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Luminescent microbial bioassays and microalgal biosensors as tools for environmental toxicity evaluation

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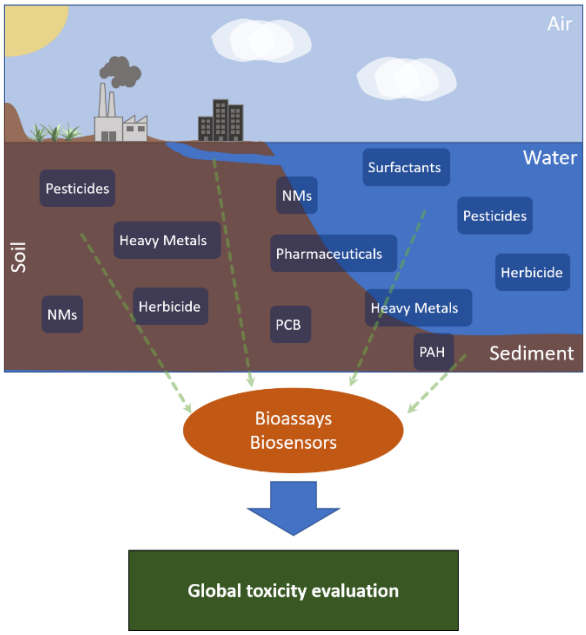
Abstract

This chapter deals with toxicity bioassays and biosensors based on luminescent microorganisms that report on global toxicity of a sample in such a way that luminescence is reduced or inhibited in the presence of toxic compounds that impair metabolism. Both natural as well as recombinant microorganisms are considered. A detailed description of their main characteristics and environmental applications is reported. A few examples of bioassays for detecting oxidative stress (both bioluminescent and fluorescent bioreporters) are also mentioned and discussed as reactive oxygen species (ROS) formation and subsequent oxidative stress if the antioxidant defenses of the cells are surpassed is one of the main mechanisms of toxicity for most pollutants. There is also a section dedicated to microalgal–based biosensors given their ecological relevance as primary producers, their easiness of culture and immobilization in different matrices, ability to acclimate to low nutrients conditions and ubiquity in aquatic environments. The most used toxicity endpoints for this type of biosensors are the alteration of photosynthetic activity (optical and amperometric biosensors) and the inhibition of enzymes such as alkaline phosphatase (APA) or sterases (mostly conductometric biosensors). The main information is shown in tables that include the microorganisms, their main characteristics (reporter gene systems, transducer types for biosensors) and their main environmental applications as well as relevant references. Although some of these bioassays have already been standardized by different international organizations, there are still many which are also promising tools for environmental global toxicity evaluation and should be fully validated and standardized for regulatory purposes.

Keywords (5-10): biosensor, bioreporter, bioluminescence, fluorescence, photosynthesis, oxidative stress.

Graphical abstract = Figure 1



Contents

Abbreviations

CAHs Chlorinated aliphatic hydrocarbons

PCP Pentachlorophenol

DCP Dichlorophenol

BTEX Benzene, toluene, ethylbenzene and xylene

NMs Nanomaterials

PAHs Polycyclic aromatic hydrocarbons

CBNs Carbon nanotubes

CAB Chlorophyll *a* binding protein

ROS Reactive oxygen species

CI Combination index

EC_{50} Effective concentration of pollutant that causes a 50% effect with respect to a non-treated control

LAS Linear alkylbenzene sulfonate surfactant

APA alkaline phosphatase activity

SAMs self-cell assembled monolayers

PAM Pulse-Amplitude-Modulated

1. Introduction

Worldwide contamination of natural resources, water, soil an even air poses a threat for human health and the environment. There is a need for real-time, *in situ* or online monitoring of pollutants. Chemical analysis techniques using chromatographic and spectroscopic methods are highly accurate and sensitive, but also costly as they require complex and analytical laboratory equipment, skilled personnel and, quite often, pretreatment of samples or extensive sample extraction are needed before measurements can be done. Biological toxicity assays should also be performed as they complement the chemical analyses and report on a simple question that chemical analysis cannot answer: is the sample toxic? Toxicity bioassays report on the bioavailability of the pollutants that is a parameter closely related to toxicity and on potential interactions of pollutants towards the tested organism, whether synergistic or antagonistic.

The information given by toxicity bioassays is very valuable for risk assessment strategies as they give information at a global scale. Representative bacteria, yeast, algae, plants and animals have been used in toxicity assays; mostly survival, growth and reproduction have been used as global toxicity endpoints; these bioassays are usually time-consuming and depending on the organisms, several days might be needed to perform a complete assay. Ethical issues might be of great concern when using higher-trophic level organisms such as fish or mammals (Farré and Barceló 2003).

Over the years, less laborious, cost-effective and with rapid toxicity endpoint bioassays have been developed. Among these bioassays, those based on microorganisms such as bacteria, yeast and algae are very useful due to their ease of cultivation and use and the possibility of mass production. Inhibition of bioluminescence or fluorescence have become the toxicity endpoint of choice (Steinberg et al. 1995). Both naturally and recombinant (transgenic) bioluminescent microorganisms have been used in assessing the toxicity of environmental samples and some of these bioassays have been commercialized (Belkin 2003). An ulterior evolution of these bioassays is the construction of the devices known as biosensors in which the biological receptor (i.e. microorganisms) is integrated within a transducer that converts the biological response (luminescence, fluorescence and others) into a quantifiable signal, which depends on the type of transducer: optical, electrochemical, thermal or piezoelectric (Bentley et al. 2001; Lei et al. 2006). Immobilization of the microorganisms is a key process for the development of biosensors (Michelini and Roda 2012).

There have been recent reviews on different bioassays and microbial biosensors covering mostly electrochemical cell-based biosensors for toxicity assessment (Ejeian et al. 2018; Hassan et al.

2016; Lagarde and Jaffrezic-Renault 2011). In addition, Kokkali and van Delft (2014) reviewed quite extensively the available commercial bioassays for toxicity in water samples. This chapter will, then, focus on toxicity bioassays and biosensors mostly based on luminescent microorganisms both natural and transgenic that report on global toxicity of an environmental sample in such a way that luminescence is reduced or inhibited in the presence of toxic compounds that impair metabolism (a schematic representation of this chapter is shown in Figure 1). This type of bioassays is usually referred as turn-off or lights-off, as there is a loss of function. A few examples of bioassays for detecting oxidative stress (both bioluminescent and fluorescent bioreporters) are also mentioned and discussed as reactive oxygen species (ROS) formation and subsequent oxidative stress if the antioxidant defenses of the cells are surpassed is one of the main mechanisms of toxicity for most pollutants.

Finally, there is a section dedicated to microalgal–based biosensors given their ecological relevance as primary producers, their easiness of culture and immobilization in different matrices, ability to acclimate to low nutrients conditions and ubiquity in aquatic environments. Despite their relevance, not many reviews have been published on this topic; in this context, (Brayner et al. 2011) wrote a rather comprehensive review but focused mainly on algal immobilization procedures and discussed just a few microalgal biosensors. The most used toxicity endpoints for this type of biosensors are the alteration of photosynthetic activity (optical and amperometric biosensors) and the inhibition of enzymes such as alkaline phosphatase (APA) or esterase (mostly conductometric biosensors).

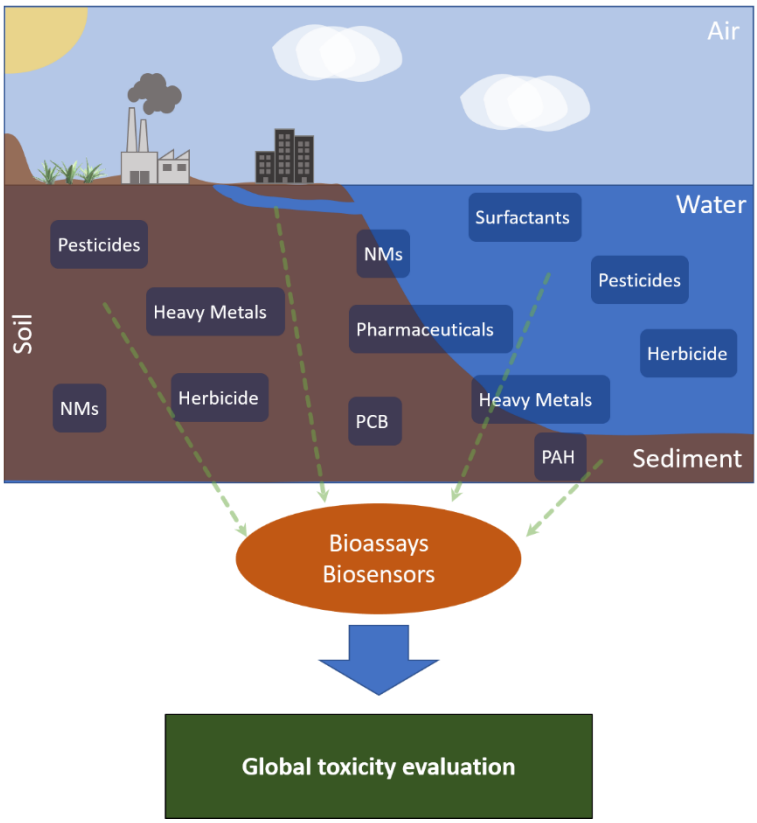


Figure 1. Schematic representation of the content of the chapter in terms of the toxicity bioassays and biosensors reporting on global toxicity. NMs: Nanomaterials; PCB: polychlorinated biphenyls; PAH: Polycyclic aromatic hydrocarbons.

2. Naturally bioluminescent microorganisms for environmental toxicity evaluation

Since the first research works during the 60's decade in which the effect of air pollutants on luminescent bacteria was determined (Serat et al. 1967; Serat et al. 1965), the use of naturally bioluminescent microorganisms has been widely expanded as a sensitive test for the quick assessment of environmental toxicity. The first organism used for this purpose was the aquatic bacterium *Photobacterium phosphoreum* (*P. phosphoreum*) (Serat et al. 1965), however other prokaryotic and eukaryotic organisms have been also used in bioluminescent assays. Table 1 provides an extensive summary of all the naturally luminescent microorganisms which have been used during last decades, featuring the type of organism, species, main applications and commercial devices if available. All these bioassays are based on the reduction of light emitted by a nonpathogenic luminescent organism upon exposure to a toxic sample. Essentially, as bioluminescence mainly depends on cell metabolism, any toxic compound that may compromise the metabolic state of cells will cause a reduction in light emission, generally, proportional to the substrate concentration.

Table 1. Naturally bioluminescent microorganisms which have been used for environmental toxicity evaluation organized by type, specie, tested pollutants and main applications (most reports before 2014 already reviewed in Fernández-Piñas et al. (2014).

Type	Microorganism	Tested pollutants	Environmental applications	Commercial test	References
Prokaryotic	*Aliivibrio fischeri* -several strains-	NMs, heavy metals, organotin compounds, PAH, PCB, Personal care products, pesticides, pharmaceuticals, plasticizers, Surfactants, allelopathic compounds, antibiotics, hydrophobic organic substances, azo-dyes, herbicide.	Freshwater, groundwater, mixtures, seawater sediment, soil, wastewater, agriculture, industrial wastewater, domestic wastewater, marine sediments.	Microtox M500 Microtox ToxAlert® 10 ToxAlert 100 LUMISTox 300	*Inorganic pollutants:* (Andreani et al. 2017; Aruoja et al. 2015; Bondarenko et al. 2016; Cukurluoglu and Muezzinoglu 2013; de OF Rossetto et al. 2014a; de OF Rossetto et al. 2014b; Fekete-Kertész et al. 2017; Gao et al. 2015; Heinlaan et al. 2008; Hjorth et al. 2017; Hsieh et al. 2004; Jemec et al. 2016; Jośko and Oleszczuk 2013; Jung et al. 2015; Kurvet et al. 2017; Mortimer et al. 2008; Nuzzo et al. 2017; Pereira et al. 2011; Ríos et al. 2018; Sajayan et al. 2017; Sanchís et al. 2015; Strigul et al. 2009; Svartz et al. 2017; Tsiridis et al. 2017; Velzeboer et al. 2008; Zabetoglou et al. 2002). *Organic pollutants:* (Aichberger et al. 2006; Burga-Perez et al. 2013; Dzyadevych and Chovelon 2002; El-Alawi et al. 2001; Farré et al. 2008; Farré et al. 2002a; Hirmann et al. 2007; Jurado et al. 2008; Kungolos et al. 2004; Lechuga et al. 2016; Li et al. 2008; Ma et al. 2005; Muneer et al. 1999; Neamtu et al. 2004; Osano et al. 2002; Papadopoulou and Samara 2002; Pérez et al. 2001; Salizzato et al. 1998a; Scholz and Liebezeit 2012; Segura et al. 2012; Yu et al. 2013). *Water :* (Burga-Perez et al. 2013; Corrêa et al. 2009; Ellouze et al. 2009; Farré and Barceló 2003; Fernández-Piñas et al. 2014; Gouider et al. 2010; Hernando et al. 2007; Katsoyiannis and Samara 2007; Kováts et al. 2012; la Farré et al. 2001; Molins-Delgado et al. 2016; Nohava et al. 1995; Ocampo-Duque et al. 2008; Saddoud et al. 2009; Somensi et al. 2010; Wang et al. 2002; Wolska et al. 2006) *Soil:* (Bispo et al. 1999). *Sediments:* (Papadopoulou and Samara 2002; Salizzato et al. 1998b; Scholz et al. 2012; Zabetoglou et al. 2002).
	Aliivibrio logei	Heavy metals, PAH, pesticides.	Industrial wastewater, seawater.		(Fernández-Piñas et al. 2014).
	Vibrio harveyi	Heavy metals, NMs.		LumiMARA	(Jung et al. 2015; Thomulka et al. 1997).
	Photobacterium leiognathi	Fuel traces, heavy metals,	Freshwater.	LumiMARA Microtox ToxScreen®	(Fernández-Piñas et al. 2014; Jung et al. 2015).

			NMs, PAH, PCBs, pesticides.			
		Photobacterium phosphoreum -several strains-	Heavy metals, NMs, PAH, pesticides.	Mixtures, sediments, freshwater, drinking water.	Microtox LumiMARA LUMISTox 300 Microbiosensor B17-677F	*Inorganic pollutants:* (Barrena et al. 2009; Deryabin et al. 2016; Fernández-Piñas et al. 2014; Hassan and Oh 2010; Jung et al. 2015; Wang et al. 2017). *Organic pollutants:* (Kahru et al. 1996; McConkey et al. 1997). *Water:* (Hermens et al. 1985). *Sediments:* (Brouwer et al. 1990).
		Photorhabdus asymbiotica	NMs.		LumiMARA	(Jung et al. 2015).
		Photorhabdus luminescens	Heavy metals, NMs.	Wastewater.	LumiMARA	(Jung et al. 2015; Schmitz et al. 1999).
		Vibrio qinghaiensis sp. Q67	Heavy metals, pesticides.	Drinking water, freshwater, mixtures.		(Ding et al. 2017; Fernández-Piñas et al. 2014; Mo et al. 2017; Wu et al. 2018; Zhang and Chen 2014).
Eukaryotic	**Dinoflagellate**	*Ceratocorys horrida*	Heavy metals, organochlorine compound.	Industrial wastewater, seawater.	QwikLite QwikLite™	(Fernández-Piñas et al. 2014; Lapota et al. 2007; Okamoto et al. 1999; Rosen et al. 2008; Sanchez-Ferandin 2015).
		Lingulodinium polyedrum	Heavy metals.	Industrial wastewater, seawater.	QwikLite QwikLite™	
		Pyrocystis fusiformis	Heavy metals.	Industrial wastewater, seawater.	LUMITOX® QwikLite™	
		Pyrocystis lunula	Heavy metals, pesticides.	Freshwater, groundwater, industrial wastewater, marine sediments, seawater.	QwikLite™	*Pollutants:* (Bao et al. 2008; Bao et al. 2011; Fernández-Piñas et al. 2014; Heimann et al. 2002; Stauber et al. 2008). *Water:* (Fernández-Piñas et al. 2014; Fetters et al. 2016; Hildenbrand et al. 2015; Lapota et al. 2007). *Sediments:* (Fetters et al. 2016).
		Pyrocystis noctiluca	Heavy metals.	Industrial wastewater, seawater.	QwikLite QwikLite™	(Fernández-Piñas et al. 2014; Lapota et al. 2007; Rosen et al. 2008; Sanchez-Ferandin 2015).
		Pyrophacus steinii	Heavy metals.	Industrial wastewater, seawater.	QwikLite™	
	Fungi	*Armillaria borealis*	Heavy metals.			(Fernández-Piñas et al. 2014).
		Armillaria gallica	Heavy metals.			

		Armillaria mellea	Heavy metals, pesticides.	Soil.		(Fernández-Piñas et al. 2014; Horswell et al. 2006; Jemec et al. 2016; Paton et al. 2006; Weitz et al. 2002).
		Gerronema viridilucens	Heavy metals.			(Fernández-Piñas et al. 2014; Mendes et al. 2010; Mendes and Stevani 2010).
		Omphalotus japonicus	Heavy metals.			(Fernández-Piñas et al. 2014).
		Mycena citricolor	Heavy metals, pesticides.			(Weitz et al. 2002).

Abbreviations. NMs: Nanomaterials; PAH: Polycyclic Aromatic Hydrocarbons; PCB: PolyChlorinated Biphenyl.

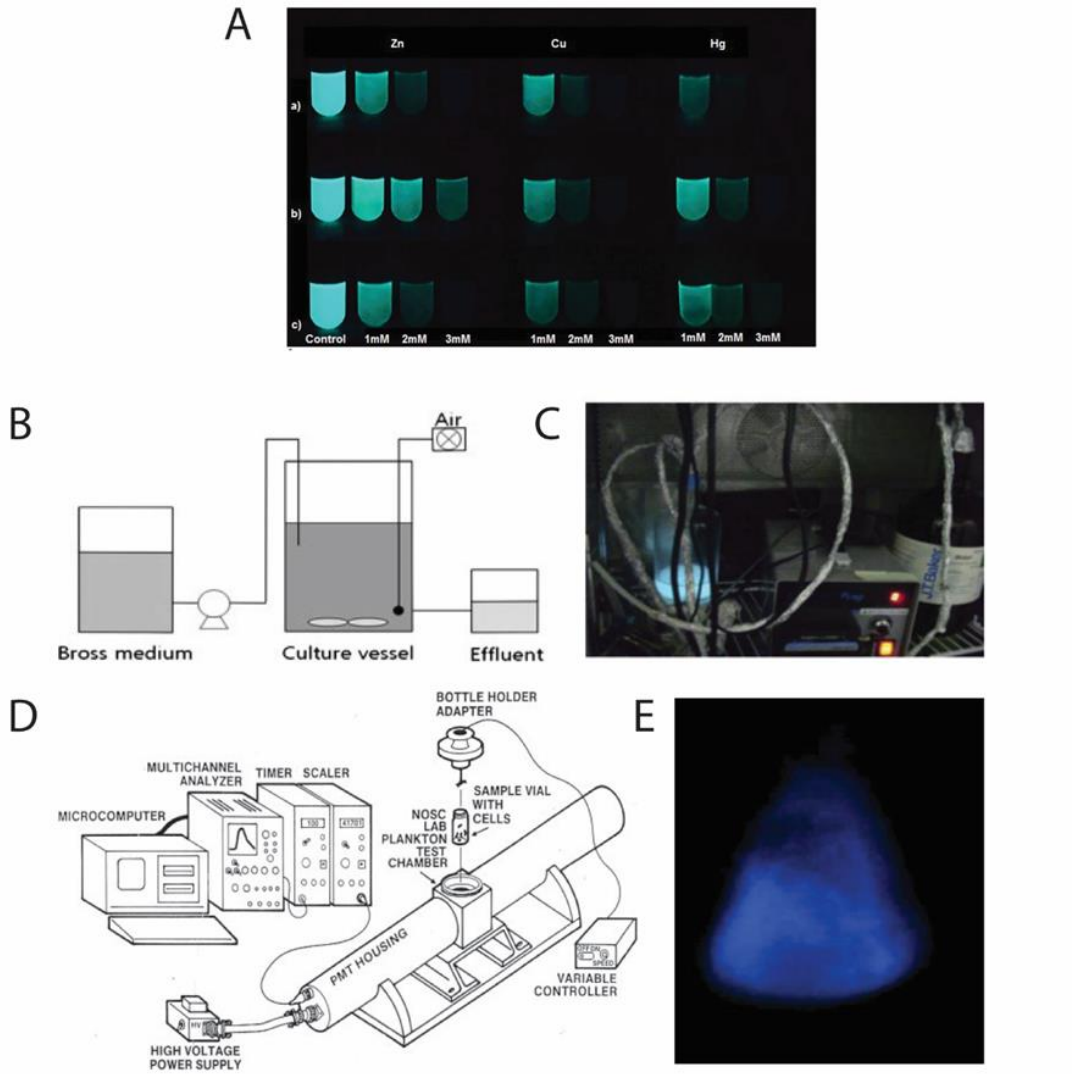


Figure 2. Examples taken from the reviewed literature of naturally bioluminescent microorganisms (see Table 1). (A) Performance of *Aliivibrio* bioluminescence reporter assay to determine efficiency of bioflocculant MSI021 on the removal of heavy metals. (a) bioluminescence observed in *Aliivibrio* cultures with 1, 2, and 3 mM heavy metals such as zinc, copper and mercury, (b) bioluminescence observed in *Alivibrio* cultures with 1, 2, and 3 mM heavy metals and 50 mg/ml bioflocculant, and (c) bioluminescence observed in *Aliivibrio* cultures with 1, 2, and 3 mM heavy metals and 100 mg/ml bioflocculant. Reprinted with permission from Sajayan et al. (2017), Copyright (2017), Elsevier. (B) A schematic diagram of continuous culture and photo (C) of continuous culture device for continuous culture of *Photobacterium phosphoreum.* Reprinted with permission from Hassan and Oh (2010), Copyright (2010), Elsevier. (D) diagram of the Laboratory Plankton Test Chamber (LPTC), the forerunner of the QwikLite™ bioluminescent toxicity test system. This system was originally designed and constructed for measuring bioluminescence from individual bioluminescent plankton isolated on-board oceanographic ships and (E) time exposure photograph of bioluminescence emitted from stirred dinoflagellate cells *Pyrocystis lunula*. The color of the light is blue-green centered a wavelength of approximately 470–490 nm. The integrated

bioluminescence is measured by the LPTC and QwikLite™. Reprinted with permission from Lapota et al. (2007), Copyright (2007), Elsevier.

