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REVIEW PAPER

Cyanobacteria as bioindicators and bioreporters of environmental analysis in aquatic ecosystems

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Abstract Knowledge of the incidence of anthropogenic pressure on water ecosystems is one of the main focus of integrated water resource management. The use of biological methods to assess water quality is of particular importance since organisms show an integrating response to their environment. Tolerances or ecological ranges of individual species can differ depending on the taxon, which leads to distinct bioindicator values of cyanobacterial taxa. In addition, a number of morphological and physiological features are known to relate with the environment in which they occur, which makes them excellent environmental indicators. Therefore, we review literature data of the main cyanobacterial methods used to obtain information about changes in running water quality, mainly related to eutrophication processes, which are found as the main cause of disturbance in rivers, with the focus on benthic cyanobacteria, as habitat recommended for monitoring studies. Further, their trophic independence and ease of cultivation make them very useful in the field of bioreporters of environmental monitoring and ecotoxicology. In fact, several cyanobacterial strains have been already genetically engineered to construct bioreporters which respond to different types of pollutants as well as limiting nutrients. The potential of cyanobacteria both as in situ bioindicators as well as bioreporters of environmental analysis in aquatic ecosystems will be discussed.

Keywords Environmental monitoring · Eutrophication · Nutrients · Toxicology · Transgenic cyanobacteria

Abbreviations

BMWPc	Biological Monitoring Working Party adapted to Catalonian streams
IBD	Biological Diatom Index (Indice Biologique Diatomées)

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Introduction

Anthropogenic pressure on water bodies which receive diverse pollutants, leading to multiple negative ecological impacts on these habitats, is a serious problem, whereby knowledge of the state of water quality is essential for water management systems. In the last decades, eutrophication problems have replaced organic pollution as the dominant chemical pressure in large river systems being caused by high nutrient (nitrogen, N, and phosphorus, P), fluxes from population pressure and intensive agriculture (Jarvie et al. 2013; Mischke et al. 2011). For watershed management, P has been regarded as the primary limiting nutrient for algal growth as nuisance in freshwaters. Accordingly, controlling P inputs has been the primary goal for resource managers, reducing freshwater P loads but this is not the case for N, which is more mobile throughout the environment, being therefore both N and P important in eutrophication processes (Paerl et al. 2011).

Traditionally the assessment of river water quality had been based on the measurement of physico-chemical characteristics, but although these measurements were efficient for regulating effluent discharges and protecting humans, they were not very useful for large-scale management of catchments or for assessing whether river ecosystems are being protected. Therefore, different biological methods were developed to assess river condition since effects on biota are usually the final point of environmental degradation and pollution of these systems (Norris and Thoms 1999). Two important arguments in favour of biological methods are (a) due to organisms having an integrating response to their environment, fluctuations in water quality, which may be missed by intermittent physico-chemical analysis, are recorded, and (b) if we wish to maintain healthy, diverse biological communities, it is more appropriate to monitor the aquatic community rather than physico-chemical variables only (Cox 1991). The advantage of monitoring with the use of bioindicators is that biological communities reflect overall ecological quality by integrating the effects of different stressors providing a broad measure of their impact and an ecological measurement of fluctuating environmental conditions. Overall routine monitoring of biological communities is reliable and relatively inexpensive compared to the cost of assessing toxicant pollutants (Iliopoulou-Georgudaki et al. 2003). Other arguments about the use of biological methods versus physico-chemical analysis are summarized in Fig. 1.

The forerunner works of bioindicator systems for surface water quality assessment started more than a century and a half ago by Kolenati (1848) and Cohn (1853), both quoted in Liebmann (1962), De Pauw and Vanhooren (1983) and Iliopoulou-Georgudaki et al. (2003). This ancient literature described that organisms occurring in organically polluted water were different from those in clean waters. Since then, the bioindicator concept has evolved substantially and is now widely applied in situations ranging from the verification of the compliance of industries to integrated water resource management, with a shift towards using environmental indicators of anthropogenic impact within a regulatory framework ((EC) 2000; Carignan and Villard 2002; USEPA 2000).

A range of biological assemblages has been used to monitor and assess environmental contamination or long-term changes. Although the methods used for a long time were based mostly on heterotrophic bacteria or macroinvertebrates, algae have long been used to assess environmental conditions in aquatic habitats (Stevenson and Smol 2003; Whitton 1999), being routinely used for monitoring rivers (Whitton 2012). Cyanobacteria, as primary producers with a key role in the N and C cycles, are useful bioindicators, given that any detrimental effect on this phototrophic community may have a negative effect on nutrient availability to organisms at higher trophic levels.

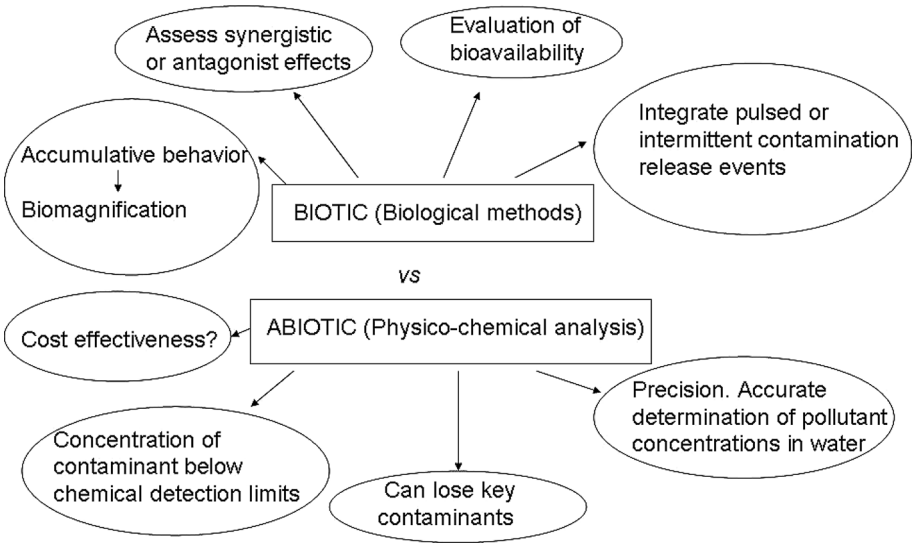

Fig. 1 Characteristics of methods for assessment of aquatic contamination: abiotic vs biotic

The present review gives a short summary of the main cyanobacterial methods that are used to obtain information about changes in running water quality, and mainly related to eutrophication processes, which are found as the main cause of disturbance in rivers. The focus will be on benthic cyanobacteria, as habitat recommended for monitoring studies (Kelly et al. 1998; Round 1991), given that rivers are characterized by their unidirectional flow along a continuum from source to entry to the sea or any other large water body, and benthic organisms, as relatively sessile, are directly in contact with the flowing water detecting the early effects of disturbances in the ecosystem that may arise. A second part of the review focuses on the use of cyanobacterial bioreporters able to sense and detect different types of pollution from general toxicity to specific pollutants, such as heavy metals or Fe, P and N chemical species that may control eutrophication processes.

Cyanobacteria as bioindicators

Although cyanobacteria have generally been regarded as a problematic symptom of eutrophic conditions, it must be emphasized that their occurrence should not be always linked to an ecological decline. On the contrary, some species are characteristics of clean waters, emerging their use as bioindicators for monitoring running waters. Besides, there are specific responses to nitrogen and phosphorus enrichment depending on the cyanobacteria, which are linked to changes in species dominance along eutrophic gradients in running waters, as will be described below.

On the other hand, cyanobacteria are also been regarded as a widely and ubiquitously distributed group. However, it must be emphasized that the occurrence and predominance of cyanobacteria in a vast array of habitats is a result of several general characteristics and of some features characterizing certain cyanobacterial species. Many species are generalists and will tolerate a great range of environmental conditions including extreme environments that usually exclude eukaryotic algae (Castenholz 2001). Thus, not all

cyanobacterial taxa are ubiquitous but have ecological niches that are constrained by their ecological properties (Komárek 1994). Tolerances or ecological ranges of individual species can differ depending on the taxon. Some broad-niched species are recognized by many authors, while others are found to be confined to a more restricted range of ecological conditions (Komárek and Anagnostidis 1999). For instance, some species are characteristic of unpolluted waters whereas other pollution-tolerant species can survive in waters so heavily contaminated with organic matter that they become deoxygenated (Fjerdingstad 1964; Fogg et al. 1973; Palmer 1969).

The first serious attempt to use cyanobacteria as bioindicators of water quality was the saprobic system (Kolkwitz and Marsson 1908), which showed that water conditions determined the composition of the algal flora. A range of methods were subsequently developed although the principal tools for monitoring rivers, which are extensively employed, are indices based on diatom communities (Stevenson et al. 2010). However, a number of researchers have suggested that the floristic composition of other groups in the benthos besides diatoms could be useful for monitoring rivers (Kelly and Whitton 1998). Methods based solely on diatoms, with no parallel assessment of non-diatom phytobenthos are potentially worrying, as it indicates that monitoring will fail to detect situations where pressures have resulted in shifts in the balance between different groups of phytobenthos (Kelly 2013). In addition, it has been pointed out that the diatom-based methodology, which has been widely developed for monitoring lowland rivers is less suited for upland rivers, partly because the individual cells are too short-lived, but also because little is known about the response of different species to nutrient types and ratios and/or other factors (Whitton 2013). However, many upland rivers have slow-growing cyanobacterial species which are colonial, so particular species may eventually prove to be good indicators of certain combinations of nutrient conditions (Kelly and Whitton 1998). Besides, in some fluvial systems lower values of biotic indices such as the BMWPc (based on macroinvertebrates) and IBD (based on diatoms) than expected have found in Spanish rivers without anthropogenic influences (Aboal et al. 2002). These results suggest a cyanotoxic effect on the communities studied and cyanobacterial dominance in such fluvial systems, and highlight the necessity to consider the cyanobacterial communities.

Some countries use the full or a wide range of periphyton taxa, including cyanobacteria, for routine monitoring programs, such as several countries in central Europe, including Austria, Germany, Czech Republic and Poland (Kelly 2013; Whitton 2013). In Austria, the use of diatoms combined with other benthic algae, including cyanobacteria, is mandatory for ecological quality impairment analyses, and the use of diatoms alone is only permitted for regional case studies or when the abundance of non-diatom benthic algae is low (Rott and Schneider 2014). In Norway and other regions of northern Europe, the Periphyton Index of Trophic status (PIT), based on presence/absence of the full range of cyanobacteria and eukaryotic algae other than diatoms, has been adopted for phytobenthos assessment (Schneider and Lindstrøm 2011). An algae Index of Biotic Integrity (IBI) using also non-diatom algae, including cyanobacteria, for bioassessment of southern California streams, was recently developed by (Fetscher et al. 2014), and species optima calculations combined with indicator species analysis identified more than 40 algal species as potential indicators of nutrient conditions in these streams (Stancheva et al. 2012, 2013). Barinova and coworkers analysed algal communities, including the cyanobacteria, in running waters of Israel, Russia and Georgia in order to select bioindicators species (Barinova et al. 2011, 2006, 2008; Barinova and Tavassi 2009). Studies of water quality in rivers of Iran confirmed the use of cyanobacterial species as bioindicators for monitoring eutrophication (Soltani et al. 2012).

Morphological and physiological indicator characteristics

Morphological and/or physiological features not restricted to a particular species, but occurring in a number of genera or species, may provide useful insights to the nutrient status of running waters. Some filamentous species form specialized cells (heterocysts or heterocytes) (Fig. 2a), which differentiate from an ordinary vegetative cell, and are the site of nitrogen fixation. The fixed nitrogen moves from heterocysts to vegetative cells. Experimental studies have shown that heterocyst formation can be prevented if there are high enough concentrations of nitrate and/or ammonium in the growth medium, so the presence of many heterocysts in a filament is an indication that the organism is growing in an environment relatively deficient in combined nitrogen compared to other nutrients (Whitton 2002; Whitton and Mateo 2012). Analyses of nitrogenase gene expression to assess N_2 fixation along a NO_3-N gradient showed that nitrogenase activities specific to *Nostoc* and *Calothrix* were only detected in streams with low values of nitrates (Stancheva et al. 2013). Similarly, Horne and Carnmiggelt (1975), as well as Grimm and Petrone (1997) documented high N_2 fixation rates in northern California and Arizona desert streams, respectively, in relation to low levels of NO_3-N found in waters. Therefore, nitrogenase activity can also be used as an indication of low levels of combined N (NO_3-N, NO_2^--N, NH_4^+-N).

