The Handbook of Environmental Chemistry 43

Series Editors: Damià Barceló · Andrey G. Kostianoy

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Antoni Ginebreda
Narcís Prat *Editors*

Experiences from Ground, Coastal and Transitional Water Quality Monitoring

The EU Water Framework Directive Implementation in the Catalan River Basin District (Part II)

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The Handbook of Environmental Chemistry

Founded by Otto Hutzinger

Editors-in-Chief: Damià Barceló • Andrey G. Kostianoy

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Aims and Scope

Since 1980, *The Handbook of Environmental Chemistry* has provided sound and solid knowledge about environmental topics from a chemical perspective. Presenting a wide spectrum of viewpoints and approaches, the series now covers topics such as local and global changes of natural environment and climate; anthropogenic impact on the environment; water, air and soil pollution; remediation and waste characterization; environmental contaminants; biogeochemistry; geo-ecology; chemical reactions and processes; chemical and biological transformations as well as physical transport of chemicals in the environment; or environmental modeling. A particular focus of the series lies on methodological advances in environmental analytical chemistry.

Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last three decades, as reflected in the more than 70 volumes of *The Handbook of Environmental Chemistry,* there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry,* the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of "pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Editors-in-Chief

Foreword

Environmental quality is going to be a crucial issue for the people in charge of public affairs in the next years. To manage an environment where air, water, and soil should be in good conditions is not only an objective but a compulsory requirement in terms of well-being and of public health.

From older times water management has been a very important issue, but recently, water managers have had to cope with new challenges arising from social demands mainly focused on ecological improvement. Flowing water in rivers, lakes, estuaries, coastal waters, or reservoirs is not only regarded as resource but as a key element for sustaining aquatic ecosystems and services they provide. Good ecological status meets services and goods sustaining human well-being as well as suitable freshwater quality for safety human uses. To take into account aquatic ecosystems, preservation requires building stronger linkages between ecological, economic, and social demands with the purpose of improving water management. This framework offers the most promising way forward for the field of conservation together with a suitable human development.

Nevertheless, this challenge requires changes. Thus, in the legal side, new laws, directives, etc., are needed and institutional changes and new administrative models (development of new agencies, water authorities) are necessary. On the other side, developing new monitoring programs in order to provide suitable and enough information on water status under an ecological integrative perspective is required. Also, water management plans should be developed which encompass a comprehensive water management combining sustainable human use together with good ecological status, economic sustainability (cost recovery strategies), and social participation. Moreover, climate change should be also considered which demonstrates the scope and complexity of this challenge.

The above mentioned target makes necessary the development of new monitoring tools for water quality assessment adapted to water ecosystem types and new quality elements must be measured. Therefore, there has been a rapid increase in the development and application of ecological indicators for water quality assessment and management in developed countries. For instance, the United States, Canada, Europe, and Australia have been developing new water monitoring programs based on biological and ecological indicators for water management purposes and planning. In the European Union (EU), the Water Framework Directive (WFD) (2000/60/EC) launched in 2000 a new framework for the protection of groundwater and inland and coastal waters. The WFD represents an opportunity for a new water resource management in Europe based on ecological and economical sustainability, with the requirement of a wide social involvement. The WFD was an important conceptual change of the way that EU Member States (MS) should consider water management by putting ecosystem integrity at the base of management decisions. Since then, all MS expended considerable time and resources to collect appropriate biological, environmental, and human pressure data to develop operative tools aiming at elaborating new monitoring programs and innovative river basin management plans. As the magnitude and difficulties of this large-scale endeavor became evident, both the European community and individual MS have funded a large number of research projects, particularly in the areas of ecological assessment for water management, to develop and improve the expert knowledge. The WFD was relevant for its innovativeness and the shift towards measuring the status of all surface and coastal waters using a range of biological communities rather than the more limited aspects applied so far.

In Catalonia, the government has been deeply involved on all this process and has been implementing the WFD soon after it was adopted. Hence the administrative institution especially devoted to water management, the Catalan Water Agency (ACA), was created in 2000. ACA is in charge of planning and carrying out water management strategies in Catalonia, taking into account both water demands and environmental protection. The ACA is nowadays in charge of building and maintaining urban wastewater treatment plans, water supply management, flooding protection plans, etc. Moreover, it has been monitoring all aquatic ecosystems, including inland and coastal waters and groundwater relationship, and has been developing new tools to ensure ecological and chemical status measurements in surface waters and chemical and quantitative status in groundwater, in accordance with the WFD requirements. Additionally, some research institutes have also been promoted mainly focused on water management. An example of this is the Catalan Institute for Water Research (ICRA), that focuses its research lines in the integral water cycle, hydraulic resources, water quality (in the broadest sense of the term: chemical, microbiological, ecological, etc.), and treatment and evaluation technologies. The research carried out at the ICRA has to do with all the aspects related with water, particularly those associated with its rational use and the effects of human activity on hydraulic resources.

Over the last decades, it has been necessary to monitor and to assess the ecological status of water bodies following the WFD guidelines. Accordingly, the ACA started a close science to policy relationship with research institutions, which have been closely involved in such development. From this collaboration novel methodologies have been proposed, and a huge amount of data has been gathered over more than a decade. Overall, this cooperation has proved to be a stimulating and fertile ground for research of the interface between science and management.

Accordingly, the Catalan Water Agency (ACA) established a new monitoring program in order to provide a proper water status diagnosis just before the water management plan's updating in the Catalan River Basin District. The ACA has now a global picture of the ecological and chemical status of all water bodies in Catalonia. The experience gained by the Agency over the last 15 years has been incorporated in these two different book volumes that I have the privilege to introduce in this preface: *Experiences from Surface Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I)* and *Experiences from Ground, Coastal and Transitional Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part II)*. Both books summarize all the findings on water monitoring for WFD purposes, and they discuss further perspectives according to the new knowledge obtained. They are devoted to such effort which has resulted in a series of protocols adapted to the aquatic ecosystem monitoring in Catalonia. Both books encompass several specific chapters focused on different aquatic systems (rivers, lakes, wetlands, reservoirs, estuaries, bays, coastal waters, and groundwater) and are written by several researchers in close collaboration with ACA's technicians. They provide good examples and suitable monitoring tools for aquatic ecosystem monitoring in Catalonia that can also be easily extrapolated to other Mediterranean river basin districts. Data analyzed and information obtained are not only useful in understanding the current quality status but also gathering the necessary knowledge to design the best tools for aquatic ecosystem management and restoration and/or conservation measures adapted to each aquatic ecosystem type, paying special attention to Mediterranean conditions which deeply affect water management in southern Europe. At that time, just to end I can say that we are proud of the work done by our community of experts in water management working in public administrations, in research centers, and in private companies. I hope that the materials and experiences enclosed in the two volumes reflect a step forward of a better management of water and stimulate new developments for the future.

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Volume Preface

Freshwater systems in Europe are threatened by a variety of stressors (chemical pollution, geomorphological alterations, changes in land uses, climate variability and change, water abstraction, invasive species, and pathogens). Chemical aquatic pollution today comprises a wide range of emerging chemical substances, such as pharmaceuticals, personal care products, or pesticides, among others. Stressors are of diverse nature but cause adverse effects on biological communities and ecosystems. It is well known that the relationship between multiple stressors might determine changes in the chemical and ecological status, which are the key objectives of the European Union Water Framework Directive (WFD). This important piece of legislation has pushed the EU River Basin Authorities to carry out advanced monitoring programs in collaboration with universities and research centers.

These two volumes of *The Handbook of Environmental Chemistry* we introduce here (Volume I: *Experiences from Surface Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I)* and Volume II: *Experiences from Ground, Coastal and Transitional Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part II)*) correspond to an excellent collaborative example between the River Basin Authority from the Catalan River Basin District (NE Spain), the so-called Catalan Water Agency (ACA), with the Catalan Universities and Research Centers. These books cover the main research outcomes achieved during the last 10 years following WFD implementation. It contains a total of 26 chapters and over 75 authors who explain how, from the interaction between the ACA and several academic centers, the different quality elements included in the WFD have been adapted to Mediterranean aquatic ecosystems. We want to remark the importance of this interaction between the members of the ACA and the members of academia or experts in a collaborative effort that probably is unique in the WFD implementation in Europe.

Why ACA has developed such collaborative effort? First of all because for most of the biological elements, no or few experience in how to use such elements existed in Spain Water authorities. ACA had more experience in the analysis of chemical parameters, i.e., priority substances. Second, the methods to be used by WFD guidelines should be inter-calibrated; therefore ACA was aware that a set of methodologies with a robust scientific background was needed, so their results could be compared to other European countries. Third, most of the streams in Catalonia are in a Mediterranean climate area, and for this reason, taxa present in aquatic ecosystems and their environmental constraints are different from those of more temperate ecosystems from Europe. Scientifically robust methodologies should be adopted by ACA to explain why our aquatic ecosystems are different and how these differences affect the way in which the water quality is measured.

The ACA has easily found the way to build up from the scientific knowledge the tools needed by the administration to measure the status of the water. Catalonia has a long tradition on water quality studies which is grounded in the shoulders of several Masters and Commanders of Science. We think that at least two of them should be quoted: the former professors of the University of Barcelona Ramón Margalef and Enric Casassas. Margalef was a well-known ecologist and the first professor of Ecology in Spain, and Cassassas was the introductor of modern analytical techniques in Spain. In a postwar situation, after Spanish civil war (1936–1939) and the second world war (1939–1945), scientific research in Spain was very poor and many times under scientifically unreliable people. The late professors Ramon and Enric were extremely clever and open-minded people, and despite many obstacles, they found a way to put the roots of what now is one of the best schools of aquatic studies in Europe. Both were excellent professors and researchers and generous people with new ideas and solutions. Certainly they were an example of scientists with a global vision but with a local action, with a real compromise with their homeland, Catalonia. This school has produced an array of young scientists (not so young anymore) that have studied in-depth many aspects of ecology or chemistry in freshwater systems with a deep vision on the Mediterranean water bodies. At the same time, most of these students formed many other students and these to other, so the first grand-grand-children are at this moment at the front line of water quality research studies. Other masters exist also in Catalonia in hydrogeology, microbiology, or fish ecology, that several of the authors of this book have taken advantage.

Thanks to the effort of Margalef, Cassasas, and others and his students; when ACA started to think what to do for the implementation of the WFD, most of the fundamentals for such work were there. But in many cases the scientific research is not applied for the administration because the two worlds are hardly in contact. The merit to understand that such relationship is necessary should be given to some of the directors of the ACA and some of the ministers of the environment of the regional government of Catalonia who recognized the importance of such collaboration. It was of help too that some of the disciples who did their Ph.D. with students of the two masters already mentioned took a position in ACA. These people are now coeditors, with Prof. Prat, of these two books: Antoni Munné and Antoni Ginebreda. Both are Ph.D. from Catalan universities and understand that without the collaboration of scientist and managers, it is almost impossible to produce enough robust tools to be compared with other well-known tools developed elsewhere. We, the scientists, should be very aware of the role of these two people because without their effort these two books could never be produced.

We hope that this book will be of much interest for many international readers too. We think that it will be a useful guide for other European river basins, as well as in other parts of the world, as a good example of the added value of collaborative research on aquatic sciences. Indeed the books contain a comprehensive list of monitoring programs of importance for WFD implementation to the Mediterranean climate aquatic ecosystems. The literature references of the different chapters contain great amount of work produced by these numerous groups of academics and managers working and publishing together in the most relevant journals of ecology, fishes, microbiology, analytical chemistry, etc. We thank all of them for their time spent writing all the different chapters and making these books unique in this series.

We, as the most senior authors and former students of Margalef and Cassasas, are very proud of this work. We thank very much the ACA and the government of Catalonia for continuously supporting such work. We encourage as well, even under the present economic difficulties, to maintain such effort. It is obvious that new methodologies and tools will need to be incorporated to monitor programs in the future. We believe that the best way to do it is by establishing bridges of collaboration between scientist and managers.

Barcelona, Spain Narcís Prat and Damià Barceló

Contents

Specific Viruses Present in Polluted Groundwater Are Indicative of the Source of Nitrates and Faecal Contamination in Agricultural Areas

Sílvia Bofill-Mas, Marta Rusiñol, Josep Fraile, Teresa Garrido, Antoni Munné, and Rosina Girones

Abstract Microbial source tracking (MST) tools are used to identify sources of faecal pollution to accurately assess public health risks and implement best management practices. Many different viruses are excreted by humans and animals and are frequently detected in water contaminated with faeces or/and urine. Because of the large degree of host specificity of each virus and the substantial stability of many excreted viruses in the environment, some viral groups are considered to be accurate MST indicators. The Laboratory of Virus Contaminants of Water and Food at the University of Barcelona has proposed the use of viral indicators as well as cost-effective methods for the concentration of viruses from water. The developed procedures have been used to determine the levels of faecal pollution in environmental samples as well as for tracing the origin of faecal contamination. Such tools were recently used by the Catalan Water Agency to identify nitrate contamination sources in groundwater.

Human adenoviruses, human polyomavirus JC, porcine adenoviruses, bovine polyomaviruses, chicken/turkey parvoviruses, and ovine polyomaviruses can be quantified in samples using molecular methods (qPCR). The selected DNA viruses specifically infect their hosts and are persistently excreted in faeces and/or urine throughout the year in all geographical areas studied. The procedures that have been developed to quantify these viruses have been applied to bathing, coastal, surface and groundwater. In this study, the source of nitrate contamination in groundwater was identified by analysing viral markers, thereby demonstrating the usefulness of

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the selected viruses for the identification of sources of contamination in water. This methodology can be used to provide information to guide the proper application of measures in place to protect water from pollution caused by nitrates from several sources and thus to facilitate the accurate application of the 91/676/EEC Directive, which is mainly focused on agricultural sources of water contamination.

Keywords Adenovirus, Faecal contamination, Microbial source tracking, Nitrate contamination, Polyomavirus, Viral markers

Contents

Abbreviations

ACA Catalan Water Agency (in Catalan)
CRBD Catalan River Basin District
WFD Water Framework Directive

1 Faecal Contamination in Groundwater

Humans, as well as farmed animals, play an important role in the microbial contamination of water, crops and food and introduce large quantities of pathogens into the environment through their excretions.

Although most pathogens could be removed if sewage, manure and slurry were appropriately treated, many are discharged into receiving waters or may be disposed of in biosolids on land. Pollutants enter the water environment from two main types of sources: point sources, which are single and identifiable sources of contamination, and nonpoint sources, which are more diffuse sources of contamination. Nonpoint sources of contamination may release pollutants intermittently and may be attributable to infiltration from farmland treated with pesticides and fertilisers. Examples of point sources are landfills, leaking gasoline storage tanks, leaking septic tanks and accidental spills. Both point and nonpoint sources of contamination may affect groundwater, and several waterborne disease outbreaks that are believed to have had viral aetiologies have been attributed to the consumption of polluted groundwater [1, 2]. Viruses (23–80 nm) are much smaller than bacteria (0.5–3 μm) and protozoa (4–15 μm) and thus move more easily through soil pores. They are highly stable at low temperatures in the darkness and survive for long periods in groundwater environments. However, relatively limited data on the level of viral contamination in groundwater are available compared with other environmental water matrices [3].

Detailed knowledge about sources of contamination is needed to develop efficient and cost-effective waste management strategies to minimise faecal contamination in watersheds and food, to evaluate the effectiveness of best management practices and to conduct system and risk assessments as part of water- and food-safety plans, as recommended by the World Health Organisation. Faecal sources of contamination have high nitrogen content, and both pathogens and nitrates present in groundwater polluted with faeces may pose a risk to human health when such groundwater is used as a source of drinking water.

Nitrate is the most widespread groundwater quality problem in many countries, and it is the most frequent cause of a groundwater body failing to meet good status under the WFD in some EU countries (http://ec.europa.eu/environment/water/water-nitrates/reports.html), including Catalonia [4]. The principal nitrogen inputs into groundwater are derived from manure, fertilisers, sewage sludge and crop residues from agricultural areas [5]. In the environment, several forms of nitrogen (NO_2, NH_4, NH_3) can potentially be transformed into nitrate (NO_3). Various activities may cause nitrate groundwater pollution in agricultural areas. The use of synthetic nitrogen fertilisers as well as the use of organic fertilisers, such as manure and slurries, is the main cause of this pollution. In some areas, high levels of nitrates in groundwater used as a source of drinking water are a consequence of the increase in livestock production that has occurred in recent years. Moreover, an absence of slurry, manure tanks or storage facilities may also contribute to this problem. The disposal of municipal or industrial effluents by spreading sludge on fields may also be a diffuse source of nitrate pollution in groundwater.

Other sources of nitrate pollution in groundwater include the following: interactions between groundwater and surface water, nitrogen-rich effluents, poorly constructed wells that allow water to be exchanged between polluted and nonpolluted aquifer layers, old and badly designed landfills, septic tanks and leaking sewerage systems (http://www.who.int/water_sanitation_health/dwq/chemicals/en/nitrateschap1.pdf).

The intensification of livestock production results in an increase in the amount of animal waste that must be managed. Catalonia, with a population of nearly 7.5 million people, has an important meat industry, with 6.8 million pigs, 0.5 million cattle and 0.6 million sheep [6]. A total of 19 out of 53 (36%) groundwater bodies in Catalonia have been classified as being of poor chemical quality as a result of high nitrate levels. Most of the affected groundwater bodies are located in agricultural areas, although not all stresses on groundwater result from agricultural activities. In some cases, urban wastewater leakage may also contribute to this problem. However, to date, agricultural sources and manure applications on fields, in particular, have been the main causes of pressure on groundwater. Together with nitrogen compounds, faecal microorganisms are released into the environment in manure in holding ponds or storage areas or are applied to pastures to fertilise crops. Most livestock manure is disposed of on the ground, depending on the crop type, and annual quantities of nitrogen that are applied per hectare are specifically restricted in vulnerable areas [7, 8]. However, microorganisms and especially viruses can still, in some cases, infiltrate groundwater. The survival, fate and transport properties of viruses in the environment vary based on the type of virus, viral inactivation kinetics at high temperatures, UV exposure, filtration or adsorption in porous media or sediments and deposition and resuspension of sediments [9, 10].Survival is likely shorter in surface water than in groundwater because of UV exposure, higher temperatures (depending on the time of year and the location) and the opportunity for more interactions with other organisms that can inactivate viruses [11] in superficial water. Tracing and identifying the sources (human and/or animal) of faecal contamination in water are therefore essential, both to improve waste management and to assess risks to human health.

2 Development of Microbial Source Tracking (MST) Techniques

Faecal pollution is a primary health concern in the environment, in water and in food; for this reason, bacterial faecal indicators have been analysed widely to assess the microbiological quality of water, and such assessments are required by water safety regulations. The use of index microorganisms, whose presence points to the possible occurrence of a similar pathogenic organism, and indicator microorganisms, whose presence represents a failure affecting the final product, to assess the microbiological quality of water or food is well-established and has been practised for almost a century.

Classic microbiological indicators, such as faecal coliform bacteria, *Escherichia coli* and enterococci, are most commonly analysed to evaluate the level of faecal contamination. However, whether these bacteria are suitable indicators of the occurrence and concentration of pathogens such as viruses and protozoan cysts has been questioned for the following reasons: (1) indicator bacteria are more sensitive to inactivation by treatment processes and sunlight than are viral or protozoan pathogens; (2) indicator bacteria may not originate exclusively in faecal sources; (3) indicator bacteria may have an ability to multiply in some environments of interest; (4) it may not be possible to identify the source of faecal contamination; and (5) the presence of indicator bacteria may be poorly correlated with the presence of other pathogens. Thus, various authors have concluded that these indicators could fail to predict the risk of contamination with waterborne pathogens, including viruses [12–16]. Therefore, the team at the Laboratory of Virus Contaminants of Water and Food at the University of Barcelona has proposed that quantitative tests of specific viruses be used as complementary indicators of faecal contamination in water.

Methods for detecting and identifying the source of faecal pollution in the environment are known as microbial source tracking (MST) tools [17, 18]. These methods mainly focus on detecting a microorganism that is intrinsically related to faeces and that thus indicates the presence of contamination and hence of potentially excreted pathogens, such as bacteria, viruses and parasites. MST can assist health and environmental agencies with the identification of sources of faecal contamination. MST tools can also be employed to help make decisions related to the management of drinking water sources, shellfish-growing waters and recreational waters.

A large body of work has been developed in the MST field over the past several years. The first reviews listing the available methods for identifying indicators of faecal pollution in water were published in 2002 [19, 20]. Three years later, the US Environmental Protection Agency published the first guide document [21], and since then, several authors have published newer methods and have compared their applicability with existing methods [18, 22–26]. MST tools can be classified into several broad categories: genotypic versus phenotypic analyses of either cultivated target organisms or indicators or cultivation-independent approaches in which samples from the environment are analysed directly.

Some of the MST methods proposed in the literature lack environmental stability, host specificity and/or global prevalence. Moreover, some MST methods are laborious; they require large and suitable databases for each context and good statistical tools to allow meaningful interpretation of the results [18]. These limitations can be overcome using molecular methods to detect and quantify host-specific viral faecal indicators in water and food. Molecular techniques, specifically nucleic-acid amplification-based assays, provide sensitive, rapid and quantitative analytical tools for studying pathogens, newly emergent strains and indicators that are examined for microbial source tracking. Such methods are used to evaluate the microbiological quality of water [27], the efficiency of virus removal in drinking water and wastewater treatment plants [28–30].

3 Viruses Used for Tracing the Sources of Contamination in Water

Considering the limitations of current standard bacterial faecal indicators, selected viral groups have been proposed as alternative or complementary indicators to improve control of the microbiological quality of water and to reduce microbiological risk. Viruses are more stable than common bacterial indicators in the environment and are usually highly host-specific; because they are host-specific, their detection helps to trace the origin of faecal contamination. The viruses most commonly used for MST to detect faecal pollution are bacteriophages and DNA viruses (Table 1).

These viruses are recognised as important waterborne pathogens that are present in faeces, and new viruses that produce both symptomatic and asymptomatic infections are currently being described by metagenomic techniques [60]. Many orally transmitted viruses produce subclinical infections, and symptoms due to

Table 1 Summary of the proposed viral MST tools for the detection of human and animal faecal contamination

Host	Viral MST tools	Genome	References
Human	Bacteriophage RNA F-specific (FRNAPH)	RNA	[31–37]
	Bacteriophage of *B. fragilis* spp.	dsDNA	[38–40]
	Adenovirus (HAdV)	dsDNA	[41–44]
	Polyomavirus (JCPyV, BKPyV)	dsDNAc	[44, 45]
	Enterovirus (HEV)	ssRNA	[46, 47]
	Tobamovirus (PMMoV)	ssRNA	[48]
Cattle	Bacteriophage RNA F-specific	RNA	[33, 34, 36]
	Adenovirus (BAdV)	dsDNA	[49, 50]
	Polyomavirus (BPyV)	dsDNAc	[50–52]
	Enterovirus (BEV-2)	ssRNA	[46, 53, 54]
Swine	Adenovirus (PAdV)	dsDNA	[49, 55]
	Circovirus (PCV2)	ssDNAc	[56]
	Teschovirus (PTV)		[53, 57]
Sheep	Polyomavirus (OPyV)	dsDNAc	[58]
Avian	Parvovirus (Ch/TyPV)	dsDNA	[59]

dsDNA double-strand DNA, *ssDNA* single-strand DNA, *dsDNA*/*ssDNAc* double- or single-strand circular DNA

these viruses are only observed in a small proportion of the population. However, some viruses may give rise to life-threatening conditions, such as acute hepatitis in adults, as well as severe gastroenteritis in small children and the elderly. Some of the most important faecal viral pathogens are noroviruses, enteroviruses, adenoviruses, rotaviruses and the hepatitis A and E viruses. Human and animal viruses, such as adenoviruses [41, 42, 52], polyomaviruses [44, 55, 61] and parvoviruses [59], are frequently asymptomatic in immunocompetent hosts and often cause persistent infections. Moreover, they are highly host-specific, highly stable in the environment and resistant to disinfection [42, 62, 63]. Thus, the identification and quantification of specific viruses using molecular assays can be used for MST [42, 44].

3.1 Adenovirus

The *Adenoviridae* family has a double-stranded DNA genome of approximately 35,000 base pairs (bp) surrounded by a 90–100 nm, non-enveloped, icosahedral capsid with fibrelike projections from each vertex. Adenovirus infection may be caused by consumption of contaminated water or food or by inhalation of aerosols from contaminated waters, such as those used for recreational purposes. HAdV comprises 7 species with 57 types, which are responsible for enteric and respiratory illnesses and eye infections [64–66]. Among animal adenoviruses, porcine adenovirus (PAdV) may cause gastroenteritis symptoms such as diarrhoea, anorexia or dehydration in piglets, while sows can suffer multifactorial respiratory diseases and even abortion [67].

- *Excretion pattern*: HAdV particles may be excreted in faeces for months or even years [49, 68]. Fifty per cent of the population has asymptomatic AdV infections at some time, and gastroenteritis occurs in 60% of children under 4 years of age [69]. HAdV40 and 41 serotypes of HAdV can be excreted at high concentrations in faeces (10^{11} viral particles per gram) and transmitted via the faecal-oral route. Other adenoviruses, such as HAdV-1, HAdV-2, HAdV-5, HAdV-7, HAdV-12 and HAdV-31, are related to respiratory diseases and have also been detected in contaminated water and shellfish [70, 71]. PAdV infections can also be asymptomatic and are detected in nearly 70% of swine faeces, with most isolates being closely related to serotype 3 [49].
- *Prevalence*: Human and porcine adenovirus (HAdV) have been detected in contaminated water samples throughout the year in all geographical areas studied [29, 44, 49, 55, 72]. HAdV has been found in nearly 100% of urban wastewater samples tested, including those from cities in Africa, the USA, Central and South America and Europe. Adenoviruses are also frequently detected in shellfish, including samples that met current safety standards based on levels of faecal bacteria [73].
- *Stability*: Adenovirus is inactivated only after 2 h at 85°C [74]. With moist heat, the time and temperature of inactivation are slightly reduced; exposure to 65°C for 30 min is then sufficient to inactivate adenovirus particles [75]. Chlorine treatment, which is very commonly used to disinfect and purify water, oxidises viral protein shells and nucleic acids [76]. Nevertheless, infectious HAdV can still be detected after chlorine treatment for 30 min (2.5 mg/L), although its concentration drops by approximately 2.7 $\log_{10}$ [77, 78].

3.2 Polyomavirus

Polyomaviruses are small, icosahedral viruses that have circular, double-stranded DNA genomes approximately 5,000 bp in length and that infect several species of vertebrates. The first human polyomaviruses, JC and BK (JCPyV and BKPyV), were identified in clinical samples from immunocompromised patients [79, 80]. The pathogenicity of JCPyV is commonly associated with progressive multifocal leukoencephalopathy (PML) in immunocompromised states, and infections with this virus have attracted new attention because of JCPyV reactivation and pathogenesis in some patients with autoimmune diseases who are being treated with immunomodulators [81, 82]. Among the known animal polyomaviruses, bovine polyomaviruses (BPyV) does not cause significant pathogenicity in cattle, and no disease has as yet been ascribed to this agent.

- *Excretion pattern*: Both human and animal PyVs are excreted in urine by healthy individuals [52, 83, 84]. JCPyVs have been detected in 40–80% of the population, and BPyV has been detected in 30% of the bovine urine samples analysed

[52, 61]. Polyomaviruses are transmitted by an unknown mechanism, although it is speculated that respiratory, cutaneous and faecal-oral routes could be involved in their transmission.

- *Prevalence*: Human JCPyV is distributed worldwide, and specific antibodies have been detected in over 80% of humans [85]. JCPyV and BKPyV were first described in environmental samples in 2000 [44]. JCPyV is frequently detected in river water, seawater, reclaimed water [72], drinking water [86] and shellfish grown in waters affected by sewage [61]. These viruses are present in nearly 100% of all sewage samples from different geographical areas [72]. BPyV has been identified as the cause of a widely disseminated infection in bovines, and it is a frequent contaminant of commercial bovine serum. BPyV has been detected in river water samples near slaughterhouses, farms and grazing areas [72].
- *Stability*: Polyomaviruses, such as SV40, are only significantly affected by exposure to a temperature of 95°C for 1 h [74]. Numbers of JC polyomavirus Mad4 viral particles were reduced by 1.5 to 1.1 $\log_{10}$ GC, as measured by qPCR after 30 min of contact (2.5 mg/L), although no infectivity assays were conducted for this virus in these studies [77].

3.3 Parvovirus

The *Parvoviridae* family comprises small animal viruses with 5 kb linear, single-stranded DNA genomes with two large open reading frames. This family of viruses is divided in two subfamilies: the *Parvoviridae*, which mainly infect vertebrates, and the *Densoviridae*, which infect arthropod hosts.

- *Excretion pattern*: Human parvoviruses have been detected in stool samples, but their transmission pathways remain unclear [87, 88].
- *Stability*: These viruses have shown high resistance to temperature and low pH [89, 90] and have been found in commercial meat samples [91]. Bovine parvovirus was not significantly affected by exposure to 95°C for 2 h [74].
- *Prevalence*: When sewage water, as a representative matrix that can be used to test large populations, was monitored, a high prevalence (81%) of parvovirus was observed [92]. Avian parvoviruses are excreted in poultry faeces and have been reported in studies from different countries [59].

4 Methods for the Use of Viral Markers for MST in Groundwater

Viruses are present in the environment in low concentrations and are distributed unevenly. To detect viruses in the environment, it is essential to collect a significant volume of sample and to concentrate the viral particles before employing a detection assay. Detection of viruses in minimally or moderately polluted waters requires that viruses from at least several litres of water be concentrated into a much smaller volume (Fig. 1).

There are several concentration methods available, and many of them include two concentration steps in series, which will affect the recovery efficiency of the whole process. The development of cost-effective methods for the concentration of viruses from water and of cost-effective molecular assays, as well, facilitates the use of viruses as indicators of faecal contamination and as MST tools. The first methods used were based on the detection of viral indicators by PCR [41, 42, 44, 49]; more recently, quantitative PCR techniques have been developed that allow not only the detection but also the quantification of these viruses in environmental samples [29, 52, 58, 59] (Fig. 2).

It has been proposed that HAdVs and JCPyVs be quantified to trace human faecal contamination. HAdVs are present in sewage samples from all geographical areas

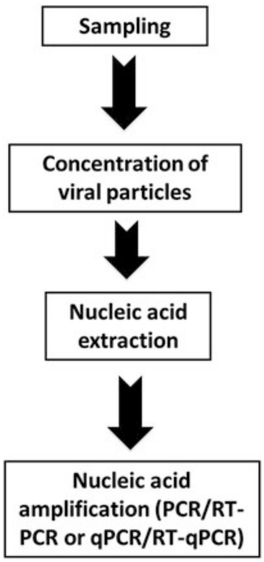

Fig. 1 Flowchart of the method used to detect and quantify viruses in water samples by PCR-based methods

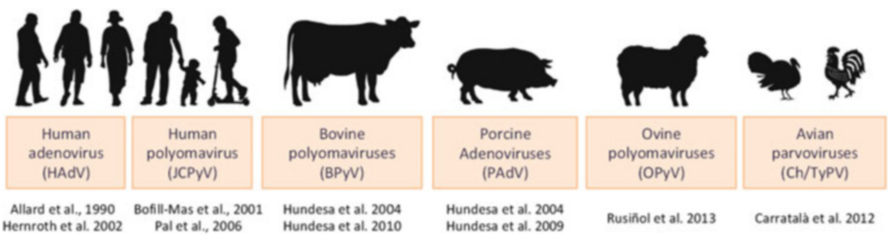

Fig. 2 Human and animal MST methods constituting a toolbox for identifying sources of faecal contamination

Table 2 Review of MST studies using HAdV to trace human sources in the environment

Country	References	qPCR	Main results
USA	[94]	[95]	16% (18/114), 1E+02-1E+04 GC/I
Japan	[96]	[95]	45% (29/64)
USA	[95]	[95]	S: 80% (4/5) 4.3E+04 GC/I; SW: 100% (11/11), 8.1E+06GC/I
Spain	[86]	[43]	R: 93% (13/14), 4E+02 GC/I; SW: 100% (10/10), 1.4E+07 GC/I
Spain	[29]	[43]	100% (6/6), 3.8E+07 GC/I
Spain	[28]	[43]	R: 90% (102/114), 1E+01-1E+04 GC/I
New Zealand	[97]	[95, 98]	R: 83% (5/6), 1.70E+01-1.19E+03 GC/I; SW: 100% (10/10), 1.87E+05GC/I
Germany	[99]	[98]	97.5% (40/41), 1.0E+04-1.7E+06 GC/I
France	[100]	[43]	100% (42/42), 1.0E+04G/I
Spain	[101]	[43]	R: 100% (7/7), 3E+03 GC/I; SW: 100% (7/7), 3.2E+06 GC/I
Japan	[102]	[95]	61.1% (11/18), 3.6E+03-1.38E+05 GC/I
Germany	[103]	[98]	96.3% (193/190), 2.9E+03-7.3E-7.3E+05GC/I
Brazil	[104]	[43]	SW 64.2% (54/84) 1E+07 GC/I
Brazil	[105]	[43]	100% (12/12); 5E+04-1.3E+07 GC/I
Spain	[106]	[43]	100% (7/7), 1E+01-1E+06 GC/I
Ghana	[107]	[98]	GW: 0% (0/4), SW: 22% (2/9)
Chad	[108]	[43]	GW: 0%, R:6% (1/16)
Germany	[109]	[98]	R: 9.3% (108/111), 3E+03 GC/I; SW: 100% (12/12), 1.0E+07 GC/I
Greece	[110]	[43]	45.8% (22/48)
Europe	[63]	[43]	R: 41% (381/928) S: 27% (132/482)
Brazil	[111]	[43]	100%, 1E+07 GC/I
Brazil	[112]	[43]	96% (46/48)
Spain	[113]	[43]	100% (44/44), 8.32E+03 GC/I
Brazil	[112]	[43]	69% (25/36) 1E+05 GC/I, 52.7% infective
Brazil	(114)	[43]	100% (24/24), 1E+05-lE+06 GC/I
New Zealand	[115]	[43]	R:86% HAdV (30/35) and 63% HAdV F (22/35), 1E+02 GC/I; S: 60% (9/15), 2.8E+02 GC/I; SW: (37/37)1E+05 GC/I
Uganda	[116]	[43]	GW: 0%; R: 70% (29/41), 2.65E+04 GC/I
Australia	[117]	[98]	91% (21/23) after sewerage overflow
Australia	[118]	[98]	100% (30/30), 1E+05-1E+06 GC/I
China	[119]	[98]	100% (24/24), 2.28E+04 GC/I
USA	[120]	[98]	40% (26/65), 2.2E+04 GC/I
Spain, Brazil, Hungary, Greece, Sweden	[72]	[43]	Spain 1.5E 03 GC/I (50/61), Greece 4.8E+02 GC/I (18/80), Brazil 3.9E+05 GC/I (253/276), Hungary 1E+04GC/I (108/109), Sweden 1.6E+02GC/I (12/108)

G groundwater, *R* river water, *S* seawater, *SW* raw sewage

that have been studied, while JCPyV is a less abundant but highly human-specific virus [93]. For this reason, the analysis of both viruses to determine the extent of human faecal pollution of environmental samples is a good approach that has a specificity of 100%. Both viruses have been evaluated in various studies in different water matrices, and their utility in MST has been demonstrated (Tables 2 and 3).

Porcine adenoviruses (PAdVs) and bovine polyomaviruses (BPyVs) have been proposed as porcine and bovine faecal indicators [49, 51], and several studies have

Table 3 Review of MST studies using JCPyV to trace human sources of contamination in the environment

Country	References	qPCR	Main results
Spain	[86]	[45]	R: 100% (9/9), 2.7E+04 GC/I
Spain	[29]	[45]	SW: 100% (6/6), 6.11E+06 GC/I
Spain	[28]	[45]	75% (18/27), 7.4E+02-1.3E+03 GC/I
United States	[121]	[121]	100% (41/41), 3.07E+07 GC/I
Germany	[99]	[122]	97% (40/41), 2.4E+04 GC/I
United States	[123]	[121]	50% (40/40)
Australia	[124]	[121]	R: 25% (5/20), 1E+03GC/I; SW: 100% (40/40), 1E+05GC/I
Spain	[71]	[45]	SW: 85% (6/7), 1E+05 GC/I; R: 100% (7/7), 1E+03 GC/I
Brazil	[125]	[45]	96% (6/7), 1.2E+06 GC/I
Japan	[102]	[45]	11% (1/18), 7.91E+02-3.42E+03 GC/I
Germany	[103]	[122]	68% (129/188), 1.4E+04 GC/I
Germany	[109]	[122]	R: (73/111) 1E+03 GC/I; SW: 100% (12/12) 1E+08 GC/I
United States	[126]	[121]	S: 3% (1/32); SW: 100% (15/15)
Greece	[110]	[121]	68% (33/48)
United States	[127]	[121]	1% (2/35), 1E+04 GC/I
Brazil	[112]	[121]	21% (10/48)
United States	[128]	[121]	12% (90/752)
Spain	[113]	[45]	100% (6/6), 5.44E+05 GC/I
United States	[129]	[121]	20% (26/130), SE+02-3.55E+05 GC/I
United States	[130]	[121]	61% (15/25)
Spain and Brazil	[131]	[45]	R: 100% (12/12), 9.38E+03 GC/I; SW: 100% (12/12), 1.05E4 GC/I
New Zealand	[115]	[121]	R: 51% (18/35), 1E+03GC/I; 5: 67% (7/15), 1E+03GC/I; SW: (36/37), 1.5E+06GC/I
Brazil	[114]	[45]	100% (24/24), 1E+05-1E+06 GC/I
Australia	[117]	[121]	52% (12/23)
Spain, Greece, Brazil, Hungary, Sweden	[72]	[45]	Spain 1.8E+03GC/I (41/61), Greece 5.6E+02 GC/I(15/80), Brazil 4.6E+03 GC/I (190/276), Hungary 2.1E+04GC/I (76/109), Sweden 7.2E+01GC/I (10/108)

G groundwater, *R* river water, *S* seawater, *SW* raw sewage

shown that these viruses are widely disseminated in swine and bovine populations, respectively, without producing clinically severe disease (Table 4) and are thus useful MST tools.

More recently, the quantification of ovine polyomaviruses and chicken/turkey polyomavirus has been suggested for tracing ovine and poultry faecal pollution, respectively [58, 59]. Quantification of each of these viruses has been used to trace the origins of nitrate pollution in groundwater in some areas of Catalonia, as described in the next section.

Table 4 Studies describing the detection of bovine and porcine faecal pollution using BPyVs and PAdVs as MST tools

Virus	References	qPCR	Matrices analysed	Main results
PAdV	[55]	[55]	River, slaughterhouse and urban sewage	100% positive samples in slaughter-house sewage (1.56E+03 GC/L) and 100% in river (8.38 GC/L)
	[133]	[133]	River	50% positive river-water samples
	[56]	[55]	Manure	66% of the samples collected in the SMTS positive and 78% of the samples collected in the manure treatment system positive
	[135]	[55]	Manure	PAdVs were more prevalent than other viruses and may possibly be considered indicators of manure contamination
	[136]	[55]	Influents and effluents from swine manure biodigester	60% (24/40) samples positive
BPyV	[134]	[134]	Sewage	100% positive for manure and waste-water samples, 5.6% positive for faecal samples
	[52]	[52]	River, slaughterhouse and urban sewage	91% positive samples in slaughter-house sewage (2.95E+03 GC/L) and 50% in river (3.06E+02 GC/L)
	[132]	[52]	Groundwater	1/4 well-water samples positive for BPyV (7.74E+02GC/L)
OPyV	[58]	[58]	River, slaughterhouse	75% (3/4) slaughterhouse samples positive 20% (1/8) river water samples positive

5 Case Study: Identification of the Sources of Nitrate Contamination in Catalonian Groundwater

Virus-detection assays have been used to detect viruses in groundwater samples from diverse areas in which nitrate levels exceeded >50 mg/L [137, 138] to trace the origins of nitrate pollution, as a collaborative study with the Catalan Water Agency and the Laboratory of Virus Contaminants of Water and Food from the University of Barcelona.