The robustness and ease of this assessment strategy was quickly implemented in other studies (Bulich and Isenberg 1981; Kaiser and Palabrica 1991) and more bioluminescent bacteria such as *Aliivibrio fischeri* (*A. fischeri*, formerly known as *Vibrio fischeri*), *Aliivibrio logei (A. logei)*, *Vibrio harveyi* (*V. harveyi*), *Photobacterium leiognathi (P. leiognathi)*, *P. phosphoreum, Photorhabdus asymbiotica*, *(P. aymbiotica), Photorhabdus luminescens* (*P. luminescens*, previously called *Xenorhabdus luminescens*) or *Vibrio qinghaiensis sp.* Q67 (V. *qinghaiensis sp.* Q67) were also included in this kind of works (Table 1; please, see Figure 2 with examples of naturally bioluminescent microorganisms). One of the best known microbial bioassay is based on the marine bioluminescent bacterial strain *A. fischeri* NRRL B-11177 which was used to develop a commercial bioluminescence test in the late 70's under the name of Microtox™ (Microbics Corporation; Carlsbad, USA), as a rapid screening substitute to toxicity tests with animals such as fish or small invertebrates (Bulich and Isenberg 1981). A wide revision on the toxicity of a large number of chemicals that have been obtained using the Microtox system was completed by Kaiser and Palabrica (1991), more recently, just based on *A. fischeri* bioluminescence inhibition assay by Abbas et al. (2018).

As this bioluminescence test emerged as a cost-effective and rapid biological test, other companies marketed the same test under the commercial name ToxAlert® (Merck, Germany), BioTox™ (Aboatox, Finland), ToxScreen^3 (CheckLight Ltd, Israel) or LUMIStox™ (Hach Lange, US). It has been proved that different bioassays generate comparable results as Jennings et al. (2001) evidenced that three different commercial bioluminescence-based toxicity assays produced good results in terms of comparability between tests. In order to extend the spectrum of organisms used at a time and to get wider and valuable information of the toxic compound, the NCIMB Company (UK) commercialized the LumiMARA assay. This assay is analogous to the other bioluminescence tests described above, but it is based on a set of 11 bioluminescence bacteria (NCIMB Company, UK); this integrated evaluation constitutes a unique toxicity profile for the sample. In general, those commercial tests consist of freeze-dried or liquid-dried bacteria that are reconstituted prior to the assay. Then, their light production and, subsequent reduction by the toxicant, is measured by a luminometer and finally, the biological effect is given as the EC_{50}, i.e. the effective concentration of the compound that decreases light emission to 50 % of the original light emission.

Although the presence of color and turbidity in the samples might interfere with the luminous intensity measurements, those restrictions have been solved along years through several

variations of the basic bioluminescence assay. After 30 years testing with different strains of *A. fischeri*, some standardized test emerged. The German Institute for Standardization was the first agency in doing so and published the first standardized bioassays through the DIN 38412-34 in 1991. Other agencies added their respective normalized tests such as the International Organization for Standardization (ISO 11348 which is divided in three parts, depending on whether the method is based on freshly prepared bacteria, liquid-dried bacteria or freeze-dried bacteria; and ISO 21338-kinetic determination of the inhibitory effects of sediment, other solids and colored samples on the light emission of *A. fischeri*), the American Society for Testing and Materials (ASTM Draft No. 8, 1995), Environment Canada (Report EPS 1/RM/24) or the National Institute for Coastal and Marine Management (NICMM RIKZ/AB-99.107x).

As can be checked along the scientific literature, bioluminescence inhibition assay can be used for toxicity measurement of single compounds and mixtures of organic and inorganic compounds (Table 1). Within the group of organic pollutants, Polycyclic Aromatic Hydrocarbons (PAH), Polychlorinated Biphenyls (PCB), herbicides and pesticides have received special attention for their detrimental effects to environment and human health. As an example of a study which tests these pollutants, Salizzato et al. (1998a) determined the concentrations of aliphatic hydrocarbons and chlorinated pesticides from the lagoon of Venice and assessed their acute toxicity using the Microtox test, indicating that PAHs were more toxic than the other pollutants in agreement with the higher concentration obtained for PAHs through chemical methods. In addition, the continuing human development inevitably brings new compounds to the market that might have a negative environmental effect as well. Different substance such as surfactants, azo dyes, organotin compounds, plasticizers, among others, derived from the anthropogenic activity, have been recently assessed using bioluminescent assays (Lechuga et al. 2016; Segura et al. 2012) (Table 1). In particular, surfactants are a wide group of organic chemicals that play important roles in a great variety of fields such as detergents, lubricants, coolants, etc. However, the number of research works studying the environmental impact of these chemicals are scarce. Lechuga et al. (2016) reviewed and tested the effect of ionic and non-ionic surfactants using *A. fischeri* and other three model organisms (the microalgae *Raphidocelis subcapitata* and *Phaeodactylum tricornutum* and the freshwater crustacean *Daphnia magna*). Their results indicated that *A. fischeri* was more sensitive to the toxic effects of the surfactants than the other organisms, highlighting the importance of this specie as suitable organism for establishing the maximum permissible concentrations in aquatic ecosystems.

Pharmaceuticals, including antibiotics, and personal care products are an important, varied and enormous group of chemical compounds that are present as micropollutants in various aquatic environments and some of them have been catalogued as pollutants or great importance by different international regulations (e.g.: European Commission 2008/105). Several authors have used the luminescent bioassays based on *A. fischeri* in order to assess the impact of triclosan (Farré et al. 2008), antibiotics (Ji et al. 2013) or nonsteroidal anti-inflammatory drugs (Yu et al. 2013) in the trophic level of bacteria, organisms of great value in terms of energy flow and nutrient cycling in the ecosystem. de García et al. (2016) performed an ecotoxicological assessment of 20 pharmaceuticals and personal care products applied both individually and in mixtures using the *A. fischeri* bioassay. Interestingly, environmental concentrations of these substances were tested and the EC_{50} for each substance was estimated and compared with other studies. The categorization of products according to their hazard levels showed that more than half of the compounds exhibited some level of hazard (harmful to aquatic organisms, toxic or highly toxic) for *A. fischeri*.

Within the group of inorganic pollutants, heavy metals and, lately, nanomaterials (NMs) have centered the investigation of many research group around the world. Several human activities including mining, smelting or agriculture, among others, have resulted in the release of important amounts of heavy metals to the environment, where they can be strongly accumulated and biomagnified in water, sediment, and the aquatic food chain (Tchounwou et al. 2012). All the prokaryotic organisms that are showed in the Table 1 have been used for assessing heavy metal pollution, indicating that the advantages of this bioassay can be also applied for this type of contaminants. However, the use of high concentration of NaCl (up to 2-3 %), a needed requirement for bioassays based on marine organisms such as *A. fischeri, A. logei, V. harveyi, P. leiognathi* or *P. phosphoreum*, may influence the speciation, bioavailability and subsequent toxicity of metals. Therefore, organisms belonging to the genera of *Photorhabdus* or, specifically, *V. qinghaiensis sp. Q67* which are natural terrestrial and freshwater luminescent bacteria, respectively, have been proposed as alternative organisms for testing heavy metals or special pollutant from these environmental compartments. Schmitz et al. (1999) designed and compared a miniaturized bioluminescence inhibition assay in microtitration plates based on the terrestrial entomopathogenic nematode symbiont *P. luminescens* with the *A. fischeri* standardized test, using heavy metals as toxic substances. The authors concluded that this method might be a reliable alternative at low osmolarities in comparison to the established protocols and suggested to employ both assays to determine the toxic potential of environmental samples in order to reduce the occurrence of false positive.

Recently, engineered nanomaterials (NMs) and eco(nano)toxicology have become the focus of considerable attention because of their wide applications in many fields. Bioassays using bioluminescence bacteria for toxicity screening have been applied widely as acute toxicity bioassays to understand the ecotoxicological impact of NMs on aquatic organisms. Due to their advantages in simplicity, rapidity, cost-efficiency, and reproducibility, several commercial test and single bioluminescence organisms have been used in order to assess the ecotoxicity of a great variety of NMs such as metallic nanoparticles/nanomaterials based on Ag (Andreani et al. 2017; Georgantzopoulou et al. 2012; Jung et al. 2015), Cu (de OF Rossetto et al. 2014a), Al (Svartz et al. 2017), Zn, Ti (Jośko and Oleszczuk 2013), lanthanides (Kurvet et al. 2017), Fe (Hjorth et al. 2017; Oleszczuk and Kołtowski 2017), Cr (Puerari et al. 2016); organic nanomaterials for example polyamidoamine dendrimers (Mortimer et al. 2008), organic vesicles (Pereira et al. 2011), nano-plastic particles (Booth et al. 2016); or carbon-based NMs e.g. fullerene, carbon nanotubes (Sanchís et al. 2015) and graphene (Pretti et al. 2014). However, one of the present problems of nanotechnology is that there is an uncountable number of distinct NMs being synthesized and entering the market, thus there is an urgent need for hazard ranking and grouping of NMs. In this sense, it is necessary to find bioassays which efficiently identify the ecotoxicity of NMs considering their technical complexity, standardization status, sensitivity of the test and required training. Seven different and well-characterized NMs were used by Bondarenko et al. (2016), aiming at generating toxicity data using a set of 15 distinct bioassays which covered a wide spectrum of organisms (6 medically important bacterial species, yeast, alga, protozoan, two crustacean species, zebrafish and three mammalian *in vitro* cell lines). From all the evaluated test, four assays were selected and the 30 minutes *A. fischeri* bioluminescence inhibition assay (ISO21338:2010) which covered the trophic level of bacteria as decomposer was included in a multitrophic selection for nanotoxicity screening. Nevertheless, it is worth noticing that *A. fischeri* bioassay is only based on a single bacterial strain, which limits the various sensitivity ranges when compared to other assessment tools with multi-species of bacteria (marine and freshwater) such as LumiMARA. In this regard, Jung et al. (2015) evaluated the toxicity of four surface-coated Ag nanoparticles using the luminous microbial array LumiMARA, demonstrating the feasibility of this test which could be an improvement of the available methods in terms of sensibility without affecting the methodologic high-throughput point of view.

Whether using a panel or a single naturally bioluminescence organism, the suitability of these bioassays is more than demonstrated, not only at level of known particular/group of pollutants, but also when a complex environmental matrix is assessed in which a range of distinct pollutants

might be contained within. Applications of direct environmental samples with luminescent prokaryotic organisms have been extensively studied during the last decades including soil (Bispo et al. 1999), marine (Hernando et al. 2007), surface (Ocampo-Duque et al. 2008) and groundwater (Molins-Delgado et al. 2016; Wolska et al. 2006) samples as well as municipal or industrial wastewater effluents (Corrêa et al. 2009; Ellouze et al. 2009; Farré and Barceló 2003; Farré et al. 2002b; Gouider et al. 2010; Katsoyiannis and Samara 2007; Kováts et al. 2012; la Farré et al. 2001; Li et al. 2008; Nohava et al. 1995; Saddoud et al. 2009; Somensi et al. 2010; Wang et al. 2002) and sediments (Papadopoulou and Samara 2002; Salizzato et al. 1998a; Scholz et al. 2012; Zabetoglou et al. 2002).

As has been widely described along this section, the use of inhibition of bioluminescence produced by prokaryotic organisms as a toxicological end point has been well documented. However, less attention has been paid to other eukaryotic organism. In this sense, several bioluminescent assays are based on different naturally bioluminescence eukaryotic organisms and contribute to expand the current available bioassays with higher taxonomic groups such as dinoflagellates or fungi.

Dinoflagellates are unicellular marine organisms that are either autotrophic, being ecologically relevant as primary producers, or heterotrophic. The most used dinoflagellate species in bioluminescence test are *Ceratocorys horrida, Lingulodinium polyedrum* (formerly *Gonyaulax polyedra*)*, Pyrocystis fusiformis, Pyrocystis lunula, Pyrocystis noctiluca* and *Pyrophacus steinii*. Lapota et al. (1993) developed a toxicity bioassay based on these organisms to evaluate toxic effects of different substances (Lapota et al. 1994). Commercial toxicity bioluminescence assays based on dinoflagellates include Lumitox® (Lumitox Gulf L.C., USA) and QwikLite®. The US Space and Naval Warfare Systems Centre developed the QwikLite® bioluminescence test, but it is now marketed by Assure Controls Inc (Carlsbad, California, USA) as the QwikLite TM 200 portable test kit. These tests are based, as other bioluminescent assays, in the decrease of dinoflagellate bioluminescence by a potential toxic sample following longer exposure time than prokaryotic bioassays which are fundamentally dedicated to assess the acute toxicity of samples (15 or 30 min of exposure). Procedure based on bioluminescence in the dinoflagellates *Pyrocystis lunula* was developed into ASTM method E1924-97 (“Standard guide for conducting toxicity tests with bioluminescent dinoflagellates”), but it was withdrawn in 2013. The application of bioluminescent dinoflagellate bioassays in environmental applications is quite extensive as they have been used to assess the toxicity of heavy metals, several pesticides and complex matrices such as industrial wastewater, seawater, groundwater or marine sediments (Bao et al. 2008; Bao

et al. 2011; Fetters et al. 2016; Heimann et al. 2002; Hildenbrand et al. 2015; Okamoto et al. 1999; Rosen et al. 2008; Sanchez-Ferandin 2015; Stauber et al. 2008).

The ecological significance of fungi as eukaryotic organisms involved in different processes such as nutrient cycling and decomposition makes them a significant taxonomic group for environmental monitoring of soil pollutants (Horswell et al. 2006; Paton et al. 2006). Although it is thought that there are at least 71 species of naturally bioluminescent fungi (Oliveira et al. 2012), only six species (*Armillaria borealis*, *Armillaria gallica*, *Armillaria mellea*, *Gerronema viridilucens*, *Omphalotus japonicus* formerly known as *Lampteromyces japonicus*- and *Mycena citricolor*) have been used in toxicity bioassays during last decades (Horswell et al. 2006; Mendes et al. 2010; Mendes and Stevani 2010; Paton et al. 2006; Weitz et al. 2002). Due to the slow growth of these organisms, a bioassay relating the inhibition of fungal bioluminescence with the pollutant biological effect provides a rapid and sensitive method to assess the toxicity and bioavailability of pollutants in different samples. Vydryakova et al. (2011) evaluated the effect of organic and inorganic toxic compounds to several species of bioluminescence fungi. Their results showed that the fungi were more sensitive to several heavy metals than a bacterial bioassay, demonstrating that the application of bioluminescence fungi for toxicity testing is a rapid and promising approach in assessing the toxic effect of different substances.

3. Recombinant optical microbial bioreporter assays and their application in toxicity evaluation.

Natural bioassays, such as Microtox® previously discussed, have been widely used in toxicity evaluation in aquatic and terrestrial environments, but they have several limitations. The main problem is the need of high salt concentration to aim optimal bioluminescence. Another restriction is the limited range of pH and temperature. Furthermore, most of these organisms are aquatic, and they are not representative of terrestrial ecosystems and could respond in a different way to pollutants than representative organisms. Due to these and other limitations, transgenic microorganisms have been developed to address the need of assessment of different environments (Fernández-Piñas et al. 2014). These transgenic microorganisms or bioreporters, harbour reporter genes from different sources such as *lux* genes from bioluminescent bacteria (*A. fischeri, V. harveyi or P. luminescens*), *luc* gene from firefly (*Photinus pyralis; P. pyralis*) or *gfp* gene (*Aequorea victoria; A. victoria*). There are different types of bioreporters depending on their specificity; the non-specific or “turn-off” bioreporters offer information about the toxicity of a contaminant or environmental sample without specifying the origin of this contaminant. They harbour a reporter gene fused to a constitutive promoter and their signal is, as in the case of naturally optical microorganisms, turned off in response to toxic analytes in the environment.

In specific bioreporters or “turn-on”, the signal increases in presence of a specific pollutant or stress. This bioreporters harbour the fusion of specific promoter gene to a reporter gene. In luminescent organisms, both kinds of bioreporters are also named lights-off or lights-on bioreporters respectively. Most of these bioreporters are used in bioassays but some of them have been immobilized in biosensor configuration (Ahn and Gu 2012; Lee et al. 2005; Mitchell and Gu 2006).

In this section, we will describe lights-off microbial bioreporters and their use in environmental applications. In addition, as primary mechanisms of action of pollutants, bioreporters that respond on oxidative stress are included. Oxidative stress bioreporters harbour the promoter of specific oxidative stress genes fused to a reporter gene. Table 2 provides a summary of recombinant optical microbial bioreporters featuring the toxicity endpoint, the species, commercial kits if are available and the environmental applications.

3.1 Turn-off bioreporters

Even though *Escherichia coli* (*E. coli*) is not an ecologically relevant organism, optical recombinant bioreporter strains have been favourably used in water, soil and air toxicity evaluation due to a better knowledge of their genetics, diversity and physiology (Fernández-Piñas et al. 2014; Robbens et al. 2010). The most used *E. coli* bioreporter strain in environmental monitoring is *E. coli* HB101 (pUCD607) bearing the plasmid pUCD607 (P*tet::luxCDABE*), giving constitutive bioluminescence expression (Rattray et al. 1990). This strain was constructed for *in situ* detection of *E. coli* in soils. Later, it has been used in toxicity assays in soil polluted or spiked with heavy metals. The bioassays have been made with resuscitated lyophilized bacteria and aqueous extracts from soils (Fernández-Piñas et al. 2014; Okonokhua 2014). Furthermore, *E. coli* HB101 (pUCD607) has been used in contaminated soils with metal(loid)s to know the fate of As, Cr, Cu and Zn which were present in the wood ashes generated from the combustion of mixed source waste wood (Mollon et al. 2016). The authors concluded that the detection of these metals even at low doses of ash in soil may increase the risk of draining of metal(loid)s and intensify the As potential transfer in the food chain. Hechmi et al. (2016) used the same bioreporter to check the toxicity in a bioremediated soil. The soil was previously artificially contaminated with the biocide pentachlorophenol (PCP) and fungal bioremediation was carried out by two fungal strains. After 28 days of exposure with the fungus, the toxicity of the bioremediated soil was measured by bioluminescence inhibition of *E. coli* HB101 (pUCD607) showing the effective fungal bioremediation. Another environmental application of this bioreporter is the potential use of the manuka (*Leptospermum scoparium*) plant in the bioremediation of microbial contaminated sites (Prosser et al. 2014). The extracted water from

the plant with potential antimicrobial properties was exposed to different bacteria including *E. coli* HB101 (pUCD607) inhibiting their growth. In the same way, Azimi and Thompson (2017) investigated the nutrient imbalance for controlling microbial growth in metal fluids using La_2O_3 (as phosphorus scavenger) and measured the bioluminescence inhibition of *E. coli* HB101 (pUCD607).

Other recombinant bacteria with the plasmid pUCD607 are *Pseudomonas fluorescens* 10586 (*P. fluorescens* 10586), *Pseudomonas putida* F1 (*P. putida* F1) *and Rhizobium leguminosarum* biovar *trifolii* (Fernández-Piñas et al. 2014). All these bioreporters have been used in soils extracts whose principal contaminants were heavy metals and PCP in different studies (Brown et al. 1996; Paton et al. 1995b). Furthermore, these bioreporters were used simultaneously for evaluating the toxicity of heavy metals in soils showing that their sensitivity were different depending of the soil matrix (Chaudri et al. 1999; Dawson et al. 2006; Flynn et al. 2002; McGrath et al. 1999; Paton et al. 1997; Paton et al. 2006).

To assess the toxicity and the course of bioremediation of groundwater boreholes contaminated with chlorinated aliphatic hydrocarbons (CAHs), *E. coli* HB101 (pUCD607), *P. fluorescens* 10586, *A. fischeri* and several lights-on bioreporters were used (Bhattacharyya et al. 2005). The most sensitive strain was *A. fischeri* and, although, the selected bioremediation strategy was air sparging, activated charcoal was necessary to eliminate all the contamination as the bioluminescence of *A. fischeri* decreased if this treatment was not applied.

Besides the studies previously described with *E. coli* HB101 (pUCD607), this strain has been also used in online biosensing with resuscitated freeze-dried cells (Turner et al. 2001) or with the cells immobilized in a polyvinyl alcohol matrix (Horsburgh et al. 2002), allowing directly the quantification of the toxicity over time.

Rhizobium leguminosarum biovar *trifolii* TA1-Tn5*luxAB* and *P. fluorescens* 10586/FAC510 tested the toxicity of heavy metals in soils and also in solution evaluating the effect of pH, complexing agents and PAHs (Amin-Hanjani et al. 1993; Paton et al. 1995a; Paton et al. 1997). These strains were less sensitive than the above-mentioned bioreporters as they do not harbour the *luxCDE* for the aldehyde synthesis. However *P. fluorescens* 8866 Tn5 *luxCDABE* and *P. putida* F1 Tn5 *luxCDABE* in applications where soils were contaminated with heavy metals have comparable sensitivity to those with pUCD607 (Fernández-Piñas et al. 2014). Both strains have been used in soils with heavy metals, dichlorophenol (DCP) in solution and benzene, toluene, ethylbenzene and xylene (BTEX) in soils (Dawson et al. 2008; Weitz et al. 2001). Recently, these bioreporters have assessed the potential ecotoxicity of biochar extracts (Knox et al. 2018).

Two plasmids that have been used to construct environmental bioreporters are pDN*lux* and pSL*lux* which differ in copy number and in the constitutive promoter used to drive *lux* expression (Fernández-Piñas et al. 2014). *P. fluorescens* OS8 (pDN*lux*) was constructed by (Ivask et al. 2009) as global toxicity bioreporter in a heavy metal quantification study in conjunction with more bioreporters. Later, it has been used to reveal the effects of rhamnolipids on Cd bioavailability in solution and soil extracts (Bondarenko et al. 2010) and as a global toxicity bioreporter along with *E. coli* MC1061 (pSL*lux*) and inducible bioreporters exposed to CuO NMs (Bondarenko et al. 2012). Another strain is *E. coli* MC1061 (pDN*lux*) used as a global toxicity bioreporter in soils, sediments of a mining area and heavy metals in solution (Ivask et al. 2007; Ivask et al. 2009). Moreover, both *E. coli* strains were compared with *A. fischeri* exposed to heavy metals and organic chemicals (Kurvet et al. 2011). The *E. coli* bioreporters showed similar sensitivity among them and higher than *A. fischeri*, concluding that they could replace the *A*. *fischeri* bioassays due to thermostability of *luxCDABE* form *P. luminescens*.

