells to detect pesticides by monitoring changes in photosynthesis, measured through variations in oxygen production or chlorophyll fluorescence. A paper-based biosensor with whole cells of the microalgae *Chlamydomonas reinhardtii* immobilized on a paper substrate for herbicide detection through chlorophyll fluorescence has been developed [48,54]. The results showed a decrease in variable fluorescence inversely proportional to the herbicide concentration (0.5–200 nmol L^{-1}), indicating a linear relationship in the measured dose–response curves and a limit of detection of 4 pmol L^{-1}. In addition, the biosensor demonstrated satisfactory storage stability for up to three weeks. Over the past two decades, microalgae-based biosensors have been extensively developed for pollutant detection due to their high sensitivity (in the μg L^{-1} range), sustainability, and ease of handling, since microalgae can be cultivated on a large scale at a low cost, and remain active in high-pH environments, reducing contamination by other microorganisms. By using bio-recognition elements specific to the target metal ion, algae-based biosensors can provide susceptible and selective detection of potentially toxic pollutants in environmental samples. This allows for rapid and reliable monitoring of water quality and the early detection of contamination events, enabling timely interventions to protect human health and the environment [54,75–78].

One of the main steps in biosensor development is the immobilization of the biological recognition element. In the case of an algae-based biosensor, the microalgae cells are immobilized, on a surface or solid matrix (as long their detection ability is maintained) or used as a cell suspension [14]. In addition, immobilization is crucial to secure the stability, reproducibility, practicability, and durability of the biosensor [78,79]. Different immobilization procedures have been adopted based on the suitability of the biological element and transducing system, involving physical methods like entrapment, encapsulation, and adsorption or chemical methods like covalent bonding and cross-linking [20,22,54,84,85].

Physical methods are primarily used to immobilize whole cells or cellular organelles. The thickness and porosity of membranes used for encapsulation and entrapment significantly affect the performance of biocomponents, often resulting in reduced sensitivity and slower response times [79,86]. In this sense, a study examining the fluorescence of various microalgae species (*C. vulgaris*, *P. subcapitata*, and *C. reinhardtii*) immobilized with a calcium alginate matrix further immobilized in an inorganic silica matrix as a biosensor demonstrated that the immobilized microalgae exhibited lower sensitivity in comparison with the algae in suspension (limits of detection 0.001 μmol L^{-1} and <0.001 μmol L^{-1}). These findings suggest that the immobilization of whole-cell biosensors does not compromise their functionality. Furthermore, photosynthetic activity measured by fluorescence decreased in a dose-dependent manner consistently across all three species [86], indicating that immobilization can protect cells (resulting in greater durability of the biosensor) and allow the study of highly toxic metal ions and in larger quantities without dilutions.

After preparation, the algae-based biosensor is exposed to the medium containing the analyte. At this stage, the immobilized algae are put directly in contact with the target substances (Figure 3). The high biological selectivity of the algal receptors enables the specific interaction of the analyte, such as metal ions, through biochemical interactions. Certain algae species demonstrate a remarkable affinity for metal ions such as Cd^{2+}, Cr^{6+}, and Zn^{2+} [46,52,55]. The interaction between the analyte and the biological components of the biosensor triggers biochemical changes in the algae, which are essential for subsequent signal transduction. These biochemical alterations, resulting from the algae–analyte interaction, are measured by detectable optical and electrochemical signals through physicochemical transducers. Electrochemical biosensors function by detecting chemical reactions through variations in the electrical properties of the solution. These systems require a three-electrode configuration: a working electrode, a counter electrode, and a reference electrode [80,82]. The working electrode serves as the transducer where the reaction takes place; the counter electrode completes the circuit and applies current to the working electrode; and the reference electrode maintains a stable potential. Electrochemical biosensors are further categorized into conductometric, potentiometric, and amperometric types [73]. Optical biosensors, on the other hand, comprise an optical transducer and bioreceptor molecules, converting biological events into electrical signals by inducing changes in the light's absorption, transmission, reflection, refraction, phase, amplitude, frequency, or polarization in response to the physicochemical alterations caused by biorecognition events [83].