To ensure the designation of vulnerable zones according to the Directive against pollution caused by nitrates from agricultural sources [138], a total of 14 different monitoring stations were evaluated (Table 5). This study aimed to determine whether the pollution sources in these areas were manure, urban wastewater sludge or chemical fertilisers applied for agricultural uses. From three to five samples were taken per well for later analysis for the presence of different human and animal viruses. Samples were assayed for the presence of human adenovirus (HAdV) and human polyomavirus (JCPyV) to detect human pollution sources (from urban wastewater sludge used in agriculture or from sewage leaks); samples were assayed

Table 5 Human and animal viruses in groundwater from wells with nitrate contamination

Groundwater monitoring station	Type	Depth (m)	*N*	Human pollution		Animal pollution		NO_3^- mg/L
				HAdV	JCPyV	PAdV	BPyV	
				Genome copies/100 mL				
Font través (ClarianaCardener)	Spring	0	5	ND	ND	ND	ND	>110
Pou Casa Lloch (Olius)	Well	5	5	ND	ND	7.74E +01	ND	>100
Pou de Ca l'Arnau (Solsona)	Well	9	5	ND	ND	ND	ND	>100
Pou Ardèvol (Pinós)	Well	40	5	ND	ND	ND	9.53E +02	30–40
Mina del Sanou (Sta Coloma Queralt)	Gallery	0	4	7.00E +02	ND	ND	ND	24–46
PouBudell (Forès)	Well	6	4	ND	ND	ND	ND	70.9
PouNou (Conesa)	Well	30	4	1.42E +02	ND	ND	ND	5.1
Pou de les Escodines (Forès)	Well	11	4	ND	ND	ND	ND	150.1
Mina Aiguadolç (Sta Coloma Queralt)	Gallery	0	4	ND	ND	ND	ND	24–46
Font de la Freixa (Argençola)	Spring	0	4	8.47E +01	ND	ND	ND	45–54
Pou de Biure (Les Piles)	Well	0	4	8.01E +02	ND	ND	ND	76
Pou de les Piles (Les Piles)	Well	65	4	1.59E +02	ND	ND	ND	100–140
PouGuialmons (Les Piles)	Well	12	4	1.19E +02	ND	ND	ND	117–122
Pou de Sant Gallard (Les Piles)	Well	40	4	ND	ND	ND	ND	80–100

for the presence of porcine adenovirus (PAdV) to detect porcine sources of pollution (from the application of pig manure); and samples were assayed for the presence of bovine polyomavirus (BPyV) to detect bovine sources of pollution (from cow manure applications; Table 5).

Viruses were concentrated using the procedure described by Calgua and coworkers [131], based on flocculation with skimmed milk. After viruses were concentrated from 10 L water samples, viral nucleic acids were extracted. Then, qPCR assays specific for human adenoviruses (HAdV), JC polyomavirus (JCPyV), porcine adenoviruses (PAdV), bovine polyomaviruses (BPyV), ovine polyomaviruses (OPyV) and chicken/turkey parvoviruses (Ch/TuPV) were used to determine the relative quantities of each of these viruses in the samples and hence to determine the source of faecal contamination. The source of faecal contamination determined in this way is then indicative of the source of nitrates in the groundwater from which samples were taken [43, 45, 52, 55].

The results obtained by qPCR were further confirmed by nested PCR and sequencing, as previously described [44, 49, 51]. The results obtained are summarised in Table 5.

The results show that in one area (Olius), faecal/urine contamination of porcine origin is clearly present (4/4 replicates tested positive), strongly suggesting that the application of swine slurries could be a significant source of nitrate contamination in the groundwater at that location. In the Pinós area, for which low levels of nitrate were measured, sporadic bovine contamination was detected (1/4 replicates tested positive), and diffuse contamination or the application of bovine manure was considered to be the potential source of the viruses that were detected. Finally human faecal pollution was detected as the main source of contamination in 4 other studied areas; further investigation is needed to identify the sources of contamination in these areas. This methodology has been tested in areas where nitrate concentrations are above the statutory limit (i.e. >50 mg/L) and thus where the use of groundwater as drinking water is compromised. A greater number of samples would be required to determine whether a relationship exists between the concentration of nitrates and the presence of the virus.

This study determines the origins of contamination of nitrates in groundwater, so that their sources (urban, animal or inorganic fertiliser use, in the case that viruses were not detected) could be established. The conclusions of this study could have implications for the future management of water in the region.

6 Conclusions and Future Trends

Groundwater is a vital source of water that provides, in Europe alone, drinking water for 300 million inhabitants. In Catalonia, over 587 hm^3/year of groundwater is used, and this amount represents close to 20% of the total water used in the region. Today, high nitrate levels in groundwater remain an important target for pollution reduction worldwide, with implications for human and environmental health [139]. Nitrate and pesticide pollution from agricultural sources are major, well-known problems with groundwater quality, and increases in water demand and population density will increase the probability of faecal contamination of groundwater. Moreover, falling groundwater levels will further endanger the quality of groundwater and its ability to clean itself. In addition to these problems, overabstraction has already begun to induce saltwater intrusion along most stretches of the Mediterranean coast, rendering the groundwater in those areas useless for drinking and most other purposes. Appropriate management of water resources and more specifically of groundwater resources requires the reliable evaluation of water quality and the identification of sources of contamination. These measures are needed to prevent further contamination, to implement remediation measures and especially to provide information that can be used to institute measures to protect waters from pollution caused by nitrates from agricultural sources [138].

Currently, microbiological quality assessments of environmental waters largely rely on detecting faecal indicator bacteria. Although this approach has clearly reduced health risks in many countries, the faecal indicator approach may be combined with monitoring of more environmentally stable viral indicators specific to human and animal sources of contamination [27, 93]. The viral MST tools developed in this study can be used to track faecal contamination of human, bovine, ovine, porcine and avian origins using specific individual assays or, in the near future, using multiplex assays. Multiplex diagnostic tools are already available, and multiplex quantitative PCR assays for MST have been described previously in a study that examined diverse human and animal viruses [133].

Human (HAdV, JCPyV), bovine (BPyV), porcine (PAdV) and ovine (OPyV) viral markers have been shown to be useful for identifying the origin of faecal contamination in river water and seawater in Brazil, Sweden, Spain, Hungary, Greece and New Zealand [58, 72]. However, more information on the environmental stability and distribution of viruses in diverse geographical areas and water matrices will be needed to validate some animal viral markers, including new viral MST tools that may be developed in the future for other animals representing other sources of contamination.

Routine quantitative PCR assays for viral indicators may also be improved and standardised, in light of new methods that have been developed that allow the absolute quantification of genome copies without requiring that independent calibration curves be generated [140]. Other technical improvements that can be expected include advances in microfluidics and nanobiotechnology, as a result of which miniaturised systems for the detection of viral indicators could be developed that are based on microchips. Several such approaches have been described [141, 142]. New technologies, such as high-throughput mass sequencing, have been used to analyse urban sewage from diverse geographical areas and have produced a wealth of data about the viruses present in wastewater [60]. However, further development of NGS techniques are still needed to provide more sensitive and affordable assays that could potentially be used for routine analyses.

Cost-effective methods for using specific DNA viruses as markers of the source of faecal (or nitrate) contamination in water have been developed and validated. These methods may be standardised to acceptable levels of cost, feasibility, sensitivity and repeatability, especially in the case of the DNA viruses selected in our MST studies. The sampling strategies should also be considered carefully to obtain samples that best represent the water in question. Ideal sampling strategies could involve the use of hydrological and physicochemical sensors and time- and flow-integrating automated sampling devices.

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Trend Assessment for Groundwater Pollutants: A Brief Review and Some Remarks

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Abstract Groundwater is a valuable natural resource that needs to be assessed and protected. The European Union (EU) adopted new water legislation that includes the Water Framework Directive (WFD) and the Groundwater Daughter Directive (GWD). Both require the identification of sustained increasing pollution *trends* and their reversal. This is the second pillar of the WFD: such trends have to be identified for any pollutants that result in groundwater being characterized as at risk of not meeting the environmental objectives. Measuring these trends is necessary to determine and understand whether changes in land use, fertilizer application, pollution history, or climate change are affecting groundwater quality. However, in many cases, groundwater data series may not meet minimum requirements for classical statistical procedures employed in trend assessment: among other obstacles, data may be sparse, with missing or extreme values, censored data, seasonal effects, and autocorrelation. The aim of this chapter is to present and review several statistical methodologies that have been proposed and applied in recent years to deal with groundwater trend assessment, discussing the relative advantages and disadvantages of each one.

Keywords Catalonia, Daughter Directive, Groundwater, Monitoring, Quality trend assessment, Water Framework Directive

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Contents

1 Introduction

Groundwater is a valuable natural resource that accounts for over 97% of all the freshwater available on Earth, excluding glaciers and ice caps (extracted from Groundwater Protection in Europe, [1]). It needs to be monitored and protected from chemical and organic pollutants, not only because groundwater is used as drinking water but also because it is an important resource for industry and agriculture and has recreational uses and environmental value [2]. Due to the fact that groundwater moves through the subsurface slowly, the impact of human activities may last for decades, and pollution events that occurred in previous years will probably continue to threaten us for several generations.

The European Union (EU) adopted new water legislation that includes the Water Framework Directive (WFD) (2000/60/EC) [3] and the Groundwater Daughter Directive (GWD) (2006/118/EC) [4]. The WFD is a regulatory framework for the protection of all natural waters which prescribes *environmental objectives* to be achieved by the end of 2015 and contemplates extensions to 2021 or 2027. The GWD for the protection of groundwater against pollution defines the specific environmental objectives of the WFD. It requires that threshold values be established (by the end of 2008) for pollutants related to the pressures identified as putting bodies of groundwater at risk (GWD, Article 3). These threshold values are quality standards, and so they are to be used to assess the chemical status of groundwater. The GWD also introduces measures to prevent or limit the introduction of pollutants into groundwater (GWD, Article 6).

Both the WFD (Article 17) and the GWD also require that *trends* must be identified for pollutants that characterize groundwater as being at risk of not meeting the environmental objectives of the WFD (WFD, Annex V 2.4.4; GWD, Article 5). According to the GWD, those trends must be statistically and environmentally significant (GWD, Article 2). The environmental significance relates to the potential future impact of an identified increasing trend in pollution. Such trends should be reversed when they reach 75% of the EU groundwater quality standard values or specific threshold values (GWD, Article 5). In many countries, the concentrations of groundwater pollutants in aquifers are already approaching or have even exceeded statutory limits for drinking water. For this and other reasons, long-term monitoring of the concentrations of groundwater pollutant by both water utilities and regulatory agencies has become widespread. The achievement of a *good status* and the reversal of *significant and sustained* upward trends in the concentration of pollutants, including nitrates, are environmental objectives set out in Article 4 of the WFD. Trend reversal is to be achieved through the implementation of the Program of Measures (WFD, Annex VI) which aims to progressively reduce pollution and prevent further deterioration of bodies of groundwater (GWD, Article 5). Therefore, the statistical assessment of *trend reversal* is another matter of concern.

The properties that determine the quality of groundwater can vary over different time scales (daily, seasonally, or annually) and depend on the characteristics of the aquifer. In fact, the concentrations of a solute in samples at a single station depend on numerous factors including land use history, groundwater flow, and local groundwater pumping regimes, as well as seasonal and climate effects. Furthermore, the monitoring itself may involve elements that could make it difficult to accurately assess trends, for example, the sampling frequency, amount of missing data, length of monitoring period, and presence of uncontrolled covariate variables.

In many cases, the characteristics of groundwater data series may not meet the minimum requirements for classical statistical procedures employed to analyze trends. Among other obstacles, the data may be sparse and gappy and include censored data, extreme values or *outliers*, seasonal effects, and autocorrelation [5–7]. For EU members, in accordance with the WFD and GWD, two technical reports [8, 9] contain a series of general recommendations for groundwater trend assessment, including monitoring requirements, how to treat censored values, the length of data series, and the statistical methods to be employed. Besides those two excellent guides, a large number of papers have been published in many different journals over the last few decades. However, in our opinion, none of those works manage to definitively determine the procedures to be applied, because a procedure that may be adequate for a certain data series may be cumbersome or inappropriate for another series.

Another concern is related to individual monitoring points and data aggregation. Every groundwater body (GWB) is usually monitored at a certain number of sampling sites (stations). As required by the GWD (Article 5) and WFD (Article 13), member states must summarize the way in which the trend assessment at individual monitoring points within a GWB or group of GWBs has contributed to identifying that those bodies are subject to a significant and sustained upward trend or are experiencing a reversal of such a trend. Furthermore, the WFD (WFD, Annex V Section 2.3) states that in assessing the status of a GWB, the results of individual monitoring points within it are to be aggregated for the body as a whole. So apart from assessing the trend at every station, we have to assess the overall trend in the GWB.

In Catalonia, a groundwater quality monitoring network has been set up in accordance with the requirements of the WFD (WFD, Article 8 and Annex V). Control networks have been designed taking into account the hydrogeological model and the pressures that have been identified on each GWB. Currently, there are some 942 stations distributed across a total of 53 GWBs. Chemical quality is analyzed annually, and quantitative analysis is performed monthly. The average spatial density of the stations in a given GWB is 0.58 stations per 10 km^2. This network for monitoring groundwater quality dates from 1994 in some strategic aquifers, and the first monitoring program in accordance with the WFD requirements began in 2007.

This chapter mainly focuses on presenting an overview of some of the main statistical techniques that have been proposed in recent years for the statistical testing of a groundwater trend. The methods presented here are rather simple and can easily be implemented using different commercial or open-source statistical software packages. In order to illustrate the procedures and highlight the main results, some of the GWBs and monitoring stations in Catalonian have been selected and the methods applied (see Sect. 3). The pollutants analyzed are nitrates and chlorides. All of the techniques are applied using packages and functions implemented in R [10].

2 An Overview of Methodologies

2.1 Individual Monitoring Points (Stations)

Groundwater monitoring networks provide observation of random variables (concentrations of pollutants) at each sampling site over time. Within the assessment of significant upward trends and trend reversal, we have to consider ([9], Sect. 6.2) the following issues: (a) a correct statistical method for assessing trends at each monitoring point, (b) how to deal with values below the limit of quantification (LOQ), (c) the appropriate length of time series, (d) how to establish baseline levels of substances, (e) what an acceptable level of confidence is in trend assessment, (f) how to establish a starting point for trend reversal, and (g) how to statistically demonstrate that a trend has been reversed.

In accordance with the GWD (Annex IV), to deal with individual concentrations below the LOQ, they are replaced by LOQ/2. Regarding the length of time series for yearly data, Grath et al. [8] recommend at least 8 measurements for the detection of an upward trend and 15 measurements in order to establish whether there is a significant breakpoint (trend reversal). In fact, that research clearly advises against long-term time series if the statistical method does not take into account a possible breakpoint. Nevertheless, we will see that a breakpoint, even within a short-term series, can seriously affect the performance of statistical methods that are recommended for trend assessment.

Reviewing the literature, the problem of detecting and estimating trends in hydrology data has a long history [5, 6, 11–27], and some of the publications report comparative analysis of different techniques. For example, Esterby [18] reviewed some parametric and nonparametric trend detection methods by applying them to water quality time series. Meanwhile, Hess et al. [21] present an overview of six linear methods used to analyze environmental time series. A comprehensive evaluation of 28 statistical methods for checking trend, homogeneity, seasonality, periodicity, and persistence in hydrologic time series was recently published [28]. So far, there is no general consensus regarding which method performs best in a given unknown situation, and few extensive comparisons of the proposed methods have been published. In this scenario, quite a lot of published work advises visual inspections by the user (among others, [29]) in order to decide which method is suitable, whether assumptions are valid, or to finally assess trends. This, however, is quite unrealistic; how many plots would the user have to examine considering all the stations and pollutants? Perhaps it would be thousands, so a robust automatic method (software) for assessing trends is absolutely vital. In this context, TTAinterfaceTrendAnalysis was developed in R package [30] to perform nonparametric trend analysis (Kendall test family) through an interactive GUI. Notwithstanding, other techniques to assess temporal trends in an automatic manner are absolutely crucial.

In this section, we review some of the more common statistical procedures used for trend assessment in groundwater quality data. The methods can be classified into two main general approaches: parametric methods (distribution dependent) and nonparametric methods (distribution free). However, the decision as to which procedures are most useful (to reveal change when it is present and not identify any change when there is no trend) depends on the characteristics of the data: the distribution (normal, skewed, symmetric, heavy-tailed, extreme values); the presence of outliers (exaggerated extreme values, perhaps due to measurement error or a one-off serious contamination event); and its seasonality, whether there are missing values (a few isolated values or large gaps), there is censored data (e.g., due to the LOQ), or there is some serial correlation and if it does contain a monotonic trend or an abrupt change [26]. If the assumptions made in order to apply a statistical test do not hold for the data, then the estimate of the significance level could be incorrect.

Finally, when we carry out a statistical test, it is necessary to define the null hypothesis H_0 (in our case, the hypothesis of no trend) and the alternative hypothesis H_1 (trend). Two types of errors can occur when we perform a test. A *type I error* occurs when the researcher rejects the null hypothesis when it is in fact true (*false positive)*. The probability of committing a type I error is called the significance level, and it is frequently denoted as α (the most common choice is $\alpha = 5\%$). A *type II error* is present when the researcher fails to reject the null hypothesis which is in fact false (*false negative*). The probability of committing a type II error is usually denoted as β, and the complementary probability, $1 - \beta$, is known as the *power of the test*. Type I and type II errors are not complementary (i.e., their sum is not one), but they do stand in a relationship: if we decrease the chance of committing a type I error (choosing a lower α value), then β increases. The decision rule for rejecting the null hypothesis is in accordance with the *p-value*. The p-value is the probability of observing a test statistic equal to or more extreme than our experimental value, assuming that the null hypothesis is true. If the p-value is less than the significance level, we reject the null hypothesis.

2.1.1 Regression Models

Linear models are the most widely used framework from a parametric perspective. The classical approach to assessing a trend is based on fitting a linear regression (LR) or quadratic regression (QR) model [8]. As is well known, the simple LR model is:

$$x_i = \beta_0 + \beta_1 t_i + e_i \quad i = 1, \ldots, n \tag{1}$$

where x_i is the value for the ith observation, t_i is the corresponding value for the independent variable (time), β_0 is the intercept, β_1 is the slope, and e_i are the residuals (assumed to be independent and identically distributed). The regression coefficients are estimated using the method of *ordinary least squares* (OLS). A t-test may be used to test that the true slope is not different from zero ($H_0 : \beta_1 = 0$), which implies that there is no correlation between the pollutant and time ($H_0 : \rho = 0$). So we can conclude that a linear trend exists if the p-value of this statistic is less than α (the significance level). Nevertheless, a new assumption is made when we apply the test: the residuals, e_i, are normally distributed. Moreover, OLS are highly sensitive to outliers. In spite of this, as reported in Grath et al. [8], LR was the most used statistical method for trend assessment in the EU in 2001.

Trends which are nonlinear (say quadratic, exponential, or with an abrupt change) will be poorly described by a linear slope coefficient. We can easily extend the linear model to QR by adding a new term to Eq. 1:

$$x_i = \beta_0 + \beta_1 t_i + \beta_2 t_i^2 + e_i \quad i = 1, \ldots, n \tag{2}$$

If the coefficient β_2 (quadratic term) is significant, we reject the null hypothesis that LR is acceptable. A quadratic fit implies a concave or convex function (second-order polynomial), and the *inflection point* is at time $t_{\mathrm{IP}} = -\beta_1/(2\beta_2)$. Nevertheless, the quadratic model has its own limitations: (a) it cannot fit other nonlinear functions; (b) it is not easy to assess the trend before and after the inflection point (recall that a second-order polynomial never remains flat, which means we cannot say “no trend”); (c) the parameter estimates are greatly influenced by outliers; and (d) hypothesis tests are sensitive to departures from normality.

Therefore, it could be useful to apply a robust procedure. The *robust LR* (RLR) model can be stated as:

$$x_i = \beta_0 + \beta_1 t_i + \sigma e_i \quad i = 1, \ldots, n \tag{3}$$

where $\sigma > 0$ is a scale parameter. There are a number of ways to perform robust regression [31–36]. To deal with outliers in the x-direction, the most commonly used methods are based on *Huber-type estimates* [31, 37], which form a class of M-estimates. An M-estimate of β_1 is a solution of:

$$\sum_{i=1}^{n} w_i e_i x_i = 0 \tag{4}$$

where $e_i = x_i - (\beta_0 + \beta_1 t_i)$ are the residuals and $w_i = W(e_i/\sigma)$ with $W(u)$ a suitable weight function. Equation 4 is the equation of a weighted OLS estimate and must be obtained by an algorithm called *iteratively reweighted least squares* (IRLS). In the first iteration, each point is assigned equal weight, and the model coefficients are estimated using OLS. For subsequent iterations, the weights are recomputed so that points farther from model predictions in the previous iteration are given lower weights. The Huber M-estimator (the default in many software packages) is defined as $W(u) = \psi_k(u)/u$, with $\psi_k(u) = \max(-k, \min(k, u))$, where k is constant that the user has to specify. The estimate of σ is usually $\hat{\sigma} = \mathrm{MAD}$ (*median absolute deviation*). This approach is fairly robust against departures from normality and outliers in the x-direction. The model presented in Eq. 3 can easily be generalized to perform robust nonlinear regression.

Another approach is *loess* or *lowess*. Cleveland [38–40] proposed the *loess* algorithm as a flexible and robust method to fit a regression function which is suitable when there are outliers and we do not know the parametric model. The name is derived from *locally weighted scatterplot smoothing*, because a weighted least squares method is used to fit linear or quadratic functions at every local predictor point, based on its neighborhood. The *smoothing parameter* controls the fraction of the data contained in each local neighborhood, and data points are weighted by a decreasing function of their distance from the center of the neighborhood. Recommended in Grath et al. [2] as a method that is much more flexible with regard to the shape of a trend, an ANOVA test based on the *loess* smoother is described [2, 41] which allows us to examine both monotonic and non-monotonic trends. In spite of that, we want to mention several pitfalls of the system. (a) The election of the smoothing parameter is not easy, but it is absolutely essential, because it crucially influences the final result. (b) To perform a *loess* fit with short data series is a risky task which can lead to overfitting or underfitting of the data and may boost data autocorrelation. (c) In our experience, the method is not very outlier resistant, so a *loess* fit can become distorted due to the presence of outliers. (d) The ANOVA test proposed considers the *loess* fit as the true function (the error sum of squares is in fact the sum of squared *loess* residuals), so the outlined test is not reliable if we fail to fit the right function. Regarding the selection of a smoothing parameter, Hurvich et al. [42] propose an interesting and automatic determination based on AICc (the corrected Akaike information criterion) [43, 44], as applied in Wen and Chen [45]. To summarize, for short data series, we prefer the robust regression approach outlined before *loess*; it is less risky and easier to perform, and we do not have to select a smoothing parameter.

Detecting step trends (an abrupt change in the mean level at a specific point in time) in a process is also an important topic. Various statistical methods are available for identifying and locating steps in a time series [46–48]. Piecewise LR is used to detect significant changes in a trend (*breakpoints*), which means there are two different linear relationships in the data with a sudden, sharp change in direction. In this case, x_i is modeled by splitting the linear predictor into two pieces:

$$x_i = \begin{cases} \beta_0 + \beta_1 t_i + e_i & t_i \leq \gamma \\ \beta_0 + \beta_1 t_i + \beta_2 (t_i - \gamma) + e_i & t_i > \gamma \end{cases} \tag{5}$$

where γ is the breakpoint [49]. The slopes of the two lines are β_1 and $\beta_1 + \beta_2$, so β_2 can be interpreted as the difference between the slopes. The model given in Eq. 5, also named *two-section LR* (2SLR), forces continuity at the breakpoint [50]. It is straightforward to see that if $\beta_2 = 0$, we are in fact fitting simple LR. As is evident, the 2SLR model can be a useful approach for detecting trend reversals. Unfortunately, it is not robust when facing outliers.

2.1.2 Nonparametric Methods

Up to now, the methods we have summarized are based on linear models. Another possible approach is to apply a nonparametric model. Nonparametric methods tend to have been favored in the analysis of large datasets from national monitoring programs [18], since these methods involve fewer assumptions and they are less sensitive to outliers. There has been widespread use of Spearman's *rho* and Mann-Kendall (MK) *tau* statistics (especially the latter) to test for the presence of monotonic trends [5, 6, 51–62] and others.

The MK test is a well-known nonparametric rank-based method. Mann [63] originally used the test, and Kendall [64–66] derived the test statistic distribution. It is a distribution-free method (it does not require, e.g., normally distributed data), and it can be useful for detecting trends in time series when the data exhibit a monotonic function of time. MK test is robust against the influence of extreme values, suitable to be used with skewed variables [67], and appropriate for data that do not display seasonal variation (or for seasonally corrected data) and have negligible autocorrelation. It has been widely used and recommended by many researchers (see, e.g., [5, 60, 68]) to detect trends in the field of hydrology and in similar applications. The *tau b* correlation coefficient [65] is the natural estimator of the strength of the trend in the case of using the MK test and is easily derived.

In accordance with MK test, the null hypothesis H_0 is that the data is a sample of n independent and identically distributed (iid) random variables [5, 69]. The test statistic, Kendall's S [65], is calculated as follows:

$$S = \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \operatorname{sign}\left(x_i - x_j\right) \tag{6}$$

where $\operatorname{sign}\left(x_i - x_j\right)$ is equal to 1, 0, or -1 according to the sign of the difference $x_i - x_j$. Under the null hypothesis, $E(S) = 0$, and

$$\operatorname{var}(S) = \frac{n(n-1)(2n+5) - \sum_{j=1}^{g} k_j\left(k_j - 1\right)\left(2k_j + 5\right)}{18} = \sigma^2 \tag{7}$$

where g is the number of tied groups and k_j is the number of observations in the jth group (if there are no ties, delete the summation in the numerator of Eq. 7). For $n > 10$, the test statistic

$$Z_S = \begin{cases} (S-1)/\sigma & \text{if } S > 0 \\ 0 & \text{if } S = 0 \\ (S+1)/\sigma & \text{if } S < 0 \end{cases} \tag{8}$$

where $\sigma = \sqrt{\operatorname{var}(S)}$ follows a standard normal distribution [65]. The significance levels (p-values) can be obtained as follows:

$$p = 2(1 - \Phi(|Z_S|)) \tag{9}$$

where $\Phi()$ is the cumulative distribution function (CDF) of a standard normal distribution.

The MK test detects monotonic trends, so it cannot assess a trend reversal. A sequential MK test, originally proposed in Sneyers [70], is used in Partal and Kahya [71] and Shifteh Some'e et al. [72]. Unfortunately, it is not a test in a statistical sense, but a heuristic visual procedure to detect a trend reversal. We consider this approach to be quite subjective, and we do not recommend it.

Hirsch et al. [5] developed an extension of Kendall's test called the Seasonal Kendall (SK) test that accounts for seasonality in the data. In order to estimate the magnitude of the trend, they developed the SK slope estimator, which is an extension of the estimator proposed by Theil [73] and Sen [74]. A modified version of the SK test that allows for both seasonal data and serial dependence has also been developed [6]. The literature contains many methods for dealing with seasonality (i.e., monthly or quarterly trends); however, the issue is beyond the scope of this review, which focuses on yearly data.

Spearman's *rho* correlation is an alternative to Kendall's *tau b*, despite being less commonly used in trend assessment. The results of the two statistics and tests are very similar; however, the faster convergence to the normal distribution of the statistic based on Kendall's *tau b* makes it slightly preferable in the case of a small dataset [75].

If a significant trend is found, the magnitude (rate of change per unit time) can be estimated using the TS slope [73, 74]. This approach involves computing slopes for all pairs of data points $\left(x_i - x_j\right)/\left(t_i - t_j\right)$ and then using the median of these slopes as an estimate of the overall slope:

$$\beta_{\mathrm{TS}} = \mathrm{Med}\left(\frac{x_i - x_j}{t_i - t_j}\right) \quad \forall\, i > j,\;\; i, j = 1, \ldots, n \tag{10}$$

Thus, it is fairly sensitive to the presence of extreme values but can handle a moderate number of values below the detection limit and missing values. The trend slope, β_{TS}, is a measure of monotonic change and represents the median rate of change for the selected period, assisting the user in comparing the magnitudes of trends for several stations. However, it must be said that it is a linear trend estimator.

Another option, which as far as we know has not been used in the area of monitoring GWBs, is RoCoCo (*robust rank correlation coefficients*), proposed by Bodenhofer [76, 77], who presents a set of measures to test for monotonic associations between two observables. These robust rank correlation measures are based on fuzzy orderings, and the tests developed seem to outperform the classical variants because they are more robust for small samples when facing noise. The MK test is ideally suited for detecting monotonic relationships, but if you have numerical data such as pollutant concentrations, they may contain noise. In that case, even small random perturbations of true values may obscure a monotonic association. The robust gamma correlation coefficient seems to overcome this problem. The basic idea behind this correlation is to replace the strict orderings in the definitions of concordant and discordant pairs by continuous functions that measure the degree to which one value is greater than another (fuzzy ordering). Given a dataset consisting of n pairs of observations, $(t_i, x_i)_{i=1}^{n}$, the scoring functions R_T and R_X are used to compute the overall score of concordant pairs, C, and the overall score of discordant pairs, D:

$$\widetilde{C} = \sum_{i=1}^{n}\sum_{j \neq i}\overline{T}\left(R_T\left(t_i, t_j\right), R_X\left(x_i, x_j\right)\right)$$

$$\widetilde{D} = \sum_{i=1}^{n}\sum_{j \neq i}\overline{T}\left(R_T\left(t_i, t_j\right), R_X\left(x_j, x_i\right)\right)$$

The function $\overline{T}$ is a triangular norm used for aggregating the relationships between t and x components. The final robust gamma rank correlation coefficient is then computed as:

$$\widetilde{\gamma} = \frac{\widetilde{C} - \widetilde{D}}{\widetilde{C} + \widetilde{D}} \tag{11}$$

in perfect analogy with Goodman's and Kruskal's gamma.

To test the significance of the robust gamma correlation coefficient, Bodenhofer and Klawonn [76] propose permutation testing. In order to calculate the correlations and to perform the tests, we use the R package RoCoCo [78].

2.1.3 Time Series Correlation

One of the underlying assumptions common to all the models and tests presented so far is that the data are serially uncorrelated. When there is serial time correlation, the performance of the tests at assessing the significance of the trend could be affected (type I error) and may give misleading results. The effects are well known [79, 80]: in the case of positive autocorrelation, the tests have a tendency to be risky (increase in false positives), while if the autocorrelation is negative, the tests perform in a conservative way (increase in false negatives). Environmental variables, such as hydrological data, frequently exhibit some form of positive autocorrelation [80, 81], and several authors have studied and quantified the type I error in a large amount of situations (both with analytical and simulation studies) and shown that they are often greater than the significance level adopted ([80, 82, 83], among others). If we are applying a parametric model, the time series statistics framework can be used to overcome the problem of autocorrelation. However, it is not easy to obtain reliable results when dealing with a small sample dataset, and probably for this reason, such a framework has not often been applied or recommended for annual data.

The most simple case is the presence of *lag-1 autocorrelation* (the reading x_i recorded at time t_i is correlated with the previous value x_{i-1}), that is to say, a first-order autoregressive process AR(1). For annual data, it may be a suitable approach, because the serial correlation with a lag bigger than 1 year will in general be low. From now on in this work, we will assume only a possible significant lag-1 autocorrelation.

Several proposals have been made to avoid false trend detections resulting from autocorrelation. Generally speaking, such efforts can be classified into two different approaches. The first modifies the statistical test to account for the presence of the serial correlation. Further information regarding this first approach, including the advantages of its use, can be found in Yue and Wang [84] and Khaliq et al. [85]. The second approach transforms the original data so that it meets the assumption of no temporal dependence [86]. This second approach is often adopted via the procedure called *pre-whitening* (PW) [79, 87, 88]. In all cases, a key step in the process is the estimation of the serial dependence.

Nevertheless, at this point, there is another obstacle to consider: the interaction between the trend and the autoregressive process [89–91]. If the data reflect a deterministic trend, the estimate of the autocorrelation will become artificially inflated. For this reason, it has been proposed that the trend be extracted from the data (*detrending*) prior to the estimation of the autocorrelation [80]. Therefore, in order to evaluate the autocorrelation, two steps are involved: (1) detrending of the data (in the case of a significant trend) and (2) estimation of the autocorrelation within the detrended data.

In order to detrend (DT) the data, the following procedure has been proposed:

1. Calculate the Theil-Sen (TS) estimator with the original data, β_{TS}.
2. Assess whether the trend is significant using a $(1-\alpha) \times 100\%$ confidence interval of the slope. At least two different procedures have been described to build this confidence interval: a procedure based on order statistics [92, 93] and a bootstrapping procedure [94]. A controversial point is the election of the significance level (type I error); $\alpha = 0.05$ ($\alpha = 0.10$ if we choose a unilateral approach) is the usual value, but it could be too conservative in this step (low test power), so we recommend a value from 0.10 to 0.20 (0.15 and 0.20 are values often recommended in many stepwise variable selection procedures).
3. If zero is inside the confidence interval, we accept the hypothesis of no trend; and therefore the data remain unchanged: $x_i^* = x_i$. Otherwise, we remove the trend, transforming the data; thus,

$$x_i^* = x_i - \beta_{\mathrm{TS}} t_i$$

At this point, we want to note an obvious fact that is poorly reported in the literature: despite TS being a robust nonparametric estimator, it simply evaluates a slope, and therefore, the detrending procedure described above only removes a linear trend. It is straightforward and easy to understand that if the data reflect a nonlinear trend, detrending with a linear function is inappropriate. Suppose we have a data series with a quadratic trend (Eq. 2); then the detrending procedure leads to:

$$x_i^* = x_i - \beta_{\mathrm{TS}} t_i = \beta_0 + (\beta_1 - \beta_{\mathrm{TS}})\, t_i + \beta_2 t_i^2 + e_i \quad i = 1, \ldots, n$$

which is another quadratic function of time (so the data reflect a new trend). Even for data with a linear trend (Eq. 1), it is not certain that detrending completely removes the trend:

$$x_i^* = x_i - \beta_{\mathrm{TS}} t_i = \beta_0 + (\beta_1 - \beta_{\mathrm{TS}})\, t_i + e_i \quad i = 1, \ldots, n$$

Can we be sure that $\beta_1 - \beta_{\mathrm{TS}} \approx 0$? It is not always, particularly with small datasets. To summarize, the detrending procedure may produce data that reflect a new trend. As far as we know, there have been no exhaustive studies of the consequences of the detrending procedure on the estimation of autocorrelation when it is applied to data with linear and nonlinear trends.

Once we establish that the data have no trend, we have to evaluate the autocorrelation. This is no easy matter, as can be seen in much of the published work

([95–98], among others). To estimate the lag-1 autocorrelation, the conventional estimator is widely used [99]:

$$r_1 = \frac{\sum_{i=2}^{n} (x_i - \overline{x})(x_{i-1} - \overline{x})}{\sum_{i=1}^{n} (x_i - \overline{x})}, \quad \overline{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \tag{12}$$

The statistic r_1 is very similar to Pearson's correlation coefficient, but the numerator only has $n - 1$ terms, and $\overline{x}$ is used instead of calculating the average for $x_1, \ldots, x_n$ and $x_1, \ \ldots, x_{n-1}$. Under the hypothesis $\rho_1 = 0$, the expected value of r_1 is $E(r_1) = -1/n$ [100]. On this basis, Huitema and McKean [101] proposed the modified estimator:

$$r_1^+ = r_1 + \frac{1}{n} \tag{13}$$

Nevertheless, r_1^+ has a negative bias if $\rho_1 > 0$ and n is small. In fact, the approximate bias of r_1 is $-(1 + 4\rho_1)/n$ [102]. To test $H_0 : \rho_1 = 0$, we use the approximation proposed by Moran [100] of var(r_1):

$$\mathrm{var}(r_1) = \frac{(n-2)^2}{n^2(n-1)}\left(1 - \rho_1^2\right)$$

which is an accurate estimation under the null hypothesis [103, 104]. So, under the null hypothesis $\rho_1 = 0$, $E_0\left(r_1^+\right) = 0$ is the expected value of r_1^+, and the variance is

$$\mathrm{var}_0\left(r_1^+\right) = \frac{(n-2)^2}{n^2(n-1)} \tag{14}$$

It is now straightforward to use the statistic

$$Z_{r_1^+} = \frac{n\sqrt{n-1}}{(n-2)} r_1^+$$

which, under the null hypothesis, asymptotically follows a standard normal distribution. Therefore, it is now easy to calculate the p-value:

$$p = 2\left(1 - \Phi\left(\left|Z_{r_1^+}\right|\right)\right)$$

As we said above, the existence of serial correlation affects the MK test [67, 79]. Autocorrelation implies a change in the variance of the statistic S, var(S). Specifically, a lag-1 positive autocorrelation $\rho_1 > 0$ increases the var(S), which will produce an increase in type I errors if we apply the usual MK test [84, 105]. In the case of a negative autocorrelation $\rho_1 < 0$, we will have exactly the opposite effect, but as we mention above, we expect a null or a positive time serial correlation with pollutants, and it is very rare to detect a negative autocorrelation. In order to solve the problem with the MK test, two strategies appear in the literature: data PW and modifying the MK test.

Modified MK Test for Autocorrelated Data

One way to modify the MK test is by modifying var(S). There are several proposals, and here we present the method described in Lettenmaier [106] and Hamed and Rao [107], reviewed and evaluated in Yue and Wang [84]. Once we have evaluated the lag-1 autocorrelation and rejected the null hypothesis $\rho_1 = 0$ (if the null hypothesis is accepted, it is recommended to apply the usual MK test), we apply the modified MK (MKM) test. The modified variance of S, var*(S), is calculated as follows:

$$\mathrm{var}^*(S) = \frac{n}{n^*}\mathrm{var}(S)$$

where n^* is the effective number of independent samples [108]. Matalas and Langbein [105] derived a formula for n^* in the case of a lag-1 autoregressive process:

$$n^* = \frac{n}{1 + 2\frac{\rho_1^{n+1} - n\rho_1^2 + (n-1)\rho_1}{n(1-\rho_1)^2}} \tag{15}$$

To arrive at our estimate of var*(S), $\hat{\mathrm{var}}^*(S)$, we have to replace ρ_1 with the estimator $\hat{\rho}_1 = r_1^+$ in Eq. 15. Next, we apply (for $n > 10$) the transformation specified in Eq. 9, replacing σ by $\hat{\sigma}^* = \sqrt{\hat{\mathrm{var}}^*(S)}$.

Finally, we note that other modifications of the MK test have been published (e.g., [67, 107]).

PW

The other approach to deal with autocorrelation is data PW [79] which means removing the serial correlation from the data prior to applying the test. This procedure reduces the occurrence of type I errors close to the adopted (nominal) significance level [86, 87]. Nevertheless, as we say above, the estimate of the autocorrelation will become artificially inflated if the data reflect a trend. For this reason, *trend-free PW* (TFPW) has been proposed [80]. TFPW is a four-step procedure: 1) DT the data; 2) estimate the autocorrelation; 3) remove the serial correlation; and 4) replace the trend in the data. According to Önöz and Bayazit

[83], TFPW is frequently more powerful than the PW at detecting trends, and it is widely used in much work [80, 109–114].