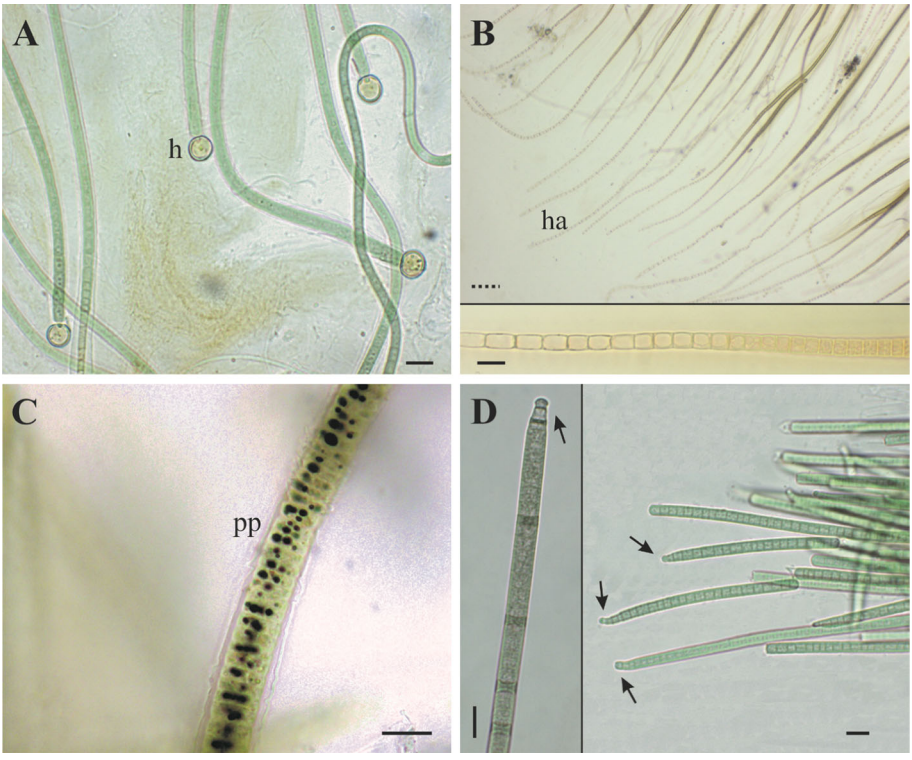

Fig. 2 Morphological indicator characteristics of cyanobacteria. **a** *Rivularia* filaments showing heterocysts (h). **b** *Rivularia* filaments showing multicellular hairs (ha). **c** *Phormidium* filament showing stained polyphosphate granules (pp). **d** *Phormidium* filaments showing calyptras (*arrows*). *Solid scale bar* = 10 μm. *Dotted scale bar* = 40 μm

Another feature of many filamentous forms is that part of the filament tapers, forming multicellular hairs (Fig. 2b), in which the cells are much narrower, elongated, highly vacuolated and usually colorless (Whitton and Mateo 2012). Phosphatase activity, which hydrolyzes organic phosphorus esters into an organic moiety and orthophosphate, being associated with P-limitation, is mainly located at hairs, when present, whereby hair frequency increases with increasing P limitation. The hairs not only enhance the surface area for phosphatase activities, but also aid in P acquisition from environments where the ambient P concentration is mostly low, but with occasional much higher pulses. A large number of hairs has been observed associated to phosphorus limitation in streams (Berrendero et al. 2008; Mateo et al. 2010; Muñoz-Martín et al. 2014a; Stancheva et al. 2013). In the same way, increased values of phosphatase activity have also been reported as good indicator of P limitation (Mateo et al. 2010; Muñoz-Martín et al. 2014a; Sabater et al. 2000; Whitton et al. 1998). Therefore, those species which have both heterocysts and well developed hairs can be used as bioindicators of environments which are deficient in combined nitrogen during part of the time, and of phosphorus for the rest of time (Whitton 2002; Whitton and Potts 2000).

On the other hand, if phosphate is added to a culture with hairs, polyphosphate (polyP) granules are formed rapidly in cells (Fig. 2c). Therefore cyanobacteria accumulate polyP granules when the cells are P-rich, being the relative abundance of these granules an indication of P-limitation or P-enrichment (Muñoz-Martín et al. 2014a; Whitton and Mateo 2012).

Furthermore, it has been suggested that the development of a thickened cap, the calyptra, which forms on the outer wall of apical cells in some cyanobacteria, (e.g. *Phormidium*) (Fig. 2d), could play a role in detecting features of their environment such as phosphate or other nutrient gradients (Whitton and Neal 2011; Whitton and Potts 2012). Muñoz-Martín et al. (2014a) found fewer filaments of *Phormidium* developing a calyptra in mats from streams sites with higher P concentrations than those of upstream oligotrophic waters.

Therefore, a programme combining a survey of macroscopically obvious phototrophs with physiological assays should provide sufficient information to assess whether conditions at the site are undergoing long-term changes regarding eutrophication.

Shifts in cyanobacterial community structure

Changes in cyanobacterial species richness, abundance, and diversity have been observed in several Spanish rivers in relation to eutrophic gradients (Douterelo et al. 2004; Loza et al. 2013a, b, c, 2014; Perona et al. 1998; Rodriguez et al. 2007). The cyanobacterial community of the upstream sites was different from that of the downstream communities, where anthropogenic influences caused an increase in nutrients. The community composition also changed with the water quality, whereby certain species were absent from more perturbed locations, while those remaining proliferated (Douterelo et al. 2004; Loza et al. 2013a, b, c, 2014; Perona and Mateo 2006). Particularly, there were greater Oscillatoriales species numbers at downstream sites with high trophic levels, while species belonging to the Nostocales were less abundant and fewer under these conditions. Heterocystous species were basically observed mainly at upstream sites where the nutrient loads were low (Douterelo et al. 2004; Perona et al. 1998; Perona and Mateo 2006; Rodriguez et al. 2007). Similar results were obtained in studies in Brazilian Rivers (Branco and Pereira 2002) where the proportion of non-heterocystous relative to heterocystous species was proposed as a good tool for environmental evaluation in tropical regions. The great majority of the

cyanobacteria present in running waters of a National Park in Northern Italy with very low nitrate values were heterocystous species (Cantonati 2008). However, the excess of N (especially nitrates) could explain the clear predominance of non-heterocystous forms in the Southern Alps (Cantonati et al. 1996, 2006). In North American flowing waters heterocystous cyanobacteria have been proposed as indicators of nutrient status, since the relative abundance of nitrogen-fixing species was negatively correlated with nitrogen concentrations (Porter et al. 2008; Stancheva et al. 2012, 2013). In addition, as stated above, nitrogen fixation could be used as an indication of low levels of combined N. These results enhance the suitability of using heterocystous cyanobacteria as bioindicators for N-limiting conditions.

Species specific responses to water quality: bioindicator values of cyanobacterial taxa

Table 1 shows a compilation of literature data for bioindicator species, in which only species with at least two references found were included. The table also lists literature values for saprobic and/or trophic status. As previously stated, the first descriptions of differential occurrence of distinct cyanobacteria depending on the water quality of running waters were those of saprobic system (Kolkwitz and Marsson 1908), in which, as well as in subsequent reviews (Fjerdingstad 1964; Rott et al. 1997; Sládecek 1973) several species, such as *Oscillatoria putrida*, *O. chlorina*, *O. splendida*, and *O. ornata* were described as bioindicators of organically polluted waters. On the other hand, others, such as *Calothrix parietina* and *C. fusca*, and *Tolypotrix distorta*, were commonly associated with clean water.

As long as organic pollution was reduced in running waters, but eutrophication problems became increasing, indices to assess trophic status were developed and applied. A number of species were recorded earlier from oligotrophic conditions: the genus *Rivularia* and other members of the Rivulariaceae, such as genera *Dichothrix* and *Calothrix* are useful as indicators of environments with waters with low levels of nutrients (Aboal 1988; Charlton and Hickman 1984; Lindstrøm and Traaen 1984). Since several species of the genus *Tolypothrix* had similar ecological optima with respect to nutrient levels (Dell'Uomo 1991; Lindstrøm and Traaen 1984), this genus has been catalogued as oligotrophic taxon (Schneider and Lindstrøm 2011). Other studies described several species characteristic of low nutrient conditions, such as species of the genera: *Nostoc* (*N. parmeloides*, *N. verrucosum*) (Aboal 1988; Dell'Uomo 1991; Sabater 1983), *Chamaesiphon* (*C. polonicus*, *C. fuscus*, *C. geitleri*, *C.investiens*, *C. minutus*, *C. starmachii*) (Aboal 1988; Rott and Pfister 1988; Sabater 1989), *Schizothrix* (*S. latierita*, *S. penicillata*, *S. tinctoria*) (Gutowski and Foerster 2009; Rott et al. 1999; Sabater 1989), and *Gloeocapsa* (*G. alpina*, *G. sanguinea*) (Rott et al. 1999, 2006), being associated all these genera with oligotrophic conditions (Schneider and Lindstrøm 2011). *Homoeothrix juliana* was recorded as occurring in pristine waters with low level of nutrients (García and Aboal 2014; Rott et al. 1999; Sabater 1989; Soltani et al. 2012), and *Phormidium corium* has also been found in unpolluted and slightly influenced environments (Gutowski and Foerster 2009; Gutowski et al. 2004; Loza et al. 2013b; Rott et al. 1999). Lindstrøm (1999) suggested that, in Norwegian streams, *Stigonema mamillosum* is especially sensitive to eutrophication and should be considered as a 'red-list' organism.

However, many data in the literature showed certain cyanobacterial taxa to be indicators of enriched waters: *Oscillatoria* species, such as *O. limosa*, *O. princeps*, and *O. tenuis* were associated to highly eutrophic waters (Gutowski et al. 2004; Palmer 1969; Sierra and

Table 1 Bioindicator cyanobacteria from literature data

Species	Bioindicator value/characteristic	Reference
Ammatoidea simplex	S: 1.4	Rott et al. (1997)
	TW: 1.2	Rott et al. (2006)
Aphanocapsa fonticola	TW: 0.6	Rott et al. (1999)
	A/B	Gutowski and Foerster (2009)
Calothrix fusca	Oligosaprobic	Dell'Uomo (1991)
	Pristine nature	Sabater (1989)
	S: 1.4	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
	TW: 1.2	Rott et al. (2006)
Calothrix parietina	Pristine nature	Sabater (1989)
	S: 1.4	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
	Oligotrophic water, low DIN	García and Aboal (2014)
Calothrix gypsophila	Oligotrophic waters	Lindstrøm and Traaen (1984)
	Low nutrient	Lindstrøm et al. (2004)
Calothrix sp.	In saprobic katharobic zone	Persoone and De Pauw (1979)
	Decreasing with eutrophication	Kann (1982)
	IV: 5.21	Schneider and Lindstrøm (2011)
Chamaesiphon confervicola	Oligotrophic waters	Lindstrøm and Traaen (1984)
	S: 1.3	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
	B	Gutowski et al. (2004)
	TW: 1.2	Rott et al. (2006)
	IV: 6.61	Schneider and Lindstrøm (2011)
Chamaesiphon fuscus	Unpolluted streams	Kann (1978)
	S: 1.6	Rott et al. (1997)
	TW: 0.7	Rott et al. (1999)
	Low nutrient	Lindstrøm et al. (2004)
	Low NO_3-N and conductivity	Schaumburg et al. (2004)
	A	Gutowski et al. (2004)
	TW: 0.7	Rott et al. (2006)
	IV: 5.09	Schneider and Lindstrøm (2011)
Chamaesiphon geitleri	Unpolluted streams	Kann (1978)
	TW: 0.6	Rott et al. (2006)
	S: 1.4	Rott et al. (1997)
	TW: 0.6	Rott et al. (1999)
Chamaesiphon incrustans	S: 2.0	Rott et al. (1997)
	TW: 1.7	Rott et al. (1999)
	B	Gutowski et al. (2004)
	IV: 20.38	Schneider and Lindstrøm (2011)