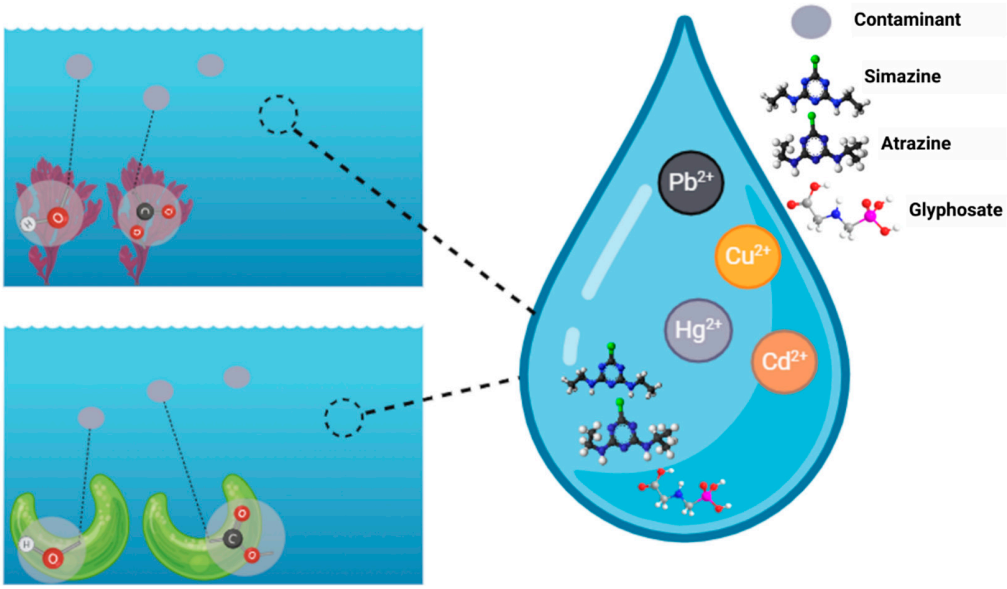

Figure 3. Chemical interaction between the algae and different contaminants.

The use of the microalgae *Chlamydomonas reinhardtii* as a bioindicator for the presence of pesticides has been investigated [82]. Electrochemical biosensors to monitor the metabolic activity of the algae through the detection of O_2, H_2O_2, and H_3O^+/OH^- (pH-related ions) were carried out. The platinum black biosensor showed the highest sensitivity for O_2 and H_2O_2, whilst Pt/IrO_2 showed the greatest sensitivity for pH. In optical biosensors, this transduction typically involves measuring changes in the natural fluorescence of the algae. The fluorescence intensity varies according to the analyte concentration, allowing the indirect quantification of the target substance. Alternatively, electrochemical biosensors can monitor changes in current or potential generated by the biological interaction. The choice of transducer type depends on the specific properties of the analyte and the characteristics of the developed biosensor [22].

Algae-based biosensors can be utilized for the specific detection of potentially polluting elements through specific response patterns. The identification of a specific element by means of a biosensor occurs through the comparative analysis of the response patterns generated by different strains of microalgae. Each element induces a distinct alteration in the fluorescence of the microalgae, which, when recorded and compared with a database of known patterns, allows for the identification of the contaminant. Podola and Melkonian (2005) studied various strains of microalgae as biosensors, among which the strains *Tetraselmis cordiformis* and *Scherffelia dubia* demonstrated high sensitivity to herbicides such as diuron and isoproturon, creating a response pattern that enabled the precise identification of these compounds in water based on prior standardization with reference substances [87]. Another alternative for specific detection of potentially polluting elements is the use of genetically modified microalgae that are resistant to certain compounds [88,89]. Haigh-Flórez et al. (2014) developed a dual-head biosensor employing strains of *Dictyosphaerium chlorelloides*, which are sensitive and resistant to simazine, immobilized in porous silicone films. The sensitive strain shows a significant decrease in O_2 production in the presence of the herbicide, while the resistant strain remains unaffected, enabling selective detection of the compound. The photosynthetic activity of the strains was monitored using an integrated luminescent O_2 sensor. The device provides in situ herbicide concentration measurements every 180 min, with a detection limit of 12 $\mu g\ L^{-1}$ and a working range of 50–800 $\mu g\ L^{-1}$ [90].

Biosensors for specific detection and biosensors for general toxicity detection differ in terms of prior preparation and analysis methods. Biosensors using specific response patterns are designed to identify and quantify individual compounds, such as herbicides, through the analysis of differentiated responses from various strains of microalgae, previously evaluated against these contaminants and thus defining a standard response. This type of biosensor utilizes more than one microalgal strain and compares the variations in chlorophyll fluorescence of these microalgae exposed to standards and environmental samples [87]. In contrast, biosensors aimed at detecting general toxicity may use only one strain and assess the overall impact of all contaminants present in a sample, without comparison to previously established response patterns. These biosensors measure total toxicity, reflecting the aggregated response of the microalgae to the environmental sample, without determining which element is responsible for the generated toxicity [14].

3.3. Optical Biosensor Using Algal Fluorescence

A bioluminescent biosensor using microalgae *Chlorella vulgaris* as a biological component for detecting several metal ions in water has been developed [20]. The tests revealed a fast detection time of 15 min, and a broad pH tolerance of 6–8, and contributed to cell growth monitoring through the analysis of cell density. Optical biosensors were also developed, exploring the inhibition of electron transfer in terms of variations in chlorophyll fluorescence emission in the presence of pollutants [91]. In this context, algal biomass can be immobilized on surfaces to measure its fluorescence when in contact with metal ions.