We propose the following TFPW algorithm that deals with missing values:

1. Apply the detrending procedure and estimate the lag-1 autocorrelation $\hat{\rho}_1 = r_1^+$.
2. Is the autocorrelation significant? If $H_0 : \rho_1 = 0$ is accepted (choose α between 0.10 and 0.20), then do not transform the data, i.e., final data are original data: $x_i^{***} = x_i$.
3. If $H_0 : \rho_1 = 0$ is rejected and β_{TS} (recall TS estimator) is not significant (the zero value is inside the confidence interval), remove the autocorrelation from the original data: $x_i^{***} = x_i - \hat{\rho}_1 x_{i-1}$.
4. If $H_0 : \rho_1 = 0$ is rejected and β_{TS} is significant, then:

   a. Remove the autocorrelation from the detrended data:

   $$x_i^{**} = x_i^* - \hat{\rho}_1 x_{i-1}^*$$

   where $x_i^* = x_i - \beta_{TS} t_i$. Here, another obstacle arises: what do we do if the data have missing values? Curiously, this is an important point that is absent from the work we have reviewed. We propose imputing them (e.g., by applying linear interpolation), only in order to be able to remove the autocorrelation; otherwise, every original missing value implies losing another final value in this step.

   b. Replace the trend in the data: $x_i^{***} = x_i^{**} + \beta_{TS} t_i$.

   Once TFPW has been performed, you can apply the usual MK test and TS estimator to the transformed data, x_t^{***}. Finally, in Önöz and Bayazit [83], a modified form of TFPW (referred to as MTFPW) that aims to reduce its probability of rejecting a true H_0 was proposed. There is still some controversy in the literature regarding the most appropriate approach to correcting for serial correlation [115, 116]. For example, we think that many researchers forget that if $\rho_1 = 0$ and we reject the null hypothesis (due to a type I error), PW introduces autocorrelation. In this unfortunate case, it is easy to calculate this serial correlation:

   $$\mathrm{corr}(x_i, x_{i-1}) = -\hat{\rho}_1 / \left(1 + \hat{\rho}_1^2\right)$$

   where $\hat{\rho}_1 = r_1^+$. In summary, we may assume that further studies are still required to evaluate the performance of both PW and TFPW techniques.

2.2 *Overall Assessment of GWBs*

In this section, we deal with how to aggregate data from individual monitoring points (spatial aggregation) in order to assess a trend in a GWB as a whole. Grath et al. [8] introduce the necessity to calculate trends for a GWB or group of GWBs. Since trends in GWBs as a whole cannot be observed directly, an approach has to be adopted to aggregating observations from individual monitoring points. Spatial correlation is a matter of concern here, because data series coming from various stations may be correlated. Although most studies ignore this fact in their interpretations of results, it must be kept in mind that the effect of cross-correlation in the data is to increase the expected number of trends [57].

Several approaches deal with overall trend assessment by grouping or blending the results from all the stations inside a GWB. Grath et al. [8] recommended carrying out the trend assessment with the mean values (average of all the stations for each period of time). An alternative is the average (or the median) of any statistic obtained from every single station within a GWB. In a case study of CIS Guidance No. 18 ([9], Sect. 10.8), two aggregation procedures are presented: (a) defining the median trend slope and (b) using age dating to aggregate time series (simply pooling data) along a standardized x-axis showing recharge time. In Douglas et al. [60], an interesting aggregation procedure based on the results of MK tests is described, and we will outline it below. However, an average or a median implies the assumption that the trend is homogeneous across all stations, which is difficult to justify. Thus, in Van Belle and Hughes [12], a preliminary test of the homogeneity of the trend direction was proposed. When the trend is heterogeneous across stations (e.g., an upward trend in one set of stations and a downward trend in another), any overall test of trend direction or slope estimator will be misleading.

2.2.1 *S*-Mean Method

The S-mean method [60] is based on the results of the MK test for every station. Specifically, we must compute:

$$\overline{S}_m = \frac{1}{m}\sum_{k=1}^{m} S_k \tag{16}$$

where S_k is the S statistic provided by the MK test for the kth station in a GWB with m stations. If the data series are cross-correlated, i.e., there is spatial correlation between stations, the variance of $\overline{S}_m$ is

$$\operatorname{var}\left(\overline{S}_m\right) = \frac{1}{m^2}\left[\sum_{k=1}^{m}\operatorname{var}(S_k) + 2\sum_{k=1}^{m-1}\sum_{j=1}^{m-j}\operatorname{cov}\left(S_k, S_{k+j}\right)\right]$$

Following Salas-La Cruz [117], the covariance between station k and station $k + j$ is:

$$\operatorname{cov}\left(S_k, S_{k+j}\right) = \sigma^2 \rho_{k,k+j}$$

where $\rho_{k,k+j}$ is the cross-correlation coefficient between the two stations. Therefore, the variance of $\overline{S}_m$ becomes:

$$\operatorname{var}\left(\overline{S}_m\right) = \frac{1}{m^2}\left[m\sigma^2 + 2\sum_{k=1}^{m-1}\sum_{j=1}^{m-j}\sigma^2\rho_{k,k+j}\right] = \frac{\sigma^2}{m}\left[1 + (m-1)\overline{\rho}_{xx}\right] \tag{17}$$

where:

$$\overline{\rho}_{xx} = \frac{2\sum_{k=1}^{m-1}\sum_{j=1}^{m-j}\sigma^2\rho_{k,k+j}}{m(m-1)}$$

is the average cross-correlation coefficient for the whole GWB. Considering that the overall GWB has no trend, it is obvious that $E\left(\overline{S}_m\right) = 0$. Therefore, asymptotically ($n > 10$ could be enough for practical purposes), the statistic:

$$Z_{\overline{S}} = \frac{\overline{S}_m}{\sqrt{\operatorname{var}\left(\overline{S}_m\right)}} \sim N(0,1)$$

follows a standard normal distribution. Obtaining p-value is straightforward:

$$p = 2\left(1 - \Phi\left(\left|Z_{\overline{S}}\right|\right)\right)$$

This procedure takes into account spatial correlation. In our opinion, it is much better than other approaches that simply mix or group data from the different stations. Nevertheless, we warn against the use of these approaches to blending the results of individual monitoring points: they implicitly assume a global trend for the whole GWB, and that is not always so. We outline below quite a different spatial aggregation method; it does not assume a common global trend.

2.2.2 Cross-Correlation: A Bootstrapping Procedure

Burn and Hag Elnur [61] propose an ingenious bootstrapping approach to determine the critical value for the percentage of stations expected to show a trend by chance. It can be summarized in the following steps:

1. Select a year at random from the entire period of time for which data are available (supposing that the sampled values are obtained yearly). Repeat this procedure for the required number of years, i.e., the number of years in the initial

dataset, so that the new dataset is in fact a resampling of the years, without altering the data from individual monitoring points.

2. Perform the MK test for every station (at a chosen significance level, α) and determine the percentage of stations with a significant trend.
3. Steps 1–2 are repeated k times ($k = 600$ in Burn and Hag Elnur [61]), to obtain the empirical distribution of the percentage of stations that are significant at the α level. Sort the k percentage values recorded and determine the percentile $(1 - \alpha) \times 100\%$, p_{crit}.
4. If the actual percentage of stations showing a trend in the GWB analyzed is greater than p_{crit}, it will be considered significant.

Note that any temporal structure (a trend) that exists in every single station will not be reproduced in the resampled datasets because of the bootstrapping process. However, the cross-correlations in the original data are preserved, allowing us to evaluate the effect of spatial correlation within the GWB. In Sect. 4, we will provide a modified procedure that outperforms the algorithm just outlined here.

3 Assayed Procedures: A Case Study

In order to illustrate the procedures and highlight the main results, some of the stations and GWBs in Catalonia were selected (Fig. 1), and some of the methods described above were applied. We first determined the trends for individual monitoring points, in accordance with nine specific methods (see below); and then we applied two distinct methods to show how data may be aggregated in order to determine the trend for a GWB as a whole. GWBs with a long monitoring period and many sampling sites were selected to be analyzed. Nitrate and chloride concentrations were analyzed in order to identify significant upward and downward trends.

Figure 2 shows the scatterplots of the twelve stations used to compare the nine methods. These stations were not selected at random, but specifically in order to show the performance of the methods in different scenarios. One possible classification of these monitoring points based on the data shown, according to a rough trend assessment, is the following:

- Linear or monotonic trends: stations N1, N2, N3 (the latter two with extreme values), and N7.
- Overdispersion: stations N4, N5, and N6. It is difficult to assess the trends due to overdispersion; perhaps the data could fit a quadratic or even a cubic curve.
- Trend reversal: evidently this is not certain, but quite possible, at N8, N9, C1, C2, and C3.

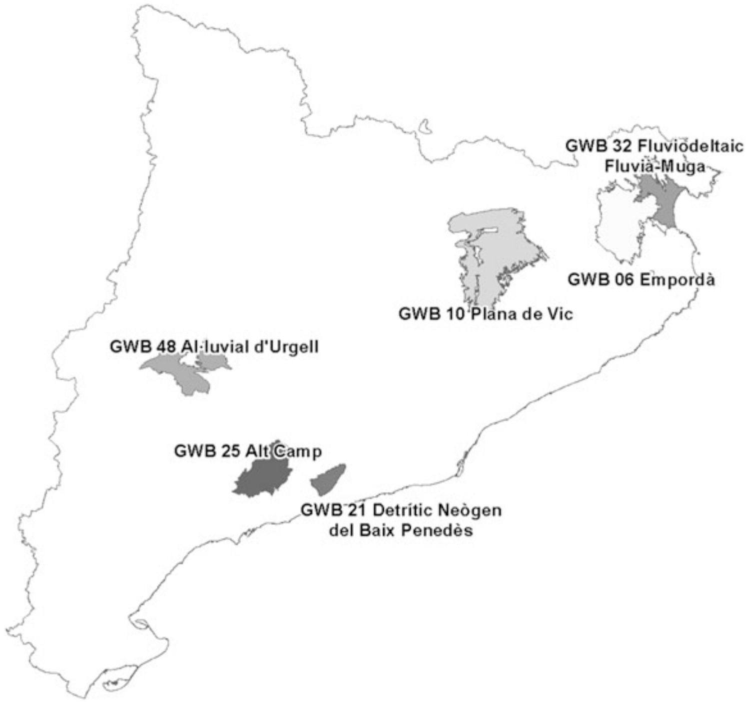


Fig. 1 GWBs located in Catalonia and sampling sites currently used to monitor chemical status

3.1 Methods Assayed for Individual Monitoring Points

For each assayed method outlined below, we describe how it was performed (using package(s), function(s), or our own scripts in R), and we highlight the main results obtained in two categories: *goodness of fit* and *trend estimation*.

1. LR, using the *lm* function. Goodness of fit: Pearson's correlation coefficient (r), R^2, and p-value. Trend estimation: $\hat{\beta}_1$ (Eq. 1) and p-value for $\hat{\beta}_1$.
2. RLR, with the *rlm* function (MASS package, [35]). Setup: Huber-type function with parameter $k = 1.345$ (default value), $\hat{\sigma} = \text{MAD}$. Goodness of fit: R^2. Trend estimation: $\hat{\beta}_1$ (Eq. 3) and p-value for $\hat{\beta}_1$.
3. MK test and TS estimator (MK&TS), with the MK function (Kendall package, [118]) and *mblm* function (package mblm, [119]). Goodness of fit: *tau b* correlation coefficient and p-value. Trend estimation: TS estimator (Eq. 10).
4. RoCoCo and TS estimator (RCC&TS), using the RoCoCo package [78] to calculate the RoCoCo. Setup: $R_T(t_i, t_j) = 1$ if $t_j > t_i$ (zero otherwise), $R_X(x_i, x_j) = \max\{0, \min((x_j - x_i)/r)\}$, respectively named classical strict ordering and truncated linear scoring, and $\overline{T}(x, y) = \min(x, y)$. Goodness of

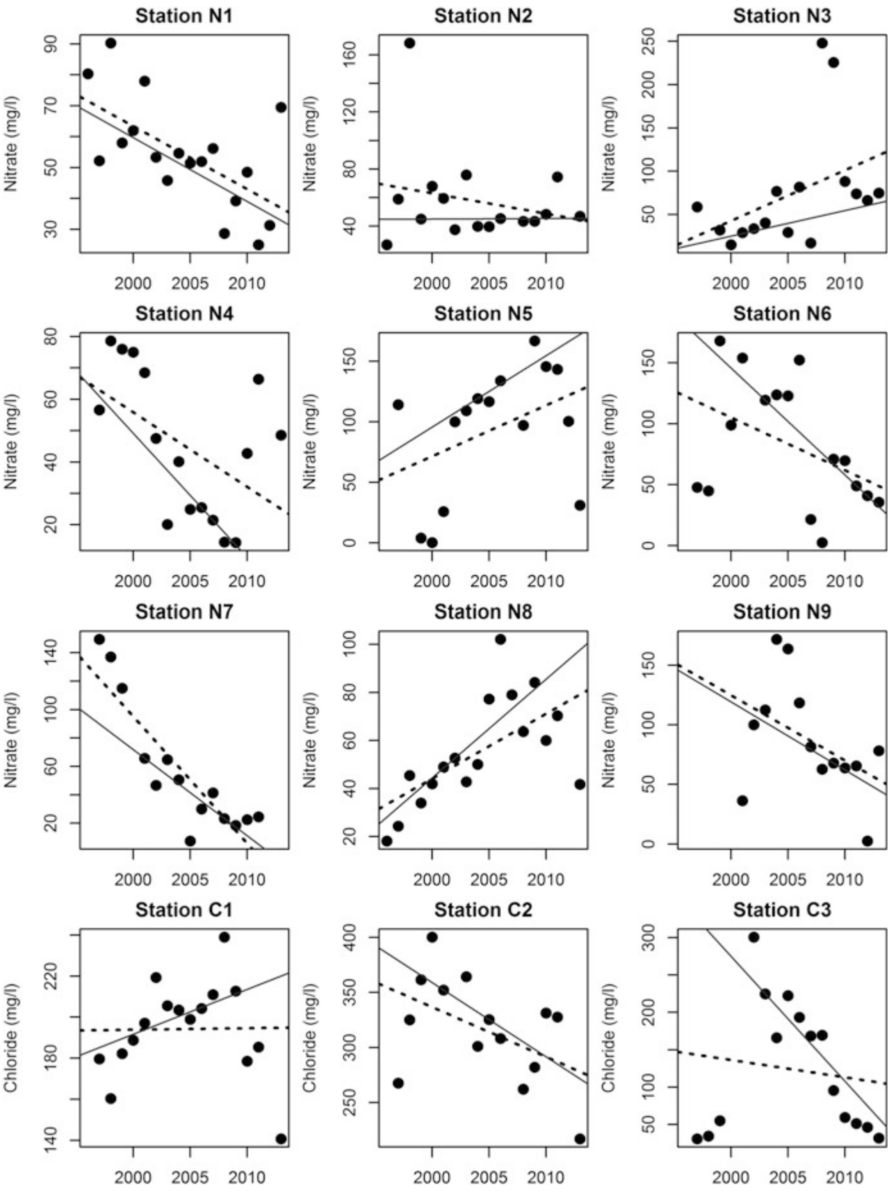


Fig. 2 Scatterplots of pollutant concentrations (nitrates and chlorides) from 1996 to 2013 for the selected monitoring points, showing the LR fit (*dotted line*) and TS fit (*solid line*)

fit: robust gamma correlation coefficient and *p*-value. Trend estimation: TS estimator (Eq. 10).

5. MK modified test for autocorrelated data and TS estimator (MKM&TS). We programmed our own code for the calculus of the modified variance estimate,

$\hat{\text{var}}^*(S)$; lag-1 autocorrelation, $\hat{\rho}_1 = r_1^+$; and p-values. If serial data had missing values, we proposed the following heuristic correction to the estimation of lag-1 autocorrelation (Eqs. 13 and 14):

$$r_1^+ = \frac{(n+g)}{n} r_1 + \frac{1}{n}, \ \text{var}_0\left(r_1^+\right) = \left(\frac{n+g}{n}\right)^2 \frac{(n-2)^2}{n^2(n-1)}$$

Goodness of fit: *tau b* correlation coefficient and p-value. Trend estimation: TS estimator (Eq. 10).

6. TFPW before applying the MK test and TS estimator (TFPW + MK&TS). We programmed our own code in order to perform the TFPW, as described in Sect. 2.1.3. A threshold value of $\alpha = 0.2$ was used to test the significance of the TS estimator and lag-1 autocorrelation. Goodness of fit: *tau b* correlation coefficient and p-value. Trend estimation: TS estimator (Eq. 10).
7. TFPW before applying RoCoCo and TS estimator (TFPW + RCC&TS). This method combines TFPW with method 4. Goodness of fit: robust gamma correlation coefficient and p-value. Trend estimation: TS estimator (Eq. 10).
8. Two-section LR (2SLR). Breakpoints detected with the *piecewise* function (SiZer package, [120]). Significance test for the difference of slopes: Davies test [121], applied with the *davies.test* function (segmented package, [122]). Goodness of fit: none. Trend estimation: $\hat{\beta}_1$ and $\hat{\beta}_1 + \hat{\beta}_2$ (Eq. 5) and p-value for $\hat{\beta}_2$ (Davies test).
9. QR, using the *lm* function. Goodness of fit: R^2 and p-value. Trend estimation: $\hat{\beta}_1$ and $\hat{\beta}_2$ (Eq. 2) and p-value for $\hat{\beta}_2$.

3.2 Methods Assayed for Overall GWB Trend Assessment

(1) S-*mean* method, described in Sect. 3.2: Nevertheless, missing values and ties are not considered in Douglas et al. [60]; thus, we propose a modified variance estimation of the statistic $\overline{S}_m$. The estimation of $\text{var}(S) = \sigma^2$ could be approximated by $\hat{\sigma}^2 \approx m\hat{\sigma}_{\overline{S}}^2$, where:

$$\hat{\sigma}_{\overline{S}}^2 = \frac{1}{m^2}\left[\sum_{k=1}^{m} \hat{\text{var}}(S_k)\right] \tag{18}$$

Therefore:

$$\hat{\text{var}}\left(\overline{S}_m\right) = \hat{\sigma}_{\overline{S}}^2[1 + (m-1)\overline{\rho}_{xx}] \tag{19}$$

(2) *Permutational* approach for testing overall GWB *trend assessment* (PTA): based on the procedure described in Burn and Hag Elnur [61], we propose a

modified method that (a) is a permutation test, so it gives a significance assessment (in terms of p-value), and (b) evaluates not only significant overall trends but upward and downward trends in a separate manner. The algorithm can be implemented in the following way:

1. For a given GWB dataset, perform trend test for every station. Given a significance level α, collect and count separately the number of significant upward and downward trends.
2. Randomly select a year from the entire period of time for which data are available (assuming a yearly sampling period). Repeat this random selection without replacement for the required number of years. The new dataset is in fact a permutation of years, without altering any data from individual monitoring points.
3. Perform the test for every station (significance level α) and determine the number of stations with significant upward and downward trends.
4. Repeat steps 2–3 k times ($k = 9,999$ permutations in our study). Sort the values of upward and downward significant trends separately, to obtain the empirical distributions of the number of stations with significant trends (upward and downward). Combine these with the actual values for the GWB obtained in step 1.
5. Finally, calculate the two percentiles, $P_{\exp}$, of the actual values obtained in step 1, so complementary values $p = 1 - P_{\exp}$ (one for upward trends and the other for downward trends) perform like p-values.

   Any of the methods summarized in Sect. 3.1 for individual monitoring points could have been applied, but for the purpose of comparing with S-mean, we performed the MK test.

4 Results and Discussion

4.1 Individual Monitoring Points

First of all, we briefly discuss the issue of autocorrelation. At nine of the twelve stations, significant positive autocorrelation was detected (Table 1). Of these nine stations, in five cases, we previously detrended (due to a significant TS slope). Therefore, because positive autocorrelation increases the likelihood of a false positive in the trend assessment, it seems absolutely essential to use a method that takes into account the autocorrelation (i.e., MKM) or to perform TFPW.

However, further analysis revealed serious flaws. Is there real autocorrelation of the data, or is it a spurious detection due to erroneous detrending? As you may recall, after linear detrending to data, if it is not appropriate (i.e., the data reflect a nonlinear trend), the transformed data will show a trend that will affect the estimate of autocorrelation. We cannot be sure exactly what happened (we are referring here to the overestimation of the autocorrelation in our case study), but it is clear that at

Table 1 Lag-1 autocorrelation (estimate and p-value) and detrending applied (yes or no) according to TS confidence interval for all individual monitoring points (stations)

Groundwater body	Station code	Station label	Detrending	Lag-1 autocorrelation	
				$\hat{\rho}_1$	p-value
GWB48	25248-0005	N1	YES	−0.2644	0.2200
GWB21	43074-0058	N2	NO	−0.0382	0.8805
GWB10	08129-0011	N3	YES	0.3677	**0.1154**
GWB21	43140-0078	N4	YES	0.5348	**0.0220**
GWB49	25152-0006	N5	YES	0.5707	**0.0247**
GWB49	25217-0009	N6	YES	0.3191	**0.1719**
GWB25	43161-0159	N7	YES	0.6785	**0.0097**
GWB21	43020-0056	N8	YES	0.2858	0.2211
GWB33	17085-0012	N9	NO	0.7203	**0.0106**
GWB39	08301-0035	C1	NO	0.4850	**0.0378**
GWB49	25242-0008	C2	NO	0.4337	**0.1187**
GWB23	08231-0025	C3	NO	0.7896	**0.0010**

Labels N and C indicate nitrate and chloride pollutants, respectively

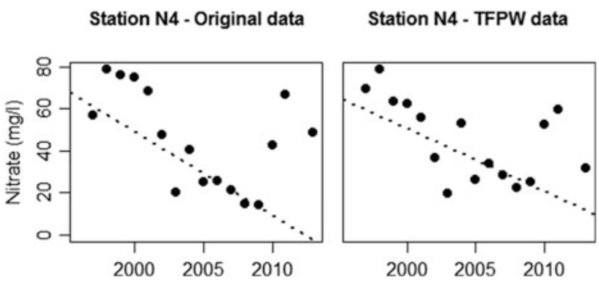


Fig. 3 Comparison plots of raw data and TFPW transformed data for station N4, with TS fitted line

various stations with significant autocorrelation, the original trend seems not to be linear, so that the detrending will not have been effective (misrepresenting the data). The nonlinear tendency is quite evident, for example, in the case of N4, N7, C1, C2, and C3. Let us look in more detail at the case of N4: the original nonlinear trend (maybe a quadratic or cubic trend) means that the detrending actually increased the linear trend (Fig. 3), so that the two methods with TFPW detect a more significant trend than the respective non-TFPW methods.

Next, we discuss the results of the nine methods in the same order as that in which they appear in Table 2 and that they were described in Sect. 3.

LR is a good method when the data fit a linear trend reasonably well (Fig. 2). However, there are several pitfalls associated with it when treating groundwater data. It can be greatly affected by extreme values; for example, consider station N3 (Fig. 4): LR fails to detect an upward trend due to the inclusion of two extreme values in the time series which increase the residual values substantially. Also, the method has difficulties in cases of overdispersion (N5, N6) or a poorly defined nonlinear trend (N4). Obviously, it is not useful in cases with a trend reversal: it was

Table 2 Goodness of fit, trend estimation, and trend assessment (upward ↑, no trend →, downward ↓) using the methods assayed (LR, RLR, MK&TS, MKM&TS, RCC&TS, TFPW + MK&TS, TFPW + RCC&TS, 2SLR, and QR) for the stations selected

		Goodness of fit			Trend estimation			Assessment
Station	Method	r	$\sqrt{R^2}$	p-value	Estimates of parameters		p-value	(↑ → ↓)
N1	**LR**	−0.6189	0.6189	**0.0062**	−2.035		**0.0062**	↓
	RLR	–	0.7043	–	−2.334		**0.0035**	↓
	MK&TS	−0.4641	–	**0.0080**	−2.136		–	↓
	RCC&TS	−0.4691	–	**0.0049**	−2.136		–	↓
	2SLR	–	–	–	2.753	40.457	**0.0373**	↓ 2012 ↑
	QR	–	0.6570	**0.0145**	−4.701	0.157	0.2755	LR
N2	LR	−0.2311	0.2311	0.3891	−1.428		0.3891	→
	RLR	–	0.3425	–	−0.315		0.7386	→
	MK&TS	0.0418	–	0.8569	0.146		–	→
	RCC&TS	0.0448	–	0.8185	0.146		–	→
	2SLR	–	–	–	−3.079	2.277	0.8809	LR
	QR	–	0.2529	0.6508	−3.690	0.136	0.7084	LR
N3	LR	0.4089	0.4089	0.1031	5.142		0.1031	→
	RLR	–	0.5321	–	3.109		**0.0492**	↑
	MK&TS	0.3235	–	**0.0765**	2.876		–	↑
	RCC&TS	0.3252	–	**0.0766**	2.876		–	↑
	MKM&TS	0.3235	–	0.2158	2.876		–	→
	TFPW + MK&TS	0.2833	–	0.1373	3.298		–	→
	TFPW + RCC&TS	0.2867	–	0.1343	3.298		–	→
	2SLR	–	–	–	−8.150	7.085	0.9447	LR
	QR	–	0.4104	0.2749	6.731	−0.092	0.8880	LR

N4	LR	−0.4080	0.4080	0.1040	−1.785		0.1040	→
	RLR	**–**	0.4081	**–**	−1.785		0.1040	→
	MK&TS	−0.3382	**–**	**0.0638**	−2.030		–	↓
	RCC&TS	−0.3502	–	**0.0573**	−2.030		–	↓
	MKM&TS	−0.3382	–	0.2837	−2.030		–	→
	TFPW + MK&TS	−0.4500	–	**0.0170**	−2.363		–	↓
	TFPW + RCC&TS	−0.4432	–	**0.0167**	−2.363		–	↓
	2SLR	–	–	–	−4.225	8.369	0.1030	LR
	QR	–	0.5221	0.1078	−6.794	0.304	0.1750	LR
N5	LR	0.3722	0.3722	0.1557	3.541		0.1557	→
	RLR	–	0.4394	–	3.774		0.1651	→
	MK&TS	0.3500	–	**0.0649**	4.089		–	↑
	RCC&TS	0.3590	–	**0.0586**	4.089		–	↑
	MKM&TS	0.3500	–	0.3033	4.089		–	→
	TFPW + MK&TS	0.2190	–	0.2763	2.581		–	→
	TFPW + RCC&TS	0.2093	–	0.2856	2.581		–	→
	2SLR	–	–	–	6.951	−69.301	0.1093	LR
	QR	–	0.4240	0.2758	10.668	−0.411	0.4330	LR
N6	LR	−0.3600	0.3600	0.1571	−3.307		0.1571	→
	RLR		0.3600	–	−3.307		0.1571	→
	MK&TS	−0.2794	–	0.1275	−4.398		–	→
	RCC&TS	−0.2882	–	0.1207	−4.398		–	→
	MKM&TS	−0.2794	–	0.2626	−4.398		–	→
	TFPW + MK&TS	−0.3167	–	**0.0957**	−4.7725		–	↓
	TFPW + RCC&TS	−0.3382	–	**0.0764**	−4.772		–	↓
	2SLR	–	–	–	19.779	−10.186	**0.0498**	↑ 2001 ↓
	QR		0.6000	**0.0452**	13.059	−0.969	**0.0424**	↑ 2003 ↓

(continued)

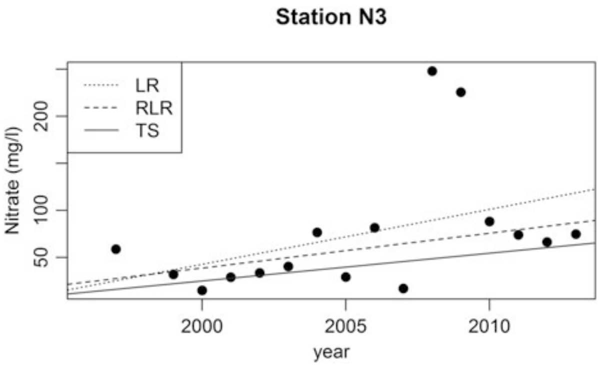
Table 2 (continued)

Station	Method	Goodness of fit			Trend estimation			Assessment
		r	$\sqrt{R^2}$	p-value	Estimates of parameters		p-value	(↑ → ↓)
N7	**LR**	−0.9041		**0.0000**	−9.738		**0.0000**	↓
	RLR	–	0.9120		−9.703		**0.0000**	↓
	MK&TS	−0.7524	–	**0.0001**	−9.650		–	↓
	RCC&TS	−0.7900	–	**0.0000**	−9.650		–	↓
	MKM&TS	−0.7524	–	**0.0602**	−9.650		–	↓
	TFPW + MK&TS	−0,7582	–	**0.0002**	−8.483		–	↓
	TFPW + RCC&TS	−0.8408	–	**0.0000**	−8.483		–	↓
	2SLR	–	–	–	−20.198	−3.678	**0.0010**	↓ 2002 ↓
	QR	–	0.9758	**0.0000**	−23.944	0.954	**0.0001**	↓ 2009 ↑
N8	**LR**	0.6271	0.6271	0.0071	2.697		**0.0071**	↑
	RLR	–	0.7411	–	3.065		**0.0008**	↑
	MK&TS	0.5147	–	**0.0045**	3.248		–	↑
	RCC&TS	0.5282	–	**0.0022**	3.248		–	↑
	2SLR	–	–	–	6.120	−5.134	**0.0042**	↑ 2007 ↓
	QR	–	0.8125	**0.0005**	10.512	−0.475	**0.0051**	↑ 2007 ↓
N9	LR	−0.3733	0.3733	0.2089	−4.536		0.2089	→
	RLR	–	0.4543	–	−4.488		0.2441	→
	MK&TS	−0.2527	–	0.2284	−4.037		–	→
	RCC&TS	−0.2599	–	0.2233	−4.037		–	→
	MKM&TS	−0.2527	–	0.5655	−4.037		–	→
	TFPW + MK&TS	−0.0769	–	0.7603	−2.831		–	→
	TFPW + RCC&TS	−0.1010	–	0.6543	−2.831		–	→
	2SLR	–	–	–	28.337	−17.15	**0.0024**	↑ 2004 ↓
	QR	–	0.7886	**0.0077**	46.354	−2.544	**0.0051**	↑ 2005 ↓

C1	LR	0.0850	0.0850	0.7462	0.381		0.7462	→
	RLR	–	0.2547	–	0.999		0.3835	→
	MK&TS	0.2500	–	0.1740	2.087		**–**	→
	RCC&TS	0.2501	–	0.1752	2.087		–	→
	MKM&TS	0.2500	–	0.4042	2.087		–	→
	TFPW + MK&TS	−0.0500	–	0.8219	−0.131		–	→
	TFPW + RCC&TS	−0.0348	–	0.8622	−0.131		–	→
	2SLR	–	–	–	4.3420	−16.363	**0.0000**	↑ 2008 ↓
	QR	–	0.7718	**0.0018**	12.514	−0.737	**0.0005**	↑ 2004 ↓
C2	LR	−0.3530	0.3530	0.1974	−3.057		0.1974	→
	RLR	–	0.3500	–	−3.014		0.2321	→
	MK&TS	−0.1429	–	0.4884	−3.156		–	→
	RCC&TS	−0.1777	–	0.3657	−3.156		–	→
	MKM&TS	−0.1429	–	0.6482	−3.156		–	→
	TFPW + MK&TS	−0.2967	–	0.1546	−3.037		–	→
	TFPW + RCC&TS	−0.3032	–	0.1579	−3.037		–	→
	2SLR	–	–	–	30.090	−9.011	**0.0205**	↑ 2000 ↓
	QR	–	0.6339	**0.0458**	13.517	−1.006	**0.0361**	↑ 2003 ↓
C3	LR	−0.0152	0.0152	0.9553	−0.243		0.9553	→
	RLR	–	0.0328	–	0.044		0.9918	→
	MK&TS	−0.1333	–	0.4995	−5.675		–	→
	RCC&TS	−0.1584	–	0.4306	−5.675		–	→
	MKM&TS	−0.1333	–	0.7862	−5.675		–	→
	TFPW + MK&TS	−0.1810	–	0.3731	−1.497		–	→
	TFPW + RCC&TS	−0.1891	–	0.3400	−1.497		–	→
	2SLR	–	–	–	45.157	−20.88	**0.0000**	↑ 2002 ↓
	QR	–	0.8494	**0.0002**	47.842	−2.863	**0.0001**	↑ 2004 ↓

If a breakpoint (2SLR) or an inflection point (QR) is detected, we show the year in the assessment column

Fig. 4 Scatterplot for station N3 showing three fitted lines, adjusted according to the LR, RLR, and TS methods assayed



not able to detect any trend (N9, C1, C2, C3), or it only detected one trend (an upward trend for station N8). In the case of N7, LR detects the downward trend perfectly, but we cannot identify the slope change. Finally, LR does not take autocorrelation into account, and as previously explained, this fact can affect the significance of the model. In this scenario, we could have applied TFPW to the data; but as we mention earlier and we will return to later, TFPW is a risky method. Another approach would be to apply any of the methodologies that have been published and implemented in a lot of software for the treatment of time series. In short, LR is highly sensitive to outliers and overdispersion; it only detects one linear trend (and therefore it cannot detect a trend reversal) and does not take autocorrelation into account. In general, we advise against the use of LR for trend detection.

RLR has proved to be resistant to extreme values. For the example station N3 (Fig. 4), LR failed to detect an upward trend due to the inclusion of two extreme values; however, RLR found the increasing trend. We suggest it as an alternative to LR, and we are a bit surprised that it has not been more widely recommended in the area of groundwater monitoring. It does still have limitations though: (1) it only works well for a linear trend; (2) it cannot detect trend reversal; and (3) it does not take autocorrelation into account.

MK test and RoCoCo (with TS estimator of the slope): as evident from Table 2, these two methods usually give almost the same final results. RoCoCo, based on fuzzy scorings and more complex than Kendall's tau b, does not improve trend detection. More theoretical and simulation studies are necessary to determine in what situations RoCoCo may become a better method to detect a trend. In particular, perhaps another choice of the fuzzy scoring function could achieve better results. Compared to RLR, the only difference is that MK test and RoCoCo detected a downward trend in N4 and an upward trend in N5. An important point to remember is that MK test and RoCoCo detect monotonic trends but are not helpful in detecting trend reversals, as we can see from the results for stations N9, C1, C2, and C3. Finally, we would like to highlight again that trend estimation with TS estimator, despite being robust, is just linear (Fig. 2).

MKM test for autocorrelated data: as we state above, this has the advantage of not manipulating the original data, but simply changing the variance of the MK S statistic. In our case study, all nine autocorrelations detected were positive, so $\mathrm{var}^*(S) > \mathrm{var}(S)$, and the MKM test offers greater p-values than MK test. As we can see, MKM test becomes a much more conservative test than MK test and only detects a significant trend in station N7. It is difficult to quantify the effect, but it is evident that the incorrect estimate (even spurious) of autocorrelation makes MKM test very conservative. Although we cannot draw definitive conclusions from just a few examples of stations, we are afraid that the MKM test is exceedingly conservative for trend detection.

TFPW with MK test or RoCoCo: as we have discussed previously, it is not clear whether TFPW is a successful technique or leads to involuntary contamination of the original data. It is certainly the latter case when the data present a nonlinear trend. If we examine stations N4 and N6 carefully, we notice a peculiar feature. In both cases, TFPW clearly increases trend detection (in N6, this represents a shift from nonsignificance to significance). The risk due to both the data detrending and the estimate of the autocorrelation can be serious, and TFPW could produce misleading results; therefore, we advise against the blind application of TFPW. In any case, we recommend applying the test without TFPW first. If the hypothesis of no trend is accepted, then do not apply TFPW to the data. If a significant trend is detected, then apply TFPW and redo the test and finally choose the larger p-value (this conservative option is suitable if we accept that there is a null or a positive autocorrelation).

2SLR displays a surprisingly high (maybe too high in some cases) capacity to detect significant breakpoints (stations N1, N6, N7, N8, N9, C1, C2, and C3), sometimes even at the end of the time series (Fig. 5). We do not know the reliability of the Davies test (used to test for different slopes and so the significance of the breakpoint) in the case of small data series and autocorrelation. However, a point of change does not necessarily indicate trend reversal: it could simply be a change in the slope, as at station N7. Finally, detecting two significantly different slopes and with different signs does not necessarily imply trend reversal: one of the two sections could represent no trend. There are several R packages that estimate breakpoints (such as SiZer and segmented), and they also calculate the bootstrap confidence intervals of the slopes, but such intervals are completely meaningless for short time series, due to their considerable length. In summary, 2SLR seems to be a versatile method that is suitable for detecting trend (which implies abandoning linear or monotonic methods), but it must be studied more carefully. On the one hand, the Davies test seems to be a bit risky (a tendency toward false positives). On the other, if a breakpoint is detected, we need a good strategy for making a decision regarding the trend: a monotonic trend (slope change detected, but no trend change), no current trend, or a trend reversal. Finally, 2SLR does not take autocorrelation into account.

QR detects non-monotonic trends in the same stations as 2SLR does (Fig. 5), although in some cases the two differ in their estimates of the breakpoint (inflection point in the case of QR). Due to the concavity or convexity of a quadratic function, it sometimes seems to detect the breakpoint too earlier or too late. It does not allow

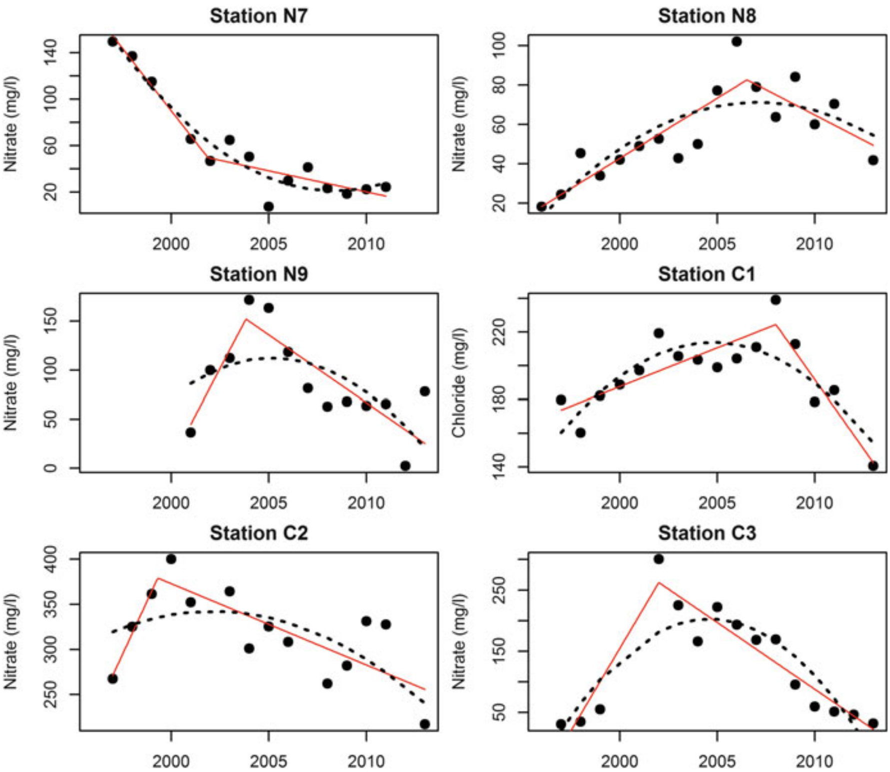


Fig. 5 Plots showing 2SLR (solid line) and QR (*dot line*) fitted models, for stations N7, N8, N9, C1, C2, and C3

for assessing the trend before and after the inflection point in an easy and automatic manner: how do we assess whether the trend is actually no trend? Finally, it does not take into account autocorrelation. Despite QR exhibiting interesting and valuable behavior, we recommend applying 2SLR to detect breakpoints.