Other global toxicity bioreporters are those based on the bacteria gram positive: *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*). These strains were transformed with a plasmid harbouring a fusion of *luxCDABE* to *lac* promoter. They have been used in soils with Hg and sediments of a mining area in both immobilized or non-immobilized format (Ivask et al. 2007); in natural waters with heavy metals (Ivask et al. 2009; Kurvet et al. 2011) and to report on oxidative stress with NMs (Ivask et al. 2010). *B. subtilis* BR151 (pCS5962/pBL1) and *S. aureus* RN44220 (pTOO02) that harbours the luciferase (*luc*) from *P. pyralis* were also used as global toxicity bioreporters by Ivask et al. (2004) in direct soil-contact and aqueous extracts of soil containing Cd and Pb. Another gram positive luminescent transgenic bioreporter used in environmental toxicity is *Streptomyces lividans* (pESK004) that constitutively expresses *luxAB* genes from *V. harveyi*. (Park et al. 2002) tested heavy metals in solution and natural samples with this bioreporter which was highly sensitive.

E. coli RFM433 GC2 constitutively expresses *luxCDABE* fused to *lac* promoter. It has been immobilized in a variety of devices constituting biosensors. Firstly, it was immobilized in Luria-Bertani agar (LB-agar) matrix deposited in propylene tubes, successfully detecting benzene in air samples (Gil et al. 2000). Later, the biosensor was immobilized in glass tubes and then in LB-agar matrix detecting BTEX and improving the sensitivity to toluene (Gil et al. 2002). Another study with the immobilized sensor detected PAHs in soil pore water (Gu and Chang 2001). The smallest portable version of this biosensor was construct by Choi and Gu (2002) detecting phenol and chlorophenols along with other lights-on bioreporters. The non-immobilized format of *E. coli* RFM433 GC2 has been used to detect the toxicity of azo dyes, gamma-radiation and coolant

water from a nuclear and thermoelectric power plant, the effluents of a wastewater treatment plant and two rivers (Kim et al. 2005; Kim and Gu 2005; Lee et al. 2003; Min et al. 2003).

Table 2. Main features and applications of recombinant optical microbial bioreporters (most reports before 2014 already reviewed in Fernández-Piñas et al. (2014).

Endpoint	Reporter system	Microorganisms	Environmental applications/ tested pollutant	References
Bioluminescence inhibition	*luxCDABE* (*A. fischeri*)	*E. coli* HB101 (pUCD607) (Prokaryotic)	Online continuous biosensing of pollutants in spiked waters, effluent of a metal plating plant and a malt distillery. Toxicity evaluation of: wastewaters from treatment stages in a coking wastewater-treatment plant; heavy metal(loid)s arising from contaminated wood ash and organic contaminants in solution, soil extracts, freshwater and remediation processes; PCP in soils; La_2O_3, La $(NO_3)_3$, and formaldehyde finding alternatives to biocides. Metals mobilization in soils. Potential use of manuka in the rehabilitation of microbial contaminated sites.	(Azimi and Thompson 2017; Fernández-Piñas et al. 2014; Hechmi et al. 2016; Mollon et al. 2016; Okonokhua 2014; Zhao et al. 2014)
	lac-luxCDABE (*P. luminescens*)	*E. coli* MC1061 (*pSLlux*) (Prokaryotic)	Global toxicity bioreporter in conjunction with inducible bioreporters in: quantification and toxicity evaluation of heavy metals in solution; detection of oxidative damage caused by NMs; toxicity evaluation of 3,5-DCP, aniline and 3,5-DCA in solution. Comparison with *A. fischeri* and *E. coli (pDNlux*). Antibacterial activity of TiO_2 NMs. Effects of silver compounds on bacterial membranes.	(Bondarenko et al. 2012; Bondarenko et al. 2018; Fernández-Piñas et al. 2014; Ivask et al. 2010; Joost et al. 2015)
	T7-*luxCDABE* (*P. luminescens*)	*E. coli* MC1061 (*pDNlux*) (Prokaryotic)	Global toxicity bioreporter in conjunction with inducible bioreporters exposed to heavy metals in soil sediments, soil solution and soil extracts. Effects of rhamnolipids on Cd bioavailability in solution and soil extracts. Heavy metals, 3,5-DCP, aniline and 3,5-DCA in solution; comparison with *A. fischeri* and *E. coli (pSLlux*). This bioreporters has been immobilized in alginate- optic fiber.	(Fernández-Piñas et al. 2014)
	lac-luxCDABE (*P. luminescens*)	*E. coli* GC2 (Prokaryotic)	Toxicity evaluation of: azo-dyes and γ radiation in solution; BTEX in air and PAH in soil extracted with rhamnolipids. Detection of phenols. Global toxicity bioreporter in conjunction with inducible bioreporters in a multichannel bioreactor to classify toxicity in rivers and wastewater treatment plant. This bioreporter has been immobilized in agar in polypropylene tubes, chip arrays and plate arrays.	(Fernández-Piñas et al. 2014)
	luxCDABE (*P. luminescens*)	*E. coli K12 MG1655 (pXen7)* (Prokaryotic)	Toxicity evaluation of CBNs.	(Deryabin et al. 2016; Zarubina et al. 2009)
	luxCDABE (*P. luminescens*)	*E. coli* RB1436 (Prokaryotic)	Toxicity evaluation of: NMs, arsenite, chromate, Cd, and Cu. Phenols and Hydroxylated Polynuclear Aromatic Contaminants, in plant-based wastes, in whole solids and solids aqueous extracts, in contaminated soils. Effects ZnO NMs.	(Deryabin et al. 2012; Kang and Kong 2016; Ko and Kong 2014; Kong 2008; Kong et al. 2018)
	luxCDABE (*P. leiognathi*)	*E. coli K12* TG1 (Prokaryotic)	Toxicity evaluation of CNBs; ZnO and CuO NMs and their mixtures. Commercial test Ecolum.	(Deryabin et al. 2012; Deryabin et al. 2016)

	luxCDABE (*A. fischeri*)	*Salmonella typhimurium* TA104 pr1 (Prokaryotic)	Global toxicity bioreporter in conjunction with inducible bioreporters in genotoxic assays. Commercial test VITOTOX®.	(Fernández-Piñas et al. 2014)
	luxCDABE (*A. fischeri*)	*P. fluorescens* 10586r (pUCD607) (Prokaryotic)	Toxicity evaluation of heavy metals in solution and soils and PCP in: effluent of a Malt whisky distillery, a papermill treatment plant, arable soils. Herbicides in freshwaters. Organotins in solution and soils. Remediation process of chlorophenols, CAHs and PAHs.	(Fernández-Piñas et al. 2014)
	tet-luxABE (*A. fischeri*)	*P. fluorescens* 10586r/FAC510 (Prokaryotic)	Toxicity evaluation of heavy metals in soil and solutions.	(Fernández-Piñas et al. 2014)
	luxCDABE (*A. fischeri*)	*P. fluorescens* 8866 Tn5l*uxCDABE* (Prokaryotic)	Toxicity evaluation of Cu in soil and solution and Zn and DCP in solution. Potential ecotoxicity of biochar extracts.	(Fernández-Piñas et al. 2014; Knox et al. 2018)
	luxAB (*A. fischeri*)	*P. fluorescens* DF57-40E7 (Prokaryotic)	Global toxicity bioreporter in conjunction with inducible bioreporters exposed to Cu.	(Fernández-Piñas et al. 2014)
	T7-*luxCDABE* *(P. luminescens)*	*P. fluorescens* OS8 (*pDNlux*) (Prokaryotic)	Effects of rhamnolipids on Cd bioavailability in solution and soil extracts. Global toxicity bioreporter in conjunction with inducible bioreporters exposed to heavy metals in solution and toxicity of CuO NMs.	(Bondarenko et al. 2012; Fernández-Piñas et al. 2014)
	luxCDABE (*A. fischeri*)	*P. fluorescens* Shk1 (Prokaryotic)	Toxicity evaluation of DCP, Cd, hydroquinone, narcotic chemicals, metal mixtures in wastewater influent and effluent. Bench scale batch experiments and continuous monitoring of wastewater.	(Fernández-Piñas et al. 2014; Ren and Frymier 2005)
	luxCDABE (*A. fischeri*)	*P. fluorescens PM6* (Prokaryotic)	Toxicity evaluation of seven metals and 25 organic compounds.	(Ren and Frymier 2005)
	luxCDABE (*A. fischeri*)	*P. putida F1 (pUCD607)* (Prokaryotic)	Toxicity evaluation of herbicides in fresh waters. Remediation assessment of soils and PAH in soil.	(Fernández-Piñas et al. 2014)
	luxCDABE (*A. fischeri*)	*P. putida* F1 Tn5l*uxCDABE* (Prokaryotic)	Toxicity evaluation of heavy metals and DCP in soil and solution. Monitoring the degradation and toxicity of BTEX in soils. Potential ecotoxicity of biochar extracts.	(Fernández-Piñas et al. 2014; Knox et al. 2018)
	luxCDABE (*P. luminescens*)	*P. putida BS566::luxCDABE* (Prokaryotic)	Effect of silver NMs on the activated sludge process, in artificial wastewater; pristine and aged silver nanoparticles in real wastewaters.	(Dams et al. 2011; Mallevre et al. 2016; Mallevre et al. 2014)
	*tet-lux*CDABE (*A. fischeri*)	*Rhizobium leguminosarum* biovar *trifolii* (pUCD607) (Prokaryotic)	Arable soils fertilized with paper mill sludge. Toxicity evaluation of heavy metals in solution.	(Fernández-Piñas et al. 2014)
	luxAB *(A. fischeri)*	*Rhizobium leguminosarum* biovar *trifolii* TA1-Tn5l*uxAB* (Prokaryotic)	Toxicity evaluation of heavy metals in soil and solutions, effect of complexing agents. PAHs in solution.	(Fernández-Piñas et al. 2014)

	lac-luxCDABE (*P. luminescens*)	*B. subtilis* BR151(pBL1/p602/22*lux*) (Prokaryotic)	Toxicity evaluation of heavy metals in solution. Global toxicity bioreporter in conjunction with inducible bioreporters.	(Fernández-Piñas et al. 2014)
	*luc*FF (*P. pyralis*)	*B. subtilis* BR151(pCSS962/pBL1) (Prokaryotic)	Used as global toxicity bioreporter in conjunction with inducible bioreporters to detect Cd in soils contaminated by metal smelters. Direct contact with soil.	(Fernández-Piñas et al. 2014)
	lac-luxCDABE (*P. luminescens*)	*S. aureus* RN4220 (p602/22*lux*) (Prokaryotic)	Toxicity evaluation of heavy metals in solution; used global toxicity bioreporter in conjunction with inducible bioreporters.	(Fernández-Piñas et al. 2014)
	*luc*FF (*P. pyralis*)	*S. aureus* RN4220 pTOO02 (Prokaryotic)	Global toxicity bioreporter in conjunction with inducible bioreporters to detect Cd in soils contaminated by metal smelters. Direct contact with soil.	(Fernández-Piñas et al. 2014)
	hao-luxAB *(A. fischeri*)	*Streptomyces lividans* (pESK004) (Prokaryotic)	Toxicity evaluation of heavy metals, chlorophenols and pesticides in solution and environmental samples.	(Fernández-Piñas et al. 2014)
	lux AB (*V. harveyi*)	*Nitrosomonas europea* ATCC 19781 (pHLUX20) (Prokaryotic)	Toxicity evaluation of LAS and Cu in soil in solid-phase contact.	(Fernández-Piñas et al. 2014)
	tet-luxCDABE (*A. fischeri*)	*Burkholderia sp.* RASC c2 (Prokaryotic)	Phenols in bioremediation processes. Toxicity evaluation of heavy metals.	(Fernández-Piñas et al. 2014)
	luxCDABE (*A. fischeri*)	*Acinetobacter* sp. DF4/pUTK2 (Prokaryotic)	Toxicity evaluation of phenol and heavy metals in water and wastewater. This bioreporter was immobilized in Ca-Alginate.	(Fernández-Piñas et al. 2014)
	luxCDABE (*A. fischeri*)	*Acinetobacter baylyi Tox2* (Prokaryotic)	Cytotoxicity detection of heavy metal in contaminated artificial seawater.	(Cui et al. 2018)
	luxCDABE (*A. fischeri*)	*Alicagenes eutrophus* BR6020 (PUTK2) (Prokaryotic)	Toxicity evaluation of polar narcotic pollutants and phenol in remediation procedures with surfactants to solubilize pollutants.	(Fernández-Piñas et al. 2014)
	luxCDABE (*A. fischeri*)	*Stenotrophomonas 664 (pUTK2)* (Prokaryotic)	Toxicity evaluation of polar narcotic pollutants and phenol in remediation procedures with surfactants to solubilize pollutants.	(Fernández-Piñas et al. 2014)
	luxAB (*V. harveyi*)	*Janthinobacterium lividum* YH09-RC (Prokaryotic)	Toxicity evaluation of phenol, benzene, toluene and heavy metals in solution. Wastewaters samples. Freeze-dried immobilized in plate. Commercial test Bactotox®.	(Fernández-Piñas et al. 2014)
	luxCDABE *(P. luminescens)*	*B. subtilis* EG168-1 (Prokaryotic)	Toxicity evaluation of metal NMs and CBNs.	(Deryabin et al. 2016)
	tac-luc (*P. pyralis*)	*Synechocystis* sp PCC6803 (Prokaryotic)	Toxicity evaluation of herbicides, heavy metals and DCP in aqueous solutions.	(Fernández-Piñas et al. 2014)

	luxCDABE (*P. luminescens*)	*Anabaena* CPB4337 (Prokaryotic)	Toxicity evaluation of priority and emerging pollutants, perfluorinated surfactants, chlorinated by-products, antibiotics and nanomaterials, in aqueous solution and in wastewater effluents. Inorganic NMs in wastewater. Pharmaceutical pollutant mixtures.	(Fernández-Piñas et al. 2014; Martín-de-Lucía et al. 2017; Pulido-Reyes et al. 2017; Rodea-Palomares et al. 2016)
	luc *(P. pyralis)*	*Saccharomyces cerevisiae W303-1B lucΔ* (Eukaryotic)	Toxicity evaluation of herbicides diuron and mecoprop, Cu in different solutions.	(Hollis et al. 2000)
	luc (*P. pyralis*)	*Ostreococcus tauri* CDK-Luc (Eukaryotic)	Toxicity evaluation of diuron, irgarol 1051, DCPMU, DCPU.	(Sanchez-Ferandin et al. 2012)
	luc (*P. pyralis*)	*Ostreococcus tauri* cyclinA-Luc (Eukaryotic)	Toxicity evaluation of diuron, irgarol 1051, DCPMU, DCPU.	(Sanchez-Ferandin et al. 2012)
	luc (*P. pyralis*)	*Ostreococcus tauri* pCAB::*luc* (Eukaryotic)	Toxicity evaluation of diuron, irgarol 1051, DCPMU, DCPU.	(Sanchez-Ferandin et al. 2012)
	luc (*P. pyralis*)	*Ostreococcus tauri* TOC1-Luc (Eukaryotic)	Toxicity evaluation of diuron, irgarol 1051, DCPMU, DCPU.	(Sanchez-Ferandin et al. 2012)
Oxidative stress	*luxCDABE* (*A. fischeri*)	*E. coli* DPD2515 (Prokaryotic)	Oxidative stress caused by H_2O_2, ethanol, MV and xanthine.	(Belkin et al. 1997)
	luxCDABE (*A. fischeri*)	*E. coli* PGRFM (Prokaryotic)	Oxidative stress caused by methyl, ethyl and benzyl viologen and H_2O_2.	(Niazi et al. 2008)
	luxCDABE (*A. fischeri*)	*E. coli* EBHJ (Prokaryotic)	Oxidative stress caused by MV, H_2O_2 and other toxicants such as potassium dichromate or ethidium. Use in an integrated simulated water toxicity monitoring system. Biological effects of wastewaters.	(Bazin et al. 2017; Lee and Gu 2003; Lee and Gu 2005)
	luxCDABE (*A. fischeri*)	*E. coli* ZWF RFM_{443} (Prokaryotic)	Oxidative stress caused by methyl, ethyl and benzyl viologen. Other toxicants such as mitomycin or ethyl methanesulfonate.	(Niazi et al. 2007)
	luxCDABE (*A. fischeri*)	*E. coli* FPR RFM_{443} (Prokaryotic)	Oxidative stress caused by methyl, ethyl and benzyl viologen. Other toxicants such as mitomycin or ethyl methanesulfonate.	(Niazi et al. 2007)
	luxCDABE (*A. fischeri*)	*E. coli* K12::*soxRSsodAlux* (Prokaryotic)	Oxidative stress caused by ZnO, CuO and Ag NMs, their bulk and solubility references.	(Ivask et al. 2010)
	gfp *(A. victoria)*	*E. coli* ro-GFP2	Oxidative stress caused by H_2O_2, menadione and other chemicals and heavy metals.	(Arias-Barreiro et al. 2010)

		(Prokaryotic)		
	luxCDABE (*A. fischeri*)	*E. coli* DPD2511 (Prokaryotic)	Oxidative stress caused by a variety of contaminants such as MV, H_2O_2 or CNBs. Biological effects of wastewaters.	(Belkin et al. 1996)
	luxCDABE (*A. fischeri*)	*E. coli* K12::*katGlux* (Prokaryotic)	Oxidative stress caused by CuO NMs.	(Bondarenko et al. 2012)
	luxCDABE (*A. fischeri*)	*E. coli* DK1 (Prokaryotic)	Oxidative stress caused by different toxicants such as Cd chloride, H_2O_2 or isopropanol. Use in an integrated water toxicity monitoring system.	(Lee and Gu 2003; Mitchell and Gu 2004)
	luxCDABE (*P. luminescens*)	*Anabaena* sp. PCC 7120 pBG2154 (Prokaryotic)	Superoxide anion produced by MV and triclosan in real water samples.	(Hurtado-Gallego et al. 2018)
	luxCDABE (*P. luminescens*)	*Anabaena* sp. PCC 7120 pBG2165 (Prokaryotic)	Superoxide anion produced by MV and triclosan in real water samples.	(Hurtado-Gallego et al. 2018)
	gfp (*A. victoria*)	*P. putida mt-2* (*katA*) (Prokaryotic)	Oxidative stress caused by the impact of desiccation.	(Svenningsen et al. 2015)
	gfp (*A. victoria*)	*P. putida mt-2* (*aphC*) (Prokaryotic)	Oxidative stress caused by the impact of desiccation.	(Svenningsen et al. 2015)
	gfp (*A. victoria*)	*P. putida mt-2* (*sodB*) (Prokaryotic)	Oxidative stress caused by the impact of desiccation.	(Svenningsen et al. 2015)
	gfp (*A. victoria*)	*P. putida mt-2* (*osmC*) (Prokaryotic)	Oxidative stress caused by the impact of desiccation.	(Svenningsen et al. 2015)

The widespread use of NMs makes necessary to evaluate their environmental effect. As previously described in section 2, several natural bioreporters have been used to test the toxicity of NMs. In the case of recombinant bioreporters, both metabolism impairment and oxidative stress (which will be described in the next section) induced by NMs have been assessed. The first recombinant lights-off bioreporter used to study NMs toxicity was *E. coli* RB1436 with pUCD615 plasmid which contains *lux* genes (Kong 2008). This strain has been applied for determining the toxicity of NMs as well as the decomposing organic wastes, papers, fruits, wood and leaves (Kong 2008); solid and aqueous soils extracts (Kong 2011) and soils with heavy metals (Kang and Kong 2016). The evaluation of the potential environmental toxicity of several metal oxide NMs (CuO, TiO_2, NiO, Fe_2O_3, ZnO, and Co_3O_4) were carried out by Ko and Kong (2014). In this study the toxicity of the NMs was compared between *E. coli* RB1436 and seeds of the plants *Lactuca sativa L*. and *Raphanus sativus L*., concluding that each bioassay has a particular "spectrum of sensitivity" to NMs: for *E. coli* RB1436 the NMs toxicity order was ZnO ≫ CuO > Co_3O_3 > NiO ≫ TiO_2, Fe_2O_3 while in the plant seeds was CuO > ZnO > NiO > Co_3O_3, TiO_2, Fe_2O_3. These differences indicate that a battery of bioassays could constitute a better tool for assessing environmental pollutants than any single one. Subsequently, Kong et al. (2018), also used *E. coli* RB1436, seeds of the plant *Lactuca sativa* and also used *Chlorella vulgaris* in order to evaluate the effect of the size of distinct ZnO NMs. The different organisms had different responses where ZnO NMs were more toxic for *E. coli* RB1436. Interestingly, the larger size of the NMs affected seed germination and algal growth but not the bioluminescence of *E. coli* RB1436, supporting the need of a set of bioassays for an accurate valuation of environmental toxicity.

P. putida BS566::*luxCDABE* (Winson et al. 1998) was used to test the toxic effects of Ag NMs on the activated sludge process (Dams et al. 2011) where Ag^+ and micro Ag were included for comparison purposes. The most toxic sample was Ag^+ followed by Ag NMs and then, Ag microparticles. Mallevre et al. (2014) brought to light the relevance of NMs ecotoxicological studies in real matrices; Ag, ZnO and TiO_2 NMs were tested with *P. putida* BS566::*luxCDABE* in culture media and in artificial wastewater and the Ec_{50} was different in each case, being in both cases Ag NMs the most toxic in the artificial wastewater. The same group evaluated the ecotoxicity of pristine and aged silver nanoparticles in real and artificial wastewater samples (Mallevre et al. 2016). Aged Ag NMs were less toxic in artificial wastewater, but not in real wastewaters where the toxicity remained despite aging.

Deryabin et al. (2016) compared the sensitivity of four different natural and recombinant bioreporters after the exposure to carbon nanotubes (CBNs) and metallic NMs. The natural and

commercial Microbiosensor B17-677F (previously described), the commercial and recombinant Ecolum (*E. coli* K12 TG1 with a *luxCDABE* cassette previously used for testing toxicity of CBNs; (Deryabin et al. 2012), the recombinant bioreporter *E. coli* K12 MG1655 (also with *luxCDABE* previously used by Zarubina et al. (2009) for testing the toxicity of CBNs) and *B. subtilis* EG168-1 harbouring the *luxCDABE* genes (Deryabin et al. 2016). All these bioreporters were exposed to CBNs and metallic NMs and *B. subtilis* EG168-1 was the most sensitive for both kinds of NMs.

Another commercial recombinant bioreporter is *Salmonella typhimurium* TApr1 which harbours the *lux* genes under a constitutive promoter. This strain has been included in the VITOTOX® test to normalize the response with respect to possible cytotoxic effects, improving the reproducibility of assays in different laboratories (Fernández-Piñas et al. 2014; Verschaeve et al. 1999).