A biosensor comprising of microalgae *Mesotaenium* sp. and cyanobacteria *Synechococcus* sp., immobilized in a 96-well microplate using silica, exhibited satisfactory performance

in detecting Cd^{2+}, $Cr^{6+,}$ and Zn^{2+} in solution, increasing the fluorescence values in 10 min of exposure. Also, the biosensors developed presented excellent storage stability of 4 weeks for microalgae and 8 weeks for cyanobacteria [46]. A whole-cell biosensor with the microalga *Scenedesmus subspicatus* was used to detect Cd^{2+}, Cu^{2+}, and Zn^{2+} in water has been reported [55]. The results revealed a satisfactory response, with limits of detection of 0.90, 0.91, and 0.88 mg L^{-1} for Cd, Cu, and Zn, respectively. Metal can influence fluorescence, making it a valuable indicator for biomonitoring environmental pollution, particularly in aquatic systems [92]. Consequently, algal biomass emerges as an economical and efficient material for the selective removal and recovery of metal ions from industrial wastewater or other sources [93,94]. The algae fluorescence can also be analyzed together with other parameters, such as cell growth and photosynthetic activity in oxygen production (Figure 4). These parameters are affected by the presence of contaminants in the cultivation medium [12].

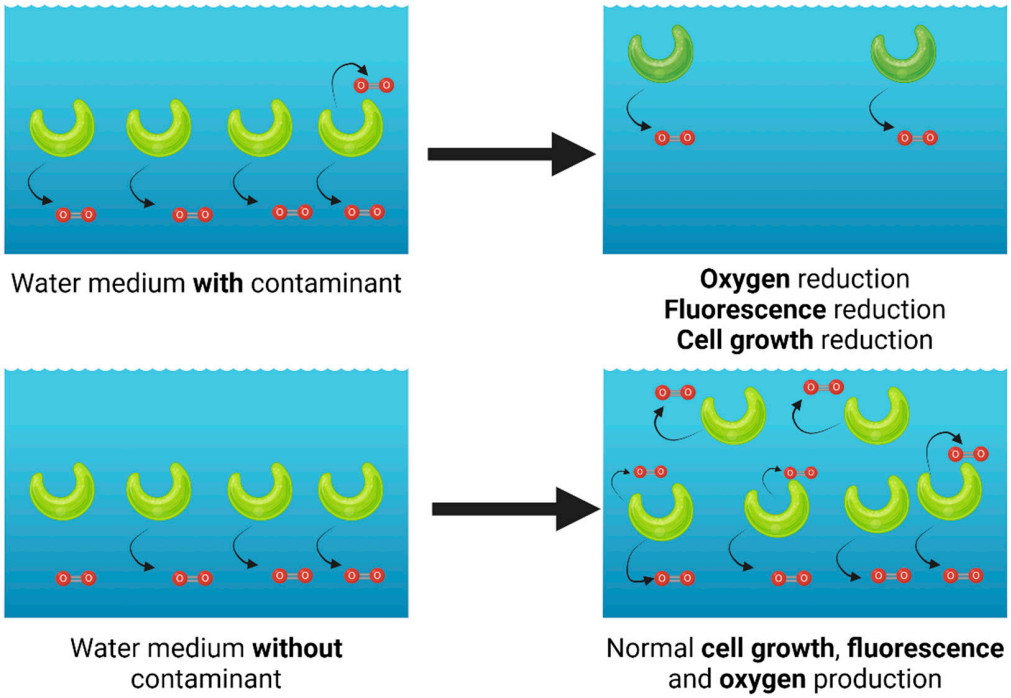

Figure 4. Example of parameters analyzed by an algae-based biosensor.

In addition to the fluorescence generated by photosynthesis, the fluorescence of other components that bind to metal ions can be quantified. A recent study developed an optical biosensor based on the intracellular fluorescence of microalgae genetically modified to facilitate the entry of metal ions into their cells [95]. The absorption and intracellular storage of metal ions in microalgae, focusing on the species *Chlamydomonas reinhardtii*, were investigated. The production of the biosensor through the insertion of a plasmid with fluorescent proteins fused to a metallothionein and in vitro metal ion binding studies was evaluated. The results highlighted the biosensor's ability to quantify free toxic metal ions in microalgae. Among the metal ions evaluated, the biosensor presented a higher sensitivity for Hg^{2+} followed by Cd^{2+}, Pb^{2+}, Zn^{2+}, and Cu^{2+}. This study demonstrates the potential of microalgae to be used in diverse and efficient ways as biosensors [95]. Along with fluorescence, dissolved oxygen is also an important parameter for evaluating the presence of pollutants, as it is directly related to the photosynthetic activity of the algae.