Table 1 continued

Species	Bioindicator value/characteristic	Reference
Chamaesiphon investiens	Low nutrient	Mulholland and Rosemond (1992)
	S: 1.4	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
	Low nutrient	Loza et al. (2013a)
Chamaesiphon minutus	Oligotrophic waters	Lindstrøm and Traaen (1984)
	S: 1.2	Rott et al. (1997)
	TW: 0.6	Rott et al. (1999)
	TW: 0.6	Rott et al. (2006)
	IV: 3.47	Schneider and Lindstrøm (2011)
Chamaesiphon polonicus	In saprobic katharobic zone	Persoone and De Pauw (1979)
	Clean waters	Aboal (1988)
	Poor in nutrients	Sabater (1989)
	S: 1.5	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
	A	Gutowski et al. (2004)
	TW: 1.2	Rott et al. (2006)
Chamaesiphon polymorphus	S: 2.4	Rott et al. (1997)
	TW: 2.3	Rott et al. (1999)
	B	Gutowski et al. (2004)
	IV: 16.11	Schneider and Lindstrøm (2011)
	Low TN	Stancheva et al. (2012)
	High nutrient	Loza et al. (2013a)
Chamaesiphon rostafinskii	S: 1.1	Rott et al. (1997)
	TW: 0.3	Rott et al. (1999)
	IV: 4.37	Schneider and Lindstrøm (2011)
Chamaesiphon starmachii	Nutrient-poor	Rott and Pfister (1988)
	S: 1.7	Rott et al. (1997)
	TW: 1.7	Rott et al. (1999)
	A	Gutowski et al. (2004)
Chamaesiphon subglobosus	S: 1.7	Rott et al. (1997)
	TW: 1.4	Rott et al. (1999)
	Nutrient poor water	Lindstrøm et al. (2004)
	B	Gutowski et al. (2004)
Chlorogloea microcystoides	S: 1.9	Rott et al. (1997)
	TW: 1.3	Rott et al. (1999)
	TW: 1.3	Rott et al. (2006)
Chroococcus turgidus	Pristine nature	Sabater (1989)
	Oligotrophic waters	Komárek and Anagnostidis(1999)
Chroococopsis gigantea	TW: 3.0	Rott et al. (1999)
	S: 1.7	Rott et al. (1997)
	B	Gutowski et al. (2004)

Table 1 continued

Species	Bioindicator value/characteristic	Reference
Clastidium setigerum	Oligotrophic waters	Lindstrøm and Traaen (1984)
	S: 1.2	Rott et al. (1997)
	TW: 0.4	Rott et al. (1999)
	TW: 0.4	Rott et al. (2006)
	Low nutrient	Lindstrøm et al. (2004)
	IV: 4.76	Schneider and Lindstrøm (2011)
Clastidium rivularis	S: 1.2	Rott et al. (1997)
	TW: 0.8	Rott et al. (1999)
	TW: 0.8	Rott et al. (2006)
Cyanophanon mirabile	Oligotrophic waters	Lindstrøm and Traaen (1984)
	S: 1.1	Rott et al. (1997)
	TW: 0.3	Rott et al. (1999)
	TW: 0.3	Rott et al. 2006)
	IV: 4.39	Schneider and Lindstrøm (2011)
Dichothrix gypsophila	S: 1.6	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
	IV: 4.20	Schneider and Lindstrøm (2011)
	Unpolluted waters	Komárek (2013)
Dichothrix orsiniana	S: 1.1	Rott et al. (1997)
	TW: 0.6	Rott et al. (1999)
	TW: 0.6	Rott et al. (2006)
	IV: 4.42	Schneider and Lindstrøm (2011)
Geitlerinema acutissimum	TW: 3.0	Rott et al. (1999)
	IV: 24.22	Schneider and Lindstrøm (2011)
Geitlerinema splendidum	TW: 3.5	Rott et al. (1999)
	IV: 43.42	Schneider and Lindstrøm (2011)
Gloeocapsopsis magma	TW: 0.6	Rott et al. (1999)
	IV: 2.74	Schneider and Lindstrøm (2011)
Gloeocapsa sanguinea	S: 1.1	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
	TW: 1.2	Rott et al. (2006)
	IV: 2.74	Schneider and Lindstrøm (2011)
Gloeocapsa alpina	S: 1.1	Rott et al. (1997)
	TW: 0.6	Rott et al. (2006)
Heteroleibleinia kuetzingii	S: 1.4	Rott et al. (1997)
	TW: 0.8	Rott et al. (1999)
	B	Gutowski and Foerster (2009)
	IV: 5.32	Schneider and Lindstrøm (2011)
Homoeothrix crustacea	Pristine nature	Sabater (1989)
	S: 1.7	Rott et al. (1997)
	TW: 2.2	Rott et al. (1999)
	A	Gutowski and Foerster (2009)
	Oligotrophic water	García and Aboal (2014)

Table 1 continued

Species	Bioindicator value/characteristic	Reference
Homoeothrix gracilis	S: 1.4	Rott et al. (1997)
	TW: 0.8	Rott et al. (1999)
	TW: 0.8	Rott et al. (2006)
Homoeothrix janthina	Poor in nutrients	Sabater (1989)
	S: 1.8	Rott et al. (1997)
	TW: 1.5	Rott et al. (1999)
	B	Gutowski et al. (2004)
	TW: 1.5	Rott et al. (2006)
	A/C	Gutowski and Foerster (2009)
	IV: 12.53	Schneider and Lindstrøm (2011)
Homoeothrix juliana	Pristine nature	Sabater (1989)
	S: 1.9	Rott et al. (1997)
	TW: 1.3	Rott et al. (1999)
	Low nutrient	Soltani et al. (2012)
	Oligotrophic water	García and Aboal (2014)
Homoeothrix varians	Unpolluted streams	Kann (1978)
	S: 1.8	Rott et al. (1997)
	TW: 1.4	Rott et al. (1999)
	B	Gutowski et al. (2004)
	TW: 1.4	Rott et al. (2006)
	B	Gutowski and Foerster (2009)
	IV: 6.14	Schneider and Lindstrøm (2011)
Hydrococcus rivularis	S: 1.5	Rott et al. (1997)
	TW: 1.7	Rott et al. (1999)
	A	Gutowski et al. (2004)
	IV: 8.5	Schneider and Lindstrøm (2011)
Hydrococcus cesatii	S: 2.2	Rott et al. (1997)
	TW: 1.8	Rott et al. (1999)
	A	Gutowski et al. (2004)
Leptolyngbya foveolarum	TW: 2.2	Rott et al. (1999)
	C	Gutowski et al. (2004)
	D	Gutowski and Foerster (2009)
	High TP	Stancheva et al. (2012)
Leptolyngbya frigida	S: 1.3	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
Leptolyngbya nostocorum	S: 1.5	Rott et al. (1997)
	Low nutrient	Loza et al. (2013a)
Leptolyngbya perforans	S: 1.4	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
Leptolyngbya tenuis	S: 2.2	Rott et al. (1997)
	TW: 3.5	Rott et al. (1999)
	Low nutrient	Loza et al. (2013a)

Table 1 continued

Species	Bioindicator value/characteristic	Reference
Merismopedia glauca	s'j: 2.04	Walley et al. (2001)
	A	Gutowski and Foerster (2009)
	IV: 5.33	Schneider and Lindstrøm (2011)
Merismopedia punctata	s'j: 2.22	Walley et al. (2001)
	IV: 3.77	Schneider and Lindstrøm (2011)
Nostoc parmeloides	S: 1.3	Rott et al. (1997)
	TW: 0.6	Rott et al. (1999)
	Very low nutrient	Mollenhauer et al. (1999)
	A	Gutowski and Foerster (2009)
	IV: 7.14	Schneider and Lindstrøm (2011)
Nostoc verrucosum	Oligosaprobic	Dell'Uomo (1991)
	Clean waters	Aboal (1988)
	S: 1.4	Rott et al. (1997)
	Low nutrient	Mollenhauer et al. (1999)
	Low nutrient	Mateo et al. (2010)
	IV: 7.34	Schneider and Lindstrøm (2011)
	Low TN	Stancheva et al. (2012)
Nostoc sp.	s'j: 1.16	Walley et al. (2001)
	TW: 0.6	Rott et al. (2006)
	IV: 7.02	Schneider and Lindstrøm (2011)
	Low nutrient	Loza et al. (2013b)
Oscillatoria chlorina	Pollution-tolerant species	Palmer 1969)
	Polysaprobic	Persoone and De Pauw (1979)
	S: 3.6	Rott et al. (1997)
	TW: 3.5	Rott et al. (2006)
	High nutrient	Soltani et al. (2012)
Oscillatoria limosa	Pollution-tolerant species	Palmer (1969)
	Waste water pollution	Rott and Pfister (1988)
	High nutrient	Sabater (1989)
	S: 2.6	Rott et al. (1997)
	TW: 3.5	Rott et al. (1999)
	s'j: 2.29	Walley et al. (2001)
	High P content	Sabater et al. (2003)
	B	Gutowski et al. (2004)
	High organic matter	Sierra and Gómez (2007)
	C	Gutowski and Foerster (2009)
	IV: 39.10	Schneider and Lindstrøm (2011)
Oscillatoria ornata	Organic pollution species	Tseng and Wang (1982)
	Organically polluted waters	Branco and Pereira (2002)

Table 1 continued

Species	Bioindicator value/characteristic	Reference
Oscillatoria princeps	Pollution-tolerant species	Palmer (1969)
	S: 2.8	Rott et al. (1997)
	TW: 3.9	Rott et al. (1999)
	TW: 3.9	Rott et al. (2006)
	C	Gutowski and Foerster (2009)
Oscillatoria putrida	Pollution-tolerant species	Palmer (1969)
	Polysaprobic	Persoone and De Pauw (1979)
Oscillatoria splendida	Pollution-tolerant species	Palmer (1969)
	IV: 40.99	Schneider and Lindstrøm (2011)
Oscillatoria tenuis	Pollution-tolerant species	Palmer (1969)
	High P content	Sabater et al. (2003)
	High organic matter	Sierra and Gómez (2007)
	IV: 44.24	Schneider and Lindstrøm (2011)
	Eutrophic conditions	Loza et al. (2013b)
Phormidium aerugineo-caeruleum	Oligotrophic conditions	Charlton and Hickman (1984), Dell'Uomo (1991), Branco and Pereira (2002), Serrano et al. (2004), Perona and Mateo (2006) and Loza et al. (2013b)
	S: 2.6	Rott et al. (1997)
	TW: 3.5	Rott et al. (1999)
Phormidium ambiguum	S: 2.1	Rott et al. (1997)
	TW: 3.0	Rott et al. (1999)
	C	Gutowski and Foerster (2009)
Phormidium atumnale	Pollution-tolerant species	Palmer (1969)
	Unpolluted streams	Kann (1978)
	Poor in nutrients	Sabater (1989)
	S: 2.7	Rott et al. (1997)
	TW: 1.7	Rott et al. (1999)
	s'j: 2.35	Walley et al. (2001)
	B	Gutowski et al. (2004)
	Nutrient poor water	Lindstrøm et al. (2004)
	Low NO_3-N and conductivity	Schaumburg et al. (2004)
	TW: 1.7	Rott et al. (2006)
	High nutrient	Loza et al. (2013b)
	Eutrophic saltwaters	García and Aboal (2014)
Phormidium corium	S: 1.3	Rott et al. (1997)
	TW: 1.6	Rott et al. (1999)
	A	Gutowski et al. (2004)
	A/B	Gutowski and Foerster (2009)
	Oligotrophic conditions	Loza et al. (2013b)
Phormidium foveolarum	S: 3.1	Rott et al. (1997)
	s'j: 2.64	Walley et al. (2001)

Table 1 continued

Species	Bioindicator value/characteristic	Reference
Phormidium hetropolare	Low nutrient	Lindstrøm et al. (2004)
	IV: 3.40	Schneider and Lindstrøm (2011)
Phormidium incrustatum	Pristine nature	Sabater (1989)
	S: 1.7	Rott et al. (1997)
	TW: 2.4	Rott et al. (1999)
	C	Gutowski et al. (2004)
	A/C	Gutowski and Foerster (2009)
Phormidium inundatum	Oligosaprobic	Persoone and De Pauw (1979)
	IV: 35.81	Schneider and Lindstrøm (2011)
Phormidium retzii	Poor in nutrients	Sabater (1989)
	S: 1.7	Rott et al. (1997)
	TW: 2.6	Rott et al. (1999)
	Low conductance (nutrient)	Branco et al. (2001)
	C	Gutowski et al. (2004)
	C	Gutowski and Foerster (2009)
	IV: 32.02	Schneider and Lindstrøm (2011)
Phormidium subfuscum	Oligo-β-mesosaprobic	Dell'Uomo (1991)
	Slightly eutrophic	Sabater (1989)
	S: 2.2	Rott et al. (1997)
	TW: 1.6	Rott et al. (1999)
	High NO_3-N and conductivity	Schaumburg et al. (2004)
	C	Gutowski et al. (2004)
	TW: 1.6	Rott et al. (2006)
	C	Gutowski and Foerster (2009)
Phormidium tenue	Enriched waters	Branco and Pereira (2002)
	High nutrient	Soltani et al. (2012)
Phormidium terebriforme	High nutrient	Kolkwitz and Marsson (1908), Fjerdingstad (1964), Sládecek (1973) and Loza et al. (2013b)
	S: 3.4	Rott et al. (1997)
	TW: 3.5	Rott et al. (1999)
Phormidium tinctorum	S: 1.6	Rott et al. (1997)
	IV: 52.77	Schneider and Lindstrøm (2011)
Phormidium uncinatum	Pollution-tolerant species	Palmer (1969)
	Oligo-β-mesosaprobic	Dell'Uomo (1991)
Plectonema tomasinianum	S: 1.5	Rott et al. (1997)
	TW: 1.7	Rott et al. (1999)
	B	Gutowski and Foerster (2009)
	IV: 17.60	Schneider and Lindstrøm (2011)
Pleurocapsa aurantiaca	Poor in nutrients	Sabater (1989)
	S: 1.1	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)