Freshwater recombinant bioreporters such us *Janthinobacterium lividum* YH9-RC are ecologically relevant microorganism from inland water. This strain harbours the *luxAB* genes integrated in the genome and was patented under the commercial name of BactoTox®. Basically, it consists of freeze-dried cells in 348-multiwell plate for continuous monitoring using a special software. This method was 7.8-8.6 times more sensitive to heavy metals and organic pollutants than Microtox® (Cho et al. 2004). Another group of freshwater recombinant bioreporters are based on cyanobacteria. Cyanobacteria are ubiquitous in freshwater environments and as primary producers, they are at the base of food chain being particularly relevant because they are representative of the health of the environment they live in. Two cyanobacterial transgenic bioreporter have been constructed until date: *Synechocystis* sp. strain PCC6803 and *Anabaena* CPB4337 (*A.* CPB4337) (Fernández-Piñas et al. 2014). *Synechocystis* sp. strain PCC6803 marked with the luciferase gene *luc* from *P. pyralis.* This bioreporter was sensitive to herbicides as well as Cu, Zn and DCP (Shao et al. 2002). The cyanobacterium *Anabaena* sp. PCC7120 (*A.* sp. PCC 7120) has been transformed with *luxCDABE* genes from *P. luminescens* and was named *A.* CPB4337 (Fernández-Pinas and Wolk 1994). This bioreporter has a high and stable luminescent signal (Figure 3), thus it has been used in different environmental matrices and in combination with several organisms such as *A. fischeri, D. magna* or *Pseudokirchneriella subcapitata* in toxicity studies of priority and emerging pollutants (Fernández-Piñas et al. 2014). *A.* CPB4337 has shown high sensitivity to emerging pollutants such as fibrates (Rosal et al. 2010a), perfluorinated surfactants and chlorinated by-products (Rosal et al. 2010b), antibiotics (González-Pleiter et al. 2013), NMs (Pulido-Reyes et al. 2017; Rodea-Palomares et al. 2010; Rodea-Palomares et al. 2012) and NMs in wastewater (Martín-de-Lucía et al. 2017). *A.* CPB4337 has been also used to evaluate mixture toxicity by Rodea-Palomares and co-workers, where

combination-index-isobologram equation (Chou 2006; Chou and Talalay 1984) was applied in order to study the toxicological interactions. This research group also tested heavy metals as well as emerging pollutants (González-Pleiter et al. 2013; Rodea-Palomares et al. 2009a; Rodea-Palomares et al. 2009b; Rodea-Palomares et al. 2016; Rosal et al. 2010b). In a more recent study, Rodea-Palomares et al. (2016) used *A.* CPB4337 to allow the identification of hidden drivers of toxicity in environmental mixtures in a high throughput study.

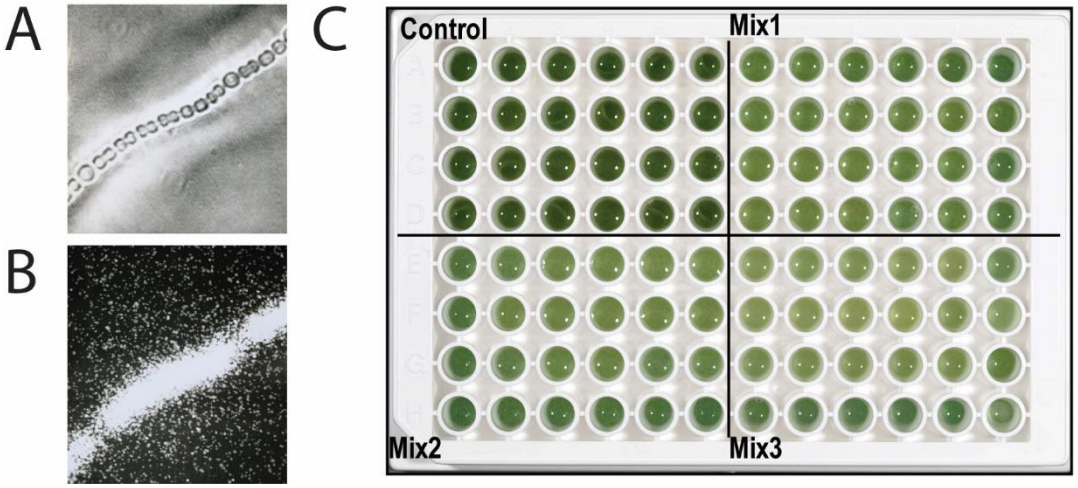


Figure 3. Recombinant cyanobacterial toxicity bioreporter *Anabaena* CPB4337 (Panel A is a bright-field image of a filament; panel B is an image of constitutive bioluminescence from the same filament with a Hamamatsu Photonics Systems, model C1966-20, mounted on a Zeiss Universal Research Microscope). Panel C shows a multiwell plate where the bioreporter is deployed to be used in a medium-to-high throughput configuration (Mix 1-3 refers to different mixtures of pollutants) (Fernandez-Piñas F., unpublished).

Nitrosomonas europaea ATCC19781 is an autotrophic ammonia-oxidizing bacterium isolated from soil and transformed with *luxAB* genes directed by hydroxylamine oxidoreductase promoter, giving constitutive light emission which is correlated with respiration (Iizumi et al. 1998). The bioreporter has been used in linear alkylbenzene sulfonate surfactant (LAS) toxicity in soil solution and in a soil-phase contact, obtaining in both cases similar toxicity (Brandt et al. 2002). Later, Ore et al. (2010) used the same strain and tested the Cu toxicity in soils concluding that toxicity depends on: the metal free ion activity in solution, the ions competing for metal sorption and the biotic ligand.

P. fluorescens DF57-40E7 has been used as a global toxicity bioreporter in conjunction with *P. fluorescens* based lights-on bioreporters in presence of Cu in soils and in solid-phase contact (Brandt et al. 2006; Brandt et al. 2008; Tom-Petersen et al. 2004). *Burkholderia* sp. RASC c2 is a 2,4-DCP-mineralizating bacteria that harbours the *luxCDABE* genes fused to *tet* promoter

obtaining constitutive light emission, so this strain can be used at the same time as bioremediation tool and to monitor the bioremediation process in DCP contaminated soils (Shaw et al. 1999). Furthermore, it has been used to detect toxicity of mono-, di-, and tri-chlorophenols (Boyd et al. 2001) and of heavy metals (Chinalia et al. 2008) in soils.

P. fluorescens strain, isolated from activated sludge, was transformed by Kelly et al. (1999) carrying the *luxCDABE* genes and named *P. fluorescens* Shk1. This bioreporter was sensitive to DCP, Cd and hydroquinone in wastewater (Kelly et al. 1999), narcotic chemicals (Ren et al. 2004) and metal mixtures (Ren and Frymier 2005). Lajoie et al. (2002) used *P. fluorescens* Shk1 to evaluate the influent and effluent of wastewater treatment plants. Furthermore, it has been used in conjunction with another strain: *P. fluorescens* PM6 expressing the *lux* genes (Ren and Frymier 2003). Toxicity of 7 metals and 25 organic compounds was found for both bioreporters and no general pattern for the sensitivity between both strains was observed. The authors concluded that the strains were appropriate for evaluating wastewater toxicity (Ren and Frymier 2005).

Another ecologically relevant microorganism is *Acinetobacter* sp. DF4, isolated from industrial wastewater and transformed with *luxCDABE* genes (in pUTK2 plasmid) fused to a constitutive promoter by Abd-El-Haleem et al. (2006) and named *Acinetobacter* sp. DF4/pUTK2. Toxicity of heavy metals has been tested in water and wastewater samples and the sensitivity order was: Zn, Cd, Fe, Co, Cr and Cu (Abd-El-Haleem et al. 2006). Later, the strain was immobilized in a matrix of Ca-alginate and the toxicity of phenolic compounds were tested (Zaki et al. 2008).

Other recombinant bioreporters with the pUTK2 (harbouring the *luxCDABE* genes behind a constitutively expressed promoter) plasmid are *Stenotrophonomas* 664 (pUTK2) and *Alicagenes eutrophus* BR6020 (pUTK2). Both strains along with *A. fischeri* were tested with the nonionic surfactant polyoxyethylene 10 lauryl ether. Both recombinant strains were 400 times more resistant than *A. fischeri*, highlighting their usefulness to evaluate toxicity of remediation processes (Layton et al. 1999).

Recently, Cui et al. (2018)have constructed the bioreporter *Acinetobacter baylyi* Tox2 from the soil bacterium *Acinetobacter babylyi* ADP1 harbouring the *luxCDABE* genes controlled by the constitutively expressed *tet* promoter. In this study heavy metals toxicity was tested with the bioreporter and with the marine fish *Mugilogobius chulae* showing correlation between these two toxicity test methods. The suitability of *Acinetobacter baylyi* Tox2 was proved as this strain was able to evaluate toxicity of real contaminated seawater.

Although the bioreporters described so far are prokaryotic bioreporters eukaryotic bioreporters have also been used in environmental applications. The main eukaryotic organism used to construct recombinant bioreporters is the yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) whose cell wall protects the organism from extreme pH, solvent exposure or osmotic changes (Fernández-Piñas et al. 2014). *S. cerevisiae* W303-1B *lucΔ* (without the peroxisome target sequence) was constructed by Hollis et al. (2000) and harbours the *lucΔ* gene from *P. pyralis* integrated into the genome, but the substrate D-luciferin had to be added for bioluminescence production. The strain responded to diuron and mecoprop herbicides in a pH range of 3 to 10, however *E. coli* HB10 (pUCD697), as a model recombinant bioreporter, did not respond to these analytes. Moreover, *S. cerevisiae* W303-1B *lucΔ* detected Cu in different solvents, but not in extremes pH due to changes in Cu speciation (Hollis et al. 2000).

The marine green alga *Ostreococcus tauri* isolated from a Mediterranean coastal lagoon was modified by Sanchez-Ferandin (2015). Four strains were constructed: the translational bioreporter TOC1-Luc (chlorophyll *a* binding protein expression (CAB)), the transcriptional bioreporter *pCAB*::*luc* (promoter of chlorophyll *a* binding protein expression) and the translational bioreporters cyclin A-Luc and CDKA-Luc (cyclin A and cyclin dependent kinase respectively). The antifouling biocides diuron and Irgarol 1015 were tested as compound toxic models. CDKA-Luc translational bioreporter was the most effective *O. tauri* bioreporter, demonstrating that it eukaryotic recombinant microorganism could be used as bioreporter of contaminated seawaters.

3.2 Oxidative stress bioreporters

Reactive oxygen species (ROS) are formed by aerobic organisms as a by-product of respiration. They have developed defences against these toxic species maintaining a balance between them (Latifi et al. 2009). In presence of a toxic compound, ROS level increases producing an unbalance called oxidative stress. Several evidences on mechanism studies denote that oxidative stress is involved in most environmental toxicity processes (Livingstone 2001; Lushchak 2011; Stone and Donaldson 2006; Valavanidis et al. 2006). At present, it is complicated to measure ROS directly, so attempts at the evaluation of free radical production and the pro-oxidant/antioxidant balance is based on markers of oxidative reactions often involving *in vitro* experiments (Betteridge 2000). As described by Monaghan et al. (2009), there are a lot of methods for oxidative stress measurement estimating the ROS production, antioxidant defences, oxidative damage and repair mechanisms. The enzymatic antioxidant defences are superoxide dismutase, peroxidase, catalase, and glutathione reductase (Gagne 2014). However, measuring oxidative stress is complex and a combination of different assays are required.

A few oxidative stress lights-on bioreporters have been constructed to know more about the mechanisms of action of different pollutants in the environment. Among the transgenic bacterial bioreporters for oxidative stress, *E. coli* still takes a prominent place (Robbens et al. 2010). The most used *E. coli* bioreporter for oxidative stress in environmental toxicity is the strain DPD2511 constructed by (Belkin et al. 1996). This strain harbour the fusion of the promoter of *katG* that encodes catalase to *luxCDABE* genes (P*katG*::*luxCDABE*) and the bioluminescence induction was checked with H_2O_2, ethanol, methyl viologen (MV) or xanthine. In addition, this bioreporter was used with other model environmental pollutants such as chromium, cadmium or ethidium bromide in order to elucidate de different toxicological mechanisms of these pollutants. Belkin et al. (1997) constructed *E. coli* DPD2515 bearing P*micF::luxCDABE* (*micF* gene encodes an outer membrane porin regulator) and was checked with the previously commented pollutants and, particularly, detected superoxide anion. With the same purpose, Lee and Gu (2003) constructed another *E. coli* bioreporter bearing the promoter of *sodA* that encodes superoxide dismutase fused to *luxCDABE* (P*sodA*::*luxCDABE*) named as EBHJ. Comparing the data of EBHJ with those of DPD2511 strain after the exposure with the pollutants (MV, potassium dichromate, cadmium chloride, bisphenol A, ethidium bromide and H_2O_2), different responses were found indicating that the mechanisms of toxicity leading to oxidative stress for different pollutants can be classified as a P*sodA*-dependent response or a P*katG*-dependent response in *E. coli.* Although no real matrices were used, the authors concluded that they may be potentially used as tools for monitoring and assessing the hazards in environmental samples in different media such as drinking water, wastewater, soil and air. Both strains EHBJ and DPD2515 were used with other strains in a panel of bioreporters to test potential environmental toxicity (Belkin et al. 1997). Furthermore, this DPD2515 strain was also used in a different panel for identification and quantification of different chemicals (Ben-Israel et al. 1998). EHBJ strain has been used in wastewater samples (Bazin et al. 2017)

E. coli EBHJ has been used in an integrated mini biosensor system for continuous water toxicity monitoring (Lee and Gu 2005). This strain with other three *E. coli* bioreporters were used in a biosensor configuration, generating 4 mini bioreactors (each one with one strain). The experiments were carried out with three chemicals: MV, H_2O_2 and mitomycin C in an experiment where the water sample was contaminated at various times during the system operation. Another strain of this mini biosensor was DK1 constructed by Mitchell and Gu (2004) bearing the fusion P*katG*::*luxCDABE* and the tested chemicals were H_2O_2, cadmium chloride and isopropanol, showing induction of bioluminescence for all of them.

More *E. coli* bioreporters to detect oxidative stress have been constructed harbouring the fusions of *zwf* (encodes a flavodoxin and ferredoxin reductase) and *fpr* (encodes a glucose-6-phosphate dehydrogenase) promoters to *luxCDABE,* namely *E. coli* ZWF RFM_{443} and *E. coli* FPR RFM_{443} respectively (Niazi et al. 2007). Both bioreporters were exposed to MV and its derivatives showing bioluminescence induction, but no response was seen after the exposure to H_2O_2, denoting their specificity to superoxide anion. *E. coli* PGRFM was constructed by the same group Niazi et al. (2008) to detect oxidative stress bearing the fusion P*pgi::luxCDABE*, where *pgi* encodes an glucose-6-phosphate isomerase. This strain was checked for MV and analogues, showing to be sensitive to superoxide anion. Furthermore, this bioreporter showed bioluminescence induction in presence of H_2O_2 and OH^-. In this case, it is worth nothing that the authors concluded that growth in minimal medium increased the sensitivity.

Different NMs such as fullerenes, CBNs or metal oxides have been shown to induce oxidative stress. As well as from surface-dependent properties, metals and chemical compounds on the NMs surface accelerate the production of ROS (Manke et al. 2013). Nowadays, a few bioreporters have been used to detect the oxidative stress caused by nanoparticles profiling their ecotoxicological properties. The first one was *E. coli K12::soxRSsodAlux* that harbour the fusion of *sodA* promoter to *luxCDABE* genes. The induction of this strain is specific for superoxide as in the presence of H_2O_2 no bioluminescence was shown. After the exposure to ZnO, CuO and Ag NMs, their bulk and ionics references, bioluminescence induction was observed for all of them except for ZnO compounds (Ivask et al. 2010). *E. coli* K12*::katGlux* was construct to study the oxidative stress induction of CuO NMs (Bondarenko et al. 2012) and also to know the mechanisms of action at sub-toxic concentrations. The authors observed biological adverse effects even at very low concentrations of CuO NMs and concluded that these effects were triggered by the solubilized copper ions.

All these aforementioned *E. coli* bioreporters harbour the fusion of a specific promoter to *luxDCABE* genes. However, *E. coli* ro-GFP2 constitutively expressed redox-sensitive green fluorescent protein roGFP2 (Arias-Barreiro et al. 2010). This strain has been tested with a variety of environmental oxidants such as H_2O_2, menadione, heavy metals or naphthalene, denoting a very rapid response and a broad specificity, because it detects both H_2O_2 and superoxide anion.

As previously described, although *E. coli* is the most used microorganism to construct bioreporters for environmental toxicity, there are more ecologically relevant microorganisms used as bioreporters. In the case of oxidative stress bioreporters, the cyanobacteria *Nostoc* sp. PCC7120 (also known as *Anabaena* sp. PCC7120) has been used to construct several strains that specifically detect superoxide anion (Hurtado-Gallego et al. 2018). Both strains harbour the

fusion of *sodA* and *sodB* promoters to *luxCDABE* operon and their names are *Nostoc* sp. PCC7120 pBG2154 and *Nostoc* sp. PCC7120 pBG2165, respectively. MV and triclosan were tested with these bioreporters showing bioluminescence induction, but not with H_2O_2 denoting their specificity. Furthermore, real water matrices were used in MV spiking experiments, showing that they could be used in real water matrices. They were the first bioreporters constructed to date which detect superoxide anion based on cyanobacteria and, also, the most superoxide anion-sensitive bioreporter strains (Hurtado-Gallego et al. 2018).

Another ecologically relevant microorganism for soil environments which has been used as oxidative stress bioreporter is *Pseudomonas putida* mt-2 (Svenningsen et al. 2015). In this work, four bioreporters bearing the *katA* (encodes catalase), *ahpC* (encodes alkylhydroperoxide reductase)*, sodB* (encodes superoxide dismutase) and *osmC* (hydroperoxide resistance gene) promoters fused to monomeric superfolder *gfp* gene were constructed. These bioreporters were used to detect the oxidative stress caused under desiccation condition in soils.

As described along this section, most bioreporters have been constructed using bacteria due to their small size, adaptability, easy driving and the great information about their genome sequences. However, there are also other bioreporters based on eukaryotic organisms. Both prokaryotic and eukaryotic bioreporters contribute with valuable information regarding the mechanisms of action and environmental toxicity of a great range of organic and inorganic pollutants. Therefore, the use of a battery of bioreporters could be a key strategy to assess the ecotoxicity of different contaminants in a more realistic scenario.

4. Microalgal-based biosensors

Microalgae-based biosensors have emerged as a valuable tool to test any pollutant that might target photosynthesis or enzymatic activities such as esterase and alkaline phosphatase activities. Within those pollutants, mostly different classes of herbicides as well as heavy metals have been assessed. Within microalgae, cyanobacteria and green algae have mostly been used; rarely, diatoms such as *Phaeodactylum tricornutum* have also been used; both groups are main components of phytoplankton in aquatic ecosystems and being phototrophic, they are critical for the health of those ecosystems and any detrimental effect on them may significantly alter the trophic chain. Green alga *Chlorella vulgaris* has been usually selected for the construction of the biosensors, its easy cultivation and immobilization in a variety of matrices make it the organism of choice. Table 3 lists the biosensors that will be discussed in this section, although not exhaustive, the table shows relevant microalgal-based biosensors constructed to date in

chronological order. We have tried to complement and update the excellent review on this subject by Brayner et al. (2011).

Most microalgal biosensors are based on the measurement of the effect of pollutants on photosynthesis either by chlorophyll fluorescence analysis (optical biosensors) or directly by measuring photosynthetic O_2 evolution (photoelectrochemical biosensors, mostly photo-amperometric). There are also a few biosensors based on the inhibition of enzymatic activities such as esterase and alkaline phosphatase activities (conductometric biosensors) (Table 3).

Most microalgal biosensors reported to date are able to detect global toxicity as any toxicant that may affect algal metabolism may impair photosynthesis, which is the main metabolic function of these organisms. As non-selective biosensors, they might be able to detect the toxicity of a variety of compounds in an environmental sample, they are adequate for testing samples from aquatic environments, soil abstract, and even aerosols (see Table 3). They have been proposed by most authors as early warning systems of water pollution. Some biosensors based on algae are Algae Toximeter II, Fluotox or Robot Luminotox (please see the review on commercially available bioassays by Kokkali and van Delft (2014).

As stated before, most biosensors have been exposed to herbicides, which are widely used in agriculture for crops, but they may remain in the soil and leach into ground and surface waters. The European Water Act of 1980 document limits the concentration of herbicides in water to less than 0.1 μg/L for individual herbicides and to 0.5 μg/L for a total herbicide class; most algal biosensors are able to detect the toxicity of herbicides in the low μg/L, so that no preconcentration of the sample should be necessary.

4.1 Electrochemical microalgal biosensors

Pollutants may alter different cell metabolic functions resulting in the consumption or generation of electroactive species that may be monitored by different electrochemical methods. Electrochemical biosensors are usually highly sensitive and maybe miniaturized without losing their properties. Depending on the detected species and the transducer type, electrochemical biosensors may be, among others, amperometric, potentiometric or conductometric. Most microalgal biosensors discussed in this section are either amperometric (usually used to monitor photosynthesis) or conductometric (used to monitor several enzyme activities).

In the eighties last century, Rawson et al. (1987) reported the construction of a mediator-assisted amperometric biosensor based on the cyanobacterium *Synechococcus* PCC6301. The

authors tested two other cyanobacteria and a green alga but the *Synehococcus* strain proved to be the most successful. The biosensor measured the photosynthetic electron transfer, so that the redox mediator is reduced and then, reoxidised at the working electrode resulting in a flow of current. Ferrycianide was used as the most appropriate redox mediator as it did not reduce the sensor life, as did p-benzoquinone. The cyanobacterial biosensor responded to herbicides (DCMU, chlortoluron and linuron) in the µg/L range in less than ten minutes.

Two amperometric microalgal biosensors were developed by Pandard et al. (1993). The authors immobilized green algae *Chlorella vulgaris*, *Scenedesmus subspicatus* and *Selenastrum capricornatum* (at present denoted as *Raphidocelis subcapitata*) onto an alumina filter disc. Both types of sensors measured photosynthetic activity, one used a redox mediator (*p*-benoquinone proved to give the best results) as a terminal electron acceptor of the photosynthetic electron transfer chain which was subsequently reoxidized at the biosensor electrode surface resulting in a flow of measurable current; and another used an oxygen electrode to measure photosynthetic oxygen evolution. Biosensors were exposed to herbicides isoproturon, chlortoluron, atrazine, propanil, Cu^{2+} and Hg^{2+}. In general, the oxygen proved to give higher sensitivity and longer operational life than the redox-mediator system.