Another advantage of biosensors lies in their versatility, as they can be effectively utilized not only for environmental analysis but also for the detection of pesticide residues in food products. Liu et al. (2023) reported the successful creation of a microalgae-based, confinement-enhanced optical biosensor that achieved accurate detection of the pesticide atrazine at remarkably low concentrations. In this study, *Chlamydomonas reinhardtii* cells

were confined in situ in microgel traps, eliminating common issues related to optical signal instability caused by cell movement and light scattering at high concentrations. This methodology facilitated spatial phase stabilization and reduced multiple scattering, enabling linear detection of atrazine over a range of 0.04–100 μg L^{-1}. Double-blind tests on commercial samples of corn and sugarcane juice demonstrated the system's high accuracy, with an average bias of 1.661 μg L^{-1} for corn and 3.144 μg L^{-1} for sugarcane samples, underscoring the biosensor's robustness for pesticide analysis in contaminated food products [96].

3.4. Electrochemical Biosensor by Algae Oxygen Production

Amperometric measurement and evaluation of photosynthetic activity based on oxygen production have proven to be a promising technique for developing algae-based environmental biosensors [97]. These biosensors measure the reduction of a redox mediator or monitor oxygen production through an electrode. Oxygen electrode-based biosensors have demonstrated good sensitivity, long operational life, and suitability for aquatic environmental monitoring [98]. Photosynthetic response as a function of light intensity, along with the amperometric activity, while the microalgae and cyanobacteria were in contact with the pollutant, has been investigated [73,78]. In this system, a sensor using *Pseudokirchneriella subcapitata*, *Desmodesmus quadricauda*, *Microcystis aeruginosa*, and *Synechococcus elongatus* in an electrochemical sensor for toxicological detection was developed, detecting the contaminant through changes in the electrical properties of the solution [80,82]. The material presented satisfactory results with a quick response time, affordability, ease of use, and the potential for development into a fully automated system [78].

Conductometric biosensors operate based on the production or consumption of ionic species during metabolic processes, causing changes in the electrical conductivity of the electrolytic solution, which can be measured to determine the sensor's response [52,80]. Potentiometric biosensors convert biological interactions between the sensor and the contaminant into measurable electrical signals [99]. On the other hand, amperometric biosensors measure changes in current resulting from the reduction or oxidation of electroactive species on the electrode surface, with the potential between the working and reference electrodes kept constant during current measurement [100].

A biosensor based on *Pseudokirchneriella subcapitata* to determine the presence of diuron, simetryn, simazine, and atrazine in water, was investigated, focused on oxygen production, along with fluorescence intensity [101]. In 10 min, the biosensor showed a limit of detection of 1.0 μg L^{-1} for diuron, and 10 μg L^{-1} for simetryn, simazine, and atrazine using a sample volume of only 200 μL, demonstrating that the material is fast, low-cost, and eco-friendly. The monitoring of atrazine in water was also investigated using a portable microalgae *Scenedesmus acutus* and *Monoraphidium contortum* sensor [86]. The microalgae were immobilized in polyelectrolyte-surfactant-carbon nanotube self-assembled material cast on a screen-printed graphite electrode, which was able to monitor the oxygen reduction through photosynthesis inhibition with a detection limit of 0.11 μmol L^{-1}, indicating a promising response for river samples. Also, the biosensor presented satisfactory stability, maintaining its integrity over 5 months of immersion in a freshwater algae medium at room temperature. The use of microbial fuel cells (MFC) to develop a photosynthetic MFC sensor for detecting the presence of formaldehyde in water has been studied [21]. To inoculate the MFCs, two species of microalgae from a pilot high-rate algal pond (HRAP) were used: *Scenedesmus obliquus* and *Chlorella luteoviridis*. The photo MFC sensor was able to demonstrate the interference of the contaminant in the photosynthetic activity of the algae, detecting the presence of formaldehyde in concentrations ranging from 0.001 to 0.02%. In addition, the electrogenic activity of the microalgae in the sensor demonstrated a cost-effective and rapid analytical method for detection compared to traditional biological analysis.