Table 1 continued

Species	Bioindicator value/characteristic	Reference
Pleurocapsa minor	Waste water pollution	Rott and Pfister (1988)
	S: 2.6	Rott et al. (1997)
	TW: 2.3	Rott et al. (1999)
	C	Gutowski et al. (2004)
	C	Gutowski and Foerster (2009)
Porphyrosiphon martensianus (Syn.: *Lyngbya martensiana*)	S: 1.5	Rott et al. (1997)
	TW: 1.9	Rott et al. (1999)
	s'j: 1.48	Walley et al. (2001)
	Low nutrient	Perona and Mateo (2006)
	B	Gutowski and Foerster (2009)
Pseudanabaena catenata	IV: 35.91	Schneider and Lindstrøm (2011)
	D	Gutowski and Foerster (2009)
	S: 3.3	Rott et al. (1997)
	TW: 1.8	Rott et al. (1999)
Pseudanabaena frigida	Oligotrophic waters	Komárek and Anagnostidis (2005)
	IV: 3.63	Schneider and Lindstrøm (2011)
Rivularia biasolettiana	Pristine nature	Sabater (1989)
	Low nutrient	Mateo et al. (2010)
	IV: 4.55	Schneider and Lindstrøm (2011)
Rivularia haematites	S: 1.4	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
	Low nutrient	Mateo et al. (2010)
	IV: 8.75	Schneider and Lindstrøm (2011)
Rivularia sp.	Decreasing with eutrophication	Kann (1982)
	s'j: 1.25	Walley et al. (2001)
	IV: 4.99	Schneider and Lindstrøm (2011)
Schizothrix lacustris	S: 1.6	Rott et al. (1997)
	TW: 0.6	Rott et al. (1999)
	IV: 4.35	Schneider and Lindstrøm (2011)
Schizothrix latierita	S: 1.5	Rott et al. (1997)
	TW: 0.3	Rott et al. (1999)
	IV: 4.29	Schneider and Lindstrøm (2011)
Schizothrix penicillata	Pristine nature	Sabater (1989)
	S: 1.3	Rott et al. (1997)
	TW: 0.6	Rott et al. (1999)
Schizothrix tinctoria	S: 1.7	Rott et al. (1997)
	TW: 0.8	Rott et al. (1999)
	B	Gutowski and Foerster (2009)
Scytonematopsis starmachii	S: 1.0	Rott et al. (1997)
	TW: 0.3	Lindstrøm et al. (2004)
	Nutrient poor water	Rott et al. (2006)
	IV: 3.08	Schneider and Lindstrøm (2011)

Table 1 continued

Species	Bioindicator value/characteristic	Reference
Scytonema crispum	S: 1.4	Rott et al. (1997)
	TW: 2.4	Rott et al. (1999)
Scytonema crustaceum	S: 1.1	Rott et al. (1997)
	TW: 0.6	Rott et al. (1999)
Scytonema myochrous	S: 1.0	Rott et al. (1997)
	TW: 0.6	Rott et al. (1999)
Stigonema hormoides	TW: 0.6	Rott et al. (1999)
	S: 1.1	Rott et al. (1997)
	IV: 1.87	Schneider and Lindstrøm (2011)
Stigonema mamillosum	TW: 0.3	Rott et al. (1999)
	Low nutrient	Lindstrøm et al. (2004)
	IV: 3.88	Schneider and Lindstrøm (2011)
Stigonema ocellatum	IV: 3.34	Schneider and Lindstrøm (2011)
	TW: 0.6	Rott et al. (1999)
	S: 1.1	Rott et al. (1997)
Tolypothrix distorta	S: 1.4	Rott et al. (1997)
	TW: 0.5	Rott et al. (1999)
	Low nutrient	Lindstrøm et al. (2004)
	IV: 7.71	Schneider and Lindstrøm (2011)
Tolypothrix distorta var. *penicillata*	Oligotrophic waters	Lindstrøm and Traaen (1984)
	S: 1.2	Rott et al. (1997)
	TW: 0.6	Rott et al. (1999)
	TW: 0.6	Rott et al. (2006)
	Low nutrient	Mateo et al. (2010)
	IV: 5.20	Schneider and Lindstrøm (2011)
Tolypothrix tenuis	S: 1.5	Rott et al. (1997)
	TW: 1.2	Rott et al. (1999)
	Low nitrate	Cantonati (2008)
	IV: 6.45	Schneider and Lindstrøm (2011)
	Low nutrient	Loza et al. (2013a)

Only species with at least two references found were included (see descriptions of bioindicator value categories below)

S Saprobic range: oligosaprobic: 1.0–1.5, oligosaprobic to β-mesosaprobic >1.5 to ≤1.8, β –mesosaprobic >1.8 to ≤2.3, β-mesosaprobic to α-mesosaprobic >2.3 to ≤2.7, α-mesosaprobic >2.7 to ≤3.2, α-mesosaprobic to polysaprobic >3.2 to ≤3.5, polysaprobic >3.5 to ≤4.0. The katharobic zone comprises the very pure waters, i.e. those waters without any trace of pollution. Oligosaprobic organisms are those indicators for scarcely polluted waters. β-mesosaprobic organisms are those indicators for moderately polluted waters α-mesosaprobic organisms are those indicators for highly polluted waters. Polysaprobic organisms are those indicators for extremely polluted waters. *s'j* revised saprobic values. *TW* trophic value: ultra-oligotraphentic all values <0.5, oligotraphentic: 0.6–1.0, oligomesotraphentic: 1.1–1.5, mesotraphentic: 1.6–2.0, meso-eutraphentic 2.1–2.5, eutraphentic: 2.6–3.0, eu-polytraphentic: 3.1–3.5. Category description of A, B, C, D—A: sensitive species, characteristic of certain types of water bodies (found at the most pristine sites), B: less sensitive species, more widely distributed, indicate good conditions, C: tolerant species, indicate eutrophication when highly abundant, D: species prefer strongly eutrophic conditions. *IV* Indicator value. IV has been calculated as the average log (TP) to the power of 10 at the sites where the taxon occurs. Indicator values range from 1.87 to 68.91. *DIN* dissolved inorganic nitrogen. *TP* total phosphorus. *TN* total nitrogen

Gómez 2007; Walley et al. 2001). *Leptolyngbya foveolarum* (Gutowski and Foerster 2009; Gutowski et al. 2004; Rott et al. 1999; Stancheva et al. 2012), *Phormidium terebriforme* (Fjerdingstad 1964; Kolkwitz and Marsson 1908; Loza et al. 2013b; Rott et al. 1997, 1999; Sládecek 1973), and *P. ambiguum* (Gutowski and Foerster 2009; Rott et al. 1999) were also associated to nutrient-rich conditions.

In general, a greater number of unicellular and heterocystous genera/species were associated with low trophic levels, while the number of non-heterocystous filamentous genera/species was greater in high nutrient conditions.

Ecophysiological strategies

Variations in benthic cyanobacterial species distribution could be explained on the basis of ecophysiological differences between cyanobacteria in which nutrient loading can be a strong selective force shaping cyanobacterial communities. Recent studies showed distinct patterns in growth and competitive response of benthic cyanobacteria under N and P regimes (Loza et al. 2014). Nutrient gradient bioassays in monoculture as well as in competition experiments with a mixture of species indicated predominance of certain cyanobacteria over others, depending on the concentration of phosphorus and nitrogen growth was either stimulated or inhibited. Some species grew better at high nutrient concentrations, while higher yields were recorded for others under low nutrient regimes, according to the differential distribution of these cyanobacteria in the river from which they were isolated in which a group of species occurred mostly in downstream nutrient-rich locations, while others were typical of upstream oligotrophic conditions (Loza et al. 2013a, b, c). Therefore, ecological ranges of individual cyanobacterial populations can differ with nutrient concentration, and the persistence of several species under low or high nutrient concentrations in running waters can be explained on the basis of their different ecophysiological properties.

A key ecological feature at upstream sites with typically low nutrients may be the ability to have nitrogenase activity. The property of field populations to show marked phosphatase activity allows their occurrence in environments where organic phosphate is an important source of phosphate, even when inorganic phosphate is below detectable levels (Whitton 1987). In addition, a possible environmental explanation for the shifts in cyanobacterial distribution could be related to differential growth and/or utilization of P and N reserves. Since P supply tends to come in pulses in running water (Bhaya et al. 2000; Turner et al. 2003) with subsequent falling in water concentration, the ability of taking up P in excess of immediate requirement and store it intracellularly in a luxury consumption (Dodds and Welch 2000), can be advantageous, allowing for minimum growth, survival, till a new P pulse arrives. Similar behaviour could be extrapolated for N in non-fixing species. Therefore, the ability to scavenge nutrients during rare periods of high concentrations, rather than a general tolerance of low-nutrient conditions, has been proposed as an adaptive strategy at upstream sites with typically low nutrients (Kelly and Whitton 1998). A greater ability to store P and slower utilization of intracellular polyP granules has been found in *Calothrix* cultures with respect to *Nostoc*, isolated from a Spanish river with eutrophic gradients (Mateo et al. 2006). Rott et al. (2000) suggested two strategies used for species to overcome resource limitations, such as rapidly growing r-strategists (e.g. *Phormidium*) with increased phosphatase synthesis during the peak growing periods, and the persistent k-strategists (e.g. *Rivularia*) showing seasonal variations in light adaptation and moderate variations in the nutrient pool/phosphatase synthesis. Other strategies exploit the presence of mucilage, which helps these organisms to survive in nutrient poor media, where they play an important role in nutrient uptake and assimilation, or the possibility of increasing

bioavailability of P at the thin layer of fine sediment, commonly observed under well-developed cyanobacterial mats (Loza et al. 2014, and references therein).

Molecular approaches

Studies on cyanobacterial diversity and community structure based mainly on the accurate taxonomic identification of the individuals found is often time-consuming due to difficulties identifying species that exhibit extreme morphological variability and whose taxonomy is controversial (Komárek and Anagnostidis 1999). Moreover, in many cases, the only means of identification is through the comparison of live material with cultured field samples, which introduces more difficulties due to culture selectivity and the fact that many species frequently develop anomalous morphological states in culture (Komárek and Anagnostidis 1999; Perona et al. 2003). Molecular techniques can obviate many of these difficulties and are especially well suited to differentiate closely related organisms, allowing morphological comparisons in parallel, and the consequent identification using both molecular and morphological data. Therefore, sequencing of ribosomal 16S rRNA genes has become a widespread practice in the detection and characterization of cyanobacteria in the environment (Komárek 2013). In addition, nucleic acid extraction from polluted locations and their subsequent amplification by polymerase chain reaction (PCR) has proved extremely useful in assessing the changes in microbial community structure by several profiling techniques (Malik et al. 2008). Among these PCR-based tools, molecular fingerprinting methods, such as Denaturing/Temperature Gradient Gel Electrophoresis (DGGE/TGGE) have been used to analyze the diversity of bacterial assemblages in different environments (Fromin et al. 2002). In particular, TGGE has been used to measure changes in cyanobacterial diversity along a pollution gradient in a Spanish river and compared it with that of using microscopic observations of field-fixed and cultured samples (Loza et al. 2013c; Rodriguez et al. 2007). These studies were based on comparisons of cyanobacterial communities in biofilms in order to evaluate and characterize their ecological responses depending on the stream water quality. The different 16S rDNA genes present in the community of each sampling point of the river were separated by TGGE, giving a characteristic pattern of bands for each site. This pattern represents a "fingerprint" of the community, allowing direct comparisons of the different samples. Band profiles differed among sampling sites depending on differences in water quality, whereby TGGE band richness decreased in a downstream direction, and there was a clear clustering of phylotypes on the basis of their origins from different locations according to their ecological requirements. Some phylotypes were associated with low nutrient concentrations and high levels of dissolved oxygen, while other phylotypes were associated with eutrophic-hypertrophic conditions. Results were consistent with those obtained from microscopic observations of field-fixed samples, and it allowed the identification of the occupants of particular ecological niches, so that the particular ecologically relevant taxa, different ecotypes, could be selected as bioindicators of water quality. Therefore, an ecotype-based model of the structure of cyanobacterial populations has been proposed in which all related inhabitants of a unique ecological niche are thought to belong to a single, stable ecotype. Results of the TGGE studies supported the view that once a community has been characterized and its fingerprint obtained, this band pattern could be used as a bar code that, like a detector or sentinel, acts as an 'early warning' device alerting us of the presence of pollutants in the environment (Loza et al. 2013c; Rodriguez et al. 2007).