In summary, we regard it absolutely necessary to incorporate methods such as 2SLR in order to detect trend reversal. We believe excessive emphasis has been placed on the detection of monotonic trends, and the broad recommendation of the MK test is not justified. We also consider the application of a 3SLR method (three-section LR) for longtime series (at least 20 years). The question is how to decide whether one or two trend reversals are present; so we have to compare several linear models using the framework of GLM (general linear models) or other criteria such as AIC [43, 44]. In the scenario of a monotonic trend, once we have abandoned the multi-section model and trend reversal, we agree that the MK test and TS slope should be recommended. As an alternative, we suggest robust linear models, which could be a very useful alternative as they can tolerate a relatively large proportion of extreme values and overdispersion.

Autocorrelation is a sensitive and controversial issue. We consider it unresolved, since MKM test seems overly conservative and TFPW is risky. As we have seen, TFPW can lead to incorrect data correction (instead of PW, we may be inducing a spurious trend due to incorrect detrending). We recommend a priori decision criteria for the expert: if it is known that annual data autocorrelation is low, it is better not to consider its existence. If medium or high autocorrelation is possible, we need to use methods that are capable of adequate detrending or a simultaneous estimation of the trend and the autocorrelation. This is not an easy matter, and we also question the reliability (efficiency and robustness) of the estimates in both detrending and the calculation of autocorrelation when time series only contain a few data points.

4.2 Overall GWB Trend Assessment

The two methods assayed produce very different results (Table 3). The method based on the *S*-mean statistic (mean of the *S* statistics from all the stations sampled from a GWB) was able to detect three significant global increasing trends (GWB21, GWB25, and GWB32), but no trend in the other three GWBs. However, the PTA method detected significant upward trends in four GWBs and two GWBs with significant upward and downward trends at the same time.

Obviously, the message sent to the management agents is quite different. See, for example, GWB48: the decision from the *S*-mean method is “no trend” (due to the conjunction of three stations showing upward trends and five showing downward trends), but the message from the PTA method is that we have both stations with significant upward trends and with significant downward trends. So we probably have two sections or areas in the GWB: one with an increasing trend and the other with a decreasing trend.

In our opinion, the *S*-mean method is only applicable if the overall GWB has the same trend; but evidently we do not know this fact a priori. So to summarize, we advise against using this approach to assess the overall GWB trend.

The PTA method appears to be a simple and effective method for global evaluation of a GWB that takes spatial correlation into account. It is necessary to consider ways to overcome the major weakness of PTA: it does not use the *p*-value of each station, that is to say, it only considers the number of stations with increasing and decreasing trends.

In short, we should abandon methods such as those that consider the mean values of all the stations. The S-mean takes into account spatial correlation, but it could offer a misleading assessment because an implicit assumption in this kind of technique is to consider GWB trends as homogeneous. We recommend an approach such as PTA to deal with overall GWB assessment.

Table 3 Overall GWB trend assessment

					GWB overall trend assessment				
		Trend assessment (# and %)			PTA (significance)		*S*-mean		
GWB	Stations (#)	↑	→	↓	↑	↓	$\overline{S}_m$	*Z*	*p*
GWB06	8	3	4	1	**0.0079**	0.3028	3.63	0.4513	0.6517
		37.5%	*50.0%*	*12.5%*					
GWB10	22	8	12	2	**0.0011**	0.2446	5.68	0.9178	0.3587
		36.4%	*54.5%*	*9.1%*					
GWB21	10	3	6	1	**0.0111**	0.3481	17.80	2.7299	**0.0063**
		30.0%	*60.0%*	*10.0%*					
GWB25	25	5	16	4	**0.0165**	**0.0456**	10.00	1.7285	**0.0839**
		20.0%	*64.0%*	*16.0%*					
GWB32	13	4	8	1	**0.0058**	0.4256	14.69	2.0778	**0.0377**
		30.8%	*61.5%*	*7.7%*					
GWB48	15	3	7	5	**0.0579**	**0.0043**	−11.20	−1.451	0.1469
		20.0%	*46.7%*	*33.3%*					

Column 2 indicates the total number of stations within the GWB. Columns 3–5 show the number and percentage of stations with upward trends ↑, no trends →, and downward trends ↓, respectively. Overall GWB trend assessment is shown in the second half of the table, for the PTA and S-mean methods assayed; in bold numbers, we highlight significance trends

5 Conclusions and Future Trends

Regarding individual monitoring points, inflection points have to be identified in a proper and efficient manner. In our opinion, this is a key step in assessing groundwater trends. On the one hand, the detection of a breakpoint is necessary to demonstrate trend reversal; on the other, techniques that detect linear or monotonic trends could be considerably affected by trend reversal. It is not true that short data series solve the problem: a breakpoint in the middle of the series could affect the performance of linear or monotonic trend techniques, and it could produce misleading results. In addition, short series imply low test power. In short, we strongly advise applying methods with the capacity to identify breakpoints (such as piecewise LR).

Researchers also need to carefully consider the techniques for dealing with outliers or extreme values. In this concern, the MK test is a good method (if no breakpoint is present); but we think much more work needs to be done in exploring robust models, such as robust regression.

Another issue for future research is autocorrelation. Though much work has dealt with this matter, we consider that it is far from resolved. PW is a risky method and can even distort the data, so we recommend that the approach only be used with great care.

In relation to overall GWB trend assessment, a data aggregation method is needed. We want to emphasize the risks of methods that consider the trend as homogeneous within the GWB, because they could hide the behavior of sets of stations with opposed trends. Instead, we recommend approaches such as PTA.

Groundwater trend assessment is certainly an exciting area of research. To date, much knowledge has been gained regarding the performance of different methods, but not enough to conclude which approach is best in daily practice without analyzing every data time series individually. Nevertheless we must remember that due to the number of GWBs, stations, and pollutants, visual inspection and decisions for each data series are unrealistic. Therefore, an expert agent (software) to make decisions and apply the proper techniques in an automatic manner is absolutely essential. We are currently working on this issue.

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Occurrence of Polar Organic Pollutants in Groundwater Bodies of Catalonia

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Abstract Currently, about half of the groundwater bodies in Catalonia present a bad status in terms of water quantity and chemical quality. The latter is linked to land use, since groundwater contamination originates from agricultural, industrial, and urban activities. This chapter reviews the monitoring programs performed in up to 18 groundwater bodies of Catalonia with the common objective of assessing the occurrence and fate of different polar organic contaminants (PolOPs) in the subsurface. They include the evaluation of polar pesticides and the veterinary antibiotics sulfonamides in rural areas and the study of pharmaceuticals, illicit drugs, and UV filters in urban areas. A review of the analytical methodologies used for the analysis of these compounds in groundwater has been provided, and PolOP concentrations measured have been discussed in terms of spatial and temporal variability and linked to potential contamination sources.

Keywords Analysis, Groundwater, Pesticides, Polar organic contaminants, Sulfonamides

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Contents

1 Introduction

Aquifers are essential to sustain water demand worldwide. Groundwater bodies are dynamic systems that adjust continually to short-term and long-term changes in climate, withdrawal, and land use. There is a direct connection between groundwater quality deterioration and land use practices and/or inadequate waste management. About 30% of Catalonia's land surface is dedicated to agriculture, which is the activity demanding more water resources (70% of the total water demand), and 6% of the land is urbanized, which is responsible for the remaining water demand [1]. According to data from 2007, up to 454 Hm^3 of groundwater are annually extracted for industrial, agricultural, and drinkable water uses in Catalonia [2]. For instance, the average use of water in agriculture equals 197 Hm^3, corresponding to 43% of the total groundwater supply [2]. Therefore, groundwater contamination by agricultural, industrial, and urban activities can be expected [3].

But not only is the quality of groundwater affected. Overexploitation of aquifers above their recharge rate is one of the main concerns regarding aquifers status, especially in areas with very high demands in Catalonia (i.e., coastal and touristic areas), which are not necessarily placed in regions with enough groundwater resources. Excessive withdrawal has led to the decrease of the groundwater table and, thus, to the salinization of coastal aquifers, i.e., *Llobregat* and *Tordera*. Some measures are being applied to reverse this situation, such as aquifer recharge with regenerated water and engineered barriers against saline intrusion [1].

The connection between agriculture and groundwater quality has also become a highly relevant issue from a scientific and management point of view. The regular application of pesticides, fertilizers, and organic amendments on very large areas, together with the use of nontreated wastewater or reclaimed wastewater for irrigation in agricultural land, has compromised the quality of this water resource, as infiltration of rainwater and irrigation return flows are the main recharge sources that aquifers feed on [4]. These diffuse contamination sources may lead to the introduction of generally low concentrations of chemicals in the groundwater body over a wide area on a regular basis.

On the other hand, point sources of contamination such as wastewater treatment plant (WWTP) discharges, seepage, or runoff from landfills and that derived from industrial facilities contribute with a wide variety of contaminants to rivers and streams which eventually may also affect groundwater quality, especially where rivers are the main source of groundwater recharge, where groundwater withdrawals induce seepage from streams, and where floods cause stream water to become bank storage [5]. These point sources of contamination can introduce high amounts of chemicals in a relative small area of the aquifer and cause groundwater contamination plumes. It has been extensively demonstrated that pharmaceuticals, illicit drugs, UV filters, and endocrine disruptors are among the most commonly organic pollutants detected in the effluents of WWTPs. Different studies dealing on the efficiency of conventional wastewater treatments (based on conventional activated sludge (CAS)) in the removal of these drugs led to the conclusion that they were not effective enough to completely eliminate these micropollutants [6–9]. This is the main reason why effluents from urban WWTPs are considered one of the main entrance pathways of pharmaceuticals into the environment and, therefore, partly responsible for the contamination of the surface, marine, and groundwater.

The occurrence of chemicals in aquifers, or their leaching potential, is related to their volumes of production and use, the compound physical-chemical properties (log K_{ow}, log K_{oc}, solubility) and biodegradability, and the nature of the aquifer in terms of its geology and hydrogeology [10, 11]. Not only the original compounds can be found in aquifers but also their transformation products generated during photo-transformation and biodegradation processes or as a result of human metabolization after drug administration [12, 13].

Aquifers should be considered as the most vulnerable water reservoirs. Despite presenting a bigger inertia to quality changes, as the soil system above may act as a "protective shield," they are especially vulnerable to pollution because once contaminated, the effects are often irreversible or of difficult and expensive remediation. Groundwater quality protection in European countries started in the 1980s through different EU directives. First, direct and indirect discharges of certain substances, such as organohalogen, organophosphorus, and organotin compounds, were limited and even prohibited, in order to prevent groundwater pollution [14]. Secondly, Directive 91/676/EEC [15], known as the "nitrates directive," was issued to prevent or reduce groundwater and surface water pollution by nitrates due to the intensive use of fertilizers during agricultural practices. As a consequence, ten nitrate vulnerable zones were established in Catalonia following this directive [16]. Increased concentrations of nitrate that result from both nitrification of ammonium and direct introduction from mineral fertilizers are commonly present in both groundwater and surface water associated with amended agricultural lands. Awareness on the potential risks and hazards that the application of plant protection products and biocidal products might pose for human and animal health, and for the environment, especially for groundwater, justified Directives

91/414/EEC [17] and 98/8/EC [18], which regulated the placing on the market of these substances. The drinking water directive [19] set, for the first time, limits for selected chemicals, including metals and organic and inorganic compounds, in waters intended for human consumption. The approval of the Water Framework Directive (WFD) in 2000 [20] was an important milestone in the EU water policy. This directive contained general provisions for the protection and conservation of groundwater. In 2006, the directive on the protection of groundwater against pollution and deterioration [21] complemented the WFD, and groundwater quality standards (GWQS) were established for the presence of nitrates (50 mg/L) and pesticides, including their relevant metabolites and degradation and reaction products (0.1 μg/L for individual compounds and 0.5 μg/L for the sum of all pesticides included in a monitoring procedure). Regarding pharmaceuticals and UV filters, there is no legislation so far establishing maximum risk concentrations in groundwater or any other environmental compartment, due mostly to the lack of data regarding their environmental impact (acute/chronic toxicity, stability, etc.).

Compliance with current groundwater policy requires the development of appropriate analytical methodologies to determine pesticides at ppt level. The recent development of highly selective and sensitive techniques has permitted not only to lower the limits of detection for these contaminants but also to confirm the presence of new classes of polar organic pollutants (PolOPs) in groundwater [22, 23]. Research on the environmental fate, toxicity, and behavior of these contaminants is essential to understand the potential risks of exposure and to establish appropriate water quality standards if needed.

In this context, this chapter compiles the most recent works related to the study of PolOPs in groundwaters of Catalonia and reviews the analytical methodologies used for their determination. Special consideration is given to polar pesticides and sulfonamides, as these compounds have been investigated more in depth in the whole region.

2 Analysis of Polar Organic Pollutants in Groundwater

Due to dilution and natural attenuation processes, PolOPs are expected to be at trace level in groundwater. Instrumental sensitivity for detecting such concentrations is currently not an issue with the available analytical technologies. However, false positives and false negatives are of concern. Therefore, the analysis of "blanks," the correct handling of the sample that prevents cross-contamination, and the evaluation performance of the analytical protocol are essential to obtain reliable results [24]. Main characteristics of the analytical methods used to evaluate the occurrence of PolOPs in groundwater of Catalonia are summarized in Table 1.

After collection, groundwater samples were filtered (0.45 μm) in order to remove suspended solids that may clog the adsorbent bed and subsequently stored in the dark at −20°C until analysis. Isotopically labeled compounds, used for internal standard calibration in the quantification process, were added to the sample before

Table 1 Analytical methodologies to analyze PolOPs in groundwaters of Catalonia

Chemical class	Sample volume (mL)	Sample pretreatment	Analyte extraction and concentration	Analyte detection	Recovery (%)	LOD (ng/L)	Reference
Illicit drugs (21 analytes)	5	Filtration (0.45 μm) and IS addition	Online SPE (PLRP-s cartridges)	LC-ESI(+)-MS/MS Mobile phase: ACN/H_2O with NH_4HCO_2/CH_2O_2	>80%	0.4–9.2	[25]
Pharmaceuticals (95 analytes)	2.5	Filtration (0.45 μm) Na_2EDTA addition (0.1%) and IS addition	Online SPE (HySphere Resin GP)	LC-ESI(+/−)-MS/MS Mobile phase: ACN/H_2O with CH_2O_2 (0.1%) for (+) and MeOH: ACN (1:1)/H_2O for (−)	9–179	0.03–78	[27]
Pesticides (22 analytes)	5	Filtration (0.45 μm) and IS addition	Online SPE (HySphere Resin GP (−) and PLRP-s (+))	LC-ESI(+/−)-MS/MS Mobile phase: ACN/H_2O	50–116	0.1–4.4	[26]
Sulfonamides (21 analytes)	40	Filtration (0.45 μm) and IS addition	Online SPE (Oasis HLB)	LC-ESI(+)-MS/MS Mobile phase: ACN/H_2O with CH_2O_2 (0.1%)	34–134	0.03–3.3	[28, 29]
UV filters (9 analytes)	5	Filtration (0.45 μm) and IS addition	Online SPE PLRP-s	LC-ESI(+/−)-MS/MS Mobile phase: ACN/H_2O with CH_2O_2 (0.1%) for (+) and ACN/H_2O with $NH_4C_2H_3O_2$ for (−)	88–114	0.3–3	[30]

SPE solid phase extraction, *LC* liquid chromatography, *MS/MS* tandem mass spectrometry, *ESI* electrospray ionization, *IS* internal standard, (−) negative ionization mode, (+) positive ionization mode, *LOD* limit of detection analyte extraction and pre-concentration. For pesticide and illicit drug analysis, isotopically labeled analogues were available for almost each target analyte [25, 26].

The achievement of low limits of detection (LODs) requires the extraction and concentration of the target analytes prior to analysis. Solid phase extraction (SPE) was the common technique used in all the studies reviewed dealing with the presence of PolOPs in groundwaters of Catalonia. This technique is easily coupled online with the analytical detector, which allows the development of fully analytical methodologies. Besides automation of the analytical process, online SPE procedures present some clear advantages over off-line SPE methods such as minimal sample handling and low sample-volume requirements that lead to improved reproducibility and accuracy. Moreover, they provide high throughput and they are less time- and labor-consuming. As it can be observed in Table 1, low LODs are achieved in online approaches with ten to hundred times less sample volume than usually is required in off-line methodologies. PolOPs were concentrated onto different reversed-phase hydrophilic-lipophilic polymeric sorbents.

Due to their polar nature, PolOPs are amenable to liquid chromatography coupled to mass spectrometry (LC-MS) analysis. Chromatographic separation was carried out in C_{18} reversed-phase columns. A Purospher Star RP-18 endcapped column (125×2 mm, 5 μm of particle size) (Merck) was used for the analysis of pesticides, drugs, UV filters, and pharmaceuticals, and an Atlantis C_{18} (150×2.1 mm, 3 μm of particle size) (Waters) was used for the separation of sulfonamides [28, 29]. Mobile phases used in each case are shown in Table 1. Acidified mobile phases were occasionally used in order to improve ionization of the compounds monitored in the positive mode [25, 27–30].

Ionization of target PolOPs was done by means of an electrospray source. Despite the fact that one of the main disadvantages of this type of ionization is the derived matrix effects that may enhance or suppress the MS analyte signal and consequently affect the analyte recoveries, these are not so pronounced in groundwater because it is one of the cleanest environmental aqueous matrices. Nevertheless, internal standard calibration was carried out in order to correct these potential matrix effects. However, due to the compromise on the analytical conditions that has to be adopted in multiresidue methods, especially in multi-class methods, some analytes were unavoidably poorly recovered [25, 27]. Low analyte extraction recoveries can be corrected with the addition of the corresponding isotopically labeled analogues at the beginning of the analytical procedure in order to generate reliable results.

3 Polar Pesticides in Groundwater of Catalonia

Spain is one of the major pesticide consuming countries in Europe. Insecticides are the main pesticide class used in Spain (31.2% of the total), followed by herbicides (30.2%) and fungicides (22.4%) [31]. Pesticide use in Spain has slightly decreased during the last decade, after an increasing trend since the mid-1990s. However, 2.7 kg of active ingredient per hectare was still used on average in 2011 [31]. The presence of these chemicals in groundwater has been investigated in different regions of Spain since 1997, and according to the reported results, their concentrations ranged from the low ppt to the ppb level [32].

Catalonia, with slightly more than 10 kg of pesticides applied per hectare [31], is among the regions with the highest use of pesticides in Spain, and consequently, aquifers in this region are vulnerable to pesticide pollution. As an implementation to the different surveillance and operational monitoring programs carried out regularly by the Catalan Water Agency (ACA) in compliance with the WFD, selected polar pesticides were monitored in 18 groundwater bodies of Catalonia subjected to significant agricultural pressure. The analyzed samples (265 in total) were collected at 112 sampling stations (wells, wellsprings, and piezometers) during four consecutive years: 2007 (June–November), 2008 (June–November), 2009 (May–October), and 2010 (April–September). Location of the investigated groundwater bodies and the amount of sampling sites investigated in each one are shown in Fig. 1. This figure also shows the status of groundwater bodies in terms of quantity and chemical quality as estimated by ACA [33]. As it can be observed in the figure, most of the sampling stations were located in aquifers featuring a bad status. Twelve out of the 18 investigated groundwater bodies were in bad chemical status, and five of these also showed a bad quantitative status (i.e., B14, B32, B33, B38, and B39). Most of the sampled aquifers were unconfined aquifers with medium to high porosity and permeability due to their sandy and gravel composition. Groundwater samples were collected in wells used for agricultural and drinking water purposes, at the minimum flow rate possible and after stabilization of the water physical-chemical parameters (i.e., temperature, pH and conductivity). More details on groundwater samples and sampling locations have been provided elsewhere [26, 34].

3.1 Occurrence of Individual Pesticides

The investigated polar pesticides, mostly herbicides, belong to seven different chemical classes. They include acidic herbicides such as phenoxy acids (2,4-D, bentazone, MCPA, and mecoprop), anilides (propanil), and chloroacetanilides (alachlor and metolachlor); organophosphate insecticides (diazinon, dimethoate, fenitrothion, and malathion); phenylureas (chlortoluron, diuron, isoproturon, and linuron); thiocarbamates (molinate); and triazine herbicides (atrazine, cyanazine, simazine, terbuthylazine, and two transformation products desisopropyl atrazine (DIA) and desethyl atrazine (DEA)) [26, 34].

All target pesticides were detected in at least four of the investigated samples. Their frequency of detection and their occurrence in groundwater are summarized in Fig. 2. With the exception of cyanazine that was detected only in 2% of the samples, triazine herbicides were among the most ubiquitous pesticides in the

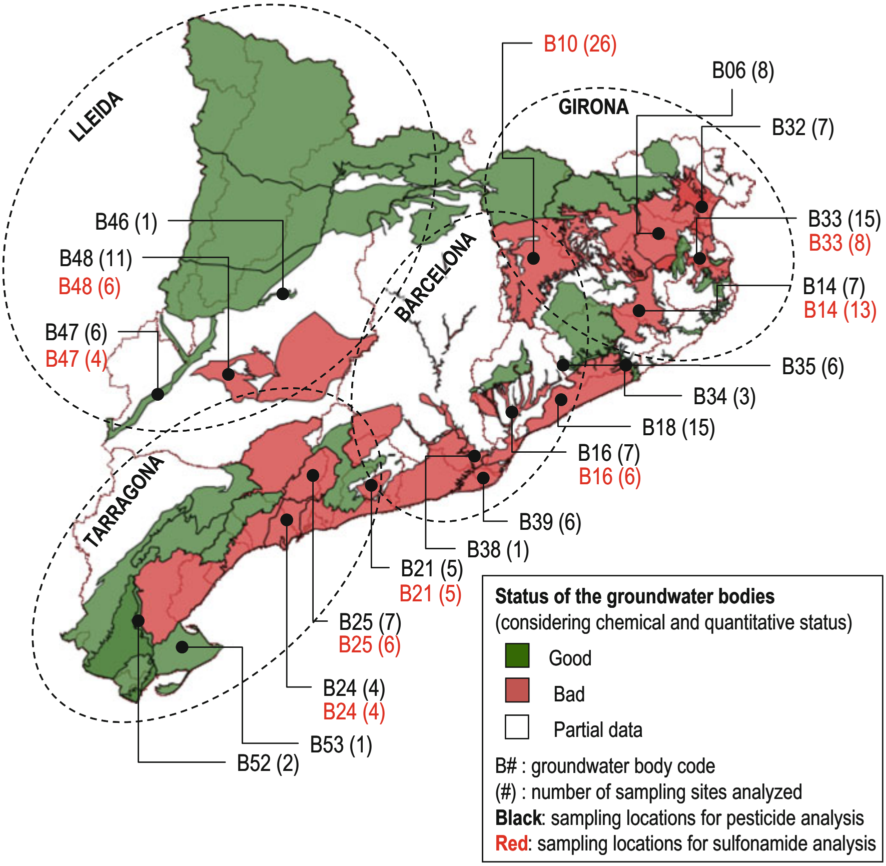

Fig. 1 Location of the groundwater bodies sampled between 2007 and 2010 to investigate polar pesticides (*black color*) and sulfonamides (*red color*) in groundwaters of Catalonia, number of sampling sites analyzed in each one, and status of the groundwater bodies, as estimated by the Catalan Water Agency (ACA) with data from 2007 to 2012 (http://goo.gl/NQqdEE)

investigated groundwater bodies (see Fig. 2a). They were not only very ubiquitous but also abundant. Besides the fact that the statistical distribution of triazine concentrations indicates that simazine, atrazine, and DEA were present on average in slightly higher concentrations than other pesticides, compounds belonging to this pesticide class were also above the GWQS of 100 ng/L set for individual pesticides in groundwater in a large number of samples (see Fig. 2a). The atrazine transformation product desethyl atrazine (DEA) was indeed the pesticide with the largest number of noncompliances, followed by atrazine and terbuthylazine. In this respect, note that some pesticide transformation products were found at higher frequencies of detection and at higher concentrations than their corresponding parent compounds in groundwater surveys carried out worldwide [35, 36]. In fact, transformation products derived from the triazine herbicides atrazine and

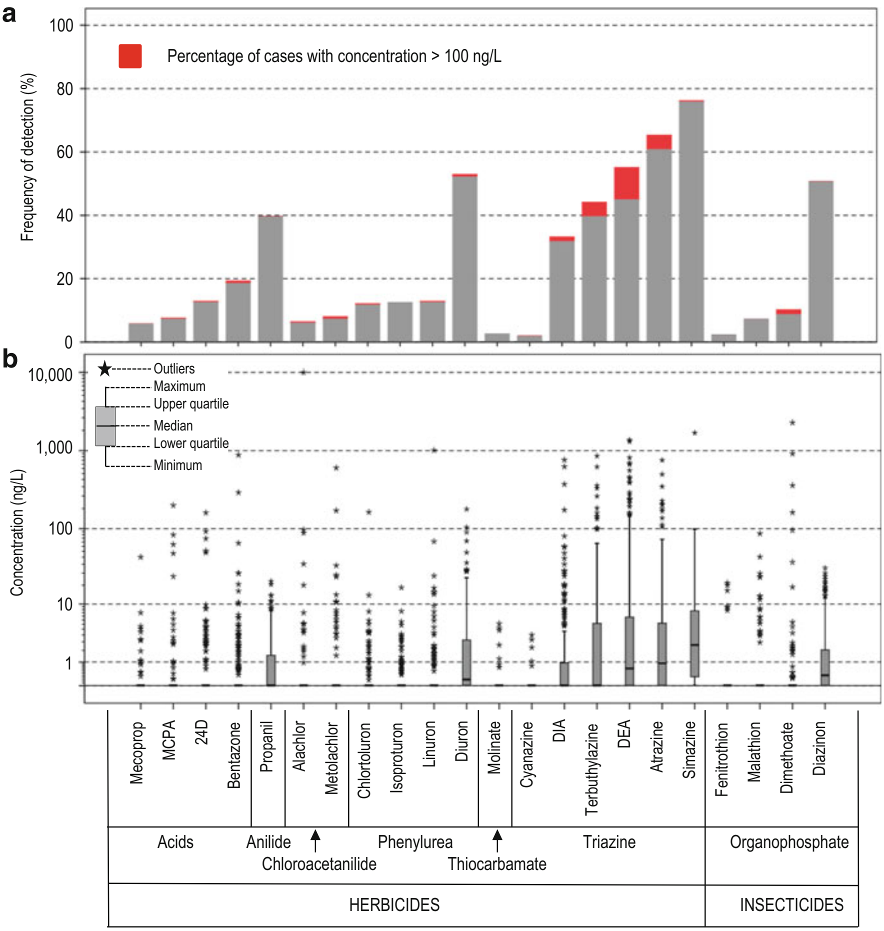

Fig. 2 Occurrence of individual pesticides in the investigated groundwater bodies: (**a**) frequency of detection and percentage of cases with concentration above the GWQS of 100 ng/L; (**b**) boxplots of the concentrations found for each individual pesticide in different samples (0.01 and 0.1 ng/L were assigned to those cases where pesticide concentrations were < LOD or < LOQ). *Outliers* (*): those values that are out of the 95% interval of confidence of the data distribution. *Maximum and minimum* indicate the highest and the lowest concentrations (outliers not included). *Upper and lower quartiles*: concentrations that leave one quarter of the data above and below these values, respectively. *Median*: concentration that falls in the middle of the dataset (outliers not included)

terbuthylazine; the chloroacetanilide herbicides acetochlor, alachlor, and metolachlor; and the herbicides chloridazon and dichlobenil could be considered as relevant polar pollutants in groundwater [11]. Taking this into account, pesticide distribution in groundwaters of Catalonia also evidences the relevance of monitoring pesticide transformation products in order not to underestimate pesticide occurrence in this environmental compartment.

Other relevant pesticides in groundwater bodies of Catalonia were the phenylurea herbicide diuron and the organophosphate insecticide diazinon, both present in about half of the investigated samples. Whereas the measured median concentration was slightly higher for diazinon than for diuron, the opposite was observed for their maximum concentrations. All investigated phenylurea herbicides but isoproturon, i.e., linuron, chlortoluron, and diuron, presented similar levels and were detected above 100 ng/L in one or two samples.

Some of the investigated pesticides were not as spread as the aforementioned chemicals in groundwater, but they were occasionally detected at maximum concentrations in the μg/L range. This was the case for the chloroacetanilide herbicide alachlor (9.9 μg/L), the phenylurea herbicide linuron (1.0 μg/L), and the organophosphate insecticide dimethoate (2.3 μg/L). Maximum concentrations of dimethoate in the μg/L range were also found in other regions of Spain due to its intensive use in agriculture [37, 38].

Alachlor, atrazine, diuron, isoproturon, and simazine are considered as priority substances in surface waters in the field of water policy [39]. All of them but isoproturon were among the most ubiquitous and/or abundant compounds in the investigated groundwater bodies. This is especially surprising for the triazines atrazine and simazine, pesticides that have been banned in the European market since 2004. These pesticides were also reported to be among the most commonly detected and abundant pesticides in European groundwaters [40]. This fact could be attributed to their long residence time in the subsurface or their slow release from the soil. Besides atrazine and simazine, other pesticides, e.g., alachlor, cyanazine, chlorfenvinphos, diazinon, fenitrothion, metolachlor, propanil, and simazine, are actually banned in the EU.

According to their GUS index (Groundwater Ubiquity Score index) [41], parameter that predicts pesticide leachability according to its K_{oc} (organic carbon-water partitioning coefficient) and $T_{1/2}$ (half-life time), high leachability could be expected for triazine herbicides (GUS index value > 3) [26]. This is in agreement with pesticide occurrence in groundwaters of Catalonia, where simazine, atrazine, terbuthylazine, and DEA, with GUS index values of 3.35, 3.75, 3.07, and 3.54, respectively, were detected in more than 44% of the investigated samples. The GUS index of those pesticides detected in less than 10% of the samples predicts a "low leachability" for fenitrothion, malathion, and dimethoate (GUS index values of 0.64, -1.24, and 1.05, respectively) or a "transition state" for cyanazine, alachlor, MCPA, mecoprop, and molinate (GUS index values of 2.07, 2.19, 2.51, 2.29, and 2.49, respectively). Although the GUS index has been commonly used to predict the behavior of pesticides in the subsurface, it indicates only the intrinsic mobility of pesticides in the water-soil system. However, pesticide occurrence in groundwater is also linked to pesticide application (nature and rate), weather, and soil properties. These are indeed decisive factors in the leachability of "transition state" pesticides.

3.2 Regional Distribution of Pesticides

Total pesticide concentration found on average in groundwater bodies of Catalonia are shown in Fig. 3a. The highest mean total pesticide concentrations were observed in groundwater bodies B33, B48, and B52 that presented 536, 621, and 452 ng/L, respectively. Total pesticide concentrations above the GWQS of 500 ng/L [21] were occasionally found in samples collected in these groundwater bodies and also in aquifers belonging to B32 and B35. However, mean total pesticide concentrations were in general below 100 ng/L, with the lowest values being found in groundwater bodies B18 (15 ng/L), B34 (3 ng/L), and B53 (0.4 ng/L).

Irrigation is one of the main factors that contribute to the presence of pesticides in groundwater, as it facilitates the transport of applied pesticides from the plant and the soil into groundwater. It also may be a source of pesticides and other contaminants when reclaimed water is used to irrigate. In this regard, high pesticide concentrations in WWTP effluents, even after tertiary treatment, have been reported elsewhere [42]. The proportion of agricultural land with and without irrigation in each investigated groundwater body is shown in Fig. 3b. More than 75% of the land above groundwater bodies B14, B21, B25, B32, B33, B47, B48, B52, and B53 is dedicated to agriculture. However, the agricultural land in B21 and B25 is mainly occupied by nonirrigated crops, e.g., vineyards, olives, and almonds,

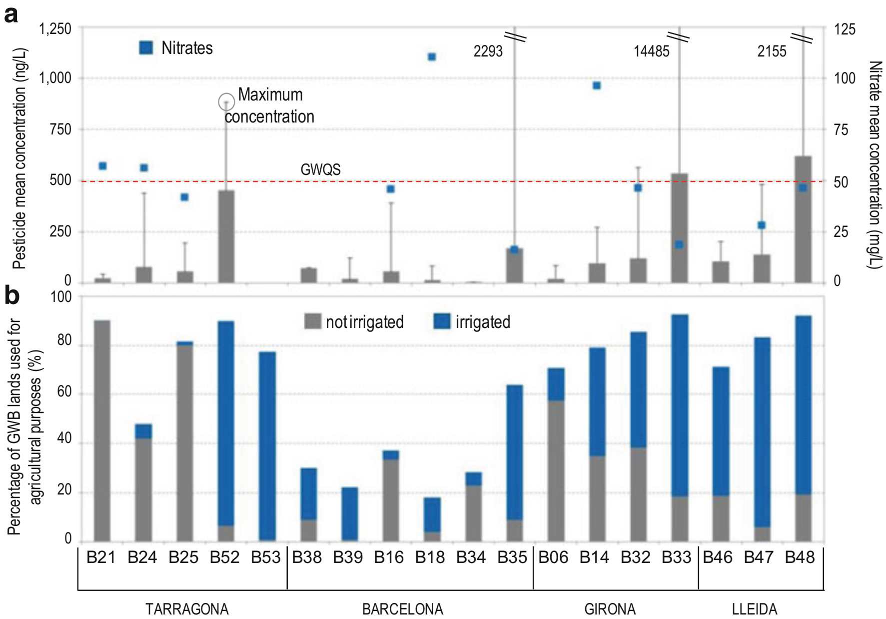

Fig. 3 Pesticide profile in the investigated groundwater bodies: (**a**) mean concentration for total pesticides and nitrates; (**b**) percentage of the groundwater body land used for agricultural purposes (%), differentiating irrigated and nonirrigated lands. *GWQS* groundwater quality standard

whereas continuous irrigation is required in most of the agricultural land in B33, B47, B48, B52, and B53, e.g., corn and fruit trees. With the exception of B53, for which only one sample was available, the amount of irrigated land is in agreement with the high pesticide levels observed (see B33, B35, B47, B48, and B52 in Fig. 3). The absence of pesticides in B53 could be attributed to a high dilution factor of pesticide concentrations occurring in the sampled aquifer due to its superficial character (1 m of unsaturated zone) and marine water intrusion. Besides B53, the lowest pesticide levels were observed in those areas with less agricultural land use and/or less irrigated surface, i.e., B21, B18, B34, and B06. Based on these results, the occurrence of pesticides in groundwater can be linked to the type of activity carried out in the area. In fact, the highest pesticide concentrations were found in groundwater bodies located in Lleida (B46, B47, and B48) and in Empordà (Girona) (B32, B33), areas known for their intensive agricultural activity.

Overall, samples containing the highest total pesticide concentrations were exclusively collected in shallow wells (up to 10 m in depth) from aquifers belonging to inland groundwater bodies, i.e., B33, B35, B47, and B48. In this case, slightly higher pesticide concentrations are expected close to the soil surface than at greater aquifer depth.

Nitrate concentration in groundwater is an indicator of agriculture activity and livestock practices in the area. Besides showing mean and maximum total pesticide concentrations, Fig. 3a also shows nitrate content in the investigated groundwater bodies as a means of evaluating their chemical status at a glance. The GWQS for nitrate concentration in groundwater is set to 50 mg/L [21]. Nitrate concentration was above this limit in 38% of the groundwater samples collected between 2007 and 2010. High nitrate concentrations (above 200 mg/L) were observed in groundwater bodies with low total pesticide concentrations B14, B16, B18, B21, B24, B25, and B32. This finding can be attributed to the fact that nonirrigated crops dominate the agricultural land in the area sampled (e.g., B14, B16, B21, B24, B25, and B32) or that there exists an additional source of nitrates other than agricultural activity (e.g., B18). High nitrate and pesticide concentrations were present in B48. Note that most of the investigated groundwater bodies (74%) include at least one municipality declared as a nitrate vulnerable zone in Catalonia [16, 43].

3.3 Temporal Distribution of Pesticides

The evaluation of groundwater pesticide pollution trends in time is not straightforward, since concentrations observed strongly depend on the amount of water abstracted and on the artificial and/or natural aquifer recharge.

The evolution of mean total pesticide concentrations in the investigated groundwater bodies of Catalonia is depicted in Fig. 4. Pesticide concentrations were observed to decrease over time in highly polluted groundwater bodies with high proportion of irrigated land, e.g., B33, B46, B47, and B48. This decrease could be attributed to a gradual implementation of good agricultural practices, the ban of

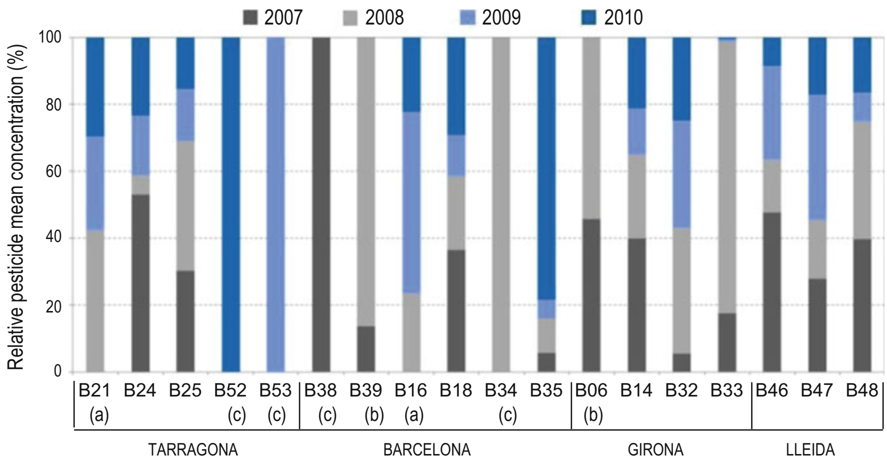

Fig. 4 Pesticide profile throughout the monitoring program. Groundwater body analyzed only in (*a*) 3, (*b*) 2, or (*c*) 1 year

several persistent pesticides in the EU in the last decade (i.e., simazine, diazinon, fenitrothion, atrazine, and alachlor), and/or an increase of the aquifer recharge.

Reduction of pesticide concentrations is the general trend observed throughout the monitoring program. However, groundwater bodies B32 and B35 experienced an increase in total pesticide concentrations. In some areas, pesticides levels were shown not to vary throughout the time. This is the case for groundwater bodies B16 and B21.

4 Sulfonamides in Groundwater of Catalonia

The global use of veterinary antibiotics in intensive animal husbandry has led to the widespread presence of these compounds in the environment, which has been extensively documented during the last two decades [44, 45]. Scientific interest has focused not only in their impact on bacteria and the spread of bacterial resistance but also in their environmental fate [46]. Sulfonamides (SAs) are one of the most important families of veterinary antibiotics, being used mainly in pig production worldwide, although they are also regularly applied in human medicine. After tetracyclines, they are the second family of antibiotics most frequently consumed, with average sales of approximately 23% in countries such as Denmark, United Kingdom, and Germany [44]. SAs are poorly absorbed by the organism, so they are excreted in varying unmetabolized amounts or as metabolites primarily via the urine and feces; still other portions are excreted as conjugates. It is a common practice to use liquid manure from livestock in agriculture with the objective of producing sustainable nutrient recycling, since nitrogen in its different species (organic, ammonium, nitrite, and nitrate) is a major constituent of manure. This application may imply the entrance of SAs and their metabolites to the soil ecosystem, together with other veterinary antibiotics contained in the manure [47–50]. Therefore, hundreds of grams of SAs per hectare may be annually spread on agricultural soils. Grazing animals can also contribute to their environmental occurrence, through direct depositions. It should not be forgotten that sewage sludge from WWTPs is also commonly applied in agricultural fields as nutrient amendment, with the corresponding loads of pollutants that have not been fully eliminated during treatment. SAs of human use such as sulfamethoxazole, sulfadiazine, or sulfapyridine have been detected in WWTP effluents in several studies [51–54]. Taking into consideration that their sorption to soil is weak and that SAs are hydrophilic compounds, it is expected that surface runoff and leaching into deeper soil layers will lead SAs to different environmental waters including groundwater.