An electrochemical biosensor based on the green microalga *Scenedesmus* sp. MN738556 aimed to evaluate the biotoxicity of Cd^{2+} has been developed [52]. The biosensor was con-

structed by immobilizing *Scenedesmus* sp. microalgae on a glassy carbon electrode surface using bovine serum albumin. Its responses were based on the chronoamperometric currents generated by the activity of alkaline phosphatase. The sensitivity was verified with a half inhibition concentration of 0.26 μg L^{-1} for Cd^{2+} at pH 8.5 and 10^7 cells mL^{-1}. Meanwhile, an electrochemical biosensor using algae (*Desmodesmus quadricauda* and *Pseudokirchneriella subcapitata*) and cyanobacteria (*Microcystis aeruginosa* and *Synechococcus elongatus*) has been studied [78]. The strategy involved immobilizing the microalgae and cyanobacteria along with a mediator in a Sephadex gel structure, allowing the detection of microbial reduction of the mediator. The results showed a good correlation with the reference analytical methods, such as the Algal Growth Inhibition Test and the Microtox Test, highlighting the ease of handling and reduced measurement time as advantages of this new biosensor.

An alternative approach to the development of algae-based biosensors involves utilizing algal biomass as a sustainable substrate for the chemical coating of silver nanoparticles, which can be employed for the electrochemical detection of pesticides. According to Ameen et al. (2023), this study used self-grown biomass of *Spirulina platensis*, modified with chemical silver, as a powder amplifier in the construction of a carbon paste electrode, with 1-hexyl-3-methylimidazolium hexafluorophosphate as a conductive ligand. The determination of atropine was conducted using voltammetric methods, demonstrating that the electrochemical behavior of atropine is pH dependent, with pH 10.0 identified as the optimal condition for analysis. The fabricated sensor exhibited a linear response within the concentration range of 0.01–800 μM, achieving an impressive detection limit of 5 nM for atropine. The results also confirmed the stability, reproducibility, and selectivity of the sensor, as evidenced by recovery percentages of 94.48–101.58% for atropine sulfate vials and 98.01–101.3% for water samples [101].

Table 1 presents the main articles focused on using algae or their components for application in biosensors for the detection of potentially toxic metal ions, covering the period from 2014 to the present. As can be seen in Table 1, the genus of algae commonly used for biosensing is *Scenedesmus*, specifically from the species *Scenedesmus obliquus*, *Scenedesmus acutus*, *Scenedesmus* sp., and *Scenedesmus subspicatus*. However, research should continue comparing the performance of the strains of algae as cell biosensors. The limits of detection using algae-based biosensors range from 0.2 mmol L^{-1} to 0.9 mg L^{-1} for different contaminants, such as diuron, Cd, Cu, and Zn. Thus, in general, algae-based biosensors offer several advantages for application in biosensors for the detection of potentially toxic metal ions, such as sensitivity and selectivity, fast response, low production cost (less than 2 USD per-sample) [53], satisfactory stability, and efficiency, indicating these devices as an effective analytical tool for environmental monitoring.

Table 1. Demonstration of different strategies of biosensor use based on reported works over the last decade (2014–2024).

Algae	Objective	Algae Preparation	Detection Method	Main Results	Reference
Chlamydomonas reinhardtii	To use biosensor as an indicator of the presence of pesticides.	Dense algal solutions	Detection of O_2, H_2O_2, and H_3O^+/OH ions, species taking part in metabolic activities of algae.	Diuron herbicide detection was achieved with a sufficiently low limit of detection—0.2 mmol L^{-1};	[82]

Table 1. *Cont.*

Algae	Objective	Algae Preparation	Detection Method	Main Results	Reference
Porphyridium cruentum	To develop biosensor based on carbon paste electrode modified with *Porphyridium cruentum* biomass for the determination of As^{3+} in contaminated water.	The microalgal biomass was well-dispersed in appropriate quantities of graphite powder and mineral oil using pestle and mortar to obtain a homogenized paste. Biomass quantity of *Porphyridium cruentum* varied from 0.5% to 7.5% optimizing maximum As biosorption.	Differential pulse anodic stripping voltammetric technique	Suitable result was obtained at pH 6.0 with 0.1 mol L^{-1} HNO_3 solution as a stripping medium, allo wing biosorption–accumulation time of 8 min using 5% *Porphyridium cruentum* biomass in graphite–mineral oil paste. Linear range for As^{3+} detection with the modified electrode–biosensor was observed between 2.5 mg L^{-1} and 20 mg L^{-1}; Efficiency of the biosensor in the presence of different interference metal ions (Na^+, K^+, Ca^{2+}, and Mg^{2+}) ions was also evaluated; The application of *Porphyridium cruentum* modified biosensor was successfully used for the detection of As^{3+} in the binary metal (Fe^{3+}, Mn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, and Pb^{2+}) contaminated system; The accuracy of application of biosorption-based biosensors for the detection of As^{3+} is as low as 2.5 mg L^{-1}.	[13]
Chlorella vulgaris, Pseudokirchneriella *subcapitata*, and *Chlamydomonas reinhardtii*	Evaluation of fluorescence of immobilized microalgae on the detection of chemicals in urban rainwaters.	Immobilized microalgae by encapsulation in a hybrid alginate/silica translucid hydrogel.	Fluorescence emission detected by a fluorometer, of chlorophyll.	Immobilized algae exhibited lower sensitivity than free algae in suspension (limit of detection 0.001 µmol L^{-1} and <0.001 µmol L^{-1}) at atrazine (pesticide), but higher fluorescence.	[84]
Paulschulzia pseudovolvox and species of cyanobacteria in the order *Chroococcales*	Investigation of the photosynthetic metabolism to be used to integrate and sense environmental signals and the effect of toxic compounds detected by the disruption of the reliable light-dependent electrogenic effect.	The algae were grown in a chamber for four weeks prior to experiments. The chamber was then converted into a Photosynthetic Microbial fuel cell by the addition of an assembly containing the cathode.	The light-dependent electrogenic activity was monitored using a potentiostat.	Initial results suggest *Paulschulzia pseudovolvox* to be more resistant to effects of the toxicants tested in *Chroococcales*, demonstrating the importance of using multiple species as they will present a different level of sensitivity to different analytes (copper, thallium, zinc, and glyphosate).	[102]