In 2005, a new technology referred to as 'Next Generation Sequencing (NGS)' was introduced that leads to the determination of millions of DNA sequences in a single

process. Through the use of the massive amounts of sequence data produced by NGS platforms, researchers have been able to observe the slight changes in community structure that may occur following anthropogenic or natural environmental fluctuations (Shokralla et al. 2012). Therefore, the use of high-throughput NGS approaches in biodiversity science has the potential to further extend the application of DNA information for routine biomonitoring applications to an unprecedented scale (Hajibabaei et al. 2011). However, in the available literature, there are few studies of microbial community composition and diversity in rivers based on NGS, and consequently, more studies are needed to better determine the cyanobacterial diversity and community structure contained within rivers (Schultz et al. 2013; Tytgat et al. 2014).

Cyanobacteria as bioreporters

The term bioreporter refers to transgenic organisms (usually microorganisms) which harbor, either in the chromosome or in a replicative plasmid, a fusion between a sensing element [a gene(s) promoter responsive to the stimulus/compound to be detected] and a promoterless reporter element encoding easily detectable output signals (i.e. light, fluorescence, color development). These recombinant organisms are designed to produce a

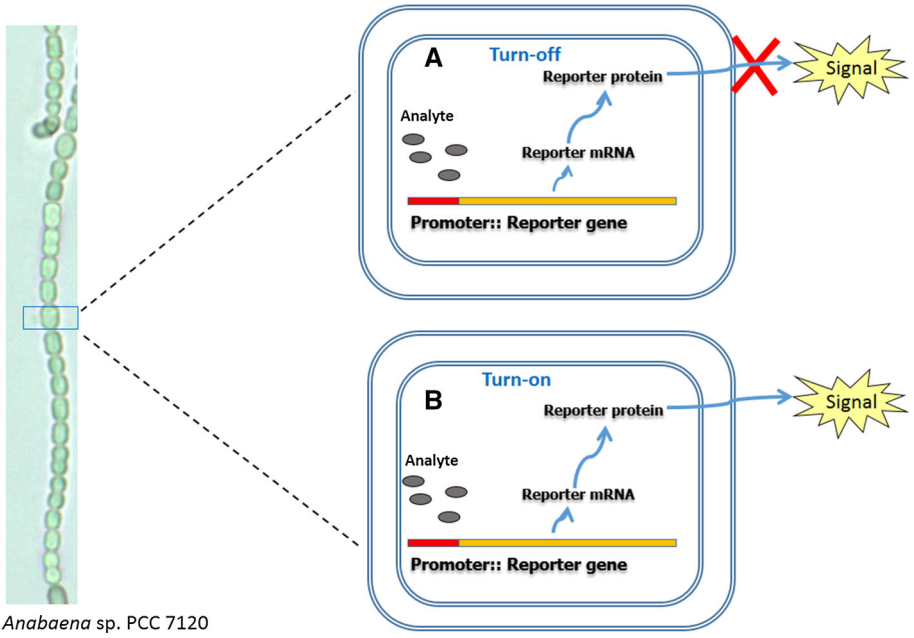

Fig. 3 Bioreporters *turn-off* and *turn-on* concepts. **a** *Turn-off* bioreporters detect general toxicity as a decrease in the reporter signal; in these bioreporters the reporter system is fused to a constitutive promoter and toxicity leads to inhibition of the reporter quantifiable product. A second class of *turn-off* bioreporters are some used to detect nutrient bioavailability which are induced in response to nutrient deficiency and gradually turned-off when the nutrient is added. **b** In *turn-on* bioreporters, the reporter system is fused to gene promoters responsive to pollutants/groups of pollutants with similar chemical structure which are turned on (induced) in response to these specific chemicals. A second class of *turn-on* bioreporters are those denoted as semi-specific where the promoter system is fused to gene promoters responsive to cellular stresses such as oxidative stress, DNA damage or membrane damage

measureable signal in response to an analyte (i.e. toxin or pollutants) or a stress situation. As depicted in Fig. 3, regarding specificity of detection, bioreporters are usually divided into three categories (Belkin 2003): non-specific, semi-specific and specific. In the field of toxicology, non-specific or ecotoxicity bioreporters, also known as general toxicity bioreporters, provide data on the toxicity of a sample but do not detect specific pollutants; they usually consist of a reporter element fused to a constitutive promoter so that the reporter product is continuously produced but in the presence of a toxin that impairs metabolism, the bioreporter response is reduced or even fully inhibited; in other words, the reporter response is turned off, so these strains may be denoted as *turn-off* bioreporters (Fig. 3a). The semi-specific bioreporters report on pollutants/stressors that cause cellular responses to stresses such as oxidative stress, genotoxicity (DNA damage) or membrane damage; the promoters are activated in response to global changes in the cell; these bioreporters harbor a fusion between the reporter element and stress responsive gene promoters; as in this case, the bioreporter response is induced (turned on), these strains may be denoted as *turn-on* bioreporters (Fig. 3b). The specific bioreporters are able to detect a specific pollutant or group of related pollutants as they have a fusion between a pollutant-responsive gene(s) regulatory element and the promoterless reporter system; they are usually *turn-on* strains but in some cases, such as some promoters responsive to nutrient deficiency used to develop bioreporters of nutrient bioavailability (see below), *turn-off* strains are constructed. In toxicology, microbial bioreporters are an interesting alternative/complement to classic toxicity bioassays as they are usually less laborious, quicker in their response and more cost-effective with no animal sacrifice (Belkin 2003; Sorensen et al. 2006; van der Meer and Belkin 2010).

There are several reporter systems that are usually used: *lacZ* encoding the β-galactosidase of *Escherichia coli* has been largely used as there are a wide range of detection methods; i.e. chemiluminescence, colorimetry, electrochemistry and fluorescence; however, it needs exogenous substrate addition which may require cell membrane permeabilization and does not allow real-time in vivo expression studies; it also may involve time and labor-consuming operations. Fluorescent proteins such as GFP encoded by genes found in the jellyfish *Aequorea victoria* and other marine invertebrate do not require the addition of exogenous substrate but lower sensitivity, autofluorescence of the organisms/samples, the need of O_2 for maturations of the fluor and lag time before expression limit their use to measure quick cellular responses although a clear advantage is that a whole myriad of mutants with different color varieties is available ((Heim et al. 1995; Heim and Tsien 1996). Finally, the luciferase reporter systems are widely used due to their high sensitivity and ease of measurement. The luciferase systems product is bioluminescence and may be based either in bacterial *luxCDABE* genes or in eukaryotic *luc* genes. Both luciferases catalyze the oxidation of a substrate, long chain aldehydes in the case of bacterial luciferases and luciferin/pholasin/coelentarazine in the case of eukaryotic luciferases, both reactions require O_2. Bacterial bioluminescence *lux* genes are present in bacteria found in marine, freshwater and terrestrial ecosystems. They are all gram negative belonging to the genera: *Vibrio*, *Aliivibrio*, *Photobacterium*, *Shewanella* and *Photorhabdus* (previously *Xenorhabdus*). The bacterial luciferase encoded by *luxAB* catalyzes the oxidation of $FMNH_2$ and a long chain fatty acid to produce the oxidized flavin (FMN) and a long chain fatty acid with the emission of blue-light (490 nm). The fatty aldehyde is produced by a fatty acid reductase complex that include a reductase, transferase and a synthetase encoded by *luxC, D* and *E* genes, respectively. A *luxCDABE* bioreporter is self-luminescent and allows for real time in vivo measurements while *luxAB*-based bioreporters need the addition of exogenous aldehyde (usually n-decanal or nonanal) to luminesce

allowing single time point measurement rather than continuous monitoring. The firefly luminescent systems is the best characterized of the eukaryotic systems (also including click beetles, railroad worms or the sea pansy); it catalyzes the O_2 and ATP dependent decarboxylation of the luciferin, emitting light in the green-yellow region of the spectrum (maximum around 560 nm). As in the case of *luxAB*-based bioreporters, this system needs the exogenous addition of the luciferin precluding in vivo continuous light monitoring; however, the quantum yield of the firefly luciferase is the highest of any of the so far characterized bioluminescence systems resulting in the highest sensitivity; besides, the different *luc* genes and mutant variants allow multicolor luciferase assays which might be interesting for developing bioreporters useful in environmental monitoring; (Branchini et al. 2005; Nakajima and Ohmiya 2010; Roda and Guardigli 2012). As the bioluminescence reactions depend on cell metabolism due to the requirement of ATP or reducing power, any pollutant affecting metabolism, may decrease light emission in a dose–response manner, being denoted as *lights-off* (from *turn-off* bioreporters, see Fig. 3a). These *lights-off* bioreporters are able to detect the general toxicity of a sample but cannot identify the pollutant(s); another kind of *lights-off* bioreporters are some that are used to detect nutrient bioavailability; while, by analogy, the *lights-on* bioreporters (from *turn-on*, see Fig. 3b) are induced by a specific stress, pollutant or group of related pollutants (Belkin 2003; Sorensen et al. 2006).

An important feature of bioreporters is that they provide complementary data of environmental samples to those obtained by analytical chemistry methods; while these give a sensitive and accurate measurements of environmental pollutants, bioreporters provide data on the bioavailability of the pollutants (the biologically relevant fraction of the chemical compound that is actually available to the reporter cell, capable of interacting/passing through cellular membranes) and/or the global toxicity of a sample.

In the field of environmental microbial bioreporters, cyanobacteria are of utmost relevance due to their photosynthetic nature, being ubiquitous primary producers in both aquatic and terrestrial ecosystems (Bachmann 2003). Cyanobacteria are at the base of trophic webs and anything having a detrimental effect on cyanobacteria may seriously affect the functioning of the whole ecosystem; in fact, reports show that marine cyanobacteria *Prochlorococcus* sp. and *Synechococcus* sp. account for up to 80 % of primary production (Goericke and Welschmeyer 1993; Liu et al. 1997). Besides, more than 100 cyanobacterial genomes have been sequenced (http://genome.microbebd.jp/cyanobase) (Shih et al. 2013) and many are genetically amenable.

To date, all reported cyanobacterial environmental bioreporters are based on luminescence systems, so that *lights-off* and *lights-on* cyanobacterial bioreporters will be described in this review; also, so far, and to our knowledge, only cyanobacterial bioreporters to detect general toxicity, nutrient bioavailability and heavy metals have been constructed; there is a lack of semi-specific bioreporters able to detect oxidative stress or genotoxicity although efforts to construct such reporters are under way (F. Fernández-Piñas; personal communication).

General toxicity or ecotoxicity bioreporters

Only two cyanobacterial general toxicity bioreporters have been described up to date; both are *lights-off*; one is based on the unicellular *Synechocystis* strain PCC 6803 (Shao et al. 2002) and one based on the filamentous heterocystous cyanobacterium *Anabaena* sp. PCC 7120 (now renamed by many authors as *Nostoc* sp. PCC 7120; both terms will appear in this review indistinctly) denoted as *Anabaena* CPB4337 (Fernandez-Piñas and Wolk 1994;

Garcia et al. 2013; González-Pleiter et al. 2013; Rodea-Palomares et al. 2009, 2010a, b, 2011).

The freshwater *Synechocystis* chromosome was double tagged with both *luc* (from firely *Photinus pyralis*) and *luxAB* genes (from marine bacterium *Aliivibrio fischeri*, formerly *Vibrio fischeri*) under the control of the *tac* promoter (Shao et al. 2002). They characterized the bioluminescence of the reporter strain with respect to batch growth and pH. As aldehyde (substrate for bacterial luciferase) addition was toxic to the culture, Shao and coworkers measured the luminescence of the *luc* gene; in this way, they tested the toxicity of four herbicides with different modes of action, two heavy metals (copper and zinc) and the organic 3,5 dichlorophenol (DCP) and compared the sensitivity of the cyanobacterial bioreporter with other heterotrophic bacterial bioreporters (based on *E. coli*; *Pseudomonas putida* or *P. fluorescens*). As expected, the cyanobacterial bioreporter proved to be more sensitive to the herbicides but in the same range of sensitivity to the heavy metals or 3,5 DCP. The *Synechocystis* bioreporter was not self-luminescent and the luciferin substrate had to be added at each time point for measurement precluding its use for continuous monitoring; besides, it does not appear to have been tested with environmental samples.