Rouillona et al. (1999) and Avramescu et al. (1999) constructed two biosensors based on unicellular cyanobacteria: *Synechococcus* PCC7942 and *Synechocystis* PCC6803, both strains were immobilized in poly(vinylalcohol) bearing styrylpyridinium. Photosynhtetic activity was measured amperometrically by the used of the redox-mediator 2,5-dichlorobenzoquinone. Biosensors were exposed to diuron and $HgCl_2$ and both were more sensitive to diuron than to $HgCl_2$; detection limits were lower for the *Synechocystis* biosensor.

Naessens and Tran-Minh (1999) developed a biosensor based on the green alga *Chlorella vulgaris* immobilized on a glass microfiber Whatman filter. Immobilized cells were associated with and oxygen Clark electrode and photosynthetic oxygen evolution measured. The biosensor device was designed to use analytes in the form of aerosols. The biosensor was exposed to perchloroethylene sprayed into the chamber and an increase in oxygen evolution was found, the authors concluded that perchloroethylene was an activator of photosynthesis with limits of detection below limits values of workers exposure, so that authors suggested the biosensor might be used as an early-warning device to determine volatile organic compounds in work-place environments.

Marine filamentous cyanobacterium *Spirulina subsalsa* was inserted into the electrode cap of a Clark-type oxygen electrode and used to assess the effects on photosynthesis (oxygen evolution)

of standardized estuarine waters spiked with heavy metals (Hg^{2+} and Cu^{2+}), the herbicide atrazine or the carbamate insecticide carbaryl (Campanella et al. 2001). The biosensor was more sensitive to the herbicide than to the carbamate insecticide or the heavy metals; issues of metal speciation in saline environments might account with the decrease of metal toxicity.

Alkaline phosphatase activity (APA) located on the cell wall has been chosen as a parameter suitable to test the toxicity of heavy metals to microalgal-based biosensors. *Chlorella vulgaris* has been the alga of choice. Chouteau et al. (2004) constructed a conductometric biosensors with the green alga immobilized inside bovine serum albumin membranes cross-linked with glutaraldehyde vapors. The biosensor was tested in the presence of Cd^{2+}, resulting in inhibition of APA activity, with a detection limit of just 1 µg/L. Ionescu et al. (2006) entrapped *Chlorella vulgaris* cells either in alginate gel or a newly synthesized pyrrole-alginate matrix. APA was measured by adding p-nitrophenol phosphate as the substrate to generate p-nitrophenol, which is electroactive and can be measured amperometrically. The authors demonstrated the higher stability of the pyrrole-alginate gel for immobilization but no test with pollutants was reported. Guedri and Durrieu (2008) constructed a novel conductometric algal biosensor to measure APA activity in the presence of metals, namely Cd^{2+}. *Chlorella vulgaris* was immobilized on self-assembled monolayers (SAMs) of alkanethiolate with the advantage that there is no physical barrier between the algae and the constituents of the reaction medium. The detection limits for the metal were in the µg/L range. Chong et al. (2008) entrapped *Chlorella vulgaris* in a bovine serum albumin membrane and immobilized directly onto the surface of a diamond electrode. APA was measured amperometrically by adding p-nitrophenol phosphate as the substrate. The biosensor was exposed to Zn^{2+} and Cd^{2+} with detection limits of 0.1 µg/L for both metals.

Shitanda et al. (2005) constructed two novel amperometric biosensors based on motility and gravitaxis of the flagellate algae *Chlamydomonas reinhardtii*. Both biosensors were exposed to toluene, copper (II) sulfate and nickel (II) chloride. The negative gravitaxis biosensors proved to more sensitive to toluene than the motility-based biosensor; to increase the sensitivity further, a thin-layer gravitaxis biosensor was developed. The authors claimed that this biosensor was much more sensitive than conventional algal biosensors, which measure photosynthetic activity.

Durrieu et al. (2011) constructed conductometric algal biosensors based on measuring the esterase activity localized on the external membrane of the algal cells. Two unicellular marine algae were used: green alga *Dunalliela tertiolecta* and diatom *Phaeodactylum tricornutum*. Algal cells were immobilized using the SAM technique already discussed above. The *Dunalliela tertiolecta* biosensor was tested with herbicides diuron and glyphosate and several of their

degradation products. Results were highly variable, so that authors conclude that it would be necessary to test a larger number of sensors and lower concentrations of the tested compounds.

Tsopela et al. (2014) fabricated an amperometric biosensor with three electrodes integrated on a silicon chip for the detection of herbicides in water. The authors used the electrochemical microsensors device in dense suspensions of the green microalga *Chlamydomonas reinhardtii*. The device could detect O_2 (photosynthetic activity of the algae), H_2O_2 and H_3O^+/OH^- ions, ionic species taking part in other metabolic activities of algae (reactive oxygen species, ROS, formation by the action of pollutants). The photosynthetic activity (O_2 monitoring) was tested in the presence of the herbicide diuron, reaching a limit of detection of 0.2 µM. In a subsequent article, Tsopela et al. (2016) developed a lab-on-chip integrated device composed of three fluidic chambers integrating electrochemical sensors and other three chambers dedicated to optical-fluorescent base detection. Algal photosynthetic activity was measured. The device was tested also with diuron achieving a limit of detection of 0.1 µM.

Recently, Buckova et al. (2017) developed an amperometric device denoted as AlgaTox which measures oxygen evolution during photosynthesis, it has been patented in Czech Republic (CZ 305687). A suspension of cells of the green algae *Scenedesmus qudricauda* is placed in the reaction vessel and the AlgaTox device measures alterations of photosynthetic oxygen production because of environmental pollution. The device has been tested with aqueous extracts of soils collected from roadsides and with waste sample from old dried-up metal processing industrial tailing pond enriched with insecticide Drevosan Profi 058/14/506. The authors claimed that the values of oxygen production alterations recorded by the device were up to six times higher than the corresponding values of alterations in growth rates determined by standard procedures, implying a higher sensitivity.

4.2 Optical microalgal biosensors

In photosynthesis, electromagnetic energy is converted into chemical energy by the absorption of light by the photosynthetic antenna systems, mainly chlorophylls that transfer that excitation energy to the reaction centers of the two photosystems, photosystemas I and II (PSI and PSII). There, the energy drives the primary photochemical reactions that initiate the photosynthetic energy conversion. Some of the absorbed energy is dissipated as heat or emitted as fluorescence at a wavelength longer than the excitation energy. Due to the high yield of the primary photochemical energy conversion (It is calculated than more than 90 % of absorbed light quanta are utilized by photosynthesis); only a small portion (around 3 % of absorbed light) is released as fluorescence (Krause and Weis 1991). At room temperature, most fluorescence is emitted by

Chlorophyll a of PS II, which is the main site highly susceptible to many environmental stresses in photosynthetic organisms (Geoffroy et al. 2007; Papageorgiou et al. 2007). Around 30% herbicides inhibit PSII, the target site is usually the QB site of the D1 protein. These herbicides, including triazines, triazinones, phenylureas, biscarbamates and phenolic herbicides (Giardi and Pace 2005), inhibit photosynthetic electron flow from the primary acceptor QA to the secondary quinone QB and thus, increase the chlorophyll a fluorescence intensity. Figure 4 shows some examples of optical microalgal biosensors.

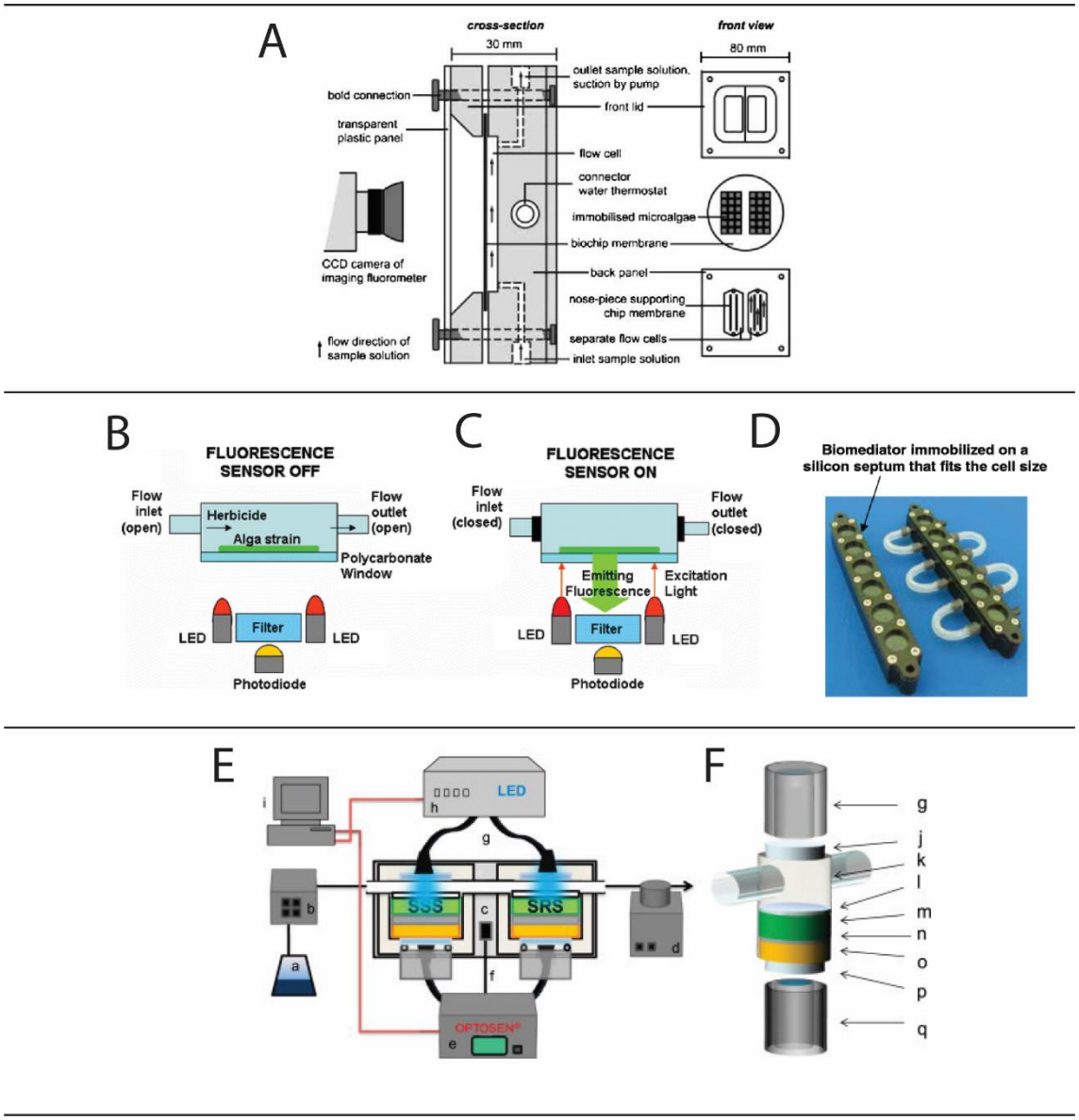


Figure 4. Examples of optical microalgal-based bioassays (see Table 3). (A) Schematic optical section of a microalgal biosensor for detection of herbicides inhibiting photosynthesis – Cross-section and front view. Reprinted with permission from Podola and Melkonian (2005), Copyright (2005), Springer Nature. B and C show a scheme of a fluorescent microalgal biosensor based on

Chlamydomonas reinhardtii. The biological container and the optical compartment are represented at the top and the bottom, respectively. (B) The sample solution containing the herbicide under test flows into the biological cell; (C) the alga strain biomediator is photoactivated and the emitting fluorescence captured by the photodetector (in static condition). (D) Two biological arrays made up by six containers (10 mm $Ø_{int}$×10 mm H, 785 µl each) for static and flow measurement mode, respectively. Reprinted with permission from Scognamiglio et al. (2009), Copyright (2009), Springer Nature. E and F depicts a microalgal dual-head biosensor for herbicide detection based on *Dictyosphaerium chlorelloides* (E) Schematic diagram of the microalgal dual head fiber-optic biosensor: a, water sample/culture medium; b, degasser; c, dual chamber measuring cell; d, peristaltic pump; e, optoelectronic O_2 transducer; f, temperature probe; g, optical fibers for actinic illumination; h, blue LED source; i, laptop computer. (F) Exploded view of a flow chamber: g, optical fiber for actinic illumination; j, sapphire window; k, flow-through chamber; l, cellulose membrane; m, microalgal membrane; n, gray silicone layer; o, O_2-sensitive film; p, polymer window; q, optical fiber to the optoelectronic O_2 transducer. Reprinted with permission from Haigh-Flórez et al. (2014), Copyright (2014), Elsevier .

Frense et al. (1998) reported the construction of an optical biosensor based on the green alga *Scenedesmus subspicatus* which was immobilized in filter paper and covered with alginate and fixed on the surface of the optical electrode. Synthetic wastewater was spiked with increasing concentrations of the herbicides atrazine and endrine, detection was possible down to the µg/L, the response time was short (10 min); the authors claimed that reproducible results could be obtained after regeneration in the nutrient medium for one hour. Immobilized algae could be stored for about six months retaining chlorophyll fluorescence.

In 2000, Naessens et al. (2000) constructed a new biosensor based on green alga *Chlorella vulgaris*. The algae were immobilized on glass microfiber Whatman filters placed in front of an optical fiber bundle inside a homemade microcell. The biosensor was exposed to herbicides and chlorophyll fluorescence intensity was monitored. An increase of fluorescence was observed after exposure to herbicides that target PSII: diuron, simazine and atrazine; alachlor, whose site of action in photosynthesis was not so clear, also increased chlorophyll fluorescence while glyphosate, an inhibitor of an enzyme involved in amino acid synthesis, decreased it. Detection limits differed with the herbicide, in the order of nM for diuron, simazine and atrazine and µM-mM for alachlor and glyphosate, respectively. The algal biosensor could be re-used with atrazine, simazine and diuron.

Sanders et al. (2001) developed two optical biosensors, one based on the cyanobacterium *Nostoc commune* and another based on the green alga *Chlorella vulgaris*. They used a pulse amplitude modulated (PAM) fluorometer to measure maximum PSII photochemical efficiency in

dark adapted cells. This parameter was calculated as Fv/Fm where the variable fluorescence (Fv) was calculated as the difference between the maximum fluorescence (Fm) and the minimum fluorescence (Fo). Fo and Fm are determined by pre- and post-saturating flash fluorescence measurement on the entrapped biosensor samples. Both biosensors were exposed to chemical warfare agents in a gas stream: Tabun, sarin, mustard agent, tributylamine (a sarin stabilizer) and the mustard agent analogue dibutyl sulfide. The same group reported in 2002 (Rodriguez Jr et al. 2002) the use of *Chlorella vulgaris* and naturally occurring populations of algae in river field samples to calculate the maximum PSII photochemical efficiency when exposed to freshwater drinking samples spiked with KCN, the organophosphorus insecticide methyl parathion and the herbicides DCMU (diuron), and MV finding a significant decrease in photochemical yields after 24-min exposure.

In 2003, Védrine et al. (2003) reported a *Chlorella vulgaris*-based biosensor entrapping the microalgae on a quartz microfiber Whatman. filter and placed in a five-membrane-home-made-flow cell. The biosensor was exposed to a range of herbicides: Atrazine, DNOC, simazine, isoproturon and diuron and alterations in chlorophyll fluorescence analyzed. Limits of detection were in the µg/L range of the herbicides, the lower detection limit was found with isoproturon and diuron (0.025 µg/L). The response of the biosensor to 1 µg/L of atrazine was claimed by the authors to be stable for over one month.

Podola et al. (2004) constructed the so-called algal sensor chip (ASC) using strains of the green algae *Klebsormidium sp*. (filamentous) and *Chlorella vulgaris*. Algae were immobilized by filtration onto a membrane filter; the multiple-strain algal biosensor was located on a glass fiber and continuously supplied with culture medium. Biosensors were exposed to vapors of formaldehyde and methanol. The toxicity endpoint was the calculation of the effective quantum yield of photochemical energy conversion in PS II (ΔF/Fm') (Genty et al, 1989). Formaldehyde vapors were detected down to the µg/L range while they were less sensitive to methanol; the biosensor response was recorded within minutes and it was reversible. In 2005, the same group reported an array-chip biosensor configuration containing nine microalgal strains (Podola and Melkonian 2005). Alterations in chlorophyll fluorescence was chosen as toxicity endpoint; the biosensor was exposed to the herbicides atrazine, simazine, diuron, isoproturon and paraquat and were detectable within minutes at concentrations in the µg/L range. Herbicide specificity was encoded in the response pattern of the algal strains to each of the five herbicides.

Nguyen-Ngoc and Tran-Minh (2007) reported the development of a *Chlorella vulgaris*-based biosensor; cells were entrapped in an inorganic translucent matrix so that the excitation light

can penetrate the membrane in contrast with immobilization on an opaque matrix. The biosensor was exposed to the herbicide diuron; changes in chlorophyll fluorescence was the measured parameter with a detection limit of 1 µg/L; the immobilized algal cells could keep over 95 % of their initial activity after a period of five weeks.

A rather different approach was undertaken by Scognamiglio et al. (2009) who used several *Chlamydomonas reinhardtii* mutants of the D1 protein of PSII as biosensors for the detection of pollutants targeting the photosystem. Mutant cells were immobilized on silicon septa attached to a portable instrument denoted as OPTICBIO-Multicell. Chlorophyll fluorescence parameters linked to PSII photochemical efficiency were calculated. The mutant strains showed different sensitivities to the different tested herbicides (atrazine, prometryn and diuron) with limits of detection (LODs) in the low nM range. The different sensitivities were useful for the development of an integrated biosensor for the detection of complex environmental samples containing mixtures of pollutants that may affect PSII (Giardi et al. 2009).

Peña-Vázquez et al. (2009) encapsulated three microalgal species, *Dictyosphaerium chlorelloides*, *Scenedesmus intermedius* and *Scenedesmus sp.*, in silicate sol-gel matrices and exposed them to triazines (simazine, atrazine, propazine and terbuthylazine), to the urea-based herbicide linuron, the hormonal herbicide 2,4D and Cu^{2+}. Chlorophyll fluorescence alterations were measured, the biosensors were sensitive to the triazines and linuron which target PSII but did not respond to 2,4 D or Cu^{2+}. The limits of detection were in the low µg/L range and the biosensors showed reversibility in their performance. Interestingly, the authors obtained a simazine resistant mutant that was key for improving selectivity towards this herbicide.

Most of the microalgal biosensors are based on freshwater species that might not be adequate for testing lagoons and coastal waters; in this context, it would be necessary to develop biosensors of environmental relevance for those ecosystems. Durrieu et al. (2011) constructed biosensors based on marine green alga *Dunaliella tertiolecta* and diatom *Phaeodactylum tricornutum*. Two biosensors were made: one based on esterase activity (conductometric, already discussed in the previous section) and an optical biosensor. The latter consisted of *Dunaliella* cells immobilized in a quartz Millipore fiber membrane; chlorophyll fluorescence was the measured parameter and the biosensor was shown to be responsive to diuron.

Ferro et al. (2012) constructed biosensors based on three species of green algae: Chlorella vulgaris, *Pseudokirchneriella subcapitata* (at present denoted as *Raphidocelis subcapitata*) and *Chlamydomonas reinhardtii*. Cells of each microalgae were immobilized in alginate and translucent silica hydrogels following a two-step procedure. Biosensors were exposed to

herbicides DCMU and atrazine, chlorophyll fluorescence was the measured parameter. *Chlamydomonas reinhardtii* was shown to be the most sensitive species with a limit of detection of 0.1µM for atrazine after 40 min exposure. Later works of the same group (Durrieu et al. 2016; Perullini et al. 2014) reported enhancements in immobilization techniques of the three algae to improve the optical quality of the biosensors.

Haigh-Flórez et al. (2014) developed a microalgal dual-head biosensor consisting in two strains of the green alga *Dictyosphaerium chlorelloides*, one sensitive and another resistant to the herbicide simazine. Cells were immobilized into porous silicone films. Cells were coupled to an optoelectronic device with an O_2 transducer based on the oxygen-sensitive luminescent dye RD3; thus, photosynthetic activity was the chosen parameter. These dual biosensors may allow for selectivity towards target waterborne pollutants.

Recently, a microfluidic device with integrated optical pH, oxygen sensors and algal fluorescence was reported (Tahirbegi et al. 2017). *Chlamydomonas reinhardtii* was suspended in buffer solution and injected into the microfluidic device to forma a biofilm at the solid-liquid interface on the bottom of the microfluidic device. The biosensor was exposed to the herbicides simazine, atrazine and diuron and algal photosynthetic/respiratory activity was measured with the optical pH and oxygen sensors and intrinsic chlorophyll fluorescence. The authors claimed that this miniaturized system could determine the pesticide concentration in the nanomolar concentration range and in less than 10 min of exposure.

Table 3. Main features and applications of algal bioassays.