Table 1. *Cont.*

Algae	Objective	Algae Preparation	Detection Method	Main Results	Reference
Chlorella vulgaris	To use biosensor to detect the presence of metal ions (Cu, Pb, Cd, Na, Al, and Li) in water.	Immobilization of microalgae in agarose solution	Bioluminescence of microalgae was used as an indication parameter (λ = 350–650 nm).	pH tolerance (pH 6–pH 8), and able to produce signals at different cell densities (1×10^6 cells mL^{-1} to 9×10^6 cells mL^{-1}) and culture ages (day 1 to day 5); The biosensor showed high sensitivity to metal ions (Cu, Pb, Cd, Na, Al, and Li); The presence of these metal ions with concentrations of 0.001 mg L^{-1} to 10.000 mg L^{-1} could be detected rapidly within 15 min of exposure.	[20]
Chlamydomonas reinhardtii	Develop an algae biosensor for the optical detection of nano-encapsulated-atrazine in agriculture.	Algae were immobilized on a paper substrate soaked with an agar thin film and placed in a glass optical measurement cell.	Detected by following the variable fluorescence	Nanoencapsulated atrazine was detected by fluorescence, with an inverse and proportional decrease to the herbicide concentrations ranging from 0.5 nmol L^{-1} to 200 nmol L^{-1} with a limit of detection of 16 h; There was slight interference in the presence of 2 mg L^{-1} for Cu and 10 µg L^{-1} for As at safe limits, a slight matrix effect, and a recovery value of 96 ± 5% for 75 nmol L^{-1} nanoencapsulated atrazine in tap water; The suitability of the proposed paper-based optical biosensor as valid support in agriculture.	[54]
A mixed culture of microalgae that predominantly contains the strains (*Scenedesmus obliquus* and *Chlorella luteoviridisI*)	Develop an innovative single-chamber air system microbial fuel cell (MFC) photosynthetic cathode and analyze electrochemical performance of the device using formaldehyde as a response meter—toxic model. Use this technology to monitor water quality.	To initiate biofilm growth, green microalgae was injected into photo MFC and allowed seating and attachment to the anode under static conditions. Two hours an open circuit potential was allowed to develop, and then the photo MFC was connected to a 1000 U resistor to trigger the development of an electroactive biofilm.	Electrochemical analysis using two-electrode mode, with the anode as the working electrode and the cathode as the counter electrode.	The photo MFC demonstrated promising proof-of-concept capability for detecting formaldehyde between 0.001% and 0.02%. Through the measurement of the electrogenic activity of micro-algae in the photo MFC, detection of these contaminants could be rapid and cost-effective compared to biological assays (given the relationship below low cost and simple treatment of materials used in manufacturing) and onsite (due to the device's small size and portability).	[21]