By contrast, the filamentous *Anabaena* (*Nostoc*) CPB4337 (Fernández-Piñas and Wolk 1994) is a self-luminescent bioreporter which bears in its chromosome a tn5 derivative with *luxCDABE* from the luminescent terrestrial bacterium *Photorhabdus luminescens*. This strain shows a high constitutive luminescence (the gene promoter has not been identified yet) and does not need the addition of exogenous aldehyde. Luminescence has been shown to be high even at increasing temperatures (up to 30 °C) in contrast with luciferases from marine organisms like *Vibrio* and *Aliivibrio* which is in agreement with *Photorhabdus* luminescence having the greatest thermal stability (Fernandez-Piñas et al. 2000; Meighen 1991). The strain has been successfully used in combination with a battery of standardized toxicity bioassays based on organisms form different trophic levels (Luminescent bacterium *A. fischeri*; the crustacean *Daphnia magna* and the green alga *Pseudokirchneriella subcapitata*) to study the toxicity of different priority (heavy metals, organic solvents) and emerging pollutants (pharmaceuticals, perfluorinated surfactants and nanomaterials) (González-Pleiter et al. 2013; Rodea-Palomares et al. 2009, 2011, 2012; Rosal et al. 2010a, b); it has also been tested in environmental matrices of different complexity (Garcia et al. 2013; Rodea-Palomares et al. 2010; Rosal et al. 2010a) and for the first time in the case of a cyanobacterial bioreporter, it has been used to study the toxicology of pollutants' mixtures by the Combination Index-Isobologram Equation method (Rodea-Palomares et al. 2010, 2012). The authors applied the Combination Index method to study the toxicological interactions of priority and emerging pollutants in the above mentioned aquatic organisms including *Anabaena* CPB4337 finding that the nature of the interaction (either synergism or antagonism) strongly depended on the test species and also in the effect level exerted by the pollutant mixture in the organism (Rodea-Palomares et al. 2010, 2012; Rosal et al. 2010b). In a study with mixtures of antibiotics, by using bioluminescent *Anabaena* CPB4337, they were able to demonstrate that certain mixtures of antibiotics could pose an ecological risk towards aquatic environments (González-Pleiter et al. 2013).

Cyanobacterial bioreporters to detect nutrient bioavailability

The environmental relevance of cyanobacteria as primary producers both in freshwater and marine environments make cyanobacterial bioreporters a useful tool to assess nutrient bioavailability in water bodies. Iron, phosphate and nitrogen are essential for primary

production and usually limiting in certain aquatic environments such as the ocean or large lakes; on the other hand, an excess of P and/or N may lead to eutrophication of water bodies and developments of algal blooms which may lead to toxin production by cyanobacteria (Dodds 2006). Bullerjahn and coworkers already published an extensive and comprehensive review on the use of cyanobacterial bioreporters as sensors of nutrient availability, namely Fe, P and N (Bullerjahn et al. 2010); in this review we shall revise all what has been done in this field highlighting and discussing the most recent reports.

Fe bioavailability

Most cyanobacterial bioreporters constructed to detect Fe have been based on the Fe-responsive *isiAB* promoter which is in part regulated by Fur (ferric uptake regulator). When cells are depleted of iron, the *isiAB* promoter is induced; when increasing concentrations of iron are supplied to the cells, the *isiAB* promoter is down-regulated in a dose–response manner; so that these bioreporters may be considered as *lights-off* strains.

The *isiAB* promoter has been fused to *luxAB* from *Vibrio harveyi* and transformed in the unicellular freshwater *Synechococcus* sp. PCC 7942 yielding strain *S.* KAS101 (Durham et al. 2002); in the unicellular marine *Synechococcus* sp. strain PCC 7002 yielding strain *S.* BM004, deposited as *Synechococcus* sp. CCMP 2669 (Boyanapalli et al. 2007) and in the model unicellular *Synechocystis* sp. PCC 6803 yielding strain S. MpILisi (Kunert et al. 2000). For strains KAS101 and BM004, bioluminescence was found to be a function of the free ferric ion concentration in metal-buffered media so that the dynamic range of Fe concentrations over which the bioreporters may be used as quantitative tools for bioavailable Fe was obtained; in the case of KAS101, the dynamic range was between free Fe^{3+} $10^{-21.1}$ to Fe^{3+} $10^{-20.6}$ M and in the case of BM004 a three parameter-sigmoidal curve was generated in a range of free Fe^{3+} between $10^{-22.4}$ and $10^{-19.4}$ M. Both reporter strains have been used in environmental systems such as the Laurentian great lakes (Hassler et al. 2008, 2009; McKay et al. 2005; Porta et al. 2003, 2005) and marine environments such as the Baltic sea or subarctic Pacific (Boyanapalli et al. 2007). These strains are not self-luminescent and aldehyde had to be added for each measurement.

Recently Zha et al. (2012) have reported a novel Fe-bioreporter based in the filamentous *Nostoc* (*Anabaena*) PCC 7120 which harbors the iron-regulated schizokinen transporter alr0397 gene promoter fused to *V. fischeri luxAB* (also non self-luminescent); The alr0397 gene is also highly inducible under iron deficiency (Dong and Xu 2009); so, it is also a *lights-off* strain. Dose–response relationships between luciferase activity and free Fe^{3+} were obtained and the dynamic range of performance of the bioreporter was found to be free Fe^{3+} $10^{-21.5}$–$10^{-19.6}$ M; this range makes the bioreporter useful in environmental samples with high bioavailable iron; the bioreporter has been tested successfully with water samples from three eutrophic Chinese lakes.

P bioavailability

In most aquatic ecosystems P is usually a limiting nutrient, barely detectable in oligotrophic systems (Whitton 2008). On the other hand, an excess of P may lead to eutrophication of water bodies and algal blooms (Conley et al. 2009). Assessing the bioavailability of P in environmental samples is not an easy issue as this element may appear in various organic and inorganic species both dissolved and particulate (Neal et al. 2010). Dissolved Inorganic Phosphorus (DIP) is a measure of dissolved orthophosphate which is probably the most readily available species to photosynthetic organisms; the

Dissolved Organic Fraction of Phosphorus (DOP) may also be available to algae and aquatic plants (Bostrom et al. 1988; Neal et al. 2010). Cyanobacteria respond to P deficiency by increasing surface phosphatase activities such as alkaline phosphatases which scavenge P from dissolved organic sources and have broad substrate specificity (Hoppe 2003); cyanobacteria may, then, be useful to determine which fraction of total P is actually detected by the phytoplancktonic cell.

At a genetic level, microorganisms sense P bioavailability and respond regulating the expression of a series of genes that constitute the Pho regulon, such as *pho* encoding the alkaline phosphatases or *pst* encoding phosphate transporters (Su et al. 2007; Van Bogelen et al. 1996). In cyanobacteria this system has been well characterized in *Synechococcus* sp. PCC 7942 and in *Synechocystis* sp. PCC 6803 (Su et al. 2007); a strain of *Synechococcus* sp. PCC 7942 in which the *phoA* promoter has been fused to the reporter genes *luxAB* from *A. fischeri* was the first P bioavailability cyanobacterial bioreporter reported (Gillor et al. 2002; Schreiter et al. 2001); the strain has been named as *S.* APL, it is not self-luminescent and needs the addition of exogenous aldehyde. The *phoA* gene is induced under P-deficiency and luminescence decreases with the addition of increasing inorganic P (DIP) in a dose–response manner so it is a *lights-off* strain. The authors tested also organic P species finding that ADP and D-fructose-6-phosphate almost completely inhibited bioluminescence and that D-glucose 6 phosphate, ρ-nitrophenol-phosphate and β-glycerol phosphate only partially inhibited luminescence; so the bioreporter is also responsive to DOP. Cells needed to be pre-starved for P during 30 h before being transferred to media with different PO_4^{3-} concentrations. The authors tested it with water samples from Lake Kinneret (Israel) finding a good correlation between luminescence measurements and total dissolved P concentrations as measured chemically; they also immobilized P-starved cells in agar in microtiter plates which could be stored fully active for three weeks at 4 °C and denoted the immobilized strain as CyanoSensor. The CyanoSensor showed a dynamic detection range of 0.3–8 μM with an incubation time of 8 h (Schreiter et al. 2001). This bioreporter has been recently used in combination with a *glnA* based N- bioreporter (see below) to measure nutrient bioavailability for over two years in water samples from the same lake collected monthly, finding that the bioavailable P fraction estimated with the bioreporter was very low indicating that in this lake P rather than N is the limiting nutrient for primary production by cyanobacteria (Gillor et al. 2010).

Synechococcus sp. PCC 7942 APL strain may be considered as representative of the picophytoplankton but not of the whole phytoplankton community; in this regard, Muñoz-Martín et al. (2011) have constructed novel self-luminescent cyanobacterial bioreporters based on the filamentous *Anabaena* (*Nostoc*) sp. PCC 7120. Three bioreporters were constructed, one denoted *Anabaena* AP harboring the *Anabaena phoA* (all2843 gene) promoter fused to the thermostable *luxCDABE* operon from *P. luminescens*; *Anabaena* AP-L with a *luxCDABE* fusion to a *phoA*-like gene (alr5291) promoter and *Anabaena* PST with a *luxCDABE* fusion to the *pst* promoter from the phosphate transport cluster *pstS1C1A1B1* (all4575 to all4572 genes). Strains *A.* AP-L and *A.* PST responded to P starvation by induction of luminescence while *A.* AP did not show any induction and was discarded for further studies; when increasing quantities of PO_4^{3-} were added to the P-starved cultures, luminescence decreased in a dose response manner both in *A.* AP-L and *A.* PST which means that both are *lights-off* bioreporters. After 24 h of incubation with PO_4^{3-}, the dynamic range of performance of both bioreporters could be calculated and was between 1 and 57 μM for the *A.* AP-L strain and between 0.5 and 57 μM for the *A.* PST strain. Re-feeding experiments with organic P-sources indicated that both strains responded to the addition of D-glucose 6-phosphate and ADP by lowering their

bioluminescence, ADP decreased bioluminescence to a higher extent than did D-glucose 6-phosphate. Both bioreporters were tested to sense P bioavailability in environmental samples from upstream and downstream course of a large river in the Madrid area (central Spain) and from the influent and effluent of an urban sewage treatment plant (STP) finding a very good correlation between the estimates of bioavailable P as measured by the two bioreporters and the chemically determined P concentrations indicating that most of the P in the samples was present as chemical species bioavailable to the cyanobacterium. The *A.* AP-L bioreporter has also been recently used in a polyphasic approach to monitor P bioavailability in five rivers of central Spain in combination with cyanobacterial morphological features related to P bioavailability (the presence of hairs, polyP granules and calyptras) and the determination in situ of alkaline phosphatases activities of cyanobacteria found at the sampling sites. As expected, there was an inverse correlation between phosphatase activity and P-bioavailability as measured by the bioreporter. The bioavailable P detected by the bioreporter was in general low and correlated with a high measurement of phosphatase activity, lower abundance of polyP granules and higher abundance of hairs and calyptra in those cyanobacteria present in the sampling sites able to develop such structures, indicating low bioavailable P concentrations in the sampling sites (Muñoz-Martín et al. 2014a). Strains *S.* APL and *A.* AP-L and *A.* PST may complement each other and provide a global picture of P bioavailability for phytoplankton in aquatic environments.

N bioavailability

As stated above, primary production, particularly in freshwater systems, is limited by P bioavailability but recent studies emphasize that N can act also as a primary limiting nutrient (Conley et al. 2009; Dolman et al. 2012; Lewis et al. 2011). As with Fe and P, chemical determination of total N concentrations does not correlate with the bioavailability of N species to photosynthetic organisms. Several cyanobacterial bioluminescent bioreporters to detect N bioavailability have been constructed. Mbeunkui et al. (2002) were probably the first to report on a cyanobacterial bioreporter to detect bioavailable nitrate, the strain N1luxKm (Richaud et al. 2001). This strain bears a *luxAB* fusion to the *nblA1* promoter in the chromosomal DNA of the unicellular *Synechocystis* sp. PCC 6803. The *nblA1* gene encodes the regulator NblA which is essential for phycobilisome degradation (Collier and Grossman 1994). Upon N deprivation, the *nblA1* gene is up-regulated; the strain is a *lights-off* bioreporter as bioluminescence is inversely proportional to nitrate concentration in the range 4–100 μM; it needs an incubation of 10 h; besides nitrate, it also responds to ammonium, so it is not really specific for nitrate. The authors immobilized the strain in a way similar to the *S.* APL strain (Schreiter et al. 2001) and denoted it as CyanoSensor N1luxkm; apparently, it has not been used to detect N bioavailability in environmental samples.