The common origin of SAs and nitrates from cattle operations and their similar physical-chemical properties regarding polarity and solubility could lead to think that vulnerable areas with high concentration of nitrates could also be polluted by SA antibiotics. On this basis, García et al. [28, 29] investigated the presence of up to 16 SAs and five of their acetylated metabolites in nine different groundwater bodies of Catalonia. Following the WFD, all the aquifers investigated were considered vulnerable in terms of contamination derived from agricultural practices, as they were all under a significant farming pressure. In fact, most of them were located in the so-called vulnerable zones by nitrate contamination, according to the provisions of the Directive 91/676/EEC [15]. Two sampling campaigns were carried out in 2008 and 2009, and a total of 39 groundwater samples were taken each year from surveillance and operative controls including monitoring wells and natural springs. The location of the investigated groundwater bodies, as well as the number of samples collected in each one, is summarized in Fig. 1. Further details on the individual samples have been provided elsewhere [28, 29]. Despite the fact that a very poor correlation was found between SAs and nitrate occurrence [28, 29], SA antibiotics were detected in all groundwater bodies under study.

4.1 Occurrence of Individual Sulfonamides

A total of 16 SAs of both veterinary and human use and five of their acetylated metabolites were investigated. The list included SAs of human consumption frequently considered in pharmaceutical multiresidue studies, such as sulfamethoxazole and sulfapyridine, as well as other veterinary SAs such as sulfadiazine and sulfamethazine. Table 2 summarizes the results obtained for each individual SA investigated. It should be mentioned that, with the exception of N^4-acetylsulfamethazine, the other four acetylated metabolites were investigated in all the groundwater bodies with the exception of B10 (*Plana de Vic*) and B14 (*La Selva*); on the contrary, sulfaguanidine and sulfacetamide were only investigated in these two groundwater bodies.

Table 2 Uses (human (H) or veterinary (V)), frequencies of detection (given as number of hits and relative frequency of detection (%)), and mean and maximum concentrations for each of the sulfonamides studied in groundwaters of Catalonia

Target sulfonamides and metabolites	Use[a]	Frequency of detection[b]		Mean concentration (ng/L) ± STD	Maximum concentration (ng/L)
		Number of hits	(%)		
Sulfadimethoxine	V	56	71.8	7.2 ± 16.2	91.5
Sulfamethoxazole	H	55	70.5	13.5 ± 48.2	312.2
Sulfaquinoxaline	V	42	53.8	13.1 ± 47.8	274.0
Sulfamethazine	V	42	53.8	7.2 ± 17.3	13.4
Sulfamerazine	V	37	47.4	42.7 ± 121.5	744.7
N^4-acetylsulfamethazine	V	36	46.2	8.9 ± 5.7	56.9
Sulfadoxine	H–V	35	44.9	5.9 ± 12	53.6
Sulfisomidine	V	34	43.6	9.5 ± 14.4	64.4
Sulfapyridine	H–V	30	38.5	4.9 ± 13.3	72.5
Sulfathiazole	V	26	33.3	3.8 ± 5.1	16.8
Sulfamethoxypyridazine	V	19	24.4	7.4 ± 17.2	68.7
Sulfabenzamide	V	19	24.4	1.7 ± 2.5	10.3
Sulfanitran	V	19	24.4	42.5 ± 124.7	568.7
Sulfisoxazole	V	13	16.7	3.8 ± 4.8	17.1
Sulfadiazine	H–V	12	15.4	1.9 ± 2.8	8.9
Sulfamethizole	V	7	9.0	2.2 ± 4.0	9.3
Succinylsulfathiazole	V	1	1.3	2.1*	2.1
N^4-acetylsulfamerazine*	V	14	35.9	5.0 ± 5.3	18.0
N^4-acetylsulfamethoxazole*	H	10	25.6	1.4 ± 1.7	5.5
N^4-acetylsulfapyridine*	H–V	9	23.1	1.6 ± 2.0	6.0
Sulfacetamide*	V	9	23.1	631.8 ± 1,205.1	3,460.4
Sulfaguanidine*	V	9	23.1	22.6 ± 29.8	91.8
N^4-acetylsulfadiazine*	H–V	3	7.7	0.9 ± 0.3	1.0

[a]H: human use/V: veterinary use
[b]Analytes were investigated in $n = 78$ samples, except those marked with * that were investigated in only $n = 39$ samples

Sulfadimethoxine and sulfamethoxazole were detected in more than 70% of all the samples investigated (71.8% and 70.5%, respectively). The high frequency of detection of the latter should be highlighted, as it is an antibiotic mostly used in human therapies and usually detected in urban wastewaters. On the contrary, the least frequently detected SAs, with detection frequencies below 10%, were succinylsulfathiazole, sulfamethizole, and the metabolite N^4-acetylsulfadiazine that were present in one, seven, and three of the investigated samples, respectively. In terms of abundance, sulfacetamide, sulfamerazine, and sulfanitran, of veterinary use exclusively, were detected at the highest concentrations (3,460.4, 744.7, and 568.7 ng/L, respectively).

Regarding the acetylated metabolites studied, N^4-acetylsulfamethazine and N^4-acetylsulfamerazine were the most frequently detected, as they are derivates of veterinary SAs. The former was detected in 46.2% of the samples, only slightly less frequently than the corresponding parent compound, sulfamethazine (53.8%), with mean and maximum concentrations (8.9 and 56.9 ng/L, respectively) higher than those of sulfamethazine (7.2 and 13.4 ng/L). N^4-acetylsulfamerazine was detected in 35.9% of the samples, with a mean concentration of 5 ng/L and a maximum concentration of 18 ng/L. These values are below those observed for its parent compound, sulfamerazine. N^4-acetylsulfamethoxazole and N^4-acetylsulfapyridine, both of human origin, were detected in 25.6% and 23.1% of the samples, respectively. Similarly as for N^4-acetylsulfamerazine, they were less frequently detected than their corresponding parent compounds and at lower concentrations.

In the sampling campaign carried out during 2009, it was observed that the number of positive findings of SAs was bigger in samples taken down to 40 m depth (89% of the investigated samples) than in those taken between 80 and 130 m [28]. The findings down to 40 m showed that most of all the SAs detected were of common veterinary use and, consequently, derived from farming and agriculture activities. However, sulfamethoxazole and sulfapyridine, used mostly in human therapies and therefore indicative of WWTP impact in groundwaters, were also present at those depths. In fact, the highest mean concentrations at 40 m corresponded to sulfamethoxazole together with N^4-acetylsulfamerazine. It should also be mentioned that the frequency of detection of the acetylated metabolites was higher than that of parent compounds at depths between 80 and 90 m, due probably to their higher solubility and mobility in the subsurface. This particular data, together with the frequencies of detection of the acetylated metabolites in the whole study, usually very similar to those of the corresponding parent compounds, highlights the importance of including the different metabolites of SAs within the scope of groundwater monitoring studies. Similarly as for pesticides, their inclusion is crucial to get a complete picture of the occurrence and fate of these PolOPs in the subsurface.

The amount of metabolized SAs released to the environment is compound dependent, and information on their stability in the different environmental compartments is lacking. For instance, it has been demonstrated through numerical models that the acetylated metabolite of sulfadiazine degrades to sulfadiazine in soil after manure application within 4 days [55] and that both compounds are highly mobile in this matrix [56]. Similar results were observed for N^4-acetylsulfapyridine, which reverted back into sulfapyridine in aerated wastewater [57] and N^4-sulfamethoxazole [52].

4.2 Regional Distribution of Sulfonamides

Figure 5 summarizes the SA findings in each groundwater body individually, in terms of the mean and maximum total SA concentrations. The SAs presenting the highest contribution to total SA concentrations in each groundwater body are shown in Table 3. The highest mean total concentration of SAs corresponded to B10 (*Plana de Vic*) and B14 (*La Selva*) with 131.40 and 481.20 ng/L, respectively. *Plana de Vic* comprises 740 km^2 and one of the areas with the most important cattle farming activity in the whole of Catalonia. There is no application of biosolids in this area. As expected, the highest contributions in this location corresponded to two veterinary SAs, sulfacetamide and sulfamerazine. The most frequently detected here, despite not at the highest concentrations, were again veterinary SA, sulfadimethoxine, and sulfamethazine (88.5% of the sampling sites in this groundwater body). On the contrary, *La Selva* has an extension of 291 km^2, with a moderate cattle farming activity but with regular biosolid application near different cities. Nevertheless, the data obtained are quite similar to those of B10; the highest contribution in La Selva corresponded also to sulfacetamide and sulfamerazine, and the highest frequencies of detection corresponded again to sulfadimethoxine and sulfamethazine. However, in both groundwater bodies the high contribution of sulfacetamide was due to an outlier value in only one of the samples; for instance, the contribution of sulfamerazine in *Plana de Vic* was much regular over the extension of the groundwater body, as it was detected in 84% of the samples, whereas sulfacetamide was detected only in 16% of the samples [29]. The situation was similar in *La Selva*, with sulfamerazine detected more frequently (54% of the samples) than sulfacetamide (23%). However, it should be mentioned that sulfamethoxazole was present in 46% of the samples in La Selva, and was one of the main contributors to total SA concentrations.

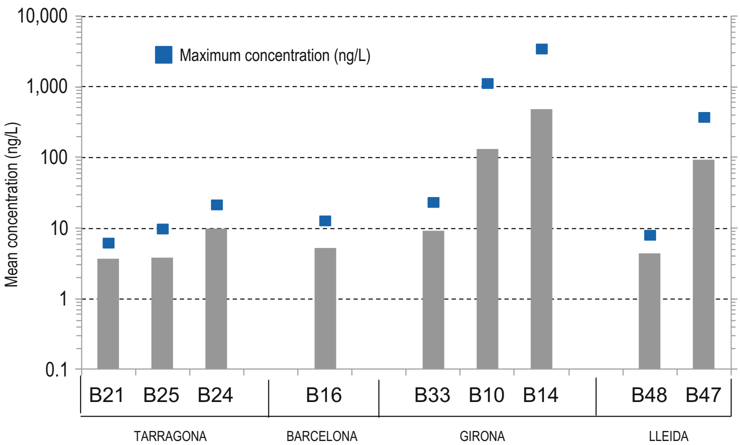

Fig. 5 Mean and maximum concentration of total sulfonamides (ng/L) in the investigated groundwater bodies

Table 3 Sulfonamides presenting the highest contribution to total sulfonamide loads and the most frequently detected sulfonamides in the groundwater bodies investigated

Groundwater body	SA with the highest contribution	Most frequently detected SAs
B10 – Plana de Vic	Sulfacetamide (4%)	Sulfadimethoxine (89%)
	Sulfamerazine (17%)	Sulfamethazine (89%)
		Sulfamerazine (85%)
B14 – La Selva	Sulfacetamide (52%)	Sulfadimethoxine (86%)
	Sulfamerazine (14%)	Sulfamethazine (86%)
B16 – Al · luvials del Vallès	Sulfamethoxazole (64%)	Sulfadimethoxine (83%)
	Sulfisoxazole (21%)	Sulfamethoxazole (83%)
B21 – Detrític neògen del Baix Penedès	N^4-acetylsulfamerazine (42%)	Sulfamethoxazole (80%)
	Sulfamethoxazole (29%)	N^4-acetylsulfamerazine (60%)
		Sulfisomidine (60%)
B24 – Baix Francolí	Sulfisoxazole (43%)	Sulfadimethoxine (100%)
	N^4-acetylsulfamerazine (37%)	Sulfaquinoxaline (75%)
B25 – Alt Camp	Sulfamethoxazole (56%)	Sulfamethoxazole (83%)
	N^4-acetylsulfamerazine (24%)	Sulfamerazine (67%)
		Sulfadimethoxine (67%)
B33 – Fluviodeltaic del Ter	N^4-acetylsulfamerazine (45%)	Sulfadimethoxine (50%)
	Sulfamethazine (19%)	Sulfamethazine (50%)
		N^4-acetylsulfamerazine (50%)
B47 – Al · luvial del baix Segre	Sulfaquinoxaline (73%)	Sulfadimethoxine (50%)
	Sulfamerazine (27%)	Sulfamethoxazole (50%)
B48 – Al · luvial d'Urgell	N^4-acetylsulfamethoxazole (37%)	N^4-acetylsulfamethoxazole (67%)
	N^4-acetylsulfapyridine (28%)	N^4-acetylsulfadiazine (50%)
		Sulfamethoxazole (50%)

High mean total SA concentrations were detected also in B47 (*Al · luvials del baix Segre*) (94.50 ng/L). The investigated aquifer in this groundwater body consists mainly of loose gravel within a matrix of thin components and is therefore very permeable. This aquifer was located below a flat area scarcely covered with vegetation, where the unsaturated zone was not very relevant, favoring the infiltration of surface waters. Pressures exerted by livestock waste and intensive agriculture (as indicated also in the pesticide section) were also high in this area, which explains the highest contribution to the total concentration of two veterinary SAs, sulfaquinoxaline (72.5%) and sulfamerazine (27.2%).

SAs of human use have frequently been reported in effluent wastewaters as they are not well removed in WWTPs based on CAS processes [58, 59]. In this regard, three of the nine investigated groundwater bodies showed a higher impact from urban activities than from intensive cattle farming activities. This was reflected in higher contributions from SAs of human consumption to the total loads. This was the case of B16 (*Al·luvials del Vallès*) and B25 (*Alt Camp*), in which the contribution of sulfamethoxazole accounted for the 63.5% and 56.1% of the total, respectively, and also from B21 (*Detrític neògen del Baix Penedès*), with a contribution from sulfamethoxazole of 28.5%. In all cases, the frequency of detection for this SA was also the highest (80–84%). The three groundwater bodies presented a high impact derived from WWTP effluents discharge and, in the case of B21, from the regular application of biosolids. However, except for B25, in which pressure derived from livestock waste is low, both intensive cattle farming and WWTP discharges are exerting a high pressure on the groundwater quality in these aquifers, and it would be complex to say which activity is the main contributor to the ubiquity of SAs in these groundwater bodies.

Again, SA contamination from urban origin was demonstrated in *Al·luvial d'Urgell*, B48, but in this occasion, two acetylated metabolites were the most relevant compounds in terms of contribution to the total concentration detected: N^4-acetylsulfamethoxazole, which was detected in four out of the six samples at concentrations between 0.88 and 5.52 ng/L, and N^4-acetylsulfapyridine, detected in two samples at concentrations of 1.41 and 6.03 ng/L, respectively. This higher occurrence of the acetylated metabolites compared to the parent compounds could be explained in terms of their solubility (as metabolites are usually more soluble in order to be excreted more easily by the organisms; this increased mobility may persist once they reach the natural media). A higher persistence of the acetylated compound could also explain this fact, although information regarding environmental half-lives is still lacking. Sulfamethoxazole and its metabolite were the two compounds detected most frequently (50% and 66.7%, respectively).

All in all, as it can be observed in Table 3, compared to the levels detected for pesticides, SA pollution in the investigated groundwater bodies was not very relevant (mean loads per sample generally < 100 ng/L except for B10 and B14), despite the geological conditions of some of the investigated aquifers that are favorable for SAs leaching to the groundwater table, e.g., sandy lithology of the unsaturated zone together with the gravel materials of the saturated zone (i.e., alluvial materials in B16, B33, B47, and B48).

5 Wastewater-Derived Polar Organic Pollutants in Urban Groundwaters

A wide range of PolOPs, such as pharmaceuticals, illicit drugs, hormones, surfactants, and personal care products, have been detected in municipal wastewaters. Consequently, untreated wastewaters and WWTP effluents are considered the main source of these compounds into the aquatic environment [60, 61]. This type of compounds may enter aquifers after water leakage from sewer and septic systems, river seepage, or aquifer recharge with reclaimed water [32, 62]. Taking this into consideration, the impact that high population density of urban areas has on water demand and waste generation makes urban aquifers extremely vulnerable to PolOP pollution. In this regard, several monitoring programs were carried out in urban groundwater of Barcelona, which is the second largest city in Spain, to evaluate the occurrence and fate of several classes of PolOPs [25, 27, 30].

The area of study included the city of Barcelona and some towns of the metropolitan area (136 km^2) and covered 2.2 million people. Samples were collected from three different aquifers within groundwater body B36. Figure 6 shows the areas where groundwater samples were collected. These sampling areas were established according to the main aquifer recharge sources, as it has been summarized in Table 4. Samples within MS and PS zones were collected from the Barcelona plain aquifer that consists of carbonated clays from Pleistocene (Quaternary) and from the underlying aquifer that consists of sandstones, marls, and sands from Miocene (Tertiary), respectively. Samples belonging to the BRD zone were collected from the superficial unconfined aquifer under the Besòs River delta that consists of gravels, sands, silts, and clays from Holocene (Quaternary). Further details on sample collection have been provided elsewhere [25, 27, 30].

The investigated families of PolOPs in urban groundwaters were pharmaceuticals (PHA) [27], drugs of abuse (DRG) [25], and UV filters (UVF) [30]. As for the target compounds analyzed, a total of 125 compounds were surveyed. This list included 34 drug transformation products. Compounds belonging to ten, six, and three different chemical classes of PHA, DRG, and UVF, respectively, were included. Occurrence in terms of mean frequency of detection of each PolOP family and mean concentration of each chemical class within the different PolOP families investigated in the zones of study is summarized in Fig. 7.

Overall, mean frequencies of detection for all investigated PolOP families were slightly higher in the BRD zone than in the MS and PS zones. The Besòs River is strongly impacted by WWTP effluents, and therefore, these compounds are expected to be continuously entering into its waters. Taking into account that aquifers underlying BRD zone are mainly recharged by natural river water bank filtration (91% of the recharge), it is not surprising that these PolOPs are frequently detected in this zone. As for the PolOPs families, PHA and DRG were more frequently detected in all zones of study than UVF.

Regarding concentrations, the same pattern, i.e., higher mean concentrations in the BRD zone than in the MS and PS zones, was also observed. Mean total concentrations of PHA were one order and two orders of magnitude higher than those measured for DRG and UVF, respectively.

Overall, the most abundant PHA classes were analgesics and anti-inflammatories and antibiotics with maximum concentrations above 100 ng/L. Macrolide antibiotics like spiramycin and azithromycin were indeed measured at concentrations higher than 1 μg/L. Despite the fact that the investigated PHA transformation products presented concentrations in general lower than their parent compounds, they significantly contributed to overall levels of PHA in

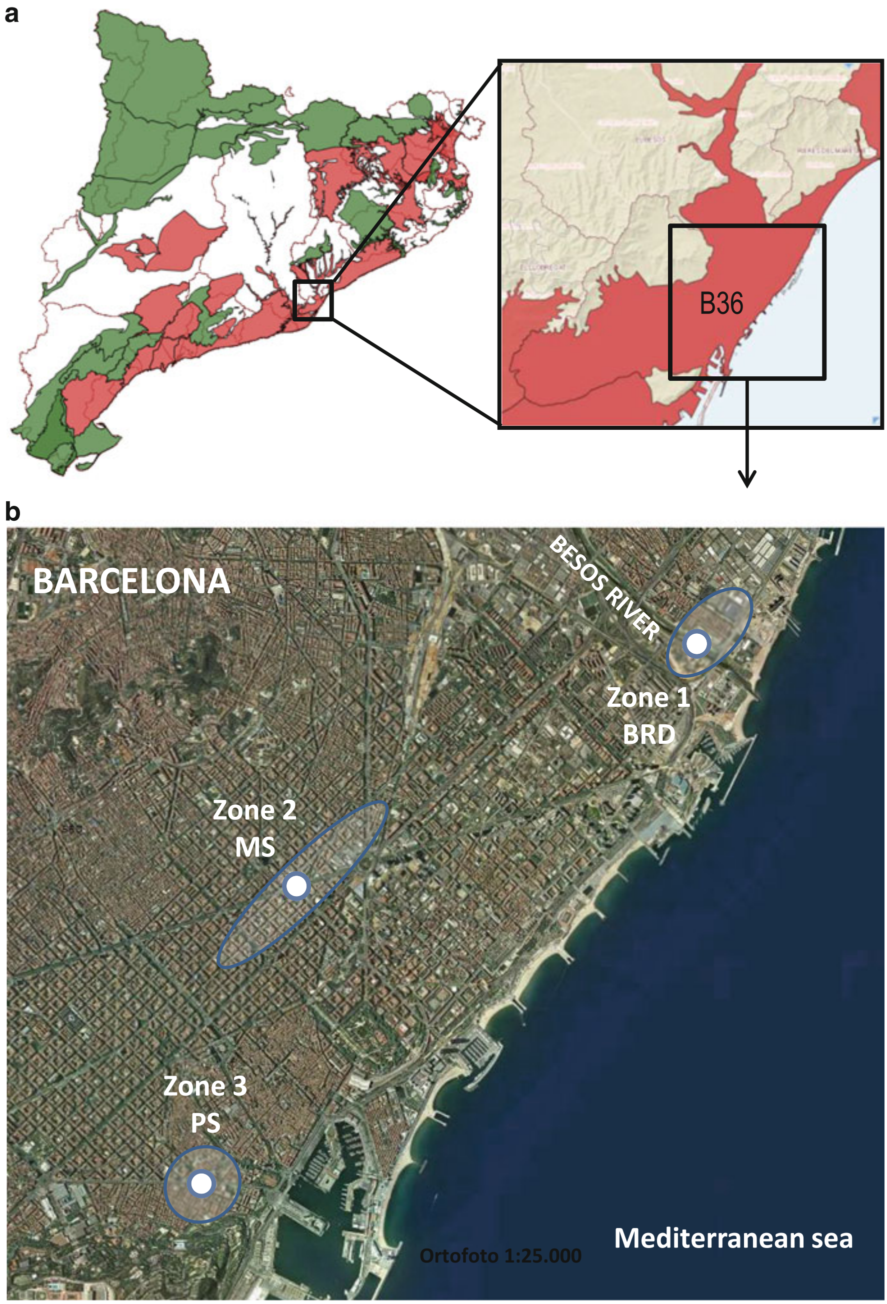

Fig. 6 Maps showing (**a**) the location of the groundwater body M36 and (**b**) sampling zones where urban groundwater samples were collected in Barcelona. © Digital map property of the Institut Cartogràfic i Geològic de Catalunya, available at www.icc.cat

Table 4 Characteristics of the zones sampled to investigate PolOPs in urban groundwaters of Barcelona

Zone	Aquifer recharge sources	Aquifer redox conditions
-1-BDR	91% river infiltration	Reducing
	9% sewage system + water supply losses	
-2-MS	60% rainfall infiltration	Oxidizing
	31% sewage system losses	
	9% water supply losses	
-3-PS	4% rainfall infiltration	Oxidizing
	50% sewage system losses	
	46% water supply losses	

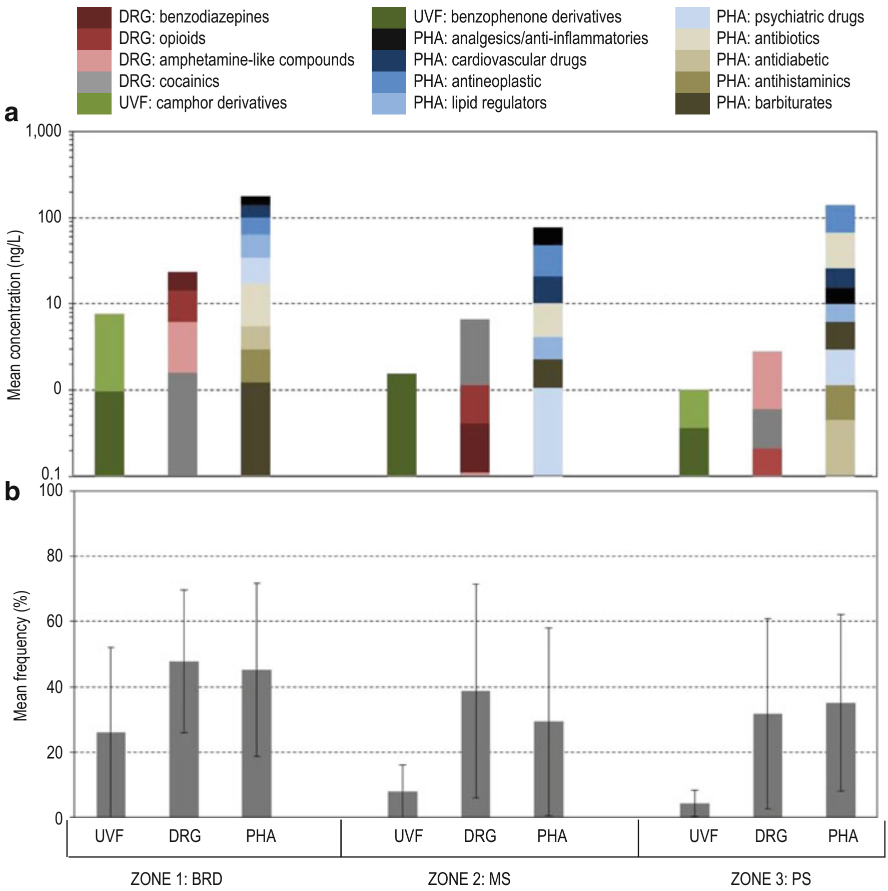

Fig. 7 Organic contaminants in groundwater bodies of Barcelona. (**a**) Mean frequency of detection of the investigated families (i.e., *DRG* drugs, *UVF* UV filters, *PHA* pharmaceuticals), in the three sampling zones, and (**b**) mean concentration of the investigated chemical classes within each PolOP family

groundwaters. The active transformation products 4-hydroxy-propanolol and enalaprilat were found at concentrations seven and three times, respectively, higher than their parent compounds. Despite the fact that the sum of the mean concentrations of each PHA class was only 180 ng/L in BRD zone, most samples collected in this zone showed total concentrations of PHA above 1,000 ng/L. In fact, the most superficial and nearest samples to the Besòs River bed presented similar concentrations to those observed in the river itself. On the contrary, samples collected in the low part of the aquifer presented lower concentrations. This could be attributed to natural attenuation of PolOP concentrations in the subsurface. However, since reducing conditions prevail in BRD zone and due to the fact that the aquifer material presents a limited sorption capacity, PolOP depletion is mainly driven by biological degradation and/or dilution effect. Oxidizing redox conditions are present in MS and PS zones; therefore, besides the aforementioned processes chemical transformation by the soil components should also contribute to natural attenuation of PolOP concentrations. However, unlike in BRD, attenuation in depth was not obvious in MS and PS zones, and measured PHA concentrations were occasionally higher in deeper sampling sites than in more superficial ones. This finding could be attributed to the presence of leaking sewage pipes in the locations with high PHA concentrations, since sewage system losses are important contributions to aquifer recharge in this MS (30%) and PS (50%) zones.

A similar situation was also observed for DRG and UVF, i.e., higher overall concentrations in BRD zone than in MS and PS zones and depletion of DRG and UVF concentrations in deeper zones of the BRD aquifer. As for DRG, the main detected compounds were MDMA or ecstasy (amphetamine-like class) and methadone and its main metabolite EDDP (opioid class). In the case of UVF, the most ubiquitous compounds in the investigated samples were benzophenone-3 (BP3) and 4-methylbenzylidenecamphor (4MBC). Individual DRG and UVF concentrations were in most cases below 10 ng/L. Mean concentrations above this level were only found for cocaine in MS zone (13 ng/L) and methadone (20 ng/L) and diazepam (16 ng/L) in BDR zone. According to the expected dilution factors, the latter may be less affected by removal processes in the subsurface. The most abundant UVF in urban groundwaters of Barcelona were the benzophenone derivatives benzophenone-1, BP3, and benzophenone-4, all of them present at maximum concentrations of 19 ng/L.

6 Conclusions

PolOPs such as polar pesticides, SA antibiotics, pharmaceuticals, illicit drugs, and UV filters have reached the water table in groundwater bodies of Catalonia. Pesticide contamination is more relevant than SA antibiotics in rural areas in terms of pollutant loads. Levels of individual and total pesticides were observed to be occasionally above the GWQS established in the regulation (100 and 500 ng/L, respectively), whereas total SA levels rarely surpassed 100 ng/L. However, a widespread presence for both pesticides and SAs was observed in the subsurface in the investigated groundwater bodies due to the extended and intensive agriculture and farming practices in Catalonia. Besides farming, SA antibiotics of human use can also reach aquifers after wastewater effluent discharges or biosolid application. Wastewater-derived PolOPs such as pharmaceuticals, illicit drugs, and UV filters are indeed commonly found in urban groundwaters of Barcelona, mainly in those areas recharged by a river highly impacted by WWTP effluents. Overall, pharmaceuticals, specifically analgesics, anti-inflammatories, and antibiotics, were the compounds most frequently detected in urban groundwaters, occasionally surpassing 100 ng/L, followed by illicit drugs and UV filters, always below that value. The monitoring studies of PolOPs carried out in groundwater bodies of Catalonia have revealed the importance of considering also the presence of their metabolites and transformation products in order to correctly assess their behavior, persistence, and fate in the subsurface. On the other hand, the main sources of PolOPs to groundwater and the main pollution hot spots, as well as the mobility of the investigated compounds within the aquifers, are crucial to understand the data. All the gathered information is very valuable in order to perform effective future monitoring studies of PolOPs in groundwaters and propose or establish prevention and correction measurements to improve groundwater quality.

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GIS-Based Software Platform for Managing Hydrogeochemical Data

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Abstract A GIS-based software platform was developed to arrange all the available hydrogeochemical data into a comprehensive structure and provide support for its proper storage, management, analysis and interpretation. This platform is composed of a geospatial database and a set of analytical instruments integrated in a graphical user interface that coordinates its activities with several software. The geospatial database was specifically developed to store and manage organic and inorganic chemical records, as well as other physical parameters. The analytical tools cover a great range of methodologies for querying, comparing and interpreting groundwater quality parameters. This tools enable us to obtain automatically several calculations such as charge balance error and ionic ratios as well as calculations of various common hydrogeochemical diagrams (e.g. Schöeller-Berkaloff, Piper, Stiff) to which the spatial components are added. Moreover, it allows performing a complete statistical analysis of the data (e.g. generation of correlation matrix and bivariate analysis). Finally, this platform allows handling

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relevant auxiliary information in an efficient way, and it is coupled to a number of technologies such as hydrogeochemical modelling or geostatistical analysis. The software platform was used in a case study involving several urban aquifers located into the metropolitan area of Barcelona (Spain) to illustrate its performance.

Keywords Catalonia, Geospatial database, GIS, Groundwater management, Hydrogeochemical data, Metropolitan area of Barcelona

Contents

1 Introduction

The availability and accessibility of water must be addressed from both qualitative and quantitative standpoints. A large number of factors may deteriorate the groundwater quality, including among others the expansion of the irrigation activities, industrialisation and urbanisation [1, 2]. A thorough and comprehensive evaluation of the negative impacts of all potentially hazardous activities is of paramount importance for the protection of groundwater bodies and ecosystems associated [3]. Compliance with standard regulatory normatives such as the European Water Framework Directive (WFD) requires continuous and intensive monitoring, thus resulting in large data sets of many potential physico-chemical parameters. Such data sets need to be routinely evaluated and interpreted by water agencies, stakeholders and assessors to provide answers to questions such as (a) the processes controlling the chemical composition of groundwater and the corresponding spatio-temporal distribution, including the delineation of the recharge area; (b) the water quality signature and how it may relate to the geological and hydrogeological setup along the travel path, as well as the soil use; and (c) the regional background composition of groundwater [4].

Water managers, stakeholders and decision-makers that are assigned with these tasks may face several difficulties. These may arise from (i) having to deal with large data sets, spanning many years; (ii) integrating data from different sources, gathered with different data access techniques and of eventually different formats; (iii) managing data with various degrees of accuracy and with different temporal and spatial extent; (iv) correlating groundwater quality information with other relevant information (e.g. geology, geophysics) so as to further investigate the underlying hydrogeochemical processes involved; and (v) integrating into the database the resulting interpretations and modelling efforts with the necessary documentation to be potentially used by third parties [5].

Handling and analysing such large amount of spatio-temporal information call for a unified database combined with a number of efficient technologies and methodologies capable of comparing, classifying and interpreting large data sets. Conventional methodologies, including preparation of hydrogeochemical diagrams, spatio-temporal representation of the data as well as uni- and multivariate analysis, are convenient tools for this purpose [6]. Nevertheless, the selection of the proper methodology for efficient chemical data handling is not straightforward and cannot be easily determined a priori because it depends on the type, quality, spatial distribution and potential use of data [7, 8]. Furthermore, the complexity and variety of processes associated with the vast amount of chemical species monitored hinder the analysis.

We present a software platform developed in a GIS environment for a comprehensive hydrogeochemical data analysis. It integrates a geospatial database that arranges all the available spatio-temporal dependent data into a coherent and logical structure and incorporates a set of analytical instruments that cover a wide range of methodologies for querying, interpreting and comparing groundwater quality parameters. It allows handling relevant auxiliary information (hydrology, geology, climate, etc.) in an efficient way, and it is coupled to a number of technologies such as hydrogeochemical modelling or geostatistical analysis. The software platform is here illustrated with a case study of the metropolitan area of Barcelona (Spain).

2 Background: Existing Software Instruments for Hydrogeochemical Analysis

Commercial and research instruments that assist the storage, handling, analysis and interpretation of hydrogeochemical data are found in different software packages. A short review of some of these existing packages is here presented.

Existing software that provide tools for correlation analysis, trend analysis and statistical analysis that enable the user to classify water samples include, without being exhaustive, SSPS [9] STATISTICA [10], SAS/STAT software [11] Stata [12], Minitab [13], Systat [14] or Microsoft Excel and MS Excel add-ins like BiPlot 1.1 [15].

Specific software packages include several methodologies to analyse and interpret hydrogeochemical data by means of the creation of classical diagrams and other calculations for ionic balance or ionic ratios. These include free software codes such as GW-Chart [16], EasyQuim [17], Piper SpreadSheet [18] or INAQUAS [19]. The last one also facilitates the classification of chemical species according to water quality norms. In particular, AqQA [20], apart from diagrams and ionic ratios, allows the comparison of samples to laboratory standards or regulatory limits. In the same line, Logicels [21] is a free software that performs convectional hydrogeochemical diagrams, calculation of ionic balance and statistical analysis but also includes additional features such as isotopic calculations and is linked to hydrogeochemical modelling software such as PHREEQC [22].

Other software includes tools and methodologies to manage and visualise hydrogeochemical data. Some examples are AQUACHEM [23], ChemPoint Professional Edition [24] and HyCA [25]. The last one includes a database and a map manager (visualisation aid) and facilitates the creation of diagrams, time series analysis and the creation of 3D and 2D maps (planar and cross sections) for all physico-chemical parameters included in the database. AQUACHEM has a fully customisable parameter database and a complete set of analysis, calculation and modelling tools. Additionally, it allows generating standard graphical plots and data visualisation by means of a combination of geological and hydrogeological maps; furthermore, it is coupled to PHREEQC. Finally, ChemPoint includes a variety of tools for entering the hydrochemical data in a structured database and allows the user to obtain different hydrochemical graphs and to obtain contour parameter concentration maps.

The need for a comprehensive management, visualisation and retrieval of spatio-temporal data has triggered the development of geographical information systems (GIS) applications to hydrogeology [26]. Due to advances in computer capabilities and data availability, a number of GIS-based applications have been developed since the turn of the century for hydrogeological analysis (e.g. [27–29]). In two recent applications, both developed in a GIS environment [8] presented a method to map groundwater contaminant concentration distribution based on different interpolation techniques and [30] developed a spatial multi-criteria decision analysis software tool to select suitable sites for managed aquifer recharge (MAR). Another example of GIS-based application is ArcHydro Groundwater tools [31], coupling the ArcHydro Groundwater data model [32] with an ArcGIS [33] platform for managing, archiving and visualising hydrogeological information.

3 The Software Platform

The software platform was intended to perform a conventional hydrogeochemical analysis, including data check, diagrams and ionic ratios, and facilitate the visualisation, processing and interpretation of the hydrogeochemical data, including a number of capabilities: (1) all tools are directly programmed in a GIS environment as in-built utilities to allow for efficiency, and (2) it incorporates new instruments for hydrochemical analysis to combine diagrams, specific queries and calculations. We first present the technical requirements and specifications and in subsequent subsections how these specifications were implemented in the final software.

3.1 Design Specifications

I. Management and storage of spatial features and time-dependent data on a geospatial database, supporting:

   1. Management of different data derived from both field data, analysis of water samples at the laboratory and groundwater models (also representative scales are quite different)
   2. Integration of different types of information (e.g. geological, meteorological, hydrological)
   3. Standardisation and harmonisation of data, including specific mechanisms for facilitating data transcription, managing different formats, editing data and dealing with unit conversion
   4. Exportation of archived hydrochemical data to be used as input in external software

II. Data processing and analysis using:

   1. GIS environment which provides tools to (1) estimate/validate the spatial distribution of the chemical/physical components; (2) generate spatio-temporal queries and calculations; (3) visualise and operate with different types of data settings; (4) create interactive mapping; (5) perform an effective assessment of the legitimacy, consistency and correlation of the input data; (6) apply index overlay techniques; and (7) allow for further analytical tools for spatial analysis, geostatistical analysis, etc.
   2. Specific tools that facilitate the hydrogeochemical analysis by using data quality control and conventional graphical analysis techniques
   3. Statistical tools to preprocess (e.g. detection and visualisation of outliers) and validate data (e.g. deletion of obvious transcription errors and of duplicates)

III. Interaction with external software for further analysis:

   1. Geostatistical software packages such as SGeMs [34] and GSLIB code [35]
   2. Groundwater modelling packages such as TRANSIN [36, 37] and Visual TRANSIN [38]
   3. Hydrogeochemical modelling packages such as PHREEQC, NETPATH [39] and MIX [40]

IV. Post-processing instruments to facilitate the integration of the results obtained from analysing and interpreting the hydrogeochemical data included in the database in the same GIS environment or in an external platform

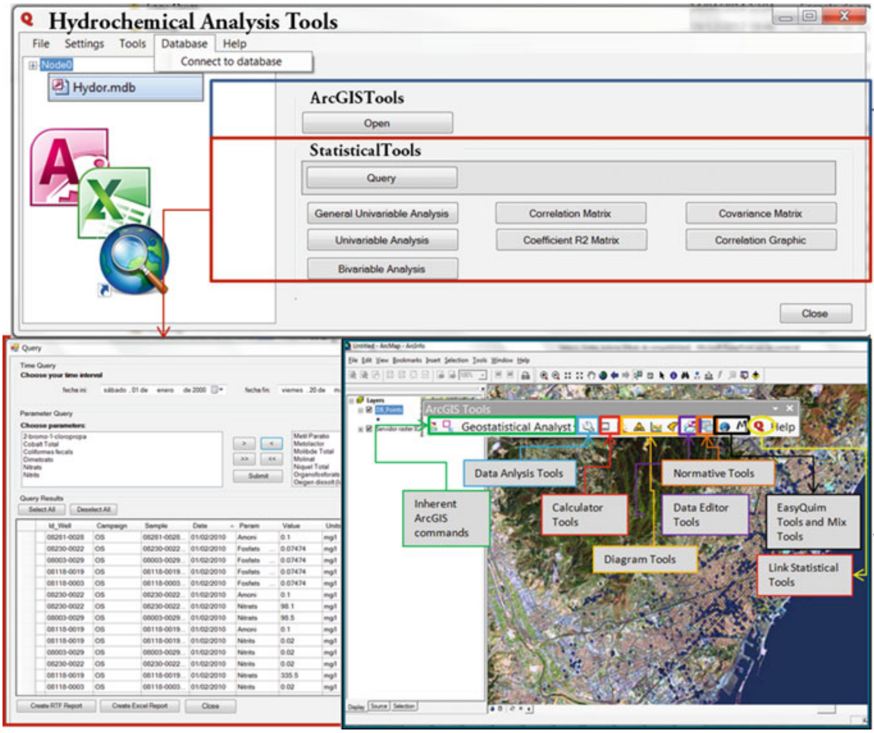

Fig. 1 Graphical user interface (GUI) of the software platform. It is composed of two main modules: ArcGIS Tools and Statistical Tools. *Lower right inset* shows a sketch representing the toolbar implemented in ArcMap (ArcGIS Tools) and its principal commands. *Lower left inset* shows the query form created to query the hydrogeochemical data of the HYDOR database for performing statistical analysis

3.2 *General Description*

The requirements enumerated in the foregoing section were adopted as guidelines during the design of the present software platform. This platform is composed of a geospatial database (termed HYDOR) and a set of tools allowing graphical and statistical analysis of hydrogeochemical parameters divided into two modules (ArcGIS Tools and Statistical Tools) integrated in a graphical user interface (GUI) that coordinates its activities with several external software (ArcGIS, MS Excel, MS Access). A sketch of the graphical interface is shown in Fig. 1

3.2.1 Geospatial Database

The database HYDOR represents geospatial information based on the Personal Geodatabase structure provided by ArcGIS [7]. This is a MS Access database that can store, query and manage a vast multiformity of attribute data, geographical features, raster data, CAD data, surface modelling or 3D data, utility and transportation network systems, GPS coordinates and survey measurements [41].