Microorganism / type	Transducer type	Environmental applications/ tested pollutants	Biosensor configuration	References
Synechococcus sp. PCC6301 / Prokaryotic	Amperometric (Photosynthetic activity)	Herbicides (DCMU, Chlortoluron, Linuron)	yes	(Rawson et al. 1987)
Chlorella vulgaris, Scenedesmus subspicatus, Selenastrum capricornutum / Eukaryotic	Amperometric (Photosynthetic activity)	Herbicides (Isoproturon, Chlotoluron, Atrazine, Propanil) Heavy metals (Cu^{2+}, Hg^{2+})	yes	(Pandard et al. 1993)
Chlorella vulgaris, Scenedesmus subspicatus, Selenastrum capricornutum / Eukaryptic	Amperometric (Photosynthetic O_2 evolution)	Herbicides (Isoproturon, Chlotoluron, Atrazine, Propanil) Heavy metals (Cu^{2+}, Hg^{2+})	yes	
Scenedesmus subspicatus / Eukaryotic	Optical (chlorophyll fluorescence)	Synthetic wastewater spiked with herbicides (Atrazine, Endrine)	yes	(Frense et al. 1998)
Synechococcus sp. PCC7942 / Prokaryotic	Amperometric (Photosynthetic activity)	Herbicides (Diuron) and mercuric chloride	yes	(Rouillona et al. 1999)
Synechocystis sp. PCC6803 / Prokaryotic	Amperometric (Photosynthetic activity)	Herbicides (Diuron) and mercuric chloride	yes	(Avramescu et al. 1999)
Chlorella vulgaris / Eukaryotic	Amperometric (Photosynthetic O_2 evolution)	Volatile Organic Compounds (VOCs; perchlororthylene))	yes	(Naessens and Tran-Minh 1999)
Marine *Spirulina subsalsa* / Prokaryotic	Amperometric (Photosynthetic O_2 evolution)	Estuarine natural waters spiked with heavy metals (copper and mercury), triazine herbicides (Atrazine) and Carbamate insecticide (Carbaryl)	yes	(Campanella et al. 2001)
Chlorella Vugaris / Eukaryotic	Optical (chlorophyll fluorescence)	Herbicides (Diron, Simazine; Atrazine, Alachlor, Glyphosate)	yes	(Naessens et al. 2000)
Chlorella vulgaris / Eukaryotic; *Nostoc commune* / Prokaryotic	Optical (fluorescence; Photosystem II photochemical efficiency)	Airborne warfare agents (Tabun, Sarin, Mustard agent, tributylamine, dibutyl sulfide)	yes	(Sanders et al. 2001)
Chlorella vulgaris / Eukaryotic	Optical (fluorescence; Photosystem II photochemical efficiency)	Freshwater drinking samples spiked with KCN, DCMU, methyl parathion and paraquat	yes	(Rodriguez Jr et al. 2002)

Chlorella vulgaris / Eukaryotic	Optical (chlorophyll fluorescence)	Herbicides (Atrazine, DNOC, Simazine, Isoproturon, Diuron)	yes	(Védrine et al. 2003)
Chlorella vulgaris / Eukaryotic	Conductometric (alkaline phosphatase activity)	Cd^{2+}	yes	(Chouteau et al. 2004)
Klebsormidium nitens / Eukaryotic; *Klebsormidium* sp. Strain M1939 / Eukaryotic; *Chlorella vulgaris* / Eukaryotic	Optical (fluorescence; Photosystem II photochemical efficiency)	VOCs (formaldehyde, methanol vapors)	yes	(Podola et al. 2004)
Nine microalgal strains Innobilised on an array biochip / Both eukaryotic and prokaryotic	Optical (chlorophyll fluorescence)	Herbicides (Atrazine, Diuron, Isoproturon, Paraquat; Simazine)	yes	(Podola and Melkonian 2005)
Chlamydomonas reinhardtii / Eukaryotic	Amperometric (changes in flagellar movement)	Toluene, copper sulfate and nickel chloride	yes	(Shitanda et al. 2005)
Chlamydomonas reinhardtii / Eukaryotic	Amperometric (changes in gravitaxis)	Toluene, copper sulfate and nickel chloride	yes	
Chlorella vulgaris / Eukaryotic	Amperometric (alkaline phosphatase activity)	No mention	yes	(Ionescu et al. 2006)
Chlorella vulgaris / Eukaryotic	Optical (chlorophyll fluorescence)	Herbicides (Diuron)	yes	(Nguyen-Ngoc and Tran-Minh 2007)
Chlorella vulgaris / Eukaryotic	Amperometric (alkaline phosphatase activity)	Zn^{2+}; Cd^{2+}	yes	(Chong et al. 2008)
Chlorella vulgaris / Eukaryotic	Conductometric (alkaline phosphatase activity)	Cd^{2+}	yes	(Guedri and Durrieu 2008)
Chlamydomonas reinhardtii photosystem II D1 protein mutants / Eukaryotic	Optical (fluorescence; Photosystem II photochemical efficiency)	Herbicides (Atrazine, Prometryn, Diuron)	yes	(Scognamiglio et al. 2009)
Chlamydomonas reinhardtii photosystem II D1 protein mutants / Eukaryotic	Optical (fluorescence; Photosystem II photochemical efficiency)	Triazines and urea-based herbicides	yes	(Giardi et al. 2009)
Dictyosphaerium chlorelloides; *Scenedesmus inermedius* and *Scenedesmus* sp. / Eukaryotic	Optical (fluorescence; Photosystem II photochemical efficiency)	Ttriazines and urea-based herbicides	yes	(Peña-Vázquez et al. 2009)
Marine *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* / Eukaryotic	Conductometric (membrane sterase activity)	Herbicides (Diuron and Glyphosate and degradation products)	yes	(Durrieu et al. 2011)

Marine *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* / Eukaryotic	Optical (chlorophyll fluorescence)	Herbicides (Diuron and Glyphosate and degradation products)	yes	
Chlorella vulgaris, Pseudokircheriella subcapitata and *Chlamydomonas reinhardtii* / Eukaryotic	Optical (chlorophyll fluorescence)	Herbicides (DCMU and atrazine)	yes	(Ferro et al. 2012)
Dictyosphaerium chlorelloides / Eukaryotic	Optical (bioluminescence-based O_2 transduction)	Herbicides (simazine)	yes	(Haigh-Flórez et al. 2014)
Chlamydomonas reinhardtii / Eukaryotic	Amperometric (Photosynthetic O_2 evolution)	Urea-based herbicides (Diuron)	No (bioassay with dense algal solutions)	(Tsopela et al. 2016; Tsopela et al. 2014)
Scenedesmus quadricauda, strain GREIFSWALD/15 / Eukaryotic	Amperometric (Photosynthetic O_2 evolution)	-Aqueous extracts of soils collected from roadsides -Waste sample from old dried-up metal processing industrial tailing pond enriched with insecticide Drevosan Profi 058/14/506	No (bioassay with algal solutions)	(Buckova et al. 2017)
Chlamydomonas reinhardtii / Eukaryotic	Optical luminescent O_2 sensor and chlorophyll fluorescence	Herbicides (Simazine, Atrazine, Diuron)	Yes (microfluidic device)	(Tahirbegi et al. 2017)

5. Conclusions and Future directions

Microorganisms–based toxicity bioassays and biosensors have emerged as a promising tool for environmental toxicity monitoring. Their ease of culture, low cost, potential of mass production have made them a good complement to chemical analyses; they report on global toxicity and most importantly, on pollutant bioavailability. There is a variety of bioassays using microorganisms of ecological relevance, which may monitor toxicity in different environmental compartments: Water, soil or the atmosphere. Some of them have been already validated and standardized by international agencies, have been commercialized and are used worldwide. Most microorganisms may be immobilized in a number of inert matrices, so that biosensors can be fabricated. There is the possibility of biosensor miniaturization and integration in microfluidics to construct lab-on-a-chip devices, so that multiplex biosensors may be made. These biosensors may allow for *in situ*, online and continuous toxicity monitoring.

Despite their advantages, some aspects should be improved before this technology can get out of the laboratory and be implemented by regulatory agencies and stakeholders:

- Validation and standardization by international agencies of microorganisms-based toxicity bioassays and biosensors should be extended.

- Most microorganisms-based toxicity bioassays and biosensors are intended to assess acute toxicity effects, which is relevant when many samples have to be tested, as early warning systems but chronic toxicity is much more realistic and efforts in that direction should be made.

- New immobilization procedures should be developed so that many toxicity bioassays may be transformed into biosensor configuration. Nanotechnology may help in this direction and in further miniaturization of the biosensor devices.

- Ecologically relevant bioassays and biosensors should be used depending on the environmental sample, e.g. bioassays based on marine microorganisms may be not completely adequate for testing freshwater samples.

- Most microorganisms–based toxicity bioassays and biosensors have not been tested with complex mixtures or real environmental samples, only with a selection of pollutants applied singly in the laboratory. This should be necessary for their implementation.

• In the case of bioluminescent recombinant bioreporters, it should be advisable the expression of the genes for aldehyde synthesis when using lux genes and to find the way for intracellular luciferin production when using eukaryotic luciferase reporter genes.

• Long-term storage and reusability of biosensors versus single-use, disposable inexpensive biosensors is an important decision to be made which may be critical for commercialization and extended use of the biosensors

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7. References

Abbas M et al. (2018) Vibrio fischeri bioluminescence inhibition assay for ecotoxicity assessment: A review Science of the Total Environment 626:1295-1309

Abd-El-Haleem D, Zaki S, Abulhamd A, Elbery H, Abu-Elreesh G (2006) Acinetobacter bioreporter assessing heavy metals toxicity Journal of basic microbiology 46:339-347

Ahn J-M, Gu MB (2012) Geno-Tox: cell array biochip for genotoxicity monitoring and classification Applied biochemistry and biotechnology 168:752-760

Aichberger H, Loibner AP, Celis R, Braun R, Ottner F, Rost H (2006) Assessment of factors governing biodegradability of PAHs in three soils aged under field conditions Soil & Sediment Contamination 15:73-85

Amin-Hanjani S, Meikle A, Glover L, Prosser J, Killham K (1993) Plasmid and chromosomally encoded luminescence marker systems for detection of Pseudomonas fluorescens in soil Molecular Ecology 2:47-54

Andreani T et al. (2017) Influence of the stabilizers on the toxicity of metallic nanomaterials in aquatic organisms and human cell lines Science of The Total Environment 607:1264-1277

Arias-Barreiro CR et al. (2010) A bacterial biosensor for oxidative stress using the constitutively expressed redox-sensitive protein roGFP2 Sensors 10:6290-6306

Aruoja V, Pokhrel S, Sihtmäe M, Mortimer M, Mädler L, Kahru A (2015) Toxicity of 12 metal-based nanoparticles to algae, bacteria and protozoa Environmental Science: Nano 2:630-644

Avramescu A, Rouillon R, Carpentier R (1999) Potential for use of a cyanobacterium Synechocystis sp. immobilized in poly (vinylalcohol): Application to the detection of pollutants Biotechnology techniques 13:559-562

Azimi Y, Thompson IP (2017) Phosphorus Depletion as a Green Alternative to Biocides for Controlling Biodegradation of Metalworking Fluids Environmental science & technology 51:5695-5702

Bao VW, Koutsaftis A, Leung KM (2008) Temperature-dependent toxicities of chlorothalonil and copper pyrithione to the marine copepod'Tigriopus japonicus' and dinoflagellate'Pyrocystis lunula' Australasian Journal of Ecotoxicology 14:45

Bao VW, Leung KM, Qiu J-W, Lam MH (2011) Acute toxicities of five commonly used antifouling booster biocides to selected subtropical and cosmopolitan marine species Marine pollution bulletin 62:1147-1151

Barrena R, Casals E, Colón J, Font X, Sánchez A, Puntes V (2009) Evaluation of the ecotoxicity of model nanoparticles Chemosphere 75:850-857
Bazin I, Seo HB, Suehs CM, Ramuz M, De Waard M, Gu MB (2017) Profiling the biological effects of wastewater samples via bioluminescent bacterial biosensors combined with estrogenic assays Environmental Science and Pollution Research 24:33-41
Belkin S (2003) Microbial whole-cell sensing systems of environmental pollutants Current opinion in microbiology 6:206-212
Belkin S, Smulski DR, Dadon S, Vollmer AC, Van Dyk TK, Larossa RA (1997) A panel of stress-responsive luminous bacteria for the detection of selected classes of toxicants Water Research 31:3009-3016
Belkin S, Smulski DR, Vollmer AC, Van Dyk TK, LaRossa RA (1996) Oxidative stress detection with Escherichia coli harboring a katG'':: lux fusion Applied and environmental microbiology 62:2252-2256
Ben-Israel O, Ben-Israel H, Ulitzur S (1998) Identification and Quantification of Toxic Chemicals by Use of Escherichia coli Carryinglux Genes Fused to Stress Promoters Applied and environmental microbiology 64:4346-4352
Bentley A, Atkinson A, Jezek J, Rawson D (2001) Whole cell biosensors—electrochemical and optical approaches to ecotoxicity testing Toxicology in vitro 15:469-475
Betteridge DJ (2000) What is oxidative stress? Metabolism 49:3-8
Bhattacharyya J, Read D, Amos S, Dooley S, Killham K, Paton GI (2005) Biosensor-based diagnostics of contaminated groundwater: assessment and remediation strategy Environmental Pollution 134:485-492
Bispo A, Jourdain MJ, Jauzein M (1999) Toxicity and genotoxicity of industrial soils polluted by polycyclic aromatic hydrocarbons (PAHs) Organic Geochemistry 30:947-952
Bondarenko O, Ivask A, Käkinen A, Kahru A (2012) Sub-toxic effects of CuO nanoparticles on bacteria: kinetics, role of Cu ions and possible mechanisms of action Environmental pollution 169:81-89
Bondarenko O, Rahman PK, Rahman TJ, Kahru A, Ivask A (2010) Effects of rhamnolipids from Pseudomonas aeruginosa DS10-129 on luminescent bacteria: toxicity and modulation of cadmium bioavailability Microbial ecology 59:588-600
Bondarenko O, Sihtmäe MM, Kuzmičiova J, Ragelienė L, Kahru A, Daugelavičius R (2018) Bacterial plasma membrane is the main cellular target of silver nanoparticles in Escherichia coli and Pseudomonas aeruginosa bioRxiv:322727
Bondarenko OM et al. (2016) Multilaboratory evaluation of 15 bioassays for (eco) toxicity screening and hazard ranking of engineered nanomaterials: FP7 project NANOVALID Nanotoxicology 10:1229-1242
Booth AM, Hansen BH, Frenzel M, Johnsen H, Altin D (2016) Uptake and toxicity of methylmethacrylate-based nanoplastic particles in aquatic organisms Environmental toxicology and chemistry 35:1641-1649
Boyd EM, Killham K, Meharg AA (2001) Toxicity of mono-, di-and tri-chlorophenols to lux marked terrestrial bacteria, Burkholderia species Rasc c2 and Pseudomonas fluorescens Chemosphere 43:157-166
Brandt KK, Holm PE, Nybroe O (2006) Bioavailability and toxicity of soil particle-associated copper as determined by two bioluminescent Pseudomonas fluorescens biosensor strains Environmental toxicology and chemistry 25:1738-1741
Brandt KK, Holm PE, Nybroe O (2008) Evidence for Bioavailable Copper− Dissolved Organic Matter Complexes and Transiently Increased Copper Bioavailability in Manure-Amended Soils as Determined by Bioluminescent Bacterial Biosensors Environmental science & technology 42:3102-3108
Brandt KK, Pedersen A, Sørensen J (2002) Solid-phase contact assay that uses a lux-marked Nitrosomonas europaea reporter strain to estimate toxicity of bioavailable linear alkylbenzene sulfonate in soil Applied and environmental microbiology 68:3502-3508

Brayner R, Couté A, Livage J, Perrette C, Sicard C (2011) Micro-algal biosensors Analytical and bioanalytical chemistry 401:581-597
Brouwer H, Murphy T, McArdle L (1990) A sediment-contact bioassay with Photobacterium phosphoreum Environmental Toxicology and Chemistry 9:1353-1358
Brown J, Rattray E, Paton G, Reid G, Caffoor I, Killham K (1996) Comparative assessment of the toxicity of a papermill effluent by respirometry and a luminescence-based bacterial assay Chemosphere 32:1553-1561
Buckova M, Licbinsky R, Jandova V, Krejci J, Pospichalova J, Huzlik J (2017) Fast Ecotoxicity Detection Using Biosensors Water, Air, & Soil Pollution 228:166
Bulich AA, Isenberg D (1981) Use of the luminescent bacterial system for the rapid assessment of aquatic toxicity ISA transactions 20:29-33
Burga-Perez KF, Toumi H, Cotelle S, Ferard J-F, Radetski CM (2013) Sensitivity of different aquatic bioassays in the assessment of a new natural formicide Journal of Environmental Science and Health, Part B 48:57-62
Campanella L, Cubadda F, Sammartino M, Saoncella A (2001) An algal biosensor for the monitoring of water toxicity in estuarine environments Water Research 35:69-76
Corrêa AX, Tiepo EN, Somensi CA, Sperb RM, Radetski CM (2009) Use of ozone-photocatalytic oxidation (O 3/UV/TiO 2) and biological remediation for treatment of produced water from petroleum refineries Journal of Environmental Engineering 136:40-45
Cui Z et al. (2018) Application of a bacterial whole cell biosensor for the rapid detection of cytotoxicity in heavy metal contaminated seawater Chemosphere 200:322-329
Cukurluoglu S, Muezzinoglu A (2013) Assessment of toxicity in waters due to heavy metals derived from atmospheric deposition using Vibrio fischeri Journal of Environmental Science and Health, Part A 48:57-66
Chaudri AM et al. (1999) Determination of acute Zn toxicity in pore water from soils previously treated with sewage sludge using bioluminescence assays Environmental science & technology 33:1880-1885
Chinalia F, Paton GI, Killham K (2008) Physiological and toxicological characterization of an engineered whole-cell biosensor Bioresource technology 99:714-721
Cho J-C et al. (2004) A novel continuous toxicity test system using a luminously modified freshwater bacterium Biosensors and Bioelectronics 20:338-344
Choi SH, Gu MB (2002) A portable toxicity biosensor using freeze-dried recombinant bioluminescent bacteria Biosensors and Bioelectronics 17:433-440
Chong KF, Loh KP, Ang K, Ting YP (2008) Whole cell environmental biosensor on diamond Analyst 133:739-743
Chou T-C (2006) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies Pharmacological reviews 58:621-681
Chou T-C, Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors Advances in enzyme regulation 22:27-55
Chouteau C, Dzyadevych S, Chovelon J-M, Durrieu C (2004) Development of novel conductometric biosensors based on immobilised whole cell Chlorella vulgaris microalgae Biosensors and Bioelectronics 19:1089-1096
Dams R, Biswas A, Olesiejuk A, Fernandes T, Christofi N (2011) Silver nanotoxicity using a light-emitting biosensor Pseudomonas putida isolated from a wastewater treatment plant Journal of hazardous materials 195:68-72
Dawson J, Iroegbu C, Maciel H, Paton G (2008) Application of luminescent biosensors for monitoring the degradation and toxicity of BTEX compounds in soils Journal of applied microbiology 104:141-151
Dawson JJC, Campbell C, Towers W, Cameron C, Paton GI (2006) Linking biosensor responses to Cd, Cu and Zn partitioning in soils Environmental Pollution 142:493-500

de García SO, García-Encina PA, Irusta-Mata R (2016) Dose–response behavior of the bacterium Vibrio fischeri exposed to pharmaceuticals and personal care products Ecotoxicology 25:141-162
de OF Rossetto AL, Melegari SP, Ouriques LC, Matias WG (2014a) Comparative evaluation of acute and chronic toxicities of CuO nanoparticles and bulk using Daphnia magna and Vibrio fischeri Science of the total environment 490:807-814
de OF Rossetto AL, Vicentini DS, Costa CH, Melegari SP, Matias WG (2014b) Synthesis, characterization and toxicological evaluation of a core–shell copper oxide/polyaniline nanocomposite Chemosphere 108:107-114
Deryabin D, Aleshina E, Efremova L (2012) Application of the inhibition of bacterial bioluminescence test for assessment of toxicity of carbon-based nanomaterials Microbiology 81:492-497
Deryabin D, Efremova L, Karimov I, Manukhov I, Gnuchikh EY, Miroshnikov S (2016) Comparative sensitivity of the luminescent Photobacterium phosphoreum, Escherichia coli, and Bacillus subtilis strains to toxic effects of carbon-based nanomaterials and metal nanoparticles Microbiology 85:198-206
Ding K et al. (2017) In vitro and in silico investigations of the binary-mixture toxicity of phthalate esters and cadmium (II) to Vibrio qinghaiensis sp.-Q67 Science of the Total Environment 580:1078-1084
Durrieu C, Ferro Y, Perullini M, Gosset A, Jobbágy M, Bilmes SA (2016) Feasibility of using a translucid inorganic hydrogel to build a biosensor using immobilized algal cells Environmental Science and Pollution Research 23:9-13
Durrieu C, Guedri H, Fremion F, Volatier L (2011) Unicellular algae used as biosensors for chemical detection in Mediterranean lagoon and coastal waters Research in microbiology 162:908-914
Dzyadevych SV, Chovelon J-M (2002) A comparative photodegradation studies of methyl parathion by using Lumistox test and conductometric biosensor technique Materials Science and Engineering: C 21:55-60
Ejeian F et al. (2018) Biosensors for wastewater monitoring: A review Biosensors and Bioelectronics
El-Alawi YS, Dixon DG, Greenberg BM (2001) Effects of a pre-incubation period on the photoinduced toxicity of polycyclic aromatic hydrocarbons to the luminescent bacterium Vibrio fischeri Environmental Toxicology: An International Journal 16:277-286
Ellouze M, Saddoud A, Dhouib A, Sayadi S (2009) Assessment of the impact of excessive chemical additions to municipal wastewaters and comparison of three technologies in the removal performance of pathogens and toxicity Microbiological research 164:138-148
Farré M, Asperger D, Kantiani L, González S, Petrovic M, Barceló D (2008) Assessment of the acute toxicity of triclosan and methyl triclosan in wastewater based on the bioluminescence inhibition of Vibrio fischeri Analytical and Bioanalytical Chemistry 390:1999-2007
Farré M, Barceló D (2003) Toxicity testing of wastewater and sewage sludge by biosensors, bioassays and chemical analysis TrAC Trends in Analytical Chemistry 22:299-310
Farré M, Gonçalves C, Lacorte S, Barcelo D, Alpendurada M (2002a) Pesticide toxicity assessment using an electrochemical biosensor with Pseudomonas putida and a bioluminescence inhibition assay with Vibrio fischeri Analytical and bioanalytical chemistry 373:696-703
Farré M, Klöter G, Petrovic M, Alonso MC, de Alda MJL, Barceló D (2002b) Identification of toxic compounds in wastewater treatment plants during a field experiment Analytica Chimica Acta 456:19-30

Fekete-Kertész I, Piszmán D, Molnár M (2017) Particle Size and Concentration Dependent Ecotoxicity of Nano-and Microscale TiO 2—Comparative Study by Different Aquatic Test Organisms of Different Trophic Levels Water, Air, & Soil Pollution 228:245

Fernández-Pinas F, Wolk CP (1994) Expression of luxCD-E in Anabaena sp. can replace the use of exogenous aldehyde for in vivo localization of transcription by luxAB Gene 150:169-174

Fernández-Piñas F, Rodea-Palomares I, Leganés F, González-Pleiter M, Muñoz-Martín MA (2014) Evaluation of the ecotoxicity of pollutants with bioluminescent microorganisms. In: Bioluminescence: Fundamentals and Applications in Biotechnology-Volume 2. Springer, pp 65-135

Ferro Y, Perullini M, Jobbagy M, Bilmes SA, Durrieu C (2012) Development of a biosensor for environmental monitoring based on microalgae immobilized in silica hydrogels Sensors 12:16879-16891