Table 1. *Cont.*

Algae	Objective	Algae Preparation	Detection Method	Main Results	Reference
Cystoseira algae	Prepare new types of electrode materials for electrochemical sensing of vardenafil as an active substance.	*Cystoseira* algae dried were dispersed in 400 mL of distilled water and transferred to autoclave. Carbon electrode material was prepared by two-step cycled hydrothermal carbonization of *Cystoseira* algae at temperatures of 180 °C and 250 °C for 8 h in the autoclave.	Cyclic voltammetry, differential pulse voltammograms, and square wave voltammetry techniques.	Material exhibited high sensitivity with the limits of detection of 96.3 pmol L^{-1} at pH 1.0 H_2SO_4 solution; The proposed sensor was successfully applied in tablet formulation, human serum, and human urine samples.	[103]
Scenedesmus acutus and *Monoraphidium contortum* strains	The construction of a portable system based on reversible photosynthesis inhibition was used as an indication parameter (λ = 350–650 nm). Produced by herbicides on microalgae, using atrazine as a model compound.	The immobilization of microalgae in a polyelectrolyte-surfactant-carbon nanotube self-assembled material cast on a screen-printed graphite electrode.	Electrochemical experiments were carried out with a purpose-built potentiostat. Oxygen production was followed by chronoamperometry. Cyclic voltammetry was carried out using the same three-electrode system.	*Monoraphidium contortum* can perform as an efficient recognition element for the construction of biosensors sensitive to atrazine; The system presents a limit of detection of 0.11 µmol L^{-1}, showing an excellent performance in river samples. The sensor maintains its integrity after five months immersed in a freshwater algae medium at room temperature.	[85]
Chlorella sp.	Developed a living sensor for metal ion detection with nanocavity-enhanced photoelectrochemistry	Mix of *Chlorella* sp. with copper nanoparticles solution.	Photoelectrochemical measurements in chronoamperometry mode.	Microalgae sensor was exploited to detect potentially toxic metal ions, Cd, Cr, Fe, and Mn with a breakthrough limit of detection of 50 nmol L^{-1}.	[86]

Table 1. *Cont.*

Algae	Objective	Algae Preparation	Detection Method	Main Results	Reference
Mesotaenium sp. and a strain of *Synechococcus* sp.	Developed an optical microalgal-cyanobacterial array biosensor using microalgae, to detect Cd^{2+}, $Cr^{6+,}$ and Zn^{2+} in aquatic systems.	Sol-gel immobilization mixture prepared by sodium silicate and colloidal silica	Optimum operational conditions for the biosensor array such as exposure time, storage stability, pH, and multiple metal ions effects.	10 min exposure time yielded optimum fluorescence values; Metal ions toxicity increased with decreasing pH, resulting in low relative fluorescence (%), and decreased with increasing pH, resulting in higher relative fluorescence (%); The optimum storage time for biosensor strains was 4 weeks for microalgal cultures and 8 weeks for cyanobacterial culture, at 4 °C storage temperature; The metal ion mixtures showed less effect on the inhibition of relative fluorescence (%) of microalgal/cyanobacterial cultures, displaying an antagonistic behavior among the metal ions tested.	[46]
Microcystis aeruginosa, *Synechococcus elongatus*, and strains *Desmodesmus quadricauda*, and *Pseudokirchneriella subcapitata*	Develop an alternative to using this electrochemical biosensor equipped with algae and cyanobacteria for toxicological investigations based on selected test chemicals.	Algae/cyanobacteria solution was centrifuged, and the pellets were diluted with 30 mL culture medium.	Potentiostatic measurement	The evaluation of the sensor signal is based on the current–time curves of a potentiostatic measurement produced by the detection of microbially reduced mediator molecules immobilized in a gel structure; The mediator molecules are reduced during the measurement process and produce a current signal, which rapidly provides information about the vigor and vitality of living bacteria, yeasts, fungi, or cells.	[78]

Table 1. *Cont.*

Algae	Objective	Algae Preparation	Detection Method	Main Results	Reference
Scenedesmus acutus and *Pseudokirchneriella subcapitata*	The developed sensor was based on green microalgae immobilized in an alginate matrix.	Immobilization in encapsulation (alginate beads)	Na Visual observation, and absorbance and fluorescence measurements.	After incubation with different pollutants for five days, naked-eye analysis by several observers proved to be a successful method for surveying algae's growth and establishing the limits of detection; Suitable limits of detection were 10 mg L^{-1} for technical-grade acid glyphosate, 15 mg L^{-1} for glyphosate-based formulation, 50 µg L^{-1} for atrazine formulation, 7.5 mg L^{-1} for Cu, and 250 µg L^{-1} for Cr; The use of the biosensor on the local samples also proved to be successful: strong intensity of green color in those samples from clean water sources.	[53]
Scenedesmus subspicatus	Develop a novel whole-cell biosensor using chlorophyll a fluorescence from a single species of microalga, Scenedesmus subspicatus, immobilized in an inorganic silica matrix, for detecting bio-availability of multi-metal ions in freshwater.	Immobilization in inorganic silica hydrogels using the sol-gel technique	Effective pH range, cell density, exposure time, and storage stability.	The optimum response for the biosensor was dependent on the pH of the matrix, cell concentration, and exposure time derived; The biosensor was operational for four weeks; The limit of detection for the algal biosensor was determined as 0.9, 0.91, and 0.88 mg L^{-1} for Cd, Cu, and Zn, respectively.	[55]
Scenedesmus sp.	Develop an electrochemical biosensor with microalgae to evaluate the biotoxicity of Cd^{2+} ions in freshwater.	This electrochemical biosensor was constructed by immobilization of microalgae–bovine serum albumin and crosslinked with glutaraldehyde in film on a glassy carbon electrode surface.	The chronoamperometric currents generated by alkaline phosphatase activity. The feasibility was evaluated, and the application of the biosensor was optimized for parameters such as pH and cell density.	*Scenedesmus* sp. biosensor is highly sensitive with a good selectivity at a 1:1 ratio for measuring the concentration of Cd^{2+} cations except in the presence of Hg^{2+}; This biosensor could respond with only one drop of an analyte (50 µL of 1000 µg L^{-1} Cd^{2+}), resulting in suitability for simple and on-site water toxicity testing.	[52]