This framework offers a comprehensive interface for geospatial data management and the possibility of exchanging geospatial data through XML, thus extending its interoperability [42]. Moreover, although the data model of the hydrogeological database described here was implemented within ArcGIS, most of these concepts are flexible enough to enable implementation into other platforms.

Description of Data Contained

The hydrogeological database is composed of different data sets that store a variety of spatial and nonspatial data necessary for a complete hydrogeological study. The data model of HYDOR is conceptualised in 8 main components: Geology (e.g. borehole lithological description, stratigraphic units, depth to bedrock), Geophysics (e.g. diagraphies), Hydrogeology (e.g. well descriptions, springs, pump rates, hydrogeochemical data), Hydrology (e.g. rivers, lakes, sea), Hydrometeorology (e.g. precipitation, temperature), Environment (e.g. protection zones), Regional Geography (e.g. topography) and Water Management Administration (e.g. River Basin Districts). Each of these components is represented in the geospatial database by a feature data set composed of a group of feature classes (points, lines and polygons). In addition, several tables are used to represent and store the feature attributes and the measurements obtained. A complete description of this database can be found in [43–45]; nevertheless a summary of the database components and their main structural characteristics is given below for illustration purposes. A sketch of some of the components of the database related with the management of hydrochemical data can be visualised in Fig. 2.

In HYDOR, each sample is associated to a point-type entity included in the feature class termed *DB_Points*. The main attributes of each point are the geographical coordinates with a description of the different names used to identify those points (potential different sources of data), the type of sampling point (e.g. well, spring, surface water body), point accessibility and other administrative information (e.g. owner).

Physico-chemical parameters together with its measured units are listed in a permissible value list (DB_LibChemParam). Organic and inorganic compounds, as well as isotopes, can be entered into the database. In addition, parameters, such as temperature, Eh, pH, electrical conductivity, alkalinity, etc., are correctly registered. Further information about existing standard normative (e.g. Water Framework Directive, Groundwater Directive, Nitrate Directive) is also included to allow classifying hydrogeochemical data according to given thresholds in the attribute tables *DB_LibNorm* and *DB_LibchemParamNorm*. Each sample is included in accordance with sampling date, campaign and depth in the table *DB_ChemSample*. Thereafter, hydrogeochemical measurements for each sample are stratified in

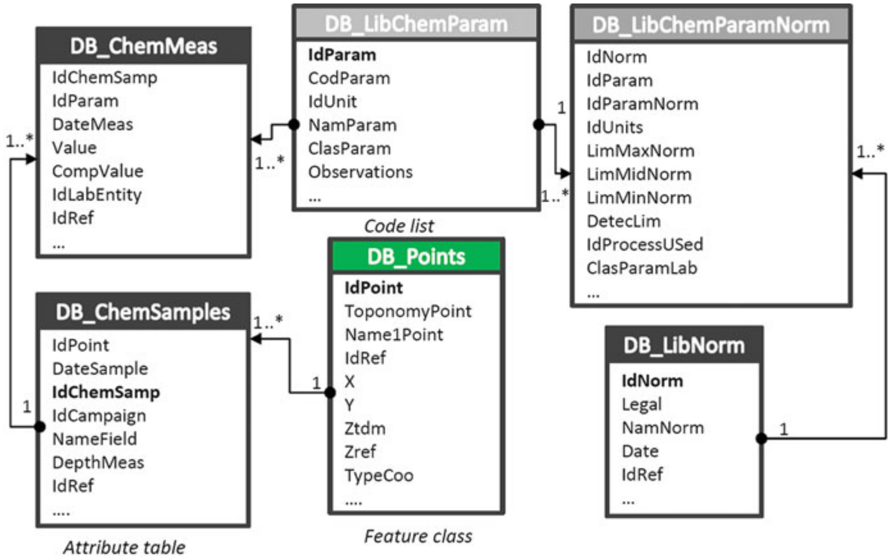

Fig. 2 Sketch showing the simplified conceptual diagram representing some contents of the HYDOR database related with hydrogeochemical data. The 1 and 1*. represent the cardinality of the relationship between tables

accordance with sampling data analysis, parameter and value and are included as a table in *DB_ChemMeasurements*.

Besides, other relevant information such as sampling methodology, the characteristics of the measurement site (e.g. well properties), analysis protocol, classification and detection limits for different laboratories can be readily included in the database. Finally, information about the campaign (*DB_Campaign*), the project (*DB_Project*) and the original source of information references (*DB_References*) are structured and stored in the database.

Incorporating Data to the Database

The management system of the geodatabase enables importing information from different spatial or nonspatial databases or spreadsheets and in different formats. Massive digital data can be transferred to the geospatial database through the use of intermediate conversion tables or existing wizards of ArcGIS following an established entry protocol. If the data are handwritten, they should be introduced manually using assisted menus.

In order to avoid errors when introducing data and to improve data harmonisation, data control procedures were developed. For instance, several permissible value lists were introduced to facilitate encoding, following recommendations of the existing standards and directives such as geological data specifications of the European Directive INSPIRE [46], the OneGeology project [47], the

Australian National Groundwater Data Transfer Standard [48], the Common Implementation Strategy for the Water Framework Directive (2000/60/EC) [49], GeoSciML [50], Water ML.2.0 [51] and the Observations and Measurements standard [52]. In addition, some classes and their attributes provided by those standards were imported to guarantee future data exchanges.

Besides, some validation checks can be performed to ensure consistency of the hydrochemical data, allowing the detection of erroneous data. Furthermore, the platform allows the visualisation and manipulation of censored values (concentrations of some compounds reported as 'non-detected', 'less than' or 'greater than' [6]). The user has the option to readily substitute the censored values by 0.5 times the detection limit (following [53]). It is noted that this procedure is not automatic and that the user can choose other methodologies to deal with censored values. Also, other utilities to facilitate the conversion of measurement units were developed to avoid inconsistencies among different data sets.

Querying the Spatio-temporal Data

To facilitate data retrieval and expedite the spatio-temporal data analysis, a set of GIS-based tools and other specific instruments were developed (see Sects. 3.2.2 and 3.2.3). Other spatial and nonspatial queries may also be generated from the geodatabase by employing the standardised MS Access query builder and/or by using the inherent capabilities of ArcGIS. Interested readers are referred to further documentation of ArcGIS and MS Access to make other queries.

3.2.2 GIS Tools

This set of analysis tools was developed as an extension of the ArcMap environment (ArcGIS; ESRI). They were created with ArcObjects, which is a developer kit for ArcGIS, based on Component Object Model (COM), and programmed in Visual Basic using the Visual Studio (Microsoft) environment (see [43–45). They were intended to manage, visualise, analyse, interpret and pre- and post-process the hydrochemical data stored in the spatial database.

The toolkit has the form of a toolbar integrated into the ArcMap environment (see Fig. 1) and consists of five instruments: (I) *Hydrogeochemical Calculator Tools*, (II) *Hydrogeochemical Diagram Tools*, (III) *Hydrogeochemical Data Analysis Tools*, (IV) *Normative Analysis Tool*, (V) *MIX Tools*, (VI) *EasyQuim Tools* and (V) *Hydrogeochemical Data Editor Tools*. In addition, ArcGIS inherent commands such as add data and select together with the full menu of the extension of *Geostatistical Analyst* are integrated into the same customised toolbar. The reported tools are presented next.

Hydrogeochemical Calculator Tools

This application consists of a query form that allows the user to perform the following operations for a preselected data set:

(a) Calculates charge balance error (CBE) for each sample stored in the database. If one of the major ions is not available for a given sample, this computation cannot be done. Nevertheless, some conventions and assumptions can be used in balancing the analysis such as the estimation of HCO3 concentration from alkalinity values.
(b) Calculates ionic ratios: Mg/Ca, Na/K, SO4/Cl and Cl/HCO3, icb index (disequilibrium chlorides and alkaline index) and SAR index (sodium adsorption ratio).
(c) Automatically converts all units to meq/L and calculates the relative percentage of a cation or anion.
(d) Displays the results of queries in a customisable table in ArcMap, containing all the aforementioned calculations, available for being exported for further analysis into MS Word or MS Excel.

The selection of the hydrogeochemical data to be analysed is made in two steps. The first one involves selecting a set of points on the screen that represents water sampling locations (points from *DB_Points*). This can be done by using any of the available select commands (e.g. select by location, by attributes, etc.) provided by ArcGIS or else the command already integrated into the toolbar (select by rectangle). In the second step the user selects the sampling period or periods to be included in the query form.

Hydrogeochemical Diagram Tools

The graphical methods are designed to simultaneously represent the total dissolved solid concentration and the relative proportion of certain major ionic species. This set of instruments (represented by different buttons in the toolbar) was designed to facilitate the creation of standard hydrogeochemical diagrams for groundwater chemical analysis and interpretation. Piper, Schöeller-Berkaloff, salinity and modified Stiff diagrams can be created automatically for the selected data set (only if the data necessary for the creation of each diagram are available).

As with the *Hydrogeochemical Calculator Tools*, the selection of the data is done by clicking several points in the map for a given interval of time or else a given point and different periods of time. The resulting diagrams and the attached information (well, data and concentration values expressed in meq/L) can be visualised on the screen in a customisable table or exported as a text file (MS Word) or into MS Excel:

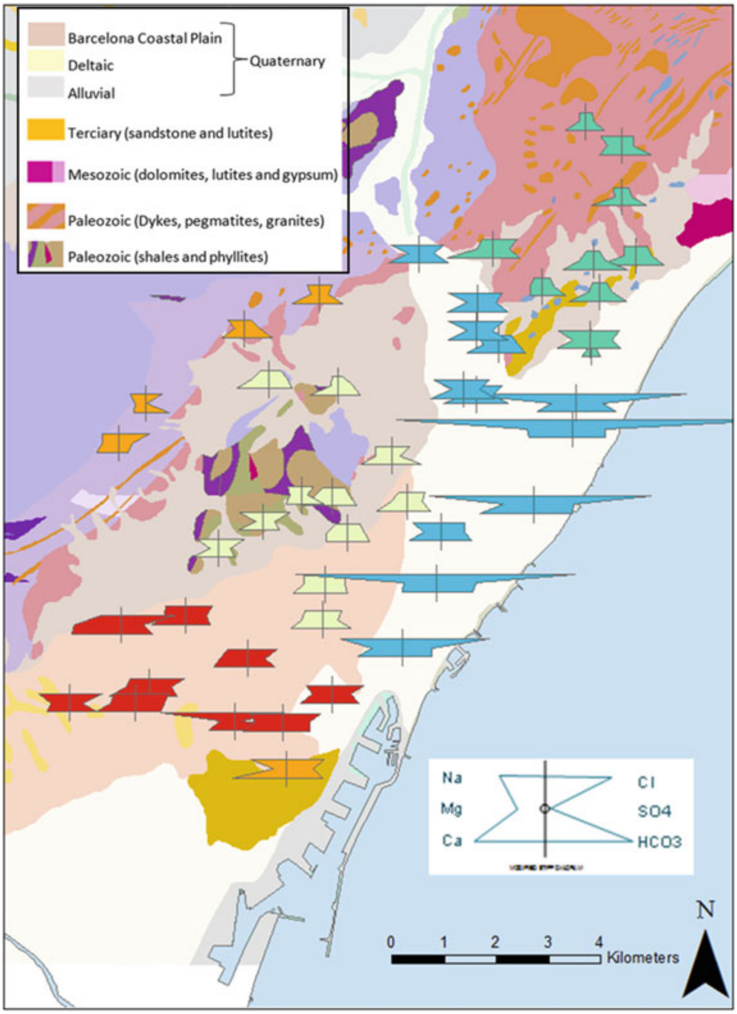

Fig. 3 Map representing the Stiff diagrams obtained for several aquifers located in the metropolitan area of Barcelona (Spain) for the samples collected during 2006–2007 (see also Sect. 4 for further explanations). This map was obtained with the aid of the Hydrochemical Diagram Tools. The base map was provided by IGC (Geological Survey of Catalonia). Coordinates are in Universal Transverse Mercator (UTM), zone 31

(a) Stiff diagram command

This command enables generating individual diagrams for a water sample or Stiff diagram distribution on maps (Fig. 3). The Stiff diagram [54] is widely used to display the variation of several ions in the same map. However, when high variability in major ion concentrations exists, a tool to harmonise the size displayed in the map is necessary [55]. Taking this into

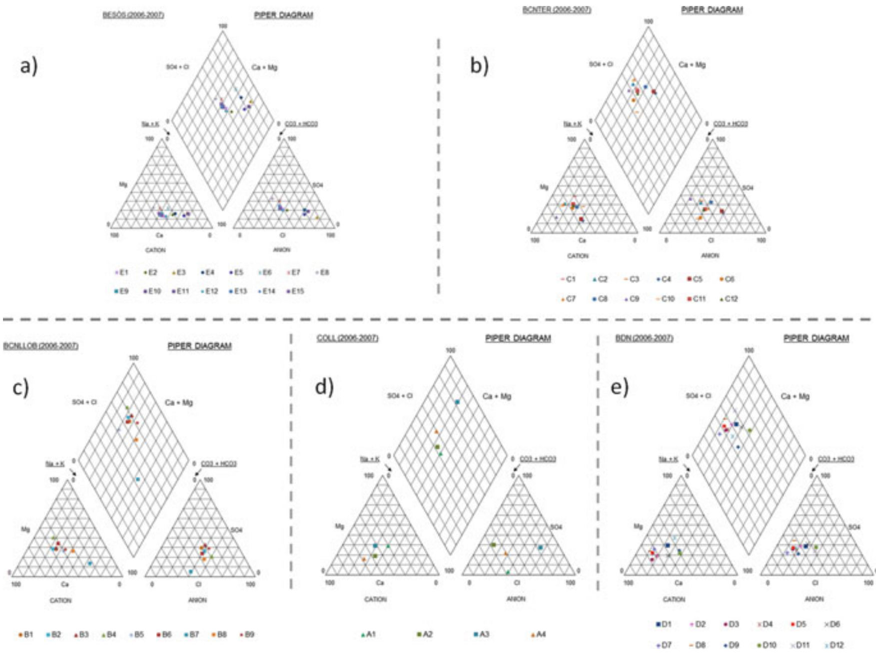

Fig. 4 Piper diagrams obtained by using Hydrochemical Diagram Tools for the samples collected during 2006–2007 and corresponding to the different study areas (see also Sect. 4 for further explanations): (**a**) BESÒS, (**b**) BCNCENTER, (**c**) BCNLLOB, (**d**) COLL and (**e**) BDN

account, the representation of the Stiff diagram in the map can be customised by selecting diverse concentration scales.

(b) Piper diagram tool

This command enables us to obtain automatically Piper [56] diagrams for the selected samples (Fig. 4). The Piper diagram is a most widely graphical form and displays relative concentration of the major anions and cations on two separate trilinear plots, together with a central diamond plot where the points from the two trilinear plots are projected [6].

(c) Schöeller-Berkaloff diagram

This tool allows us to generate the Schöeller-Berkaloff logarithmic diagram (Fig. 5) for the selected samples. This diagram allows the major ions of many samples to be represented on a single graph, in which samples with similar trends can be detected.

(d) Salinity diagram

This command generates automatically salinity diagrams. This diagram joins the calculated values of SAR index with the electrical conductivity and represents them in a single graph on a logarithmic scale.

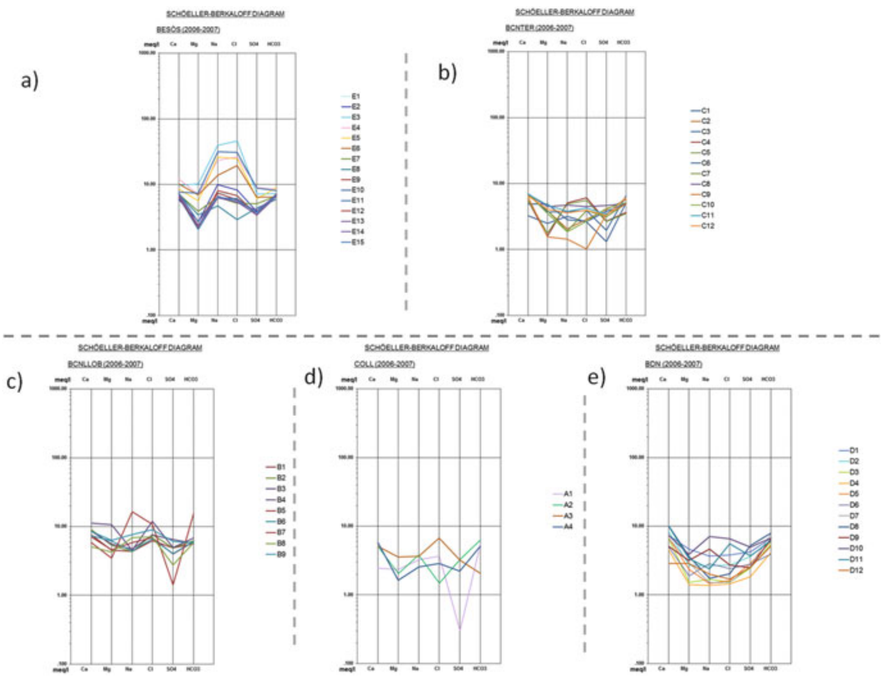

Fig. 5 Schöeller-Berkaloff diagrams obtained by using Hydrochemical Diagram Tools for the samples collected during 2006–2007 and corresponding to the different study areas (see also Sect. 4 for further explanations): (**a**) BESÒS, (**b**) BCNCENTER, (**c**) BCNLLOB, (**d**) COLL and (**e**) BDN

Hydrogeochemical Data Analysis Tools

This tool allows using a set of methodologies for querying, interpreting and comparing physico-chemical parameters measured for the selected samples:

(a) *Analysing data*. The query form enables the user to apply one or several query criteria (sampling point, sample, campaign, time interval, physico-chemical parameter) and to combine them for advanced queries on the data stored in the HYDOR geospatial database. Results of the query are shown in a list form where the user can also select the desired data for further queries (see following paragraphs) or else can be exported for further calculations or reporting into MS Excel or MS Word.

(b) *Generating maps*. This command allows us to obtain the minimum, maximum, average and standard deviation for each selected parameter, for a given interval of time and for a point or a group of selected sampling points, and to represent these values in a map in *shapefile* format. The number of samples used to compute the statistics is also displayed in the map. Results can be used for further statistical and geostatistical analyses in the same ArcMap environment (using *Geostatistical Analyst menu* already integrated into the toolbar) or can be exported to other external platforms. Additionally, further useful representations such as maps of *Pie* diagram or *Stacked* charts for the selected parameters can be obtained by using the inherent commands of ArcMap.

(c) *Plotting graphs*. This tool enables the user to explore whether correlations exist between two or more physico-chemical parameters, generating graphs where the temporal component is also added.

Normative Analysis Tool

This allows the user to obtain thematic maps for the queried parameters, classified according to the threshold approach established by a given guideline (e.g. Water Framework Directive). This enables identifying areas where some chemical species exceed a prespecified limit.

MIX Tool

MIX is an external code that allows the evaluation of mixing ratios using the concentration of samples assuming that these come from a mixing of recharge sources (known as end members) in an unknown proportion and fully accounting for data uncertainty.

The tool command allows obtaining the necessary information for the selected points and time intervals and transfers automatically all the required information to the MIX software (see Fig. 6). The selection of hydrogeochemical data to be analysed by this software is performed in three steps: (1) point(s), (2) sampling campaigns and (3) end members.

EasyQuim Tool

This command enables retrieving the information and exporting data into program EasyQuim, which is a free software developed as a plug-in in MS Excel (thus offering a great portability) to draw convectional graphical methods (Piper, salinity, Schöeller-Berkaloff and modified Stiff diagrams) as well as tables for CBE, icb index, SAR index and ionic ratios. Finally, the code supplies input data exportable to other GIS platforms to visualise Stiff diagrams.

Hydrogeochemical Data Editor Tools

This instrument enables visualising and editing information corresponding to a given sampling point by selecting it in the map. As a result, the user can consult and edit the type of sampling site (well, spring, river, etc.), the characteristics of the campaign (e.g. date, observations) and the measurements available at this point

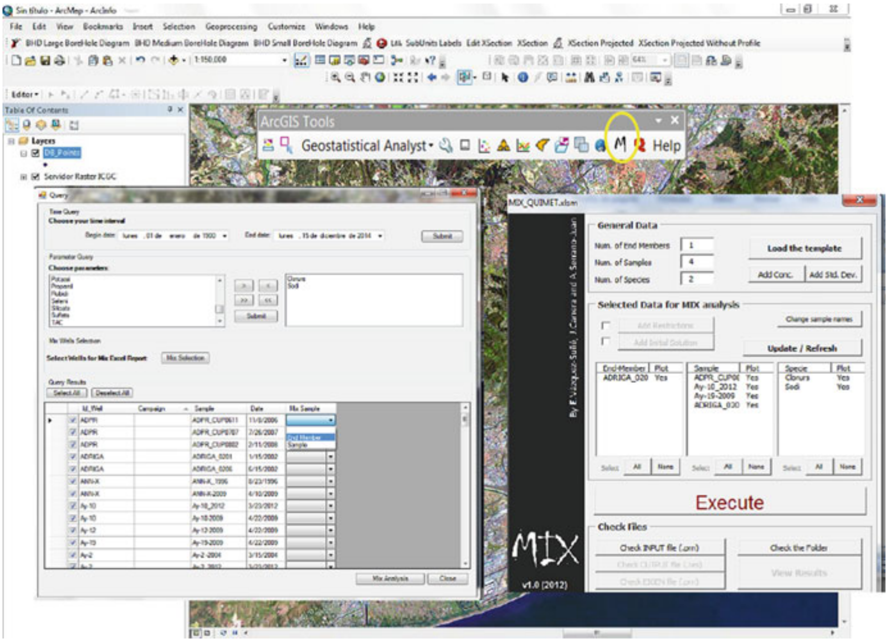

Fig. 6 *Sketch* showing the query form of the command MIX Tools

(data of sampling, parameters, etc.). The user can add new campaigns and new measurements associated to this sampling point using the same query form. The introduction of these data can be done massively using CVS files or 'one by one'.

3.2.3 Statistical Tools

This set of tools enables the user to perform a complete statistical data analysis. Again, it offers a query form that allows choosing a time interval and a set of parameters or else an entire time series for one or more parameters (Fig. 1). The result of this query is automatically exported to an MS Excel spreadsheet. By using a set of commands, the following calculations can be generated for the selected data set:

(a) *Standard statistical analysis*. This command provides a statistical univariate analysis: mean, standard deviation, minimum, maximum, variance, quartiles, kurtosis and skewness coefficient. It also creates histogram, scatter plots and box plots.
(b) *Parameter correlation matrix*. This creates the R2 correlation coefficient as well as the covariance and correlation matrices of the selected parameters analysed two by two.

(c) *Bivariate statistical analysis*. This generates correlation graphics for each pair of selected parameters.

Although this module operates independently (even though this module can be accessed directly from ArcMap using the tool termed *Link to Statistical Tools* included in the toolbar), the results obtained here can be exported to ArcGIS to perform additional analyses. Moreover, all the output from the *Hydrochemical Data Analysis Tools* and exported to MS Excel can be processed here for a more complete statistical analysis.

4 Application to the Metropolitan Area of Barcelona (Spain)

This software platform was used for the management and evaluation of the quality of groundwater in several study areas (e.g. [44, 45]) located in the metropolitan area of Barcelona (MAB), which is on the Mediterranean coast in NE Spain (Fig. 7). Geologically, MAB is formed by a coastal plain bounded by two deltaic formations and an elevated area, the Catalan Coastal Ranges. The Catalan Coastal Ranges (mainly Palaeozoic rocks) in this area display a NE-SW direction and are limited by NE-SW and NW-SE normal and directional faults [57].

The actual plain mainly consists of Quaternary formations that overlie the Pliocene series, mainly composed of marine blue marls and sandy marls [58, 59]. The Quaternary formation can be divided into lower Quaternary (locally termed *tricicle*) and upper Quaternary. The *tricicle* is made up of three cycles, from bottom to top, red clays, yellow silts and calcareous muds and calcrete [60]. The upper Quaternary is mainly constituted of torrential, alluvial and foothill deposits, where gravels and sands with a high proportion of clay matrix are present. Hydrogeologically, the Barcelona Coastal Plain can be regarded as an aquifer with a high vertical heterogeneity.

The Barcelona Coastal Plain separates the two deltaic formations (corresponding to rivers Besòs and Llobregat), which consist of two Holocene depositional systems that were also active during the Pleistocene [61–63]. In general, these deltaic formations consist of Quaternary materials and have similar characteristics consisting of several aquifer units separated by less permeable units. Quaternary materials in the Besòs Delta overlie a substratum formed by rocks of Palaeozoic (slates and granite) and Tertiary (matrix-rich gravels and sandstones of Miocene age and massive grey marls attributed to the Pliocene) age. The Quaternary of the Llobregat Delta River mainly rests unconformably on Paleozoic to Pliocene deposits [62].

The aquifers of the MAB have been used for irrigation and for industrial purposes in recent decades, posing a serious threat to the quantity and quality of the groundwater resources of the study area. Moreover, this region presents a large

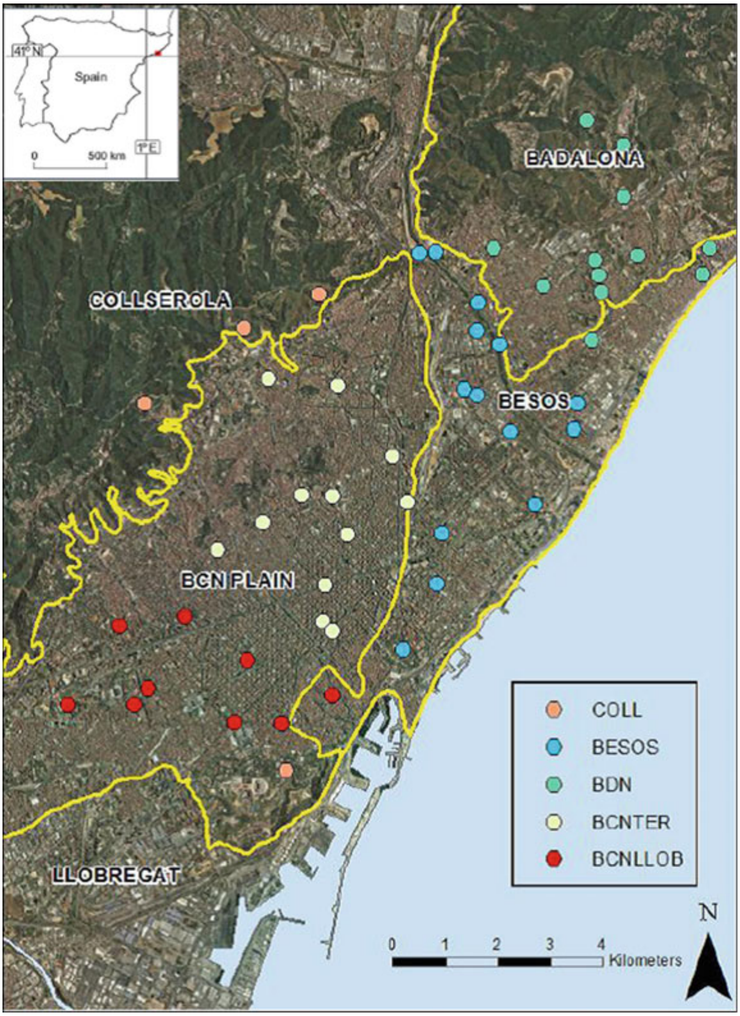

Fig. 7 Orthophotograph of the northeastern Mediterranean coast of Spain covering the extent of the study area (see *red point* in *upper left inset* for general location). The different sampled areas are also shown in the map together with the sampling points. Coordinates are in Universal Transverse Mercator (UTM), zone 31

number of underground infrastructures which compromises the quantity and quality of groundwater resources.

As an illustration of the potential of this software platform, a general analysis of the quality of the aquifers located in the MAB such as the Barcelona Plain, Badalona and Besòs Delta (see Fig. 7 for location) was chosen, based on a larger study funded by the Catalan Water Agency [64]. The application includes 56 samples (51 obtained from groundwater points) collected during 2006–2007 and distributed over the aforementioned study area. The sampling points can be grouped into five zones attending to its location in the aquifers of the study area as shown in Fig. 7: (1) Barcelona Llobregat (BCNLLOB), (2) Barcelona Ter (BCNTER), (3) Collserola (COLL), (4) Besòs (BESÒS) and (5) Badalona (BDN).

Among the 110 hydrogeochemical variables in the compiled database (physical parameters, organic and inorganic species), we selected only those with the highest frequency for a detailed evaluation (EC, pH, major ions—Ca, Mg, Na, K, Cl, SO4, HCO3, PO4, NO3, NH4—and some minor compounds). In addition, the geospatial database includes information on head measurements, pumping test results, geological description and meteorological and hydrological information.

The first step was to test the chemical analysis for charge balance error (CBE) by using the *Hydrogeochemical Calculator Tools*. In 95% of the samples, CBE was less than or equal to $\pm 10\%$, an error found acceptable for the purpose of this study. Indexes such as SAR, icb and ion ratios were also calculated for each selected point by using this command.

The next step was to analyse the hydrogeochemical data by using geochemical techniques including spatio-temporal representation of the data, correlations of different species and graphical diagrams (Piper, Stiff, Schöeller-Berkaloff). This was accomplished by using the different commands of the *Hydrogeochemical Diagram Tools* and of the *Hydrogeochemical Data Analysis Tools*. The Stiff map (Fig. 3) shows that the samples present similar characteristics according to the hydrogeological zonation. Additionally, this map shows that in general terms the mineralisation of the water increases seawards.

From the analysis of the Schöeller-Berkaloff and Piper diagrams corresponding to the different zones (see Figs. 4 and 5), it can be concluded that:

- The groundwater samples collected from springs and wells located at the high topographies (COLL) are low mineralised and present low contents of most compounds, due to the proximity of the recharge area and the possible contact with soils with low Mg and Ca content (see Figs. 4d and 5d).
- The groundwater from the Barcelona Plain (grouped into BCNTER and BCNLLOB) can be classified as Cl-SO4-Na and becomes of Ca-Mg type, probably as a result of the interaction with the different geological formations (see Figs. 4b, c and 5b, c).
- The samples from the Badalona area (BDN) are less mineralised in the higher areas. In the northern part of Badalona, mainly constituted by granite, the water can be classified as HCO3-Ca type. In the southern and central areas, the water can be classified as HCO3/Cl-Ca/Na type, probably as a result of cation exchange between sodium and calcium in the finer Quaternary alluvial deposits and an enrichment in Na in the granitic structures (see Figs. 4e and 5e).
- The water from the wells located in the Besòs Delta (BESÒS) can be classified as Cl-SO4-Na. The samples collected from wells located near the sea show the highest content in Cl and Na, suggesting seawater intrusion (see Figs. 4a and 5a).

In order to evaluate the groundwater quality of the study area and to detect possible sources of contamination, several spatial distribution maps of a number of

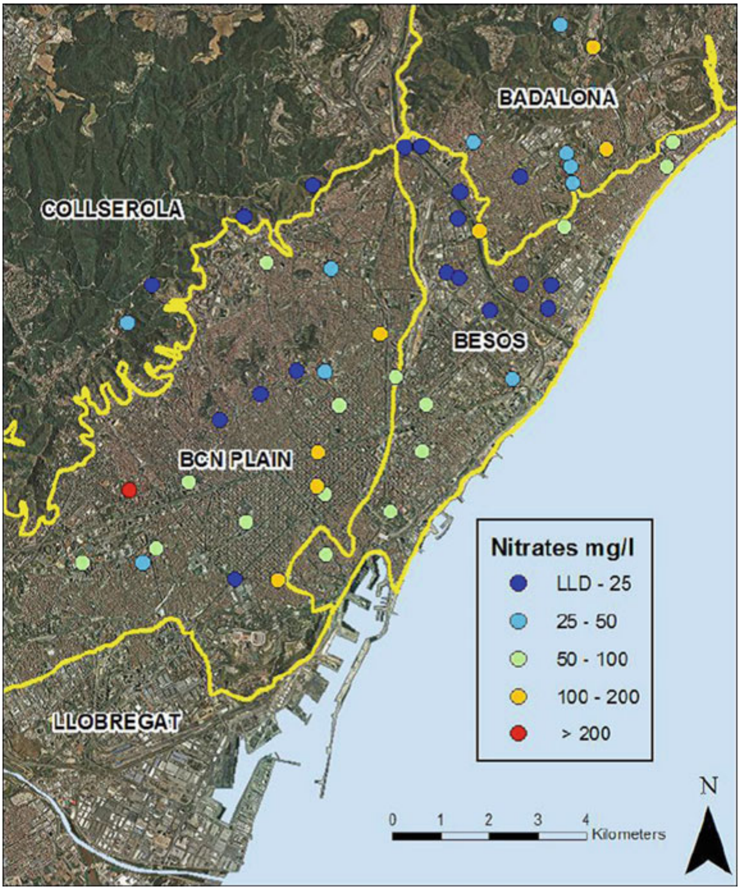

Fig. 8 Map of nitrates concentration for the period 2006–2007

parameters were obtained by using other command of GIS Tools (see Fig. 8). They show that the concentrations in residual water contamination markers, such as nitrate and phosphate, are higher in the urbanised areas except for samples collected from deeper aquifers or in the proximity of the Besòs Delta, suggesting biodegradation in a reducing environment.

Additionally, further maps of minor compounds such as sum of pesticide concentrations or LAS (compounds found in detergents) were obtained (see Fig. 9). Pesticides were only detected in the proximity of Besòs Delta and in one sample in the Barcelona Plain area. Despite of the sum of pesticide concentrations not exceeding the limit established by the Water Framework Directive in any of the observed points, that of terbuthylazine exceeded the limits established for an individual substance in one point close to the river and in another sample collected directly from the river (for further information, see [65]).

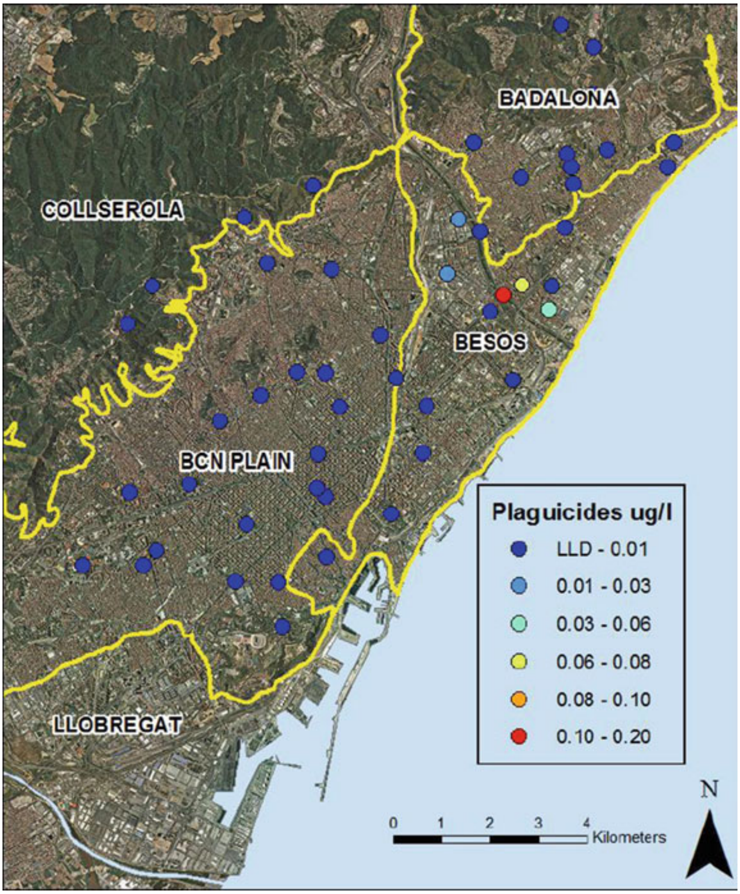

Fig. 9 Map of pesticides concentration for the period 2006–2007

In contrast with pesticides, LAS were detected in the majority of the samples analysed, including those collected from non-urban or high topographic areas, where the maximum detected was 0.8 μg/L. On the other hand, in the urban area the maximum value detected was 5.06 μg/L. Additionally, the values of LAS measured in the Besòs River (48 μg/L) and in the water treatment plant (from 147.34 to 117.62 μg/L) are even higher, suggesting that (1) aquifer recharge comes mainly from losses in the sewage system and the influence of the river and (2) the degradation process of these compounds is quite significant.

5 Conclusions and Discussion

The GIS-based software platform presented in this chapter offers a user-friendly environment with a wide range of automatic tools designed especially for the management, analysis and interpretation of hydrogeochemical data.

A key element of this platform is the HYDOR geospatial database that provides the following advantages: (1) a comprehensive storage and management of different types of hydrogeological spatio-temporal data, (2) the possibility of querying and visualising data simultaneously and (3) an efficient preprocessing of the hydrogeochemical data. The design of the database offers considerable flexibility since it may be extended and customised to other environments.

Despite the capacity of the database to store a vast amount of data, its consultation is made simple by using different multi-criteria query forms (ArcGIS Tools and Statistical Tools), which enhances the visualisation and analysis of hydrogeochemical data. The ArcGIS Tools module integrates a wide range of specific methodologies for hydrogeochemical analysis into a single GIS integrated framework. This includes: (1) multiple queries for comparing temporal and spatial groundwater parameters, (2) tools for calculating useful hydrogeochemical parameters, (3) instruments that enable the user to generate thematic maps for the parameters measured in the queried area classified according to the threshold values provided in a given guideline, (4) generation of plots with temporal evolution of preselected data for further geostatistical analysis and (5) creation of traditional hydrogeochemical diagrams, adding the spatio-temporal component, thus allowing the combined analysis of sampling points and campaigns.