Fetters KJ, Costello DM, Hammerschmidt CR, Burton Jr GA (2016) Toxicological effects of short-term resuspension of metal-contaminated freshwater and marine sediments Environmental toxicology and chemistry 35:676-686

Flynn HC, Mc Mahon V, Diaz GC, Demergasso CS, Corbisier P, Meharg AA, Paton GI (2002) Assessment of bioavailable arsenic and copper in soils and sediments from the Antofagasta region of northern Chile Science of the Total Environment 286:51-59

Frense D, Müller A, Beckmann D (1998) Detection of environmental pollutants using optical biosensor with immobilized algae cells Sensors and Actuators B: Chemical 51:256-260

Gagne F (2014) Biochemical ecotoxicology: principles and methods. Elsevier,

Gao L, Chen J, Tang C, Ke Z, Wang J, Shimizu Y, Zhu A (2015) Distribution, migration and potential risk of heavy metals in the Shima River catchment area, South China Environmental Science: Processes & Impacts 17:1769-1782

Geoffroy L et al. (2007) Effect of selenate on growth and photosynthesis of Chlamydomonas reinhardtii Aquatic toxicology 83:149-158

Georgantzopoulou A et al. (2012) Ag nanoparticles: size-and surface-dependent effects on model aquatic organisms and uptake evaluation with NanoSIMS Nanotoxicology 7:1168-1178

Giardi MT, Pace E (2005) Photosynthetic proteins for technological applications TRENDS in Biotechnology 23:257-263

Giardi MT et al. (2009) Optical biosensors for environmental monitoring based on computational and biotechnological tools for engineering the photosynthetic D1 protein of Chlamydomonas reinhardtii Biosensors and Bioelectronics 25:294-300

Gil GC, Kim YJ, Gu MB (2002) Enhancement in the sensitivity of a gas biosensor by using an advanced immobilization of a recombinant bioluminescent bacterium Biosensors and Bioelectronics 17:427-432

Gil GC, Mitchell RJ, Chang ST, Gu MB (2000) A biosensor for the detection of gas toxicity using a recombinant bioluminescent bacterium Biosensors and Bioelectronics 15:23-30

González-Pleiter M et al. (2013) Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: implications for environmental risk assessment Water research 47:2050-2064

Gouider M, Feki M, Sayadi S (2010) Bioassay and use in irrigation of untreated and treated wastewaters from phosphate fertilizer industry Ecotoxicology and environmental safety 73:932-938

Gu MB, Chang ST (2001) Soil biosensor for the detection of PAH toxicity using an immobilized recombinant bacterium and a biosurfactant Biosensors and Bioelectronics 16:667-674

Guedri H, Durrieu C (2008) A self-assembled monolayers based conductometric algal whole cell biosensor for water monitoring Microchimica Acta 163:179-184

Haigh-Flórez D, de la Hera C, Costas E, Orellana G (2014) Microalgae dual-head biosensors for selective detection of herbicides with fiber-optic luminescent O2 transduction Biosensors and Bioelectronics 54:484-491
Hassan SH, Oh SE (2010) Improved detection of toxic chemicals by Photobacterium phosphoreum using modified Boss medium Journal of Photochemistry and Photobiology B: Biology 101:16-21
Hassan SH, Van Ginkel SW, Hussein MA, Abskharon R, Oh S-E (2016) Toxicity assessment using different bioassays and microbial biosensors Environment international 92:106-118
Hechmi N, Bosso L, El-Bassi L, Scelza R, Testa A, Jedidi N, Rao MA (2016) Depletion of pentachlorophenol in soil microcosms with Byssochlamys nivea and Scopulariopsis brumptii as detoxification agents Chemosphere 165:547-554
Heimann K, Matuszewski JM, Klerks PL (2002) Effects of metals and organic contaminants on the recovery of bioluminescence in the marine dinoflagellate Pyrocystis lunula (Dinophyceae) Journal of phycology 38:482-492
Heinlaan M, Ivask A, Blinova I, Dubourguier H-C, Kahru A (2008) Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus Chemosphere 71:1308-1316
Hermens J, Busser F, Leeuwangh P, Musch A (1985) Quantitative structure-activity relationships and mixture toxicity of organic chemicals in Photobacterium phosphoreum: the Microtox test Ecotoxicology and Environmental safety 9:17-25
Hernando M, De Vettori S, Bueno MM, Fernández-Alba A (2007) Toxicity evaluation with Vibrio fischeri test of organic chemicals used in aquaculture Chemosphere 68:724-730
Hildenbrand ZL et al. (2015) Rapid analysis of eukaryotic bioluminescence to assess potential groundwater contamination events Journal of Chemistry 2015
Hirmann D, Loibner AP, Braun R, Szolar OH (2007) Applicability of the bioluminescence inhibition test in the 96-well microplate format for PAH-solutions and elutriates of PAH-contaminated soils Chemosphere 67:1236-1242
Hjorth R, Coutris C, Nguyen NH, Sevcu A, Gallego-Urrea JA, Baun A, Joner EJ (2017) Ecotoxicity testing and environmental risk assessment of iron nanomaterials for sub-surface remediation–Recommendations from the FP7 project NanoRem Chemosphere 182:525-531
Hollis R, Killham K, Glover L (2000) Design and application of a biosensor for monitoring toxicity of compounds to eukaryotes Applied and environmental microbiology 66:1676-1679
Horsburgh AM et al. (2002) On-line microbial biosensing and fingerprinting of water pollutants Biosensors and Bioelectronics 17:495-501
Horswell J, Weitz HJ, Percival HJ, Speir TW (2006) Impact of heavy metal amended sewage sludge on forest soils as assessed by bacterial and fungal biosensors Biology and fertility of soils 42:569-576
Hsieh C-Y, Tsai M-H, Ryan DK, Pancorbo OC (2004) Toxicity of the 13 priority pollutant metals to Vibrio fisheri in the Microtox® chronic toxicity test Science of the total environment 320:37-50
Hurtado-Gallego J, Martín-Betancor K, Rodea-Palomares I, Leganés F, Rosal R, Fernández-Piñas F (2018) Two novel cyanobacterial bioluminescent whole-cell bioreporters based on superoxide dismutases MnSod and FeSod to detect superoxide anion Chemosphere 201:772-779
Iizumi T, Mizumoto M, Nakamura K (1998) A bioluminescence assay using nitrosomonas europaeafor rapid and sensitive detection of nitrification inhibitors Applied and environmental microbiology 64:3656-3662
Ionescu RE, Abu-Rabeah K, Cosnier S, Durrieu C, Chovelon JM, Marks RS (2006) Amperometric algal Chlorella vulgaris cell biosensors based on alginate and polypyrrole-alginate gels Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis 18:1041-1046

Ivask A, Bondarenko O, Jepihhina N, Kahru A (2010) Profiling of the reactive oxygen species-related ecotoxicity of CuO, ZnO, TiO2, silver and fullerene nanoparticles using a set of recombinant luminescent Escherichia coli strains: differentiating the impact of particles and solubilised metals Analytical and bioanalytical chemistry 398:701-716

Ivask A, François M, Kahru A, Dubourguier H-C, Virta M, Douay F (2004) Recombinant luminescent bacterial sensors for the measurement of bioavailability of cadmium and lead in soils polluted by metal smelters Chemosphere 55:147-156

Ivask A, Green T, Polyak B, Mor A, Kahru A, Virta M, Marks R (2007) Fibre-optic bacterial biosensors and their application for the analysis of bioavailable Hg and As in soils and sediments from Aznalcollar mining area in Spain Biosensors and Bioelectronics 22:1396-1402

Ivask A, Rõlova T, Kahru A (2009) A suite of recombinant luminescent bacterial strains for the quantification of bioavailable heavy metals and toxicity testing BMC biotechnology 9:41

Jemec A et al. (2016) An interlaboratory comparison of nanosilver characterisation and hazard identification: Harmonising techniques for high quality data Environment international 87:20-32

Jennings VL, Rayner-Brandes MH, Bird DJ (2001) Assessing chemical toxicity with the bioluminescent photobacterium (Vibrio fischeri): a comparison of three commercial systems Water research 35:3448-3456

Ji J-Y, Xing Y-J, Ma Z-T, Zhang M, Zheng P (2013) Acute toxicity of pharmaceutical wastewaters containing antibiotics to anaerobic digestion treatment Chemosphere 91:1094-1098

Joost U et al. (2015) Photocatalytic antibacterial activity of nano-TiO2 (anatase)-based thin films: effects on Escherichia coli cells and fatty acids Journal of Photochemistry and Photobiology B: Biology 142:178-185

Jośko I, Oleszczuk P (2013) The influence of ZnO and TiO 2 nanoparticles on the toxicity of sewage sludges Environmental Science: Processes & Impacts 15:296-306

Jung Y, Park C-B, Kim Y, Kim S, Pflugmacher S, Baik S (2015) Application of multi-species microbial bioassay to assess the effects of engineered nanoparticles in the aquatic environment: potential of a luminous microbial array for toxicity risk assessment (LumiMARA) on testing for surface-coated silver nanoparticles International journal of environmental research and public health 12:8172-8186

Jurado E, Fernández-Serrano M, Núñez J, Lechuga M, Luzón G (2008) Evolution of acute toxicity of non-ionic surfactants over the biodegradation process Environmental Toxicology II 110:11101

Kahru A, Tomson K, Pall T, Külm I (1996) Study of toxicity of pesticides using luminescent bacteria Photobacterium phosphoreum Water Science and Technology 33:147-154

Kaiser KL, Palabrica VS (1991) Photobacterium phosphoreum toxicity data index Water Quality Research Journal 26:361-431

Kang I-M, Kong IC (2016) Effects of properties of metal-contaminated soils on bacterial bioluminescence activity, seed germination, and root and shoot growth SpringerPlus 5:272

Katsoyiannis A, Samara C (2007) Ecotoxicological evaluation of the wastewater treatment process of the sewage treatment plant of Thessaloniki, Greece Journal of Hazardous Materials 141:614-621

Kelly C, Lajoie C, Layton A, Sayler G (1999) Bioluminescent reporter bacterium for toxicity monitoring in biological wastewater treatment systems Water Environment Research 71:31-35

Kim B, Gu M, Hansen P (2005) Evaluation of Water-borne toxicity using bioluminescent bacteria. In: Environmental Chemistry. Springer, pp 699-705

Kim BC, Gu MB (2005) A multi-channel continuous water toxicity monitoring system: its evaluation and application to water discharged from a power plant Environmental monitoring and assessment 109:123-133
Knox OG, Weitz HJ, Anderson P, Borlinghaus M, Fountaine J (2018) Improved screening of biochar compounds for potential toxic activity with microbial biosensors Environmental Technology & Innovation
Ko K-S, Kong IC (2014) Toxic effects of nanoparticles on bioluminescence activity, seed germination, and gene mutation Applied microbiology and biotechnology 98:3295-3303
Kokkali V, van Delft W (2014) Overview of commercially available bioassays for assessing chemical toxicity in aqueous samples TrAC Trends in Analytical Chemistry 61:133-155
Kong I-C (2011) Comparative Studies on the Acute Toxicities of Whole Solids and Solids Aqueous Extracts Based on the Inhibition of Bacterial Bioluminescence Production Environmental Engineering Research 16:41-45
Kong IC (2008) Microbial characteristics associated with six different organic wastes undergoing anaerobic decomposition in batch vial conditions Waste Management & Research 26:261-266
Kong IC, Raliya R, Ko K-S, Biswas P (2018) ZnO Nanoparticles: Effect of Size on Bacterial Bioluminescence, Seed Germination, Algal Growth, and Gene Mutation Environmental Engineering Science 35:231-239
Kováts N, Refaey M, Varanka B, Reich K, Ferincz Á, Ács A (2012) Comparison of conventional and Vibrio fischeri bioassays for the assessment of municipal wastewater toxicity Environmental Engineering and Management Journal 11:2073-2076
Krause G, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics Annual review of plant biology 42:313-349
Kungolos A, Hadjispyrou S, Petala M, Tsiridis V, Samaras P, Sakellaropoulos G (2004) Toxic properties of metals and organotin compounds and their interactions on Daphnia magna and Vibrio fischeri Water, Air and Soil Pollution: Focus 4:101-110
Kurvet I, Ivask A, Bondarenko O, Sihtmäe M, Kahru A (2011) LuxCDABE—Transformed constitutively bioluminescent escherichia coli for toxicity screening: Comparison with naturally luminous Vibrio fischeri Sensors 11:7865-7878
Kurvet I, Juganson K, Vija H, Sihtmäe M, Blinova I, Syvertsen-Wiig G, Kahru A (2017) Toxicity of Nine (Doped) Rare Earth Metal Oxides and Respective Individual Metals to Aquatic Microorganisms Vibrio fischeri and Tetrahymena thermophila Materials 10:754
la Farré M, García Ma-J, Tirapu L, Ginebreda A, Barceló D (2001) Wastewater toxicity screening of non-ionic surfactants by Toxalert® and Microtox® bioluminescence inhibition assays Analytica Chimica Acta 427:181-189
Lagarde F, Jaffrezic-Renault N (2011) Cell-based electrochemical biosensors for water quality assessment Analytical and Bioanalytical Chemistry 400:947
Lajoie CA, Lin S-C, Nguyen H, Kelly CJ (2002) A toxicity testing protocol using a bioluminescent reporter bacterium from activated sludge Journal of microbiological methods 50:273-282
Lapota D, Moskowitz GJ, Rosenberger DE, Grovhoug JG (1993) The use of stimulable bioluminescence from marine dinoflagellates as a means of detecting toxicity in the marine environment. In: Environmental Toxicology and Risk Assessment: Second Volume. ASTM International,
Lapota D, Osorio AR, Liao C, Bjorndal B (2007) The use of bioluminescent dinoflagellates as an environmental risk assessment tool Marine Pollution Bulletin 54:1857-1867
Lapota D, Rosenberger D, Duckworth D (1994) A bioluminescent dinoflagellate assay for detecting toxicity in coastal waters Bioluminescence and chemiluminescence: fundamentals and applied aspects Wiley, Chichester:156-159

Latifi A, Ruiz M, Zhang C-C (2009) Oxidative stress in cyanobacteria FEMS microbiology reviews 33:258-278
Layton A, Gregory B, Schultz T, Sayler G (1999) Validation of genetically engineered bioluminescent surfactant resistant bacteria as toxicity assessment tools Ecotoxicology and environmental safety 43:222-228
Lechuga M, Fernández-Serrano M, Jurado E, Núñez-Olea J, Ríos F (2016) Acute toxicity of anionic and non-ionic surfactants to aquatic organisms Ecotoxicology and environmental safety 125:1-8
Lee H, Gu M (2003) Construction of a sodA:: luxCDABE fusion Escherichia coli: comparison with a katG fusion strain through their responses to oxidative stresses Applied microbiology and biotechnology 60:577-580
Lee HY, Choi SH, Gu MB (2003) Response of bioluminescent bacteria to sixteen azo dyes Biotechnology and Bioprocess Engineering 8:101-105
Lee JH, Gu MB (2005) An integrated mini biosensor system for continuous water toxicity monitoring Biosensors and Bioelectronics 20:1744-1749
Lee JH, Mitchell RJ, Kim BC, Cullen DC, Gu MB (2005) A cell array biosensor for environmental toxicity analysis Biosensors and Bioelectronics 21:500-507
Lei Y, Chen W, Mulchandani A (2006) Microbial biosensors Analytica chimica acta 568:200-210
Li K, Yediler A, Yang M, Schulte-Hostede S, Wong MH (2008) Ozonation of oxytetracycline and toxicological assessment of its oxidation by-products Chemosphere 72:473-478
Livingstone D (2001) Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms Marine pollution bulletin 42:656-666
Lushchak VI (2011) Adaptive response to oxidative stress: Bacteria, fungi, plants and animals Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 153:175-190
Ma M, Wang C, Wang Z (2005) Assessing toxicities of hydrophobic organic pollutants in Huaihe River by using two types of sampling Journal of Environmental Science and Health 40:331-342
Mallevre F, Alba C, Milne C, Gillespie S, Fernandes TF, Aspray TJ (2016) Toxicity testing of pristine and aged silver nanoparticles in real wastewaters using bioluminescent Pseudomonas putida Nanomaterials 6:49
Mallevre F, Fernandes TF, Aspray TJ (2014) Silver, zinc oxide and titanium dioxide nanoparticle ecotoxicity to bioluminescent Pseudomonas putida in laboratory medium and artificial wastewater Environmental pollution 195:218-225
Manke A, Wang L, Rojanasakul Y (2013) Pulmonary toxicity and fibrogenic response of carbon nanotubes Toxicology mechanisms and methods 23:196-206
Martín-de-Lucía I et al. (2017) Reverse Trojan-horse effect decreased wastewater toxicity in the presence of inorganic nanoparticles Environmental Science: Nano 4:1273-1282
McConkey BJ, Duxbury CL, Dixon DG, Greenberg BM (1997) Toxicity of a PAH photooxidation product to the bacteria Photobacterium phosphoreum and the duckweed Lemna gibba: Effects of phenanthrene and its primary photoproduct, phenanthrenequinone Environmental toxicology and chemistry 16:892-899
McGrath SP, Knight B, Killham K, Preston S, Paton GI (1999) Assessment of the toxicity of metals in soils amended with sewage sludge using a chemical speciation technique and a lux-based biosensor Environmental Toxicology and Chemistry 18:659-663
Mendes LF, Bastos EL, Stevani CV (2010) Prediction of metal cation toxicity to the bioluminescent fungus Gerronema viridilucens Environmental toxicology and chemistry 29:2177-2181
Mendes LF, Stevani CV (2010) Evaluation of metal toxicity by a modified method based on the fungus Gerronema viridilucens bioluminescence in agar medium Environmental toxicology and chemistry 29:320-326

Michelini E, Roda A (2012) Staying alive: new perspectives on cell immobilization for biosensing purposes Analytical and bioanalytical chemistry 402:1785-1797
Min J, Lee CW, Gu MB (2003) Gamma-radiation dose-rate effects on DNA damage and toxicity in bacterial cells Radiation and environmental biophysics 42:189-192
Mitchell RJ, Gu MB (2004) An Escherichia coli biosensor capable of detecting both genotoxic and oxidative damage Applied microbiology and biotechnology 64:46-52
Mitchell RJ, Gu MB (2006) Characterization and optimization of two methods in the immobilization of 12 bioluminescent strains Biosensors and Bioelectronics 22:192-199
Mo L-Y, Liu J, Qin L-T, Zeng H-H, Liang Y-P (2017) Two-Stage Prediction on Effects of Mixtures Containing Phenolic Compounds and Heavy Metals on Vibrio qinghaiensis sp. Q67 Bulletin of environmental contamination and toxicology 99:17-22
Molins-Delgado D, Gago-Ferrero P, Díaz-Cruz MS, Barceló D (2016) Single and joint ecotoxicity data estimation of organic UV filters and nanomaterials toward selected aquatic organisms. Urban groundwater risk assessment Environmental research 145:126-134
Mollon L, Norton G, Trakal L, Moreno-Jimenez E, Elouali F, Hough R, Beesley L (2016) Mobility and toxicity of heavy metal (loid) s arising from contaminated wood ash application to a pasture grassland soil Environmental Pollution 218:419-427
Monaghan P, Metcalfe NB, Torres R (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation Ecology letters 12:75-92
Mortimer M, Kasemets K, Heinlaan M, Kurvet I, Kahru A (2008) High throughput kinetic Vibrio fischeri bioluminescence inhibition assay for study of toxic effects of nanoparticles Toxicology in vitro 22:1412-1417
Muneer M, Theurich J, Bahnemann D (1999) Formation of toxic intermediates upon the photocatalytic degradation of the pesticide diuron
Naessens M, Leclerc JC, Tran-Minh C (2000) Fiber optic biosensor using Chlorella vulgaris for determination of toxic compounds Ecotoxicology and Environmental Safety 46:181-185
Naessens M, Tran-Minh C (1999) Biosensor using immobilized Chlorella microalgae for determination of volatile organic compounds Sensors and Actuators B: Chemical 59:100-102
Neamtu M, Yediler A, Siminiceanu I, Macoveanu M, Kettrup A (2004) Decolorization of disperse red 354 azo dye in water by several oxidation processes—a comparative study Dyes and pigments 60:61-68
Nguyen-Ngoc H, Tran-Minh C (2007) Fluorescent biosensor using whole cells in an inorganic translucent matrix Analytica Chimica Acta 583:161-165
Niazi JH, Kim BC, Ahn J-M, Gu MB (2008) A novel bioluminescent bacterial biosensor using the highly specific oxidative stress-inducible pgi gene Biosensors and Bioelectronics 24:670-675
Niazi JH, Kim BC, Gu MB (2007) Characterization of superoxide-stress sensing recombinant Escherichia coli constructed using promoters for genes zwf and fpr fused to lux operon Applied microbiology and biotechnology 74:1276-1283
Nohava M, Vogel WR, Gaugitsch H (1995) Evaluation of the luminescent bacteria bioassay for the estimation of the toxicological potential of effluent water samples—comparison with data from chemical analyses Environment international 21:33-37
Nuzzo A, Hosseinkhani B, Boon N, Zanaroli G, Fava F (2017) Impact of bio-palladium nanoparticles (bio-Pd NPs) on the activity and structure of a marine microbial community Environmental pollution 220:1068-1078
Ocampo-Duque W, Sierra J, Ferré-Huguet N, Schuhmacher M, Domingo JL (2008) Estimating the environmental impact of micro-pollutants in the low Ebro River (Spain): An approach based on screening toxicity with Vibrio fischeri Chemosphere 72:715-721
Okamoto OK, Shao L, Hastings JW, Colepicolo P (1999) Acute and chronic effects of toxic metals on viability, encystment and bioluminescence in the dinoflagellate Gonyaulax

polyedra Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 123:75-83
Okonokhua BO (2014) Bioavailability of Cu in freshly spiked, leached and field-contaminated soils Soil & Environment 33
Oleszczuk P, Kołtowski M (2017) Effect of co-application of nano-zero valent iron and biochar on the total and freely dissolved polycyclic aromatic hydrocarbons removal and toxicity of contaminated soils Chemosphere 168:1467-1476
Oliveira AG, Desjardin DE, Perry BA, Stevani CV (2012) Evidence that a single bioluminescent system is shared by all known bioluminescent fungal lineages Photochemical & Photobiological Sciences 11:848-852
Ore S, Mertens J, Brandt KK, Smolders E (2010) Copper toxicity to bioluminescent Nitrosomonas europaea in soil is explained by the free metal ion activity in pore water Environmental science & technology 44:9201-9206
Osano O, Admiraal W, Klamer H, Pastor D, Bleeker E (2002) Comparative toxic and genotoxic effects of chloroacetanilides, formamidines and their degradation products on Vibrio fischeri and Chironomus riparius Environmental Pollution 119:195-202
Pandard P, Vasseur P, Rawson DM (1993) Comparison of two types of sensors using eukaryotic algae to monitor pollution of aquatic systems Water Research 27:427-431
Papadopoulou D, Samara C (2002) Polycyclic aromatic hydrocarbon contamination and LUMIStox® solvent extract toxicity of marine sediments in the North Aegean Sea, Greece Environmental Toxicology: An International Journal 17:556-566
Papageorgiou GC, Tsimilli-Michael M, Stamatakis K (2007) The fast and slow kinetics of chlorophyll a fluorescence induction in plants, algae and cyanobacteria: a viewpoint Photosynthesis research 94:275-290
Park HJ, Hwang KO, Kim ES (2002) Construction and characterization of a recombinant bioluminescence streptomycetes for potential environmental monitoring Journal of microbiology and biotechnology 12:706-709
Paton G, Campbell C, Glover LA, Killham K (1995a) Assessment of bioavailability of heavy metals using lux modified constructs of Pseudomonas fluorescens Letters in Applied Microbiology 20:52-56
Paton G, Palmer G, Burton M, Rattray E, McGrath S, Glover L, Killham K (1997) Development of an acute and chronic ecotoxicity assay using lux-marked Rhizobium leguminosarum biovar trifolii Letters in applied microbiology 24:296-300
Paton G, Palmer G, Kindness A, Campbell C, Glover L, Killham K (1995b) Use of luminescence-marked bacteria to assess copper bioavailability in malt whisky distillery effluent Chemosphere 31:3217-3224
Paton GI, Viventsova E, Kumpene J, Wilson MJ, Weitz HJ, Dawson JJ (2006) An ecotoxicity assessment of contaminated forest soils from the Kola Peninsula Science of the Total Environment 355:106-117
Peña-Vázquez E, Maneiro E, Pérez-Conde C, Moreno-Bondi MC, Costas E (2009) Microalgae fiber optic biosensors for herbicide monitoring using sol–gel technology Biosensors and Bioelectronics 24:3538-3543
Pereira R et al. (2011) Screening evaluation of the ecotoxicity and genotoxicity of soils contaminated with organic and inorganic nanoparticles: the role of ageing Journal of hazardous materials 194:345-354
Pérez S, la Farré M, García MJ, Barceló D (2001) Occurrence of polycyclic aromatic hydrocarbons in sewage sludge and their contribution to its toxicity in the ToxAlert® 100 bioassay Chemosphere 45:705-712
Perullini M, Ferro Y, Durrieu C, Jobbágy M, Bilmes SA (2014) Sol–gel silica platforms for microalgae-based optical biosensors Journal of biotechnology 179:65-70
Podola B, Melkonian M (2005) Selective real-time herbicide monitoring by an array chip biosensor employing diverse microalgae Journal of Applied Phycology 17:261-271