Gillor et al. (2003) reported the construction of a *Synechococcus* strain bearing a fusion between the *glnA* promoter and *luxAB* which was denoted as strain *S.* GSL to detect N bioavailability; in the unicellular non-diazotrophic *Synechococcus* sp. PCC 7942, the *glnA* gene encodes a glutamine synthetase (GS) involved in the assimilation of ammonium by amidation of glutamate to yield glutamine; the global regulator NtcA regulated *glnA* expression which is severely repressed by ammonium and activated in the absence of a combined nitrogen source. Strain *S.* GSL luminescence was indeed induced in response to N deprivation and increasing ammonium concentrations progressively decreased bioluminescence (also a *lights-off* strain); the dynamic range of performance was between 1 μm

and 1 mM of ammonium; it was also found that the reporter strain also responded to nitrate and nitrite with a limit of detection (LOD) of 1 μM but also to organic compounds with LODs 10–50-fold higher. The bioreporter strain was tested with samples of the upper and lower layers of Lake Kinneret and the bioreporter response correlated well with the levels of Total Dissolved Nitrogen (TDN); so it seems that this bioreporter is non-specific and may be useful to assess total N bioavailability in aquatic environments (Gillor et al. 2003, 2010).

A different approach was used by Ivanikova et al. (2005) who constructed a luminescent cyanobacterial bioreporter using the unicellular *Synechocystis* sp. PCC 6803 based on the NtcA/B dependent nitrate/nitrite activated *nirA* promoter fused to *V. harveyi luxAB*. The fusion was integrated in the chromosome and the strain was denoted as AND100. It is a *lights-on* strain whose luminescence increases with increasing nitrate concentration and has a dynamic range of performance between 10 and 50 μM of nitrate. Although the strain might also respond to nitrite, as nitrate concentrations in most freshwater systems are considerably higher than those of nitrite, the bioreporter may be considered specific to detect nitrate bioavailability. The bioreporter has been tested in field samples from Lake Superior where it underestimated the level of nitrate; the authors amended the environmental samples with P and Fe that were apparently limiting in these samples and the bioreporter was able to detect nitrate accurately (Ivanikova et al. 2005, 2007). The above mentioned N-responsive bioreporters are all non-self-luminescent and based on unicellular cyanobacteria; recently, Muñoz-Martín et al. (2014b) constructed a battery of self-luminescent bioreporters of N bioavailability based on the filamentous diazotrophic heterocystous *Nostoc* (*Anabaena*) sp. PCC 7120 and also tested the usefulness for N detection of an alternative GS gene (*glnN*) present in the unicellular *Synechococcus* sp. PCC 7942 that encodes a class II GS strongly activated under nitrogen starvation (Aldehni and Forchhammer 2006). The three *Nostoc* (*Anabaena*) strains were named as *N.* GLA bearing the promoter of the *Nostoc glnA* gene to *P. luminescens luxCDABE*; *N.* NIR which bears a P*nirA*::*luxCDABE* fusion and *N.* GIF which bears the promoter of the *gifA* gene also fused to *luxCDABE*, the *gifA* gene encodes IF7A, a post translational regulator of GS, it is activated when there is an excess of ammonium, such that increased levels of IF7A binds to GS and inactivate it (Galmozzi et al. 2010); the *Synechococcus* strain sp. FAM431 harbors the *glnN* gene fused to *luxAB* (Aldehni and Forchhammer 2006; Leganes et al. 2009) and has been named as *S.* GLN.

The *N.* GLA and *S.* GLN bioluminescence was significantly activated in N-deplete medium and when cultures were re-supplemented with combined N sources (nitrate or ammonium), bioluminescence decreased in a dose–response fashion (*lights-off* bioreporters). Calibration curves (24 h incubation time) were calculated and the dynamic range of performance of *N.* GLA was 50–500 μM of nitrate or ammonium and for *S.* GLN was 10–500 μM of nitrate or ammonium; *S.* GLN was more sensitive as its LOD was considerably lower. *N.* NIR bioluminescence was induced in the nitrate replete medium and *N.* GIF bioluminescence activated in ammonium replete medium (both *lights-on* bioreporters). A calibration curve (6 h incubation time) was calculated for the *N.* NIR finding a dynamic range for nitrate concentrations between 10 and 100 μM. As ammonium inhibits *nir* operon expression (Cai and Wolk 1997), the bioreporter performance was studied in the absence or presence of increasing concentrations of ammonium, finding that concentrations of 125 μM and above completely inhibited bioluminescence. A calibration curve (24 h incubation time) was also calculated for the *N.* GIF strain, the dynamic range was between 100 and 600 μM of ammonium, the presence of nitrate up to 150 μM did not affect *N.* GIF

bioluminescence but the addition of 300 μM of nitrate enhanced bioluminescence so that the dynamic range shifted to 50–1000 μM of ammonium.

The bioreporters were tested with environmental samples form upstream and downstream courses of two rivers in central Spain whose concentrations of ammonium, nitrate and dissolved inorganic nitrogen (DIN; the sum of both) was determined chemically and compared to those estimated by the bioreporters. *S*. GLN detected both nitrate and ammonium (DIN) with the detected amounts very similar to the chemically determined values. The *N*. GLA strain detected also both N species but only in two samples as the others were below its LOD, The *N*. NIR strain which detects nitrate estimated quite well the amount of this N species except in samples with high ammonium concentrations as expected. The *N*. GIF strain only detected ammonium in two of the samples with the highest ammonium concentrations as the other samples had ammonium levels outside its dynamic range. When used in combination these bioreporters enabled the measurement of specific N-species as well as total N bioavailability in the wide range of concentrations present in the tested samples; besides, due to its detection range, the *N*. GIF bioreporter may be particularly useful to predict blooms in eutrophic environments. As stated for P bioreporters above, the use of both unicellular and filamentous cyanobacterial bioreporters is a powerful tool to assess the capacity of phytoplankton to assimilate various N species in aquatic ecosystems.

Cyanobacterial bioreporters responsive to heavy metals

Heavy metal responsive bioreporters are engineered to provide information on the bioavailable fraction of the metal(s) which is the fraction that may exert toxicity to the biota in the environment. The sensor element is usually the promoter of a metal(s) responsive gene fused to a promoterless reporter gene and a gene encoding a transcriptional regulator that when a certain metal(s) is present in the cell activates the promoter (Hynninen et al. 2010; Osman and Cavet 2010). Based on this concept, all the heavy metal bioluminescent bioreporters are *lights-on* as bioluminescence is induced in the presence of the metal(s) but it should be taken into account that at a certain heavy metal concentration, bioluminescence might decrease due to toxicity; it is, then, important to clearly define the dynamic range of operation of these bioreporters.

To our knowledge, the first cyanobacterial bioreporter able to detect heavy metals was constructed by Erbe et al. (1996). It was based on the *smt* locus of the unicellular *Synechococcus* sp. PCC 7942, this locus consists of the cyanobacterial metallothionein gene *smtA* and *smtB*, a divergently transcribed gene encoding the transcriptional repressor of *smtA*; binding of metal ions by SmtB induces conformational changes in it that promote RNA polymerase accessibility to *smtA* (Huckle et al. 1993; Morby et al. 1993). *smtA* transcription is induced in the presence of several metals: Zn (as preferred metal), Cd, Cu, Hg, Co and Ni (Huckle et al. 1993; Osman and Cavet 2010). The authors fused part of the *smt* locus including *smtB*, the *smt* operator/promoter region and the first codon of *smtA* to *luxCDABE* of *A. fischeri*. The strain, although self-luminescent, did not generate enough endogenous aldehyde and dodecanal was added at each sample before measurement. They tested the performance of the bioreporter in the presence of the metal salts $ZnCl_2$, $CuSO_4$ and $CdCl_2$. $ZnCl_2$ induced bioluminescence in the range 0.5–2, 4 μM $ZnCl_2$ was already toxic as luminescence began to decline; $CdCl_2$ also induced luminescence in a shorter concentration range: 0.5–1.5 μM; $CuSO_4$ was less effective in inducing luminescence than the other metal salts and toxicity was found also at higher concentrations, 15 μM. Apparently, there are no further reports in the literature concerning this particular bioreporter.

Peca et al. (2008) constructed self-luminescent novel cyanobacterial bioreporters able to detect nickel, cobalt and zinc. They fused the *coaT* promoter which is inducible by Co and Zn and the *nrsBACD* Ni responsive promoter to *luxAB* from *V. harveyi*; the constructions were transformed into a *Synechocystis* sp. PCC 6803 strain harboring the fatty acid reductase complex genes *luxCD,E* from *V. harveyi*. The constructs were integrated into the chromosome; the regulatory genes *coaR* and *nrsRS* controlling the expression of the *coaT* and *nrsBACD* genes were cloned in the same vectors. The resulting bioreporters were denoted as *coaLux* and *nrsLux* strains.

The bioreporters were tested for specificity with a battery of metal salts with an incubation time of 3 h. Form the tested metals, only Zn and Co significantly induced the bioluminescence of the *coaLux* strain while the *nrsLux* strain was specific to Ni. The dynamic detection range for Co of the *coaLux* was 0.3–6 and 1–3 μM for Zn; the *nrsLux* strain detected Ni in the range 0.2–6 μM. When the performance of the bioreporters was tested in darkness, the detection range of the *coaLux* strain for Co and Zn increased to 26 and 13 μM, respectively; the *nrsLux* strain showed no detectable luminescence. The *coaLux* bioreporter was tested for quantification of bioavailable Zn in soil samples; the concentration of Zn estimated by the bioreporter represented about 90 % of the atomic absorption spectroscopy measured Zn concentrations indicating a high bioavailability of the metal.

Aequorin-based cyanobacterial bioreporters

Cyanobacteria are ancient organisms that date from the Precambrian era and during the course of evolution have developed signal transduction systems to sense and respond to changes in their environment, including anthropogenic pollution. Free Ca^{2+} is a common intracellular messenger in eukaryotes (Berridge et al. 2000, 2003; Clapham 1995) and reported evidences indicate that it also has this role in prokaryotes (Dominguez 2004). Increases in intracellular free Ca^{2+} have been shown to be induced in response to a wide range of abiotic and biotic stimuli; in fact, there are solid evidences that indicate that the specificity of the Ca^{2+} response is encoded in the recorded Ca^{2+} signal in response to the stimulus, the so-called Ca^{2+} signature, according to the amplitude, duration, frequency, rise time, time to return to basal Ca^{2+} levels, source and intracellular location of the Ca^{2+} signal (Kudla et al. 2010; Whalley and Knight 2013).

The photoprotein apoaequorin is a sensitive Ca^{2+} indicator whose gene has been cloned form the jellyfish *Aequorea victoria* which has been expressed in a number of cell systems, both prokaryotic and eukaryotic, to measure Ca^{2+} signals in response to a variety of environmental stresses (Dominguez 2004; Torrecilla et al. 2000; Whalley and Knight 2013). Apoaequorin can be fully reconstituted to aequorin by the addition of the hydrophobic luminophore coelenterazine. In Fig. 4a the reaction of this protein with Ca^{2+} is depicted; as shown, once Ca^{2+} ions are bound to aequorin, the photoprotein catalyzes the oxidation of coelenterazine by oxygen resulting in blue light luminescence; Fig. 4 also shows some relevant parameters taken into account to define the specificity of the Ca^{2+} signatures (Fig. 4b).

Torrecilla et al. (2000) were the first to clone and express apoaequorin in a cyanobacterium, the filamentous *Anabaena* (*Nostoc*) sp. PCC 7120, denoted as *Anabaena* pBG2001a, which enables continuous and in vivo monitoring of intracellular free Ca^{2+} concentrations. The strain has been used to record and analyze Ca^{2+} signatures in response to a wide spectrum of environmental stimuli (Torrecilla et al. 2000, 2004a, b). The same group has also expressed apoaequorin in the unicellular *Synechococcus* sp. PCC 7942 under the control of the promoter of the *petJ* gene (encoding cytochrome c553) and so far, it has been used to record Ca^{2+} signatures in response to N deprivation (Leganes et al. 2009).