These tools were implemented in the same ArcGIS software package, and their analysis potentially makes the most of the additional in-built instruments of this platform (e.g. geostatistical analyst tools, spatial analysis tools). Furthermore, ArcGIS fosters a shallow learning curve, easy maintenance and interoperability among different tools owing to its widespread adoption.

The platform integrates Statistical Tools that offer the possibility of performing a complete statistical analysis of data, including descriptive statistical analysis, bivariate analysis, generation of correlation matrix and correlation graphics. It also offers interoperability with external platforms such as EasyQuim or MIX. Moreover, with adequate adjustments this software platform could be easily linked to other programs such as PHREEQC or SGeMs, considerably increasing the variety of hydrogeochemical calculations.

The application of the database model (HYDOR) for the urban environment of Barcelona together with the hydrogeochemical analysis tools proved to be an efficient framework for groundwater studies, which can be easily updated and downscaled.

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Groundwater Vulnerability Mapping Assessment Using Overlay and the DRASTIC Method in Catalonia

Xavier Carreras, Josep Fraile, Teresa Garrido, and Carles Cardona

Abstract There are different vulnerability methods to evaluate groundwater potential pollution. One of them is the DRASTIC model, a well-known and widely used parametric method based in the analysis of seven hydrogeological factors. The applicability of the DRASTIC method was tested in the area of Catalonia, covering all territory (more than 31,000 km^2). Available information related to groundwater characteristics was selected and taken from geological and hydrogeological cartographies, groundwater database, and bibliography. A cartography of 199 aquifers were used to group and to extrapolate the information when only spared data were available. Afterwards, data were processed in order to be adapted to the DRASTIC parameters. The outcome of the vulnerability map was a raster file with a 100 × 100 m pixel resolution. Two different coverages of vulnerability were calculated from different weightings according to the DRASTIC parameters: generic pollutants and pesticides. This analysis was made both in confined and non-confined aquifers. Resulting maps were considered very satisfactory, and they were compared with other existing vulnerability works in more local areas with high similar results. These vulnerability layers have constituted different groundwater management tools. In this project, we applied this approach in order to assess the risk of non-achievement of the Water Framework Directive's (WFD) objectives for Catalan groundwater. Pressure and subsequent risk analyses were carried out from overlaying human activity areas with groundwater chemical data. For instance, nitrogen load from agriculture sources, contaminated soil areas, or sewage sludge application was overlaid with vulnerability map in order to obtain the global pressure.

Keywords Catalonia, DRASTIC, Groundwater, Vulnerability

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Contents

Abbreviations

ACA	Catalan Water Agency
ICGC	Cartographic and Geological Institute of Catalonia
IMPRESS	Impact and human pressure analysis
WFD	Water Framework Directive (2000/60/EC)

1 Introduction

One of the earliest definitions of groundwater vulnerability came from Albinet and Margat [1, 2] as the possibility of percolation and diffusion of contaminants from the ground surface into water table reservoirs under natural conditions. Subsequent proposals defined vulnerability as an intrinsic property of a groundwater system that depends on the sensibility of that system to human and/or natural impacts [3]. Vulnerability could be considered a relative characteristic that indicates where contamination is most likely to occur, but cannot be directly measured in the field. The assessment of groundwater vulnerability is not always an easy task, and difficulties in obtaining reliable field data constitute usually an important limitation. However, it is an effective tool to delimitate areas affected by groundwater diffuse contamination, such as intensive fertilizer application, defining a control network, and also applying vulnerability maps as a management tool.

There are several methodologies for the evaluation of groundwater vulnerability. Most of them use overlays and index methods consisting in algebraic operations of hydrogeological parameters. The DRASTIC method, developed by Aller et al. (1987) [4] for the US Environmental Protection Agency (EPA), is one of the most widely and contrasted tools used in several countries for this purpose. DRASTIC and other simplified methods such as GOD [5], EPIK [6], COP [7], SINTACS [8], or CRIPTAS [9] are also used to assess groundwater vulnerability.

2 DRASTIC Methodology Applied at Groundwater of Catalonia

DRASTIC methodology was developed with the purpose of creating a systematic regional evaluation using major hydrogeological factors to infer the potential for contaminants to enter groundwater. DRASTIC was initially designed to use existing information available from a variety of sources. It was initially applied to evaluate potential groundwater pollution in different locations of the United States and requires a high degree of information, which often entails great difficulties of application. DRASTIC methodology was developed in other countries, but usually in a more reduced scale [10–13].

The DRASTIC method has four main assumptions: (1) the pollutant is introduced into the ground surface; (2) the contaminant is transported throughout the saturated zone by precipitation recharge; (3) the contaminant has the mobility of water; (4) and the minimum study area required is about 40 km^2. This system is used to estimate the intrinsic vulnerability basically in granular porosity media, although in karst and fractured environments, a modified DRASTIC has been used [7, 14, 15]. This is a parametric method, based on weighting and sorting. The DRASTIC Index considers seven hydrogeological properties (acronym of DRASTIC): D, depth to water; R, net recharge; A, aquifer media; S, soil type; T, topography of the terrain; I, impact of the vadose zone; and C, hydraulic conductivity of the aquifer. Ratings for each DRASTIC variable are assigned to a value that ranges from one to ten in an increasing order of impact to vulnerability and a weighting value from one to five (Eq. 1):

$$\text{DRASTIC Index} = \text{Dr} * \text{Dw} + \text{Rr} * \text{Rw} + \text{Ar} * \text{Aw} + \text{Sr} * \text{Sw} + \text{Tr} * \text{Tw} + \text{Ir} * \text{Iw} + \text{Cr} * \text{Cw} \tag{1}$$

where r = rating and w = weight.

The applicability of DRASTIC method was tested in order to assess the groundwater vulnerability in Catalonia (Fig. 1), covering a total area over 31,000 km^2.

The main effort in this regional work was the compilation and interpretation of all parameters and their extrapolation into areas with less information. A direct transposition was done when information covered all the territory, whereas if sparse information was available, it was just necessary to aggregate it. In this last case, the reference cartography was the “Aquifers map of Catalonia” [16], where a total of 199 aquifers were defined (see reference map in Fig. 2a). Among these, 13 confined aquifers are differentiated, mainly in delta areas where a silt and clay formations are clearly acting as an aquitard, differentiating two aquifers in the same vertical.

A second challenge was the transposition of selected information into DRASTIC parameters. As a general rule, classification of parameters has followed the proposal defined by Aller et al. [4]. Despite this, a specific conversion from this initial rating was applied to the vadose zone (I) and soil media (S) parameters according to

Fig. 1 Location of Catalonia

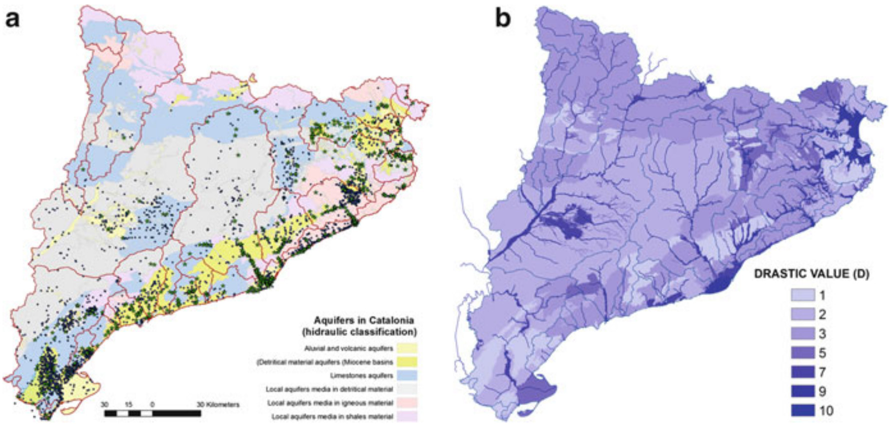

Fig. 2 Groundwater level data available (ACA); *star figures* represent points included in the monthly piezometric monitoring network. Reference map: aquifer delimitation in Catalonia (*left*). Depth to water (D) ranges according to DRASTIC method in Catalonia (*right*)

the information available in Catalonia and following the maximum accuracy possible.

Information to elaborate the seven DRASTIC hydrogeological factors came from a great variety of sources, specifically from geological and hydrogeological cartographies, from different groundwater database, and from local studies. As a result, each DRASTIC parameter was finally aggregated in layers set at different scales depending on the information source: 100×100 m cells, hydrographical basin, 199 aquifers previously defined, geological 1:50,000, and hydrogeological 1:250,000 or soil-type parcel. In order to create, treat, and overlay all these layers of information, an important development of geographic information system (GIS) tool was required. A schematic summary of the ranges and ratings finally used is showed in Table 1, while a brief description for each DRASTIC parameter is explained.

2.1 Depth of Water (D)

Depth of water (D) refers to the distance from the surface to the water table, which corresponds to the depth of material through which a contaminant must travel before reaching the aquifer. This parameter is related to the transit time of the contaminant in the unsaturated zone. It is assumed that the shallower the depth, the more vulnerable is the aquifer pollution.

Background information of water levels came mainly from the ACA hydrogeological database. It has more than 60,000 points, among which more than 3,000 have information about groundwater level. Besides, 510 points are included in the quantity monitoring network (some data from the 1970s) [17], with a monthly sampling that provides knowledge on potential variations of annual levels (Fig. 2a). Additionally, bibliographic information, mathematical models available, and other local studies (some of them inedited) [18, 19] were used in this analysis, especially in areas where groundwater level information is not so extensive. The ranges and ratings applied follow the approach defined by Aller et al. [4]. Despite that from a hydrogeological point of view it is important to know precisely the value of water level, the DRASTIC range values (Table 1) show that the influence of this parameter is relevant up to 30 m, because far from these depths the DRASTIC value was set to 1. Finally, (D) parameter was mapped, with range values grouped by each aquifer. In unconfined aquifers, this parameter corresponds to the water table, while in confined aquifers the depth to the top of the aquifer was estimated. In these cases, the knowledge of the structure and aquifer thickness was absolutely necessary. Results are showed in Fig. 2b.

Table 1 Range and rating of the DRASTIC parameters

D (m)		R (mm)		A	
0–1.5	10	0–51	1	Gravels, sands (alluvial and deltaic fm.)	6–8
1.5–5	9	51–102	3	Massive conglomerates and sands	6–8
5–10	7	102–178	6	Massive limestone and dolomite	6–9
10–15	5	178–254	8	Marl and detrital limestone	4–7
15–20	3	>254	9	Conglomerates, sands, and marl	3–5
20–30	2			Marl and evaporate formations	3–5
> 30	1			Metamorphic formation	4
				Granite formation	5–7
				Mixed formation and Neocene basins	4–8
S				T (%)	
Urban and industrial zones			1	0–2	10
Forest land, meadows, bushes			4	2–6	9
Urban soils			6	6–12	5
Riparian forests			5	12–18	3
Non-riparian forests			7	> 18	1
Beach			9		
Rocks, round dish			10		
Agricultural land (depending slope value)			2–8		
I				C (m/day)	
Silt, clay, gypsum, and sales			1	0.04–4	1
Shale			3	4–12	2
Igneous rocks and local shale			4	12–28	4
Marl/silty limestone			4	28–40	6
Dolomite			5	40–80	8
Limestone, sand, and conglomerate			6	> 80	10
Travertine and reef limestone			7		
Sand, gravel, travertine, and carbonated roof			8		
Basalts			9		

D depth to water, *R* net recharge, *A* aquifer media, *S* soil type, *T* topography of the terrain, *I* impact of the vadose zone, *C* hydraulic conductivity of the aquifer

2.2 Net Recharge (R)

Considering the precipitation as the most important source of recharge in groundwater, this parameter refers to the total quantity of precipitation (per unit area), which infiltrates and percolates to the water table. This recharge of water is the main vehicle to leach and transport a contaminant vertically to the water table and horizontally within the aquifer. Therefore, the vulnerability to pollution is enhanced by high recharge rates. Practices like irrigation or artificial recharges could be other sources that may enhance net recharge and could be taken into account, but they haven’t been considered in this project.

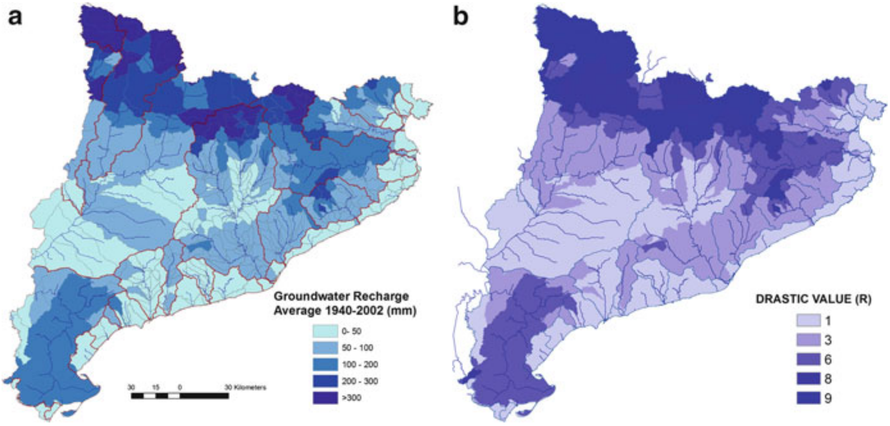

Fig. 3 Average groundwater recharge (mm) for hydrographical subbasin defined in Catalonia (506) with the hydrometeorological Sacramento model (*left*). Net recharge (R) ranges according to DRASTIC method in Catalonia (*right*)

Information used for this parameter came from an estimation of groundwater recharge in Catalonia [20]. This subterranean recharge was calculated from a hydrometeorological model named "Sacramento Soil Moisture Accounting (SAC-SMA)," from the US National Weather Service and California Department of Water Resources [21, 22]. It's a contrasted model applied in several countries and used also in ACA to assess the total water resources and as a water management tool. The work scale in this project was 506 hydrographical basins defined, with an average area of 91 km^2 (minimum 1.3 km^2, maximum 1,650 km^2). With a monthly discretization, the model was finished and calibrated with flow data from gauging stations (more than 70). The analyzed period ranges from 1940 to 2002, with an average groundwater recharge calculated near 130 mm, which represents 16% of the rain (between 10 and 20%) and around 60% of total water contribution. This average value calculated in all 506 basins was the value adopted for the calculation of this DRASTIC parameter (Table 1 and Fig. 3a). In unconfined aquifers, the ranges and ratings applied follow the proposal defined by Aller et al. [4]. In confined aquifers, a value between 0 to 3 points was subtracted to the parameter value from the upper aquifer, based on expert criteria and knowledge of the depth to the top of the aquifer and the aquitard developed. The resulting map is showed in Fig.3b, where a clear relation between the upper basin areas and the groundwater recharge is observed. Also the areas of limestone and karstification formations have a high groundwater recharge.

2.3 Aquifer Media (A)

This parameter refers to the properties of the rock which serves as an aquifer. That includes basically the degree of consolidation of the subsurface environment and also the lithology and the structure of the aquifer, the nature of porosity, and the

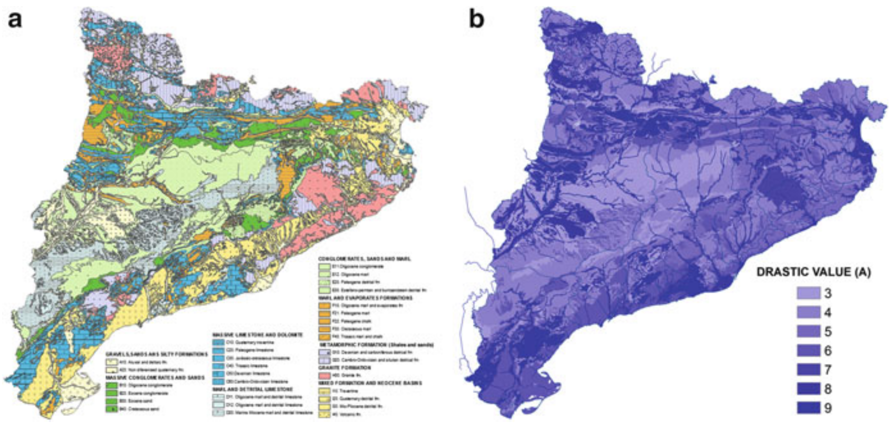

Fig. 4 Map of hydro-areas of Catalonia 1:250.000 (*left*). Aquifer media (A) ranges according to DRASTIC method in Catalonia (*right*)

potential karstification (porosity secondary). These properties control the transport of pollutants within the aquifer. In general, the larger the grain size and the more fractures or openings within the aquifer, the higher the permeability and the lower the attenuation capacity and, consequently, the greater is the potential pollution. The two hydrogeological parameters that are related to this property are the permeability (property of a rock to be pervaded by a fluid) and the porosity (fraction of void space in the material). Therefore, some authors [11] decide to join together this DRASTIC parameter with the "C" DRASTIC parameter (conductivity) and treat them as a single parameter.

In this project, it was opted to use the available cartography of the "Map of hydro-areas of Catalonia 1:250,000" [23] (Fig. 4a). DRASTIC ranges of Aller et al. [4] were adapted from the hydrogeological classification of defined geological formations in this cartography (lithology type and geological ages) (Table 1). Also, an expert criteria allowed to differentiate in each hydrogeological class some aquifers with different behaviors (knowledge of the dominant lithologies, karstification, etc.), being able to associate different DRASTIC values. The resulting map is showed in Fig. 4b. The same methodology was applied in unconfined and confined aquifers.

2.4 Soil Type (S)

Soil type (S) refers to the uppermost portion of the vadose zone, commonly considered the first 1.5 m, which has a significant biological activity. Soil has a significant importance because it is related with the amount of recharge that can infiltrate. Also, where soil zones are well developed, the first attenuation processes of filtration, sorption, volatilization, or biodegradation can be done before the contaminant penetrates into the vadose zone. The amount of clay, granulometry, and organic matter are the relevant parameters associated to the soil zone that control the pollutant infiltration. In general, the presence of clay and small grain size, along with the potential presence of organic matter, reduces the vulnerability of the groundwater to pollution.

Information about soil characteristics is in general difficult to obtain; hence, this is the most difficult DRASTIC parameter to assess. Due to the lack of a soil map at regional scale, this DRASTIC setting was assessed with the "Land cover map of Catalonia 1:250,000, v. 4" [24] (Fig. 5a). The land cover classification (level 2) (further information can be found in [25]) was used for the assessment of the potential development of soil. Then, the DRASTIC ranges were defined taking into account the lithological soil classification of the land cover map and adopting the proposal of Aller et al. [4] (Table 1). For example, rock areas have a value of 10, while urban and industrial zones, which have lost most of their natural soil and have been replaced by all sorts of artificial covers, have a value of 1. On agricultural land zones, a specific analysis was undertaken. The slope, as well as the lower proportion of fine material in deltaic areas, was taken into account (see more information in [26]). Then, depending on the slope percentage, the range of the adopted values was 2 if the slope was less than 2%, 3 if the slope ranged between 2 and 5%, 5 if the slope ranged between 5 and 10%, 7 if the slope ranged between 10 and 20%, and 8 if the slope was higher than 20%. Additionally, in deltaic areas, S DRASTIC value had an increase of 2 points. The same methodology was applied in unconfined and confined aquifers. The resulting map is showed in Fig. 5b, where urban areas (metropolitan area of Barcelona) and agricultural zones with a low slope (in general with a soil zone well developed) can be differentiated from forest areas with higher rating values. An exhaustive analysis of the proposed values in these forests areas and in agricultural areas is clearly one of the main improvements to develop.

2.5 Topography (T)

Topography (T) refers to the slope of the land surface. This parameter has an influence on the runoff capacity of the media; so, typically, vulnerability to contamination is reduced as the slope increases.

Information of slope values was determined directly from the Topographic map of Catalonia 1:50,000, v. 1 [27] generating a slope map raster 100×100 m (Fig. 6a). Then, the ranges and ratings applied are exactly to those proposed for Aller et al. [4] (Table 1). The same methodology was applied in unconfined and confined aquifers. The resulting map is showed in Fig. 6b where clearly defined mountainous areas with a general range of 1 are differentiated from plain areas with DRASTIC ranges around 9 and 10.

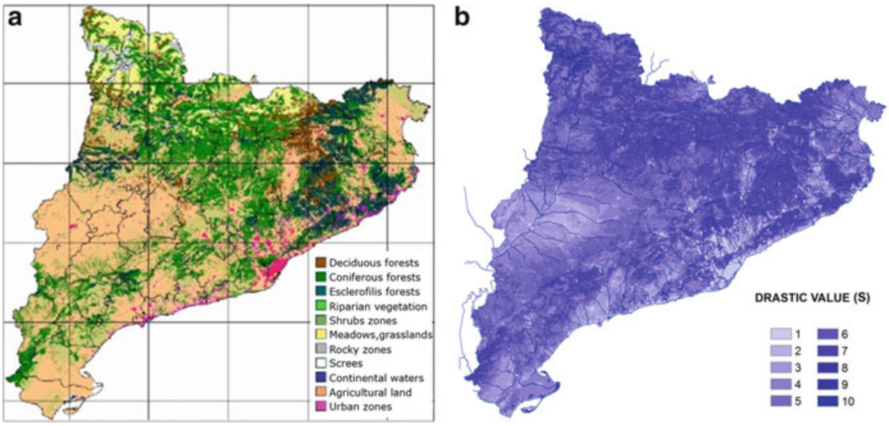

Fig. 5 Land cover map of Catalonia 1:250.000, v. 4 (CREAF, 2009) (*left*). Soil type (S) ranges according to DRASTIC method in Catalonia (*right*)

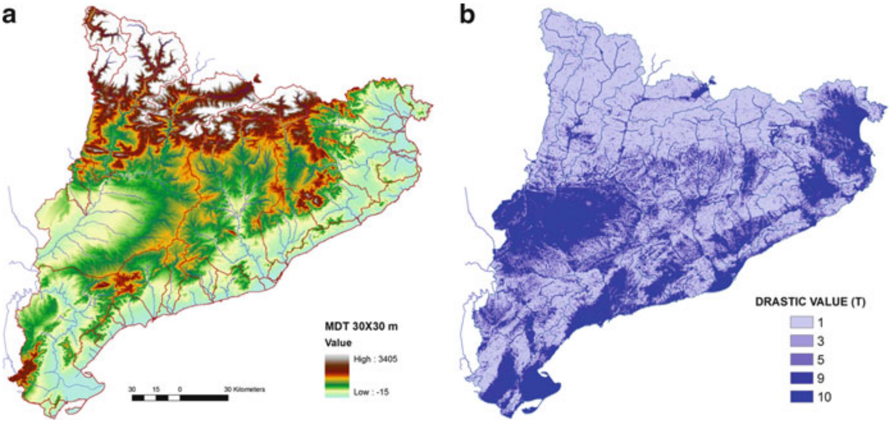

Fig. 6 Digital model topography (raster 30×30 m) (ICGC) (*left*). Topography of the terrain (T) ranges according to DRASTIC method in Catalonia (*right*)

2.6 Impact of the Vadose Zone (I)

Impact of the vadose zone (I) refers to the unsaturated zone above the water table. Although usually it could be considered the upper layer from which the soil is derived, it also must take into account other formations that can interfere when the upper layers are thin and the groundwater levels are deep. The characteristic of the vadose zone determines the attenuation processes that could occur, such as biodegradation, chemical reactions, and volatilization. Like soil type, the analysis of this parameter depends on granulometry, organic matter, and primary or secondary porosity.

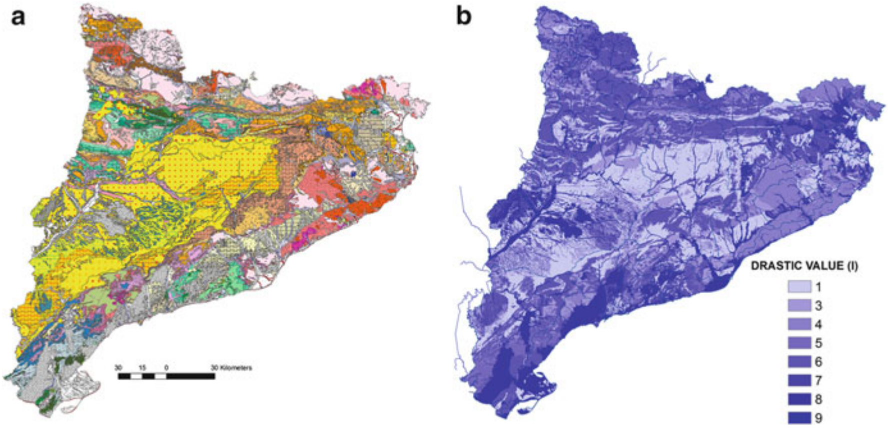

Fig. 7 Geologic map of Catalonia 1:250.000 (ICGC, 2010; legend consultable at www.icgc.cat) (*left*). Impact of the vadose zone (I) ranges according to DRASTIC method in Catalonia (*right*)

The information used to evaluate this parameter came from the "Geologic map of Catalonia 1:50,000" [28]. Figure 7a shows the geological map at a scale of 1:250,000 [29]. DRASTIC ranges provided by Aller et al. [4] were adapted from the lithological classification of this cartography (Table 1). In confined aquifers, the assessment of this parameter was extended to include the vadose zone and any saturated zone which overlie the aquifer. In this case, it was adopted that up to 3 points have been subtracted to the parameter value from the non-confined aquifer, depending if a clay/silt aquitard formation was developed (mainly deltaic aquifers). The resulting map is showed in Fig. 7b. Limestone and granular-sized lithologic units, with ratings of 7–9, are well defined and highly differentiated from zones associated to clay, silt, and shale formations, with range values of 1–2 (Oligocene central area and some Miocene formations).

2.7 Hydraulic Conductivity of the Aquifer (C)

Hydraulic conductivity of the aquifer (C) refers to the ability of aquifer materials to transmit water. The rate at which the groundwater flows is directly related with the rate that a contaminant moves into saturated zones. Hydraulic conductivity is an intrinsic aquifer characteristic that depends on intergranular porosity, fracturing, and bedding planes. As mentioned, this DRASTIC setting is treated sometimes together with the aquifer media (A) because these data are normally scarce and have a large spatial variability (especially in limestone aquifers). Thus, the analysis of this parameter is supported with bibliographic information, such those provided by Davis [30] and Freeze and Cherry [31], that relates conductivity values to aquifer lithology.

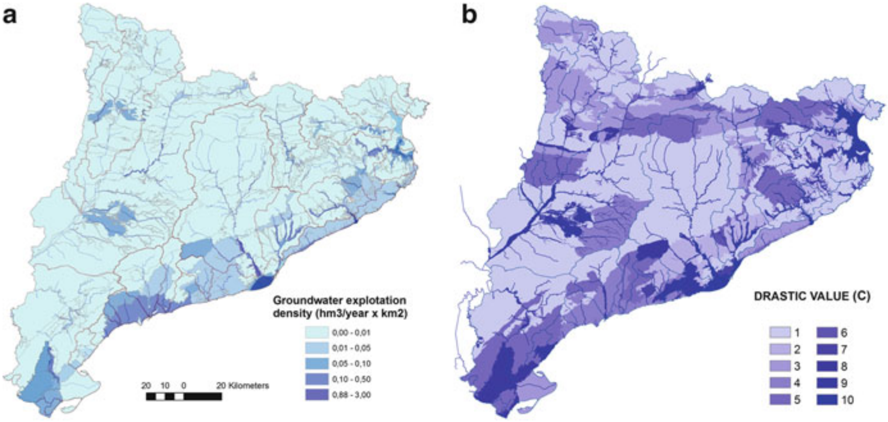

Fig. 8 Groundwater exploitation density map of Catalonia (ACA) (*left*). Hydraulic conductivity of the aquifer (C) ranges according to DRASTIC method in Catalonia (*right*)

A range of hydraulic conductivity was estimated for each of the 199 confined and unconfined aquifers defined in Catalonia, taking into account information about aquifer lithology and bibliographic information from local studies [18, 32] that include sometimes some data pumping tests and expert criteria. Additionally, information of groundwater exploitation in some cases was contrasted to decide some range values. As a general view, Fig. 8a shows the density of exploitation of groundwater (hm^3/year $\times$ km^2) and how the almost 570 hm^3/year total of groundwater extraction estimated in Catalonia is distributed. Although the effect of demographic distribution and water uses is evident, this information reveals the importance of an aquifer and consequently their hydraulic conductivity. The ranges and ratings applied were defined by Aller et al. [4] (Table 1). In Fig. 8b, the resulting map shows that the most relevant aquifers are in deltaic and limestone formations, where the density of groundwater exploitation is also significant.

3 Results of DRASTIC Methodology in Catalonia

Vulnerability maps were obtained, transposing on a fine mesh with a grid spacing of 100 m, by overlaying each individual maps under a GIS environment. Two different coverages of vulnerability were calculated according to different weightings exposed by Aller et al. [4]: for generic pollutants and for aquifer exposed to pesticide pollution. DRASTIC weights finally adopted are shown in Table 2.

For each grid cell, the two indices (generic and pesticide pollution) were calculated. With the ranges defined in Table 2, pesticide values are higher than generic, with maximum values that reach 234, while the maximum value of generic vulnerability is 204. Statistics analysis for generic and pesticide DRASTIC results is shown in Table 3.

Table 2 DRASTIC weights by Aller et al. [4]

Feature	Generic	Pesticide
Depth to water	5	5
Net recharge	4	4
Aquifer media	3	3
Soil media	2	5
Topography	1	3
Impact of the vadose zone	5	4
Hydraulic conductivity	3	2

Table 3 Statistics parameter values for generic and pesticide DRASTIC analysis

Statistics	Generic	Pesticides
Numbers of cells	3,181,185	3,181,185
Minimum value	32	35
Maximum value	204	234
Mean value	93	109
Standard deviation	28	26
Q_{25}	71	90
Q_{75}	111	127

After an analysis of the statistical parameters and taking into account different bibliography and some local works [4, 10, 33], values were finally distributed among five classes, which are attributed to a qualitative degree of vulnerability, ranging from "very low" to "very high." These range values were defined in order to evaluate several pressures that could affect groundwater quality. For this reason, one should consider that range values of the indices could not be compared directly with other groundwater vulnerability analyses. Resulting maps with range values are shown in Figs. 9 and 10 for generic and pesticide analysis, respectively.

Results from the generic vulnerability map are very satisfactory. The highest values were calculated in alluvial aquifers, with high permeability, and where the groundwater level is near the surface. Also, high values were shown in main header basin aquifers associated with aquifer media made up of calcareous and karstified limestone, while the lower values were associated to tertiary (Oligocene) silt and clay units, with a flat topography and a soil generally well developed. In other cases like sedimentary Miocene basins and shale or igneous areas, a moderate or low vulnerability was assessed.

Comparing generic versus pesticide indices (Table 2), in the pesticide case, the specific hydrogeological settings (hydraulic conductivity and aquifer media) do not have a significant importance, whereas soil media parameter has the most influence. Also the topography parameter has a different weight between the two indices. The results shown in Figs. 9 and 10, considering the range scale difference, are considered quite similar, and only with a detailed analysis some differences could be assessed. Then, in areas with a well-developed soil, DRASTIC values from pesticide analysis are lower than the generic values: central-west area (Lleida Plain) and south area (Plana de la Galera) of Catalonia. On the contrary, only in local areas in

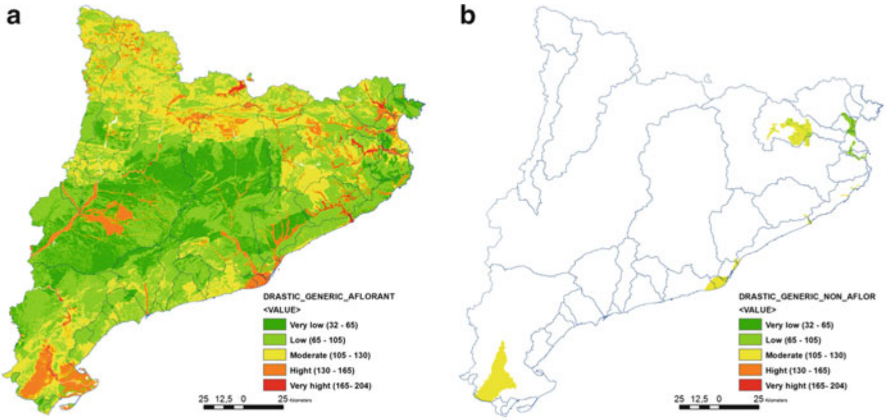

Fig. 9 Generic groundwater vulnerability result of Catalonia: non-confined aquifers (*left*) and confined aquifers (*right*)

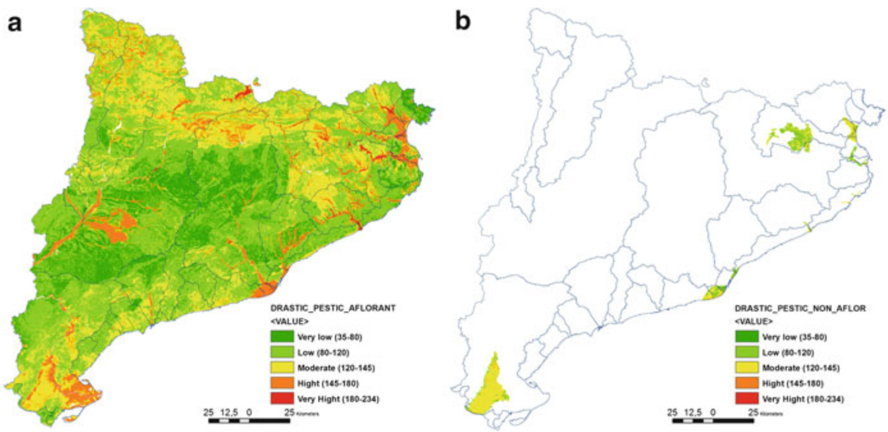

Fig. 10 Pesticide groundwater vulnerability result of Catalonia: non-confined aquifers (*left*) and confined aquifers (*right*)

alluvial aquifers, with a very flat topography and soil development assessed as very low, values from pesticides are higher (local alluvial in north and northeast of Catalonia).

Comparing these results with other local works in Catalonia such as the vulnerability map included in the published "Hydrogeological Maps 1:25,000 of Catalonia" [33], they have a significant similarity. Figure 11 shows an example of this comparison, although with a different map scale, the results are clearly concordant always taking into account the differences in range values (note the difference range adopted especially in moderate, high, and very high values). Currently, in order to improve this 1:25,000 groundwater vulnerability map, a review of the

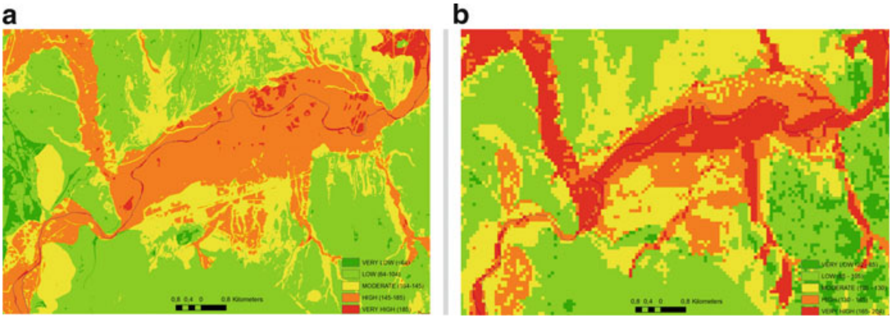

Fig. 11 Comparison between groundwater vulnerability results in local area of "Sarrià de Ter." Hydrogeological map Sarrià de Ter (ICGC, 1:25.000) (*left*). Groundwater generic vulnerability results in same area (*right*)

source of the information and methodological aspect of the DRASTIC parameter assessment has been done by ICGC.

4 Applications and Discussion

These groundwater vulnerability maps constitute a helpful groundwater management tool in a regional scale. As an example, they were applied in order to assess the risk of non-achievement of the WFD's [34] objectives for Catalan groundwater. In detail, groundwater vulnerability maps were applied to groundwater bodies pressures analysis, included in the IMPRESS document [35]. It has to be pointed that although the vulnerability map covers all the Catalan territory, the Catalan River Basin Management Plan is restricted in a half part of Catalonia (where the Catalan Government has full competence on water planning). One of the main objectives of the IMPRESS work was to evaluate the principal human activities that affect the chemical status in groundwater bodies, according to the European methodological guide [36]. In particular, with the aim to assess a pollution pressure, vulnerability maps were overlaid with several groundwater driving forces: contaminated soils, agricultural livestock, urban discharges, sewage sludge application, linear infrastructure (pipelines), gas station locations, etc. Figure 12 shows the example of nitrogen load from agriculture sources that have been overlaid with vulnerability map in order to obtain the pressure to agricultural activities. A subsequent risk analysis was carried out from overlaying each pressure with the results of chemical data. In addition, another direct application of these maps is the impact assessment where potential contaminant activities are being developed (agricultural activity areas, potentially dangerous industrial sites, sewage, water recharge, etc.).

Groundwater vulnerability maps are the result of treating and aggregating information related to hydrogeological properties. Finally, this information is

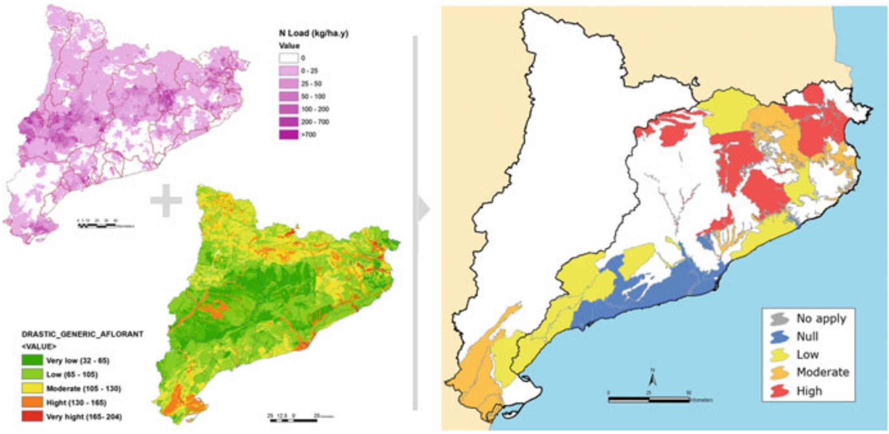

Fig. 12 Example of overlaying maps: nitrogen load in groundwater in Catalonia (*left*); generic groundwater vulnerability result of Catalonia (*central*); potential pressure for N application in groundwater bodies in Catalan Fluvial District

summarized in seven DRASTIC parameters. Some of these layers of information could be applied easily to other sectorial projects. In this sense, it could be considered that the final result is as important as the partial results of each parameter.

The results displayed represent an initial assessment of vulnerability. The analysis carried out could have some limitations that might influence the results obtained. A major restriction could be the absence of information of some DRASTIC parameters in cases where a local-scale analysis of the groundwater vulnerability is needed. Especially important is obtaining detailed groundwater levels that could corroborate vulnerability "D" parameter value, which has a very high influence in the DRASTIC method (Table 2). Similarly, a comprehensive analysis of the development and type of soil could be a very useful task to calibrate and improve the vulnerability results. In this sense, from the ICGC is planned to develop a cover of soil map at a scale of 1:250,000 that could be published by the year 2016. Finally, a more exhaustive validation of the vulnerability map with some impacts measured can be done (e.g., nitrates and pesticides).