Table 1. *Cont.*

Algae	Objective	Algae Preparation	Detection Method	Main Results	Reference
Chlamydomonas reinhardtii	Use a biosensor with transgenic microalgae for metal ion detection and quantification.	Suspension of cells in the exponential growth phase	Detection by fluorescence measurements using a spectrofluorometer.	Detection of various metal ions at low limits of detection—0.93 nmol L^{-1}; Algae have substantial buffering capacity for free potential metal ions in their cytosol, even at high external metal ions concentrations.	[95]

4. Challenges and Perspectives in the Development and Implementation of Biosensors

The application of algae-based biosensors for detecting potentially toxic pollutants is quite promising, and a key challenge is the attainment of high sensitivity, specificity, stability, and selectivity of the electrode response. The production costs of biosensors in large quantities must also be considered. It should be noted that the commercialization of biosensor technology has lagged significantly behind research results, as reflected by publications with various methods and materials that can be used. The logic behind the slow and limited transfer of technology can be attributed to cost considerations and some obstacles related to the regulation and compliance with health legislation.

Meanwhile, analytical techniques such as spectrometric techniques guarantee excellent results in the detection of potentially toxic metal ions, with some of them allowing the quantification of toxic metal ions at trace concentrations. However, there is a growing interest in developing alternative analytical methods, offering a sustainable, efficient, and cost-effective solution for potential toxic element detection, as is the case with metal ions. Biosensors emerge as an excellent alternative considering their costs, versatility, and robustness for the quantification of metal ions in environmental samples. In particular, optical and electrochemical biosensors exhibit significant analytical performance characterized by sensitivity, stability, and detection limits, making them promising tools for environmental monitoring. Optical biosensors have demonstrated high sensitivity, with detection limits ranging from 0.001 to 0.11 μmol L^{-1} for various contaminants, while electrochemical biosensors display detection limits as low as 0.11 μmol L^{-1} for atrazine, with long-term stability of up to five months [21,84,85].

Algae-based biosensors exhibit notable versatility, allowing for application in diverse environmental contexts, while their potential for low-cost production enhances their accessibility. However, limitations in reproducibility and specificity restrict their effectiveness in detecting multiple pollutants, and the complexity of optimizing immobilization techniques and simplified transducers presents additional challenges for performance improvement. Advances in nanotechnology offer promising avenues to improve biosensor functionality and facilitate the creation of innovative sensing platforms.

In terms of future perspectives, the success of using whole algae in biosensor production is noteworthy, but other new materials to optimize the performance of metal ion sensors for specific applications are still necessary. Notably, advances in nanotechnology show great promise in enhancing biosensor performance and enabling the development of novel materials and detection platforms. To address this issue, there is a growing trend toward developing user-friendly biosensors that can be easily assembled and utilized by individuals without a scientific background [53]. This shift towards more accessible technology signals a promising direction for the future of biosensor development. In addition, bioinformatics tools and data analysis algorithms can improve the accuracy and reliability of biosensor measurements, and artificial intelligence facilitates the analysis of complex data sets generated by biosensors, leading to faster and more accurate results.

5. Conclusions

In summary, algae represent a promising component of life and biomass on our planet. In addition to being promising in chemical and pharmaceutical biotechnology, algae offer a unique range of bio-compounds that enable this biomass to work within a fully sustainable plan, producing oxygen alongside biosensor performance. This alternative could overcome some disadvantages of powerful and well-consolidated analytical techniques, such as spectrometric and electrochemical techniques, as well as the recent integration of artificial intelligence to support quantitative or qualitative analytical profiles. Improving environmental quality has become a primary objective of research worldwide, inherently tied to controlling and monitoring environmental parameters. These issues demand continuous, rapid, and sensitive monitoring systems capable of detecting toxic pollutants in water and sediment samples, given the extremely negative effects on the environment and living organisms. In this regard, with the advances and issues of sustainable biosensors, algae biomass holds significant potential for new technologies and innovative applications.

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