Podola B, Nowack EC, Melkonian M (2004) The use of multiple-strain algal sensor chips for the detection and identification of volatile organic compounds Biosensors and Bioelectronics 19:1253-1260
Pretti C et al. (2014) Ecotoxicity of pristine graphene to marine organisms Ecotoxicology and environmental safety 101:138-145
Prosser J, Anderson C, Horswell J, Speir T (2014) Can manuka (Leptospermum scoparium) antimicrobial properties be utilised in the remediation of pathogen contaminated land? Soil Biology and Biochemistry 75:167-174
Puerari RC et al. (2016) Synthesis, characterization and toxicological evaluation of Cr2O3 nanoparticles using Daphnia magna and Aliivibrio fischeri Ecotoxicology and environmental safety 128:36-43
Pulido-Reyes G, Martín E, Coronado JG, Leganes F, Rosal R, Fernández-Piñas F (2017) Physicochemical and biological interactions between cerium oxide nanoparticles and a 1, 8-naphthalimide derivative Journal of Photochemistry and Photobiology B: Biology 172:61-69
Rattray E, Prosser JI, Killham K, Glover LA (1990) Luminescence-based nonextractive technique for in situ detection of Escherichia coli in soil Applied and environmental microbiology 56:3368-3374
Rawson DM, Willmer AJ, Cardosi MF (1987) The development of whole cell biosensors for on-line screening of herbicide pollution of surface waters Toxicity assessment 2:325-340
Ren S, Frymier PD (2003) Use of multidimensional scaling in the selection of wastewater toxicity test battery components Water research 37:1655-1661
Ren S, Frymier PD (2005) Toxicity of metals and organic chemicals evaluated with bioluminescence assays Chemosphere 58:543-550
Ren S, Mee RW, Frymier PD (2004) Using factorial experiments to study the toxicity of metal mixtures Ecotoxicology and environmental safety 59:38-43
Ríos F, Fernández-Arteaga A, Fernández-Serrano M, Jurado E, Lechuga M (2018) Silica micro- and nanoparticles reduce the toxicity of surfactant solutions Journal of hazardous materials 353:436-443
Robbens J, Dardenne F, Devriese L, De Coen W, Blust R (2010) Escherichia coli as a bioreporter in ecotoxicology Applied microbiology and biotechnology 88:1007-1025
Rodea-Palomares I, Boltes K, Fernández-Pinas F, Leganés F, García-Calvo E, Santiago J, Rosal R (2010) Physicochemical characterization and ecotoxicological assessment of CeO2 nanoparticles using two aquatic microorganisms Toxicological Sciences 119:135-145
Rodea-Palomares I, Fernández-Piñas F, González-García C, Leganés F (2009a) Use of lux-marked cyanobacterial bioreporters for assessment of individual and combined toxicities of metals in aqueous samples Handbook on Cyanobacteria: Biochemistry, Biotechnology and Applications:283-304
Rodea-Palomares I, González-García C, Leganés F, Fernández-Piñas F (2009b) Effect of pH, EDTA, and anions on heavy metal toxicity toward a bioluminescent cyanobacterial bioreporter Archives of environmental contamination and toxicology 57:477
Rodea-Palomares I et al. (2016) Hidden drivers of low-dose pharmaceutical pollutant mixtures revealed by the novel GSA-QHTS screening method Science advances 2:e1601272
Rodea-Palomares I, Gonzalo S, Santiago-Morales J, Leganés F, García-Calvo E, Rosal R, Fernández-Pinas F (2012) An insight into the mechanisms of nanoceria toxicity in aquatic photosynthetic organisms Aquatic toxicology 122:133-143
Rodriguez Jr M, Sanders CA, Greenbaum E (2002) Biosensors for rapid monitoring of primary-source drinking water using naturally occurring photosynthesis Biosensors and Bioelectronics 17:843-849
Rosal R, Rodea-Palomares I, Boltes K, Fernández-Piñas F, Leganés F, Gonzalo S, Petre A (2010a) Ecotoxicity assessment of lipid regulators in water and biologically treated wastewater

using three aquatic organisms Environmental Science and Pollution Research 17:135-144
Rosal R, Rodea-Palomares I, Boltes K, Fernández-Piñas F, Leganés F, Petre A (2010b) Ecotoxicological assessment of surfactants in the aquatic environment: combined toxicity of docusate sodium with chlorinated pollutants Chemosphere 81:288-293
Rosen G, Osorio-Robayo A, Rivera-Duarte I, Lapota D (2008) Comparison of bioluminescent dinoflagellate (QwikLite) and bacterial (Microtox) rapid bioassays for the detection of metal and ammonia toxicity Archives of environmental contamination and toxicology 54:606-611
Rouillona R, Tocabens M, Carpentier R (1999) A photoelectrochemical cell for detecting pollutant-induced effects on the activity of immobilized cyanobacterium Synechococcus sp. PCC 7942 Enzyme and microbial technology 25:230-235
Saddoud A, Abdelkafi S, Sayadi S (2009) Effects of domestic wastewater toxicity on anaerobic membrane-bioreactor (MBR) performances Environmental technology 30:1361-1369
Sajayan A, Kiran GS, Priyadharshini S, Poulose N, Selvin J (2017) Revealing the ability of a novel polysaccharide bioflocculant in bioremediation of heavy metals sensed in a Vibrio bioluminescence reporter assay Environmental pollution 228:118-127
Salizzato M, Bertato V, Pavoni B, Ghirardini AV, Ghetti PF (1998a) Sensitivity limits and EC50 values of the Vibrio fischeri test for organic micropollutants in natural and spiked extracts from sediments Environmental toxicology and chemistry 17:655-661
Salizzato M, Pavoni B, Ghirardini AV, Ghetti P (1998b) Sediment toxicity measured using Vibrio fischeri as related to the concentrations of organic (PCBs, PAHs) and inorganic (metals, sulphur) pollutants Chemosphere 36:2949-2968
Sanchez-Ferandin S (2015) Assessing Ecotoxicity in Marine Environment Using Luminescent Microalgae: Where Are We At? American Journal of Plant Sciences 6:2502
Sanchez-Ferandin S, Leroy F, Bouget F-Y, Joux F (2012) A new, sensitive marine microalgal recombinant biosensor using luminescence monitoring for the toxicity testing of antifouling biocides Applied and environmental microbiology:AEM. 02688-02612
Sanchís J, Olmos M, Vincent P, Farré M, Barceló D (2015) New insights on the influence of organic co-contaminants on the aquatic toxicology of carbon nanomaterials Environmental science & technology 50:961-969
Sanders CA, Rodriguez Jr M, Greenbaum E (2001) Stand-off tissue-based biosensors for the detection of chemical warfare agents using photosynthetic fluorescence induction Biosensors and Bioelectronics 16:439-446
Scognamiglio V et al. (2009) Chlamydomonas reinhardtii genetic variants as probes for fluorescence sensing system in detection of pollutants Analytical and bioanalytical chemistry 394:1081
Schmitz RP, Kretkowski C, Eisenträger A, Dott W (1999) Ecotoxicological testing with new kinetic Photorhabdus luminescens growth and luminescence inhibition assays in microtitration scale Chemosphere 38:67-78
Scholz B, Liebezeit G (2012) Screening for biological activities and toxicological effects of 63 phytoplankton species isolated from freshwater, marine and brackish water habitats Harmful Algae 20:58-70
Scholz B, Ziehe D, Pivetta LA, Pielok N, Liebezeit G (2012) Toxicity of East Sumatra River Sediments–Bacterial Luminescence, Brine Shrimp and Acetylcholinesterase Inhibition Tests Asian Journal of Water, Environment and Pollution 9:1-9
Segura PA, Kaplan P, Erythropel HC, Yargeau V (2012) Comparative rapid toxicity screening of commercial and potential “green” plasticizers using bioluminescent bacteria Industrial & Engineering Chemistry Research 51:11555-11560
Serat WF, Budinger Jr FE, Mueller P (1967) Toxicity evaluation of air pollutants by use of luminescent bacteria Atmospheric Environment (1967) 1:21-32

Serat WF, Budinger Jr FE, Mueller PK (1965) Evaluation of biological effects of air pollutants by use of luminescent bacteria Journal of bacteriology 90:832
Shao C, Howe C, Porter AJR, Glover LA (2002) Novel cyanobacterial biosensor for detection of herbicides Applied and environmental microbiology 68:5026-5033
Shaw L, Beaton Y, Glover L, Killham K, Meharg A (1999) Development and characterization of a lux-modified 2, 4-dichlorophenol-degrading Burkholderia sp. RASC Environmental microbiology 1:393-399
Shitanda I, Takada K, Sakai Y, Tatsuma T (2005) Amperometric biosensing systems based on motility and gravitaxis of flagellate algae for aquatic risk assessment Analytical chemistry 77:6715-6718
Somensi CA, Simionatto EL, Bertoli SL, Wisniewski Jr A, Radetski CM (2010) Use of ozone in a pilot-scale plant for textile wastewater pre-treatment: physico-chemical efficiency, degradation by-products identification and environmental toxicity of treated wastewater Journal of Hazardous Materials 175:235-240
Stauber JL, Binet MT, Bao VW, Boge J, Zhang AQ, Leung KM, Adams MS (2008) Comparison of the Qwiklite™ algal bioluminescence test with marine algal growth rate inhibition bioassays Environmental Toxicology: An International Journal 23:617-625
Steinberg SM, Poziomek EJ, Engelmann WH, Rogers KR (1995) A review of environmental applications of bioluminescence measurements Chemosphere 30:2155-2197
Stone V, Donaldson K (2006) Nanotoxicology: signs of stress Nature Nanotechnology 1:23
Strigul N, Vaccari L, Galdun C, Wazne M, Liu X, Christodoulatos C, Jasinkiewicz K (2009) Acute toxicity of boron, titanium dioxide, and aluminum nanoparticles to Daphnia magna and Vibrio fischeri Desalination 248:771-782
Svartz G et al. (2017) Monitoring the ecotoxicity of γ-Al2O3 and Ni/γ-Al2O3 nanomaterials by means of a battery of bioassays Ecotoxicology and environmental safety 144:200-207
Svenningsen NB, Pérez-Pantoja D, Nikel PI, Nicolaisen MH, de Lorenzo V, Nybroe O (2015) Pseudomonas putida mt-2 tolerates reactive oxygen species generated during matric stress by inducing a major oxidative defense response BMC microbiology 15:202
Tahirbegi IB et al. (2017) Fast pesticide detection inside microfluidic device with integrated optical pH, oxygen sensors and algal fluorescence Biosensors and Bioelectronics 88:188-195
Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. In: Molecular, clinical and environmental toxicology. Springer, pp 133-164
Thomulka KW, Schroeder JA, Lange JH (1997) Use of Vibrio harveyi in an aquatic bioluminescent toxicity test to assess the effects of metal toxicity: treatment of sand and water–buffer, with and without EDTA Environmental Toxicology and Water Quality: An International Journal 12:343-348
Tom-Petersen A, Hansen HCB, Nybroe O (2004) Time and moisture effects on total and bioavailable copper in soil water extracts Journal of environmental quality 33:505-512
Tsiridis V, Petala M, Koukiotis C, Darakas E (2017) Implications of handling practices on the ecotoxic profile of alumina nanoparticles towards the bacteria Vibrio fischeri Journal of Environmental Science and Health, Part A 52:15-22
Tsopela A et al. (2016) Development of a lab-on-chip electrochemical biosensor for water quality analysis based on microalgal photosynthesis Biosensors and Bioelectronics 79:568-573
Tsopela A et al. (2014) Integrated electrochemical biosensor based on algal metabolism for water toxicity analysis Biosensors and Bioelectronics 61:290-297
Turner NL, Horsburgh A, Paton GI, Killham K, Meharg A, Primrose S, Strachan NJ (2001) A novel toxicity fingerprinting method for pollutant identification with lux-marked biosensors Environmental toxicology and chemistry 20:2456-2461

Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants Ecotoxicology and environmental safety 64:178-189
Védrine C, Leclerc J-C, Durrieu C, Tran-Minh C (2003) Optical whole-cell biosensor using Chlorella vulgaris designed for monitoring herbicides Biosensors and Bioelectronics 18:457-463
Velzeboer I, Hendriks AJ, Ragas AM, Van de Meent D (2008) Nanomaterials in the environment aquatic ecotoxicity tests of some nanomaterials Environmental toxicology and chemistry 27:1942-1947
Verschaeve L, Van Gompel J, Thilemans L, Regniers L, Vanparys P, Van der Lelie D (1999) VITOTOX® bacterial genotoxicity and toxicity test for the rapid screening of chemicals Environmental and molecular mutagenesis 33:240-248
Vydryakova G, Gusev A, Medvedeva S (2011) Effect of organic and inorganic toxic compounds on luminescence of luminous fungi Applied biochemistry and microbiology 47:293-297
Wang C, Yediler A, Lienert D, Wang Z, Kettrup A (2002) Toxicity evaluation of reactive dyestuffs, auxiliaries and selected effluents in textile finishing industry to luminescent bacteria Vibrio fischeri Chemosphere 46:339-344
Wang D, Zhao L, Ma H, Zhang H, Guo L-H (2017) Quantitative analysis of reactive oxygen species photogenerated on metal oxide nanoparticles and their bacteria toxicity: the role of superoxide radicals Environmental Science & Technology 51:10137-10145
Weitz HJ, Campbell CD, Killham K (2002) Development of a novel, bioluminescence-based, fungal bioassay for toxicity testing Environmental microbiology 4:422-429
Weitz HJ, Ritchie JM, Bailey DA, Horsburgh AM, Killham K, Glover LA (2001) Construction of a modified mini-Tn 5 lux CDABE transposon for the development of bacterial biosensors for ecotoxicity testing FEMS microbiology letters 197:159-165
Winson MK et al. (1998) Engineering the luxCDABE genes from Photorhabdus luminescens to provide a bioluminescent reporter for constitutive and promoter probe plasmids and mini-Tn 5 constructs FEMS microbiology letters 163:193-202
Wolska L, Kuczyńska A, Namieśnik J (2006) Quality of groundwater contaminated by leachates from seven Polish landfills–chemical and ecotoxicological classifications Toxicological and Environ Chemistry 88:501-513
Wu S, Zhang X, Yang P, Li L, Tang S (2018) Rapid detection and toxicity assessment of citreoviridin using luminescent Vibrio qinghaiensis sp.-Q67 in drinking water International Journal of Food Science & Technology
Yu H, Nie E, Xu J, Yan S, Cooper WJ, Song W (2013) Degradation of diclofenac by advanced oxidation and reduction processes: kinetic studies, degradation pathways and toxicity assessments Water research 47:1909-1918
Zabetoglou K, Voutsa D, Samara C (2002) Toxicity and heavy metal contamination of surficial sediments from the Bay of Thessaloniki (Northwestern Aegean Sea) Greece Chemosphere 49:17-26
Zaki S, Abd-El-Haleem D, Abulhamd A, Elbery H, AbuElreesh G (2008) Influence of phenolics on the sensitivity of free and immobilized bioluminescent Acinetobacter bacterium Microbiological research 163:277-285
Zarubina A, Lukashev E, Deev L, Parkhomenko I, Rubin A (2009) Biotesting the biological effects of single-wall carbon nanotubes using bioluminescent bacteria test-system Nanotechnologies in Russia 4:871-875
Zhang J, Chen Q (2014) Predicting the long-term toxicity of five-antibiotic mixtures to Vibrio qinghaiensis sp. Q67 Pakistan journal of pharmaceutical sciences 27:1605-1609
Zhao JL, Jiang YX, Yan B, Wei C, Zhang LJ, Ying GG (2014) Multispecies acute toxicity evaluation of wastewaters from different treatment stages in a coking wastewater-treatment plant Environmental toxicology and chemistry 33:1967-1975

Figure legends:

Figure 1. Schematic representation of the content of the chapter in terms of the toxicity bioassays and biosensors reporting on global toxicity. NMs: Nanomaterials; PCB: polychlorinated biphenyls; PAH: Polycyclic aromatic hydrocarbons.

Figure 2. Examples taken from the reviewed literature of naturally bioluminescent microorganisms (see Table 1). (A) Performance of *Aliivibrio* bioluminescence reporter assay to determine efficiency of bioflocculant MSI021 on the removal of heavy metals. (a) bioluminescence observed in *Aliivibrio* cultures with 1, 2, and 3 mM heavy metals such as zinc, copper and mercury, (b) bioluminescence observed in *Alivibrio* cultures with 1, 2, and 3 mM heavy metals and 50 mg/ml bioflocculant, and (c) bioluminescence observed in *Aliivibrio* cultures with 1, 2, and 3 mM heavy metals and 100 mg/ml bioflocculant. Reprinted with permission from Sajayan et al. (2017), Copyright (2017), Elsevier. (B) A schematic diagram of continuous culture and photo (C) of continuous culture device for continuous culture of *Photobacterium phosphoreum.* Reprinted with permission from Hassan and Oh (2010), Copyright (2010), Elsevier. (D) diagram of the Laboratory Plankton Test Chamber (LPTC), the forerunner of the QwikLite™ bioluminescent toxicity test system. This system was originally designed and constructed for measuring bioluminescence from individual bioluminescent plankton isolated on-board oceanographic ships and (E) time exposure photograph of bioluminescence emitted from stirred dinoflagellate cells *Pyrocystis lunula*. The color of the light is blue-green centered a wavelength of approximately 470–490 nm. The integrated bioluminescence is measured by the LPTC and QwikLite™. Reprinted with permission from Lapota et al. (2007), Copyright (2007), Elsevier.

Figure 3. Recombinant cyanobacterial toxicity bioreporter *Anabaena* CPB4337 (Panel A is a bright-field image of a filament; panel B is an image of constitutive bioluminescence from the same filament with a Hamamatsu Photonics Systems, model C1966-20, mounted on a Zeiss Universal Research Microscope). Panel C shows a multiwell plate where the bioreporter is deployed to be used in a medium-to-high throughput configuration (Mix 1-3 refers to different mixtures of pollutants) (Fernandez-Piñas F., unpublished).

Figure 4. Examples of optical microalgal-based bioassays (see Table 3). (A) Schematic optical section of a microalgal biosensor for detection of herbicides inhibiting photosynthesis – Cross-section and front view. Reprinted with permission from Podola and Melkonian (2005), Copyright (2005), Springer Nature. B and C show a scheme of a fluorescent microalgal biosensor based on *Chlamydomonas reinhardtii*. The biological container and the optical compartment are represented at the top and the bottom, respectively. (B) The sample solution containing the herbicide under test flows into the biological cell; (C) the alga strain biomediator is photoactivated and the emitting fluorescence captured by the photodetector (in static condition). (D) Two biological arrays made up by six containers (10 mm $Ø_{int}$×10 mm H, 785 µl each) for static and flow measurement mode, respectively. Reprinted with permission from Scognamiglio et al. (2009), Copyright (2009), Springer Nature. E and F depicts a microalgal dual-head biosensor for herbicide detection based on *Dictyosphaerium chlorelloides* (E) Schematic diagram of the microalgal dual head fiber-optic biosensor: a, water sample/culture medium; b, degasser; c, dual chamber measuring cell; d, peristaltic pump; e, optoelectronic O_2 transducer; f, temperature probe; g, optical fibers for actinic illumination; h, blue LED source; i, laptop computer. (F) Exploded view of a flow chamber: g, optical fiber for actinic illumination; j, sapphire window; k, flow-through chamber; l, cellulose membrane; m, microalgal membrane; n, gray silicone layer; o, O_2-sensitive film; p, polymer window; q, optical fiber to the optoelectronic

O_2 transducer. Reprinted with permission from Haigh-Flórez et al. (2014), Copyright (2014), Elsevier .