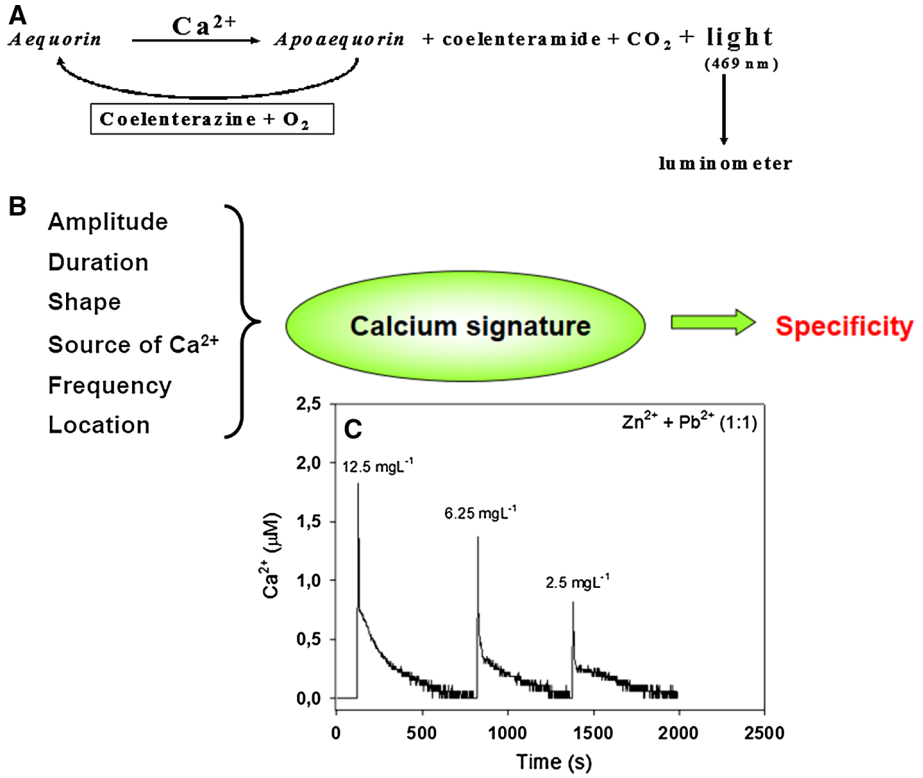

Fig. 4 **a** Aequorin reaction showing the emission of bioluminescence. **b** Relevant parameters that encode the specificity of the recorded Ca^{2+} signatures in response to environmental stimuli or pollution. **c** Ca^{2+} signature induced in *Anabaena* pBG2001a in response to binary mixtures of Zn^{2+} + Pb^{2+} at constant ratio (1:1)

It is known that many pollutants interfere with Ca^{2+} homeostasis in eukaryotic organisms (Kozlova et al. 2005; Ogunbayo et al. 2008; Ohta and Suzuki 2007; Wang et al. 2007); however such studies were lacking in prokaryotes. Barran-Berdon et al. (2011) used the apoaequorin expressing *Anabaena* (*Nostoc*) pBG2001a strain to systematically record and analyze the Ca^{2+} signatures elicited by a variety of environmental pollutants: cationic and anionic heavy metals, the metalloid As, naphthalene, organic solvents (acetone, ethanol, toluene) and pharmaceuticals such as lipid regulators (fibrates) and antibiotics (fluoroquinolones); also the Ca^{2+} signatures induced by binary mixtures of some of these pollutants and the signature induced by a real wastewater sample which could be mimicked by mixing it main constituents at environmental concentrations were recorded. The results indicated that all the tested pollutants elicited a fast and specific Ca^{2+} signature which was highly reproducible and dose-dependent; the Ca^{2+} signatures were previous to toxicity as measured by the *lights-off* bioreporter *Anabaena* CPB4337. The Ca^{2+} signatures induced by binary mixtures of pollutants could predict the nature of pollutants interactions (Fig. 4c). It was concluded that intracellular free Ca^{2+} signatures elicited by pollutants could serve as an early biomarker of exposure to environmental pollution.

Table 2 provides a summary of main features of cyanobacterial bioreporters described to date.

Table 2 Main features of cyanobacterial bioreporters

Cyanobacterium (name of strain if indicated)	Promoter-reporter fusion	Type of bioreporter	Targets and dynamic range of detection (if indicated)	References
Synechocystis sp. PCC 6803	P*tac-luc-luxAB*	Lights-off	General toxicity bioreporter. Tested with herbicides, Cu, Zn and 3,5DCP. Not tested in environmental samples	Shao et al. (2002)
Anabaena (*Nostoc*) sp. PCC 7120 (*Anabaena* CPB4337)	*Non-identified. Constitutive P-luxCDABE*	Lights-off	General toxicity bioreporter. Tested with heavy metals, organic solvents, pharmaceuticals, perfluorinated surfactants and nanomaterials. Tested with pollutants mixtures and in environmental matrices of different complexity	Fernández-Piñas and Wolk (1994), Rodea-Palomares et al. (2009), Rosal et al. (2010a, b) Rodea-Palomares et al. (2010, 2011, 2012), Garcia et al. (2013) and González-Pleiter et al. (2013)
Synechococcus sp. PCC 7942 (*S.* KAS101)	P*isiAB-luxAB*	Lights-off	Fe^{3+}; $10^{-21.1}$–$10^{-20.6}$ M. Tested with environmental samples	Durham et al. (2002), McKay et al. (2005), Porta et al. (2003, 2005) and Hassler et al. (2008, 2009)
Synechococcus sp. PCC 7002 (*S.* BM004 or *S.* CCMP 2669)	P*isiAB-luxAB*	Lights-off	Fe^{3+}; $10^{-22.4}$–$10^{-19.4}$ M. Tested with environmental samples	Boyanapalli et al. (2007)
Synechocystis sp. PCC 6803 (*S.* MpILisi)	P*isiAB-luxAB*	Lights-off	Fe^{3+}. Not tested with environmental samples	Kunert et al. (2000)
Anabaena (*Nostoc*) sp. PCC 7120	Palr0397-*luxAB*	Lights-off	Fe^{3+}; $10^{-21.5}$–$10^{-19.6}$ M. Tested with environmental samples	Zha et al. (2012)
Synechococcus sp. PCC 7942 (*S.* APL)	P*phoA-luxAB*	Lights-off	DIP (PO_4^{3-}) and DOP (ADP; D-fructose-6-phosphate; D-glucose-6-phosphate; ρ-nitrophenol-phosphate). Tested with environmental samples	Schreiter et al. (2001) and Gillor et al. (2002, 2010)
Anabaena (*Nostoc*) sp. PCC 7120 (*A.* APL-L)	P*phoAlike-luxCDABE*	Lights-off	DIP (PO_4^{3-}) and DOP (ADP; D-glucose-6-phosphate). 1–57 μM PO_4^{3-}. Tested with environmental samples	Muñoz-Martin et al. (2011, 2014a)
Anabaena (*Nostoc*) sp. PCC 7120 (A. PST)	P*pst-luxCDABE*	Lights-off	DIP (PO_4^{3-}) and DOP (ADP; D-glucose-6-phosphate). 0.5–57 μM PO_4^{3-}. Tested with environmental samples	Muñoz-Martín et al. (2011)

Table 2 continued

Cyanobacterium (name of strain if indicated)	Promoter-reporter fusion	Type of bioreporter	Targets and dynamic range of detection (if indicated)	References
Synechocystis sp. PCC 6803 (*S.* N1luxkm)	P*nblA1-luxAB*	Lights-off	4–100 μM nitrate; it also responds to ammonium. Not tested with environmental samples	Mbeunkui et al. (2002)
Synechococcus sp. PCC 7942 (*S.* GSL)	P*glnA-luxAB*	Lights-off	Ammonium, nitrate and nitrite. 1 μM to 1 mM ammonium. Nitrate and nitrite with LOD of 1 μM. Organic N compounds with LODs 10–50-fold higher. Tested with environmental samples	Gillor et al. (2003, 2010)
Synechocystis sp. PCC 6803 (*S.* AND100)	P*nirA-luxAB*	Lights-on	10–50 μM nitrate. Tested with environmental samples	Ivanikova et al. (2005, 2007)
Nostoc (*Anabaena*) sp. PCC 7120 (*N.* GLA)	P*glnA-luxCDABE*	Lights-off	50–500 μM nitrate or ammonium. Tested with environmental samples	Muñoz-Martín et al. (2014b)
Nostoc (*Anabaena*) sp. PCC 7120 (*N.* NIR)	P*nir-luxCDABE*	Lights-on	10–100 μM nitrate. Concentrations of 125 μM ammonium and above inhibit bioluminescence. Tested with environmental samples	Muñoz-Martín et al. (2014b)
Nostoc (*Anabaena*) sp. PCC 7120 (*N.* GIF)	P*gifA-luxCDABE*	Lights-on	100–600 μM ammonium. The addition of 300 μM nitrate shifted the dynamic range of detection of ammonium to 50–1000 μM. Tested with environmental samples	Muñoz-Martín et al. (2014b)
Synechococcus sp. PCC 7942 (*S.* GLN)	P*glnN-luxAB*	Lights-off	10–500 μM nitrate or ammonium. Tested with environmental samples	Muñoz-Martín et al. (2014b)
Synechococcus sp. PCC 7942	*smtB*-P*smtBA-luxCDABE*	Lights-on	0.5–2 μM $ZnCl_2$ 0.5–1.5 μM $CdCl_2$ $CuSO_4$ lees effective as inducer. Not tested with environmental samples	Erbe et al. (1996)
Synechocystis sp. PCC 6803 (S. *coaLux*)	*coaR*-P*coaT-luxAB* transformed in a *luxCD,E* *S.* expressing strain	Lights-on	0.3–6 μM Co 1–3 μM Zn. In darkness, the detection range for Zn increased to 13 μM and to 26 μM for Co. Tested with environmental samples	Peca et al. (2008)
Synechocystis sp. PCC 6803 (S. *nrsLux*)	*nrsRS*-P*nrsBACD-luxAB* transformed in a *luxCD,E* *S.* expressing strain	Lights-on	0.2–6 μM Ni. Not tested with environmental samples	Peca et al. (2008)

Table 2 continued

Cyanobacterium (name of strain if indicated)	Promoter-reporter fusion	Type of bioreporter	Targets and dynamic range of detection (if indicated)	References
Anabaena (*Nostoc*) sp. PCC7120 (*A.* pBG2001a)	Apoaequorin gene expressed in an *Anabaena* (*Nostoc*) replicative plasmid (pDU1 replicon)	Lights-on	Specific Ca^{2+} signatures in response to a variety of stimuli/pollutants. Tested with environmental samples	Torrecilla et al. (2000, 2004a, b) and Barran-Berdon et al. (2011)

Concluding remarks

Cyanobacteria are ecologically relevant organisms, ubiquitous in aquatic and terrestrial environments; as primary producers they are a fundamental component of trophic webs and have a crucial role in the N and C cycles. Biological methods based on benthic cyanobacteria to assess environmental contamination in running waters include different levels of organization, such as cellular, populations and communities, with the corresponding use of the changes in morphological and/or physiological characteristics, use of specific autecological data of cyanobacterial taxa, with a specific value of bioindicators populations, and/or shifts in the community structure, as indicator of nutrient status. Cyanobacterial identification performed by light microscopy, while remains commonplace, requires trained taxonomists with the ability to distinguish differences between taxa or morphological variations. Therefore, lately, molecular methods, which can obviate many of these difficulties, are being developed and used to analyse changes in the communities in relation to variations in water quality. New technology referred to as 'NGS', which leads to the determination of millions of DNA sequences in a single process, has the potential to further extend the application of DNA information for routine biomonitoring applications to an unprecedented scale.

Regarding cyanobacteria as bioreporters, they offer a low cost, low maintenance alternative to heterotrophic bacterial bioreporters. Due to their role in nutrient cycling and in the formation of algal blooms under eutrophic conditions in water reservoirs, the majority of cyanobacterial bioreporters constructed to date have focused on the detection of Fe, N and P chemical species; many of these bioreporters have also been validated with actual environmental samples; however, regarding other sources of pollution, cyanobacteria have been very little exploited as bioreporters: only two cyanobacterial strains have been constructed and used for general ecotoxicity testing but only one of them, *Anabaena* CPB4337 has been tested in environmentally realistic scenarios with real samples and complex mixtures of pollutants; also an *Anabaena* strain which allows in vivo monitoring of intracellular calcium levels and which is responsive to a broad range of pollutants has been constructed. With respect to specific pollutants, only two cyanobacterial bioreporters have been constructed able to detect heavy metals and none to detect specific organic pollutants. In this regard, there is much to be done in the field of cyanobacterial bioreporters, they could serve as hosts for sensing elements from other bacteria, new reporter systems should be evaluated (most of them are bioluminescent, with fluorescence/other reporter elements seldom used) and also, given the fact that over 100 cyanobacterial

genomes have been sequenced, novel genetic elements responsive to pollutants could be identified and used to construct new and useful bioreporters.

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