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Pros and Cons of Biological Quality Element Phytoplankton as a Water-Quality Indicator in the NW Mediterranean Sea

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Abstract The Water Framework Directive (WFD) mandates the use of biological quality element (BQE) phytoplankton to assess the ecological status of coastal and transitional water bodies (WB). Here, we present (i) a critique of the general ecological assumptions of the WFD, (ii) a review of the ecological features of coastal phytoplankton dynamics, (iii) several approaches to establish a methodology to assess water-quality along the Catalan coast (NW Mediterranean Sea) based on BQE phytoplankton, and (iv) a critical examination of the use of phytoplankton as a BQE. Since 2005, we have followed several approaches aimed at assessing water-quality based on BQE phytoplankton and linking this indicator to a proxy to a costal pressure index. We have therefore studied phytoplankton communities at three different levels: as potentially harmful species, as functional or taxonomic groups, and with respect to their bloom frequency. Despite intense efforts, none of these fulfilled the WFD's management requirements, which in this context were found to contain several inherent flaws. As an alternative, we propose a methodology to assess water-quality based on the use of chlorophyll-*a* (Chl-*a*), as a proxy of phytoplankton biomass. The Chl-*a* concentration offers a very simple and representative measure of the phytoplankton community, and, importantly, it is used worldwide in water-quality studies, thus allowing not only regional but also cross-country comparisons. Moreover, because Chl-*a* concentrations clearly respond to nutrient enrichment, we were able to establish a BQE-specific typology for water bodies based on salinity, which is linked to nutrient loads. Using a newly developed

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coastal pressure index (Land Use Simplified Index, LUSI) that also reflects nutrient inputs, we demonstrated a significant pressure–impact relationship, as required by the WFD for management purposes. Based on this relationship, we were able to define reference conditions and water-quality boundaries for each type. We conclude our discussion with a consideration of the pros and cons of the use of phytoplankton as a BQE.

Keywords Biological quality element, Chlorophyll-*a*, Coastal waters, Continental pressures, NW Mediterranean Sea, Phytoplankton, Pressure–impact relationship, Water-quality assessment

Contents

1 Ecological Systems, Biological Communities, and the Water Framework Directive

Coastal waters are the most productive and diverse areas of the global ocean. Their unique structural properties include continental shelves, benthic–pelagic coupling, strong gradients, terrestrial inputs, geomorphic effects, and the broad spectrum of oceanographic conditions. Moreover, coastal waters are also strongly influenced by human activities, which result in the enrichment of coastal areas with organic and inorganic nutrients, such as carbon, nitrogen, and phosphorus, and therefore in unprecedented increases in eutrophication. Indeed, the deterioration of water-quality, understood as the loss of desirable (near pristine) conditions, has mainly been due to anthropogenic pressures, most of which originate on land. Identification of the causal links between these pressures and ecosystem status is therefore a fundamental step in any policy aimed at improving the environmental quality of coastal waters.

The purpose of the Water Framework Directive (WFD) is to establish a framework for the protection of freshwaters, marine waters, and groundwater. The main environmental objective of the WFD is the achievement by all European water bodies (WBs) of a Good Ecological Status (GES) by 2015. The ecological status is used to define the water-quality of a WB and is based on hydromorphological and physico-chemical criteria as well as biological quality elements (BQEs) and quantified by an ecological quality ratio (EQR). The EQR is a relative measure that compares the structural and compositional features of an ecosystem with those of a reference system characterized by a low level of anthropogenic pressure and therefore with good water-quality. Any deterioration or improvement in ecological status, and hence in water-quality, is reflected in the responses of these BQEs in the EQR. In the case of coastal and transitional waters, BQEs are presumed to respond to the effects of the main pressures, especially eutrophication. For example, the WFD recognizes that nutrient enrichment and changes in the stoichiometry of nutrient elements can give rise to shifts in the composition and biomass of phytoplankton species and to increases in the frequency, magnitude, and duration of phytoplankton blooms. It has therefore included phytoplankton as a BQE and mandated determinations of its taxonomic composition, abundance, biomass, and bloom frequency to assess water-quality.

While the WFD emphasizes sustainable use, its GES criteria assume that a manageable relationship exists between the structure and function of ecological systems that can be evaluated by quantifying the designated BQEs. However, questions regarding the validity of this assumption and whether a BQE-based approach is robust enough to support the goals of the WFD have generated intense controversy within the scientific community. A major concern is that the proposed methodology for the achievement of GES relies on an outdated interpretation of ecology and on a highly idealized pristine state free of any type of human impact [1]. In addition, the WFD's goals are based on the concept of a balanced (or climax) community and what nowadays is recognized as an overly simplistic view of the equilibrium of biological communities. That line of thinking was developed by Clements [2], who defined a climax community as "*a biological community of plants and animals which, through the process of ecological succession has reached an equilibrium in response to climate, soil and other environmental factors. In the absence of human interference, this state is self-maintaining*." The directive has adopted this view even though the recent scientific literature contains strong evidences that it is an inappropriate model for ecosystem management [1].

The WFD is also founded on several other assumptions, which can be summarized as follows: (1) ecological systems have a clear identity; they are recognizable and spatially clearly delimited. (2) In the absence of pressures, ecological systems achieve a steady state and are both temporally and spatially stable. (3) Ecological systems have "memory" and undergo structural changes in response to sustained pressure. (4) Changes caused by anthropogenic pressures can be distinguished from those resulting from natural causes, which in turn imply known pressure–impact relationships. (5) Ecological systems will return to an initial reference state if the pressures ceased. (6) Any changes in an ecosystem will be reflected by corresponding changes in each of its components or at least, per their definition, in its BQEs.

However, the WFD's assumptions are highly contestable and largely outdated, such that today none would withstand rigorous scientific evaluation. Consequently, the validity of the entire WFD regarding its reliance on BQEs must be questioned. It is beyond the scope of this chapter to discuss the weaknesses of each of the above-listed assumptions in detail. A summary of the opposing arguments would show that the WFD's assumptions are valid only if we impose appropriate spatio-temporal restrictions on the use of a particular BQE. This approach requires in-depth knowledge of the BQE's function in the target ecosystem and a recognition of the limitations and potential artifacts of the various methods used to sample BQEs, neither of which has been sufficiently evaluated.

In the following, we examine the limitations of phytoplankton as a BQE. We begin with a review of the ecological features of coastal phytoplankton and its dynamics. We then present some of the findings of our 10-year experience in the use of BQE phytoplankton to assess the water-quality of the Catalan coast (NW Mediterranean Sea) – as required by the WFD – which illustrate several of the problems inherent to the directive. As will become apparent in this chapter, for BQE phytoplankton, none of the tested parameters, i.e., taxonomic composition, abundance, biomass, and bloom frequency, are sufficiently reliable to establish ecological status when used on their own, as they do not fulfill the management requirements demanded by the directive. Instead, we show that the chlorophyll-*a* (Chl-*a*) concentration, which serves as a proxy measure for phytoplankton biomass, clearly responds to nutrient enrichment. Accordingly, we were able to establish a positive pressure–impact relationship between a coastal pressure index (Land Uses Simplified Index, LUSI) and Chl-*a*. Based on this relationship, we developed a methodology, discussed herein, in which water-quality can be determined by measuring phytoplankton biomass. Moreover, our approach complies with the WFD's requirements regarding ecosystem management applications.

2 Is Phytoplankton an Adequate Bioindicator?

2.1 Causes of Variability in Phytoplankton Communities

The growth and distribution of phytoplankton species follow seasonal cycles that depend on latitude and on the distance of the respective community to the coast. The fundamental causes of this variability have been well studied and include nutrient availability [3–8]. Nutrient elements are essential for the growth and maintenance of photoautotrophic organisms, which use light to fix carbon dioxide, and are responsible for the vast majority of primary production in the ocean.

Phytoplankton and other microbes take up nutrients and assimilate them into macromolecules, resulting in the formation of particulate organic matter which is then integrated into food webs and, thus, into higher trophic levels. Along with nutrients and their stoichiometry, several chemical and physical factors affect the phytoplankton community, including salinity, turbulence, the stability of the water column, the degree of water confinement, water residence time, temperature, tidal mixing, and the availability of light [3, 9–12]. Additionally, the phytoplankton community is composed of many different species, whose survival is favored by differences in their ecological requirements and by their distinct life strategies based on nutritional diversity (autotrophy vs. mixotrophy), different modes of competition, adapted life cycles, and differences in growth rates [13–15]. These processes account for the highly dynamic nature of phytoplankton, their rapid response to changes in environmental conditions, and their ability to inhabit a geographically broad range of coastal environments. However, they also underlie the complex relationship between environmental conditions and both the abundance of phytoplankton [16, 17] and the unpredictable structure of their communities. Thus, one of the main drawbacks of the WFD in its designation of phytoplankton as a BQE can be summarized as follows: phytoplankton communities are highly diverse and well adapted not only to nutrient fluctuations but also to physical parameters that change over time, all of which preclude the identification of clear-cut relationships between BQE phytoplankton and environmental pressures.

2.2 *Phytoplankton Communities: An Indicator Without Memory*

To establish reference conditions, as mandated by the WFD, phytoplankton communities must be described based on their state under completely or nearly completely undisturbed conditions, with little or no impact from human activities. The WFD also assumes that the nature of phytoplankton communities reflects the "memory" of sustained pressure. However, as noted above, even in the absence of anthropogenic pressure, phytoplankton communities are highly dynamic. Marine phytoplankton communities respond to the physico-chemical properties of their environment and, therefore, do not temporally integrate environmental changes. Indeed, even within a single seasonal cycle, phytoplankton communities will be highly variable [18, 19] and will not give rise to a climax community. An effective and accurate assesment of the status of marine ecosystems and the disturbances to them requires recognition of the dynamic nature not only of phytoplankton but also of ecosystems and their communities in general. In other words, the status of a phytoplankton community should not, and cannot, be evaluated by comparing its composition and relative abundances with a static "reference" assemblage of species that, even if it existed, would by no means be representative.

2.3 Phytoplankton Species as a Bioindicator

Among the various methods for evaluating the effects of human perturbations on coastal ecosystems, the use of specific species, rather than assemblages, as indicators of ecological status has been proposed. However, in the context of eutrophication, the proliferation of a particular species of phytoplankton in direct response to a disturbance in the balance of an aquatic ecosystem is by no means certain [20]. For example, species belonging to the genus *Phaeocystis* are regarded as a nuisance in the coastal waters of the North Sea. Yet, following anthropogenically derived nutrient enrichments of Belgian coastal waters, there was little change in the respective ecosystem despite a considerable increase in *Phaeocystis* spp. [21] In the Mediterranean Sea, there is no evidence of opportunistic phytoplankton species or of a significant indicator species in the sense relied upon by the WFD in its definition of a BQE. Similar conclusions have been reached in studies conducted in other European regions.

2.4 Phytoplankton Biomass as a Bioindicator

The immediate biological response to nutrient inputs is an increase in primary production, which manifests as an increase in phytoplankton and/or macroalgal abundances [22–25]. Accordingly, Chl-*a* is commonly accepted as a proxy for phytoplankton biomass, and extensive literature supports its use as an indicator of eutrophication in coastal waters [26–34]. In relation to the WFD, this assertion supports the mandatory inclusion of a pressure–impact relationship in each methodology used to assess water-quality.

There is general agreement on the relationship between the mean concentrations of nutrients and Chl-*a* in coastal waters. However, to assess this pressure–impact relationship, Chl-*a* data must be statistically integrated with respect to time, due to the highly dynamic behavior of phytoplankton communities. Therefore, a suitable temporal database is necessary, which in turn implies the need for sufficiently frequent sampling over an adequate period of time. Thus, the WFD mandates a 6-year period to assess the ecological status of a WB. Nonetheless, in some cases and in certain places, there will be no obvious relationship between the concentrations of nutrients and Chl-*a*. This could be due to a temporal or spatial mismatch of nutrients and Chl-*a*. For example, nutrients reach coastal waters at a certain time, and some time later, Chl-*a* will be generated but it also may be the case that the nutrients are further transported before Chl-*a* can be generated. Therefore, the physical and biological processes that modulate Chl-*a* production in turbulent and dynamic environments, such as coastal waters, may also disrupt its relationship to nutrient levels.

3 The Mediterranean Sea and the Catalan Coast as a Case Study

The Mediterranean Sea is a valuable paradigm to assess anthropic pressure, because of the contrasting nature of its offshore and coastal areas. The offshore waters of the Mediterranean Sea are among the most oligotrophic areas of the world. In these waters, nutrient availability is low and inorganic phosphorus concentrations limit primary production [35]. Consequently, large areas of the sea's surface waters are characterized by low amounts of phytoplankton biomass. Modest late-winter/early-spring increases of biomass are observed in some areas, such as in the northwest basin, associated with increasing daily irradiances and a greater stability of the surface layers after winter mixing brings nutrients to the surface. Relatively high-biomass peaks also occur in fronts, upwellings, and cyclonic gyres. By contrast, coastal areas are nutrient rich, as they receive river discharges, runoff from populated areas, and submarine groundwater, but they are also influenced by offshore oceanographic conditions. The coastal marine zone is therefore a transitional area characterized by strong physical, chemical, and biological gradients that extend from land to sea. Here, biological production is closely coupled to processes that deliver nutrients to surface waters. Anthropogenic forcing clearly influences the absolute availability of these nutrients and their stoichiometry, both of which impact phytoplankton productivity and species composition.

The Catalan coast is representative of the NW Mediterranean coast in terms of its geography, demographics, and socio-economic activity [36]. The climate in this area is typically Mediterranean, with moderate temperatures and irregular precipitation throughout the year. The continental topography ranges from rocky and steep to sandy and flat, with deltaic areas, the most important of which is the Ebro delta. The tidal range is small. The sea weather is typically mild but occasionally rough or very rough, with most storms occurring during autumn and winter. Catalan watersheds consist of ephemeral streams, nine medium to small rivers, and the Ebro River in the south, all of which feed directly into the Mediterranean Sea. The Ebro River drains a watershed of 84,230 km^2, with a mean water discharge at the river's mouth of 416 m^3/s [37]. Other major rivers in the region drain an area of 13,400 km^2 and have a mean water discharge of 0.3–16.3 m^3/s [38]. Land use differs along the river basins, with agriculture accounting for 9.6–51%, forests for 18.5–56.6%, and urban areas for 1–19.1%. Agricultural land use is relatively important in southern river basins and urbanization in central ones. In terms of surface area, 10.7% of the total coastal zone is urbanized with 3,9 milions inhabitants [39]. However, the population density along the coast is highly variable, with only 33 inhabitants/km^2 in the Ebro basin but 1,425 habitants/km^2 in Metropolitan Barcelona. During the tourist season, the population density in some areas increases by up to tenfold.

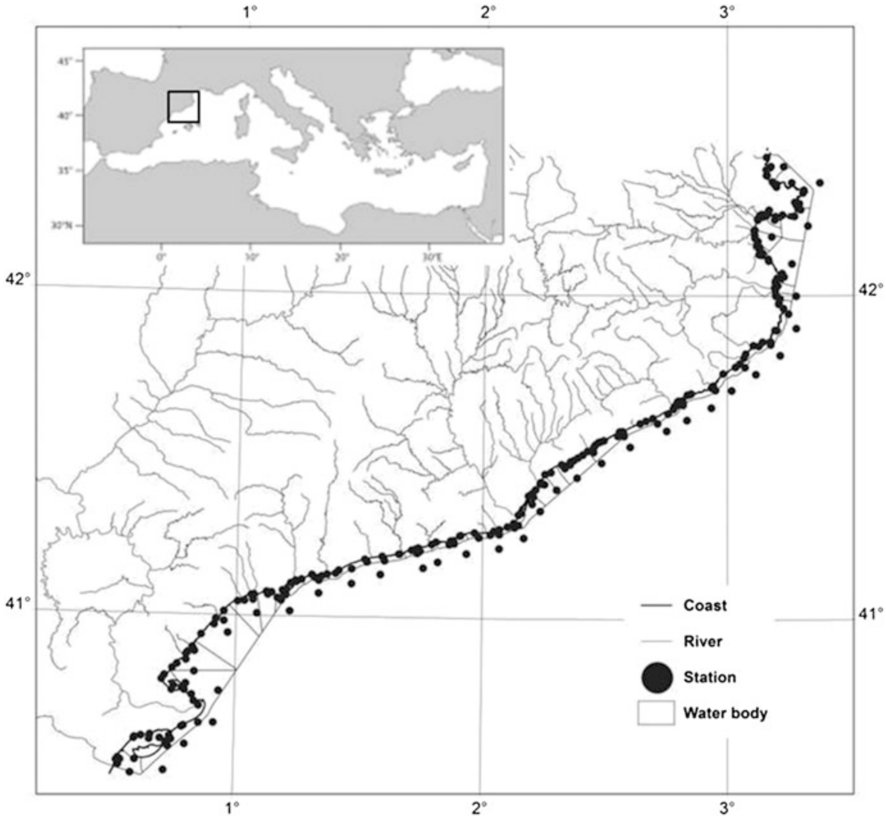

Fig. 1 Map of the Catalan coast. The coastline and main rivers are shown, together with the sampling stations and water bodies

3.1 Water-Quality Surveys at the Catalan Coast

Two time series were carried out along the Catalan coast with the aim of assessing water-quality with respect to phytoplankton: a physico-chemical and biological survey and a survey to monitor phytoplankton. Both were the result of several agreements between the Catalan Water Agency (ACA) and the Institut de Ciències del Mar (ICM-CSIC).

The physico-chemical and biological survey of Catalan coastal waters was initiated in 1990 and is ongoing. It consists of the sampling of 252 stations at specific distances from the shoreline: 35 stations at 5,000 m from the shore, 81 stations at 1,500 m, and 136 stations between 0 and 200 m, depending on the water depth (Fig. 1). The stations nearest to the coast, which are located within the sea, are representative of coastal nearshore waters (CNW) and coastal inshore waters (CIW) [36]. CNW stations are sampled every 3 months and CIW stations every 3 months, monthly, or weekly, depending on the season. At each of these stations, in situ salinity and chemical (inorganic nutrients) and biological (Chl-*a*) parameters are measured in surface waters. Dissolved inorganic nutrient (nitrate, nitrite, ammonia, phosphate, and silicate) concentrations are determined using colorimetric techniques [40] and total Chl-*a* by a fluorometric method [41].

In parallel with the above-described survey, phytoplankton monitoring was initiated in 2000 and is also ongoing. Water samples are obtained weekly, fortnightly, or monthly from 14 to 20 stations, depending on the station and year. The Utermöhl method is used to identify and count phytoplankton species [42].

3.2 WFD Implementation at the Catalan Coast

Implementation of the WFD along the Catalan coast is based on several procedures:

i. Adaptation of surveys. Catalan coastal water time series were modified in 2004 to fulfill the requirements of the WFD. Several stations were added to the surveys and sampling frequencies were in some cases adjusted. The most important change with respect to phytoplankton monitoring was to record the main taxonomic groups and not only the potentially harmful species, as was done at the beginning of the survey.
ii. WB delimitation. Thirty-six WBs located along the coast were defined: 34 coastal waters and two transitional waters (the northern and southern bays of the Ebro delta). The coastal lengths of these WBs and thus the number of stations per WB differ considerably. In the physico-chemical survey, there are between 2 and 30 stations per WB, such that all WBs are covered. For phytoplankton monitoring, 13 WBs are covered, with 1–2 stations per WB.
iii. Definition of a specific typology for BQE phytoplankton. The standard typology of Mediterranean WBs is based on the nature of the bottom substrate and the depth, which are irrelevant for BQE phytoplankton. As an alternative, a specific typology was proposed and subsequently accepted by the Mediterranean Geographical Intercalibration Group (Med-GIG). This typology is based on the degree of freshwater influence that the WB receives from land and is therefore related to nutrient loads. The three WB types are described in Table 1. Their freshwater influences are determined by the annual mean salinity.

    These types can also be subdivided into subtypes to differentiate among biogeographic areas with similar freshwater influence. For example, type II is subdivided into type II-A and type II-B to differentiate the moderate influence

Table 1 Specific WB typologies relevant for BQE phytoplankton and their annual mean salinity

Type	Description	Annual mean salinity
I	Highly influenced by freshwater inputs	<34.5
II	Moderately influenced by freshwater inputs	≥ 34.5 and <37.5
III	Not affected by freshwater inputs	≥ 37.5

of freshwater due to continental inputs from inputs coming from the Atlantic Ocean; and type III is subdivided into western (W) or eastern (E) basins of the Mediterranean Sea. More detailed information can be found in the European technical reports [43].

iv. Establishment of a method to assess continental pressures on coastal waters. Every methodology to assess water-quality must be supported by a clearly defined pressure–impact relationship whose underlying mechanisms are known. The establishment of such relationships, and therefore the assessment of anthropogenic pressures, is crucial for the development of the River Basin Management Plans required by the WFD. In coastal systems, these assessments must be focused on inland pressures, as the directive requires the assessment of pressures outside the WBs. However, while human activities are known to cause multiple pressures on different components of the marine ecosystem [44], their quantification and proof of their impacts required sophisticated, integrated tools that currently are not available. Instead, several indices have been proposed to estimate the quantity and distribution of anthropogenic pressures and their potential impacts, including BiPo [45, 46], BSPI, and BSII [47], but their use is either very complicated or requires large amounts of data. We therefore developed the Land Use Simplified Index (LUSI) [48] to simply and cost-effectively assess continental pressures on coastal waters. The rationale for the LUSI is based on the following assumptions: (1) Coastal waters receive pressures only from continental fluxes. (2) Coastal land uses determine the amount and the nutrient richness of continental fluxes. (3) An area of coastal water receiving river flows is therefore influenced by the respective watershed. (4) Coastal morphology has an effect on coastal water confinement and therefore on received pressures. LUSI integrates information regarding the specific continental pressures that influence a WB with information about the morphology of the coastal region involved, which can enhance or diminish those pressures once they reach the coast. The nature of these continentally derived inputs reflects the main characteristics of the land and its uses: urban, industrial, or agricultural. The intensity of the effects of each one on a given WB depends on the amount of land involved, which can be estimated using land use maps. The degree of riverine pressure is estimated based on the specific WB typology for BQE phytoplankton, as described in Table 1. Depending on these characteristics, a score is assigned to each WB and then combined within an algorithm to obtain a unique unitless LUSI value. A low LUSI value indicates that the coastal water is not or only slightly influenced by continental pressures, whereas a high LUSI value indicates a very strong influence of continental pressures on coastal waters. This distinction was validated by measurements of dissolved inorganic nutrient concentrations in coastal waters of the Catalan coast. All these characteristics make LUSI an important tool not only within the WFD but also within the context of DPSIR (driving forces–pressures–states–impacts–responses) models in general. To demonstrate a significant pressure–impact relationship for BQE phytoplankton within the Catalan coast, LUSI values were calculated for the entire WB by using the CORINE land cover map from 2006.

4 Sustainability of the BQE Phytoplankton to Assess the Water-Quality at Catalan Coast

We tested four different phytoplankton-related approaches to evaluate the water-quality of the Catalan coast: (i) the harmful algal bloom (HAB) index; (ii) the diatom/dinoflagellate ratio; (iii) the bloom frequency index; and (iv) measurement of Chl-*a* concentrations. These approaches meet the WFD's requirements with respect to the taxonomic composition and abundance (i and ii), bloom frequency (iii), and biomass (iv) of phytoplankton.

Each approach complied with the WFD's intercalibration process (IC), the aim of which is to ensure the comparability of biological monitoring results obtained by the member states, as required by the directive. Our group was assigned to the Med-GIG from 2005 to 2015. All four approaches were statistically tested following the guideline of the WFD and the directions of the Joint Research Centre. The statistical tests were carried out by selecting several subsets of the two Catalan coastal water time series databases, depending on the BQE parameter. One of the subsets belonged to the common dataset from Med-GIG, which was used to establish a method to assess water-quality based on Chl-*a* (iv). To test the sustainability of the diatom/dinoflagellate ratio (ii), selected stations were classified into two groups, impacted and reference stations (sites with undisturbed conditions), depending on the degree of human disturbance. All the approaches were tested against LUSI values.

i. HAB index. As previously discussed, the assumption that the presence and/or abundances of a particular species of phytoplankton can be used as an indicator of water-quality has been strongly questioned [20]. However, because HAB species are toxin producers or cause other harmful environmental effects, their presence is considered as an environmental disturbance. The HAB index [49] integrates data on the taxonomic composition and abundances of HAB species. In a study conducted at 17 stations sampled monthly during two annual cycles (2005–2006) and covering 13 WBs along the Catalan coast, HAB species were grouped into six different categories based on their toxicity and harmful effects: paralytic shellfish poisoning, diarrheic shellfish poisoning, amnesic shellfish poisoning producers, benthic species, bloom-forming microplankton, and bloom-forming nanoflagellates. Depending on the cellular abundances of these species, they were assigned a score of 0, 1, or 2 such that the cumulative scores ranged from 0 to 12 for each sample. The HAB index was then calculated for each station according to the following formula:

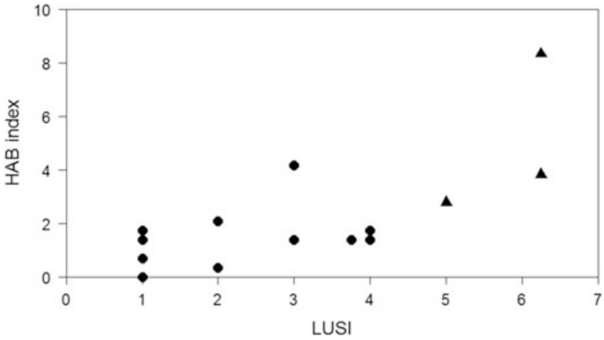

Fig. 2 HAB index and LUSI relationship for the 17 stations studied. *Triangles* represent stations with riverine influences (types I and II in Table 1) and *dots* represent type III stations (as defined in Table 1)

$$\text{HAB index } (\%) = [\text{Sum of scores}/(\text{Number of samples} \times \text{F})] \times 100$$

where $F = 12$, which is the maximum potential score for each sample. Next, the water-quality of each WB was assigned a value according to its HAB index. To confirm the accuracy of the classification, HAB index results were compared with the anthropogenic pressures defined by LUSI for each WB (Fig. 2).

The correlation between the HAB index and LUSI was significant ($R^2 = 0.5366$; $p < 0.001$) when all of the data were included. Stations with higher LUSI values were those affected by river inputs (types I and II), which in turn were prone to developing high-biomass blooms. However, because these stations clearly dominated the HAB index vs. LUSI relationship, it was no longer significant when they were excluded. These results clearly demonstrated that the HAB index is not an accurate indicator of eutrophication, given that the presence and abundances of toxic species were not significantly related to the degree of anthropogenic pressures. In fact, the proliferative potential of a harmful-producing species depends not only on the eutrophication of the WB in which that species resides but also on the species' physiological characteristics, its life cycle, the presence of its competitors and predators, and the properties of the WB itself, such as water motion, the stability of the water column, and the water residence time.

Similar conclusions were reached by other authors [50], who in devising a quality index removed the cell counts of harmful species as they did not provide any relevant information about the study area (Basque coast, NEA region). Other authors have similarly concluded that "HABs are not related to eutrophication of the Mediterranean zone" given that some toxic species are mixotrophic and can bloom even in areas with nutrient limitations [51].

ii. Diatom/dinoflagellate ratio. A second approach was based on the taxonomic composition of phytoplankton, specifically, on the ratio of the two main groups of phytoplankton: diatoms and dinoflagellates. This ratio was expected to be

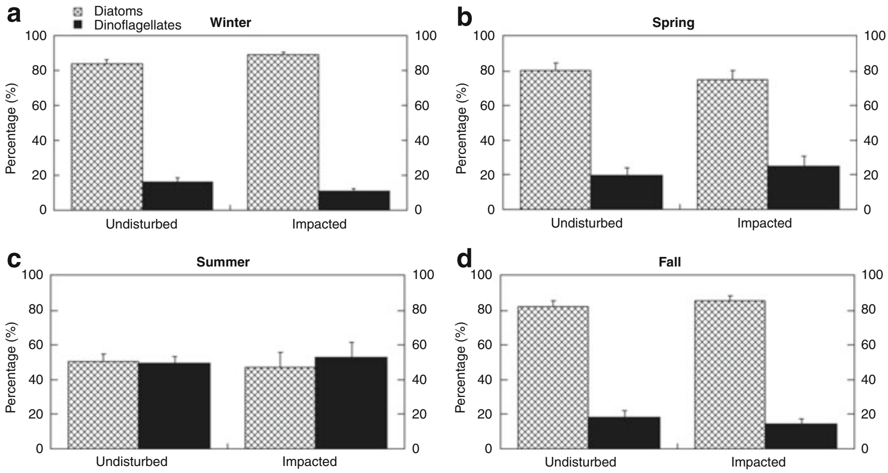

Fig. 3 Diatoms and dinoflagellates percentage at eight undisturbed (median LUSI = 1) and six impacted (median LUSI = 4) stations during (**a**) winter, (**b**) spring, (**c**) summer, and (**d**) fall. These stations were sampled monthly during a period of 2 years

responsive to changes in nutrient ratios induced by human eutrophication, as reported by some authors [52]. Anthropogenic activities increase nitrogen and phosphorous inputs into coastal waters but have little impact or even diminish silicate levels. Nitrogen and phosphorous are inorganic nutrients essential to the growth of all phytoplankton groups, whereas silicate is required by diatoms for the elaboration of their frustules. Accordingly, the growth of diatoms, but not dinoflagellates, is limited when silicate is deficient. We calculated the diatom/dinoflagellate ratio for a total of 14 stations previously evaluated using LUSI. Thus, eight of the stations were undisturbed (little or no impact from human activities; median LUSI = 1) and six were strongly impacted (high pressure from human activities; median LUSI = 4). Our hypothesis was that their LUSI-defined differences would be reflected in the diatom/dinoflagellate ratio. However, despite the clear difference in their LUSI values, there were no differences in the diatom/dinoflagellate ratios of the undisturbed vs. impacted stations (Fig. 3).

Instead, the changes in the diatom/dinoflagellate ratio mainly indicated the seasonal pattern of phytoplankton that is typical of the NW Mediterranean [53]. Therefore, the presence of diatoms and dinoflagellates depends not only on anthropogenic pressures related to nutrient inputs into WBs but also on the hydrographic characteristics of the respective water column and the general patterns of the seasonal succession of phytoplankton [3]. The inability of the diatom/dinoflagellate ratio to serve as a measurement tool of water-quality is consistent with published reports on similar problems encountered during attempts to implement other taxonomic-based indicators specified by the WFD [54–58].

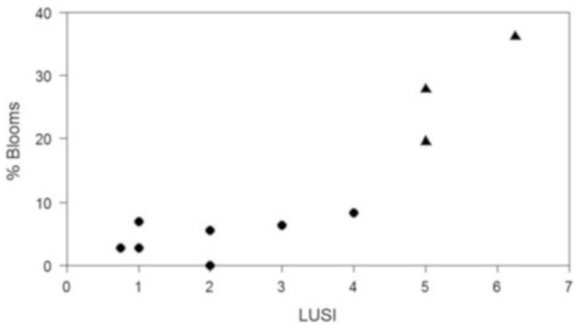

Fig. 4 Relationship between bloom frequency (%) and LUSI for the ten stations analyzed. *Triangles* represent stations with riverine influence (types I and II) and *dots* represent type III stations (see Table 1)

iii. Bloom frequency index. We measured phytoplankton bloom frequency at ten selected stations subject to different levels of anthropogenic pressure. Abundance thresholds were defined for the major phytoplankton groups (diatoms, dinoflagellates, coccolithophorids, and nanoflagellates) to estimate bloom frequency as a percentage of the total number of samples. These percentages were then used to assign water-quality categories. The correlation between the bloom frequency index and LUSI was significant ($R^2 = 0.795$; $p < 0.001$) when all of the data were included (Fig. 4). As in the case of the HAB index, the stations with higher LUSI values were those affected by river inputs, which in turn gave rise to high-biomass blooms. This sequence of events dominated the relationship such that when the respective data were excluded, the relationship was no longer significant. These results clearly demonstrate a nonlinear relationship between bloom frequency and anthropogenic pressures as well as an as-yet undefined pressure–impact relationship. Therefore, the bloom frequency index is not a good indicator of eutrophication.

In summary, the first three BQE-based approaches, which considered phytoplankton community composition and bloom frequency, are inadequate in terms of achieving the objectives of the WFD. Moreover, they reflect the broader problems regarding the use of phytoplankton indexes that rely on phytoplankton composition (whether of species or of functional groups) to classify water-quality in terms of eutrophication pressure. The absence of a direct relationship between blooms or HABs and eutrophication is in line with the current view of the scientific community, that algal blooms, including those that are toxic, can also be natural phenomena [59]. Our findings are also concordant with those reported by researchers in other European Union member states, in which national indices were developed to comply with the WFD but failed to demonstrate a relationship between BQE phytoplankton and water-quality [31, 50, 60].

iv. Chlorophyll-*a*. We followed several approaches to establish and test a methodology to assess water-quality using Chl-*a* as a proxy of phytoplankton biomass. All of them were based on the same assessment concept, in which nutrient concentrations are linked with those of Chl-*a*, which are higher in coastal areas that receive freshwater discharges of nutrients than in those that do not receive continental nutrient loads. On a practical level, this assumption is translated using the BQE-specific typology based on salinity, which recognizes at least three possible degrees of riverine influence on a WB. Thus, a WB with high river influence (type I) will have higher Chl-*a* concentrations than a WB without riverine influence (type III). In addition, all of the methodologies tested determine whether the water-quality of a WB is acceptable by comparing its Chl-*a* concentrations with a reference level, taking into account the typology. Thus, the water-quality of a WB with a Chl-*a* concentration similar to its reference, which in turn implies similarity with respect to salinity and nutrient concentrations, will be acceptable. Conversely, the water-quality of a WB with a Chl-*a* concentration that is much higher than the reference level will be unacceptable. In the latter, the difference between the measured WB and the reference WB is presumably due to an extra nutrient load related to human activities, i.e., eutrophication. In such cases, actions should be implemented to achieve the GES of that WB.

The following section provides a description of our methodology to assess water-quality based on Chl-*a*. The method was accepted by the European Commission in 2015, within the third phase of the WFD IC. After a description of the characteristics of the database, we describe the three steps that comprise the methodology: (i) establishment of the pressure–impact relationships, (ii) calculation of the reference conditions, and (iii) setting of the boundaries between water-quality categories. Finally, we provide an example by applying this approach to the Catalan coast.

4.1 Database

The methodology was developed using a subset of the common dataset from the Med-GIG, specifically, data from the NW Mediterranean (the coastal waters of France and Spain). As Spain has data from both CIW and CNW, all CIW data were transformed to CNW data, according to the first IC Med-GIG Technical Report, Section 3 Annex I Spain (Mediterranean Geographical Intercalibration Group, 2007), as shown in Eq. (1):

$$\text{CNW Chl-}a = 1/2{*}\text{CIW Chl-}a \tag{1}$$

The subset contained information from 71 WBs (23 of type II-A and 51 of type III-W). Type I was omitted from the subset as it was only present in Spanish waters, which prevented its intercalibration with similar data from France. The subset included information from each WB regarding estimated anthropogenic pressures (LUSI), their potential impacts (90th percentile of Chl-*a* values, in μg/L), and salinity (annual mean values), in order to specify its typology. Chl-*a* and salinity statistics were calculated over a 2 to 6-year period depending on the region and are representative of the CNW of each WB. As Chl-*a* values do not show a normal distribution, they were transformed according to Eq. (2):

$$v' = \log 10(v + 1) \tag{2}$$

More detailed information on the sampling and analytical methods and on the common data set can be found in European technical reports [43].

4.2 Pressure–Impact Relationships

This is the first step in obtaining a valid methodology to assess water-quality, as stated by the WFD. Thus, a linear model was fit for each WB type, using a data doubling step and the *R* software package [61]. Due to the data doubling step, the goodness of fit values, but not the *p*-values, was reliable. The linear models for type II-A and type III-W are described by Eqs. (3) and (4):

$$\text{Type II-A}: \quad \text{Chl-}a \text{ Transformed} = 0.05{*}\text{LUSI} + 0.26 \tag{3}$$

$$\text{Type III-W}: \quad \text{Chl-}a \text{ Transformed} = 0.06{*}\text{LUSI} + 0.19 \tag{4}$$

Both show a positive relationship between LUSI and Chl-*a*, indicating that the greater the continental anthropogenic pressure received by a WB, the higher the Chl-*a* concentration, and therefore the stronger the impact on that WB (Fig. 5). Differences between the linear model coefficients of both types are consistent with

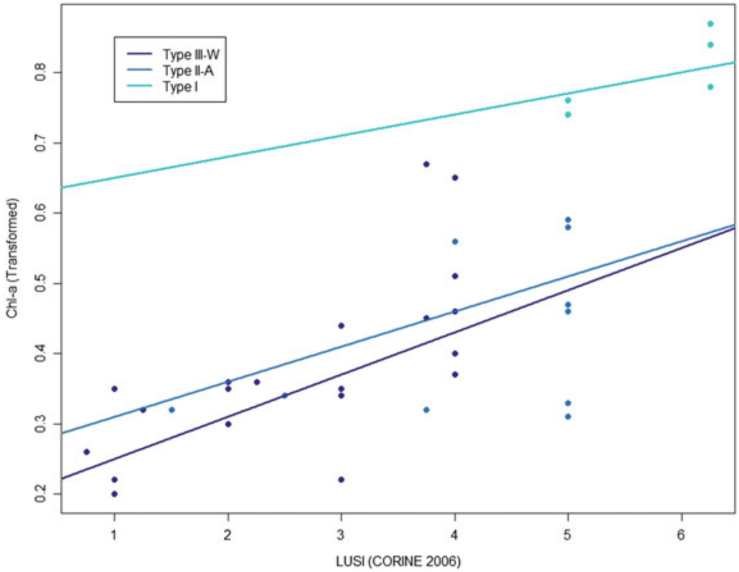

Fig. 5 Relationship between pressure (LUSI) and impact (transformed Chl-*a*) for type III-W, type II-A, and type I WBs. Linear models for type III-W and type II-A were obtained from the Med-GIG data subset. The *dots* represent data from Catalan WBs

Table 2 Reference conditions and boundaries for the assessment of water-quality based on Chl-*a* determinations along the Catalan coast

Type		Type III-W	Type II-A	Type I
Reference conditions (90th percentile Chl-*a*, μg/L)		0.79	1.28	4.13
Boundaries (90th percentile Chl-*a*, μg/L)	H/G	1.18	1.92	6.19
	G/M	1.89	3.5	13.01
	Failed	>1.89	>3.50	>13.01
Boundaries (EQR)	H/G	0.67	0.67	0.67
	G/M	0.42	0.37	0.32
	Failed	<0.42	<0.37	<0.32

Note that type II-A and type III-W also apply to the NW Mediterranean

the assessment concept. Thus, type III-W WBs have a higher slope and lower intercept than type II-A. In other words, for the same amount of pressure, a type III-W WB generates more Chl-*a* than a type II-A WB and is therefore more sensitive to pressures and will be impacted more rapidly. The intercepts provide information about the lowest Chl-*a* concentrations in the absence of pressures (theoretical value of LUSI = 0, which in practice does not exist) in type III-W WBs but not in type II-A WBs, since the latter are naturally affected by freshwater inputs and will, therefore, have a higher Chl-*a* concentration. Goodness of fit values (R^2) are 0.25 and 0.40 for type II-A and type III-W, respectively. These values are not high but they are acceptable, as they reflect the variability of the NW Mediterranean coast.

4.3 Reference Conditions

According to the WFD CIS Guidance Document No. 5, the reference condition must be derived from an undisturbed site or a site with only very minor disturbances. In accordance with this rule and considering that pressure is measured by means of the LUSI values, the minimum LUSI values for each type were selected to calculate the corresponding reference condition by using the previously established pressure–impact relationship. For a type II-A WB, the minimum LUSI value is 2 (regardless of the shape of the coastline); thus, the reference condition for this type is 1.28 μg Chl-*a*/L, expressed as a 90th percentile value. For a type III-W WB, the minimum LUSI value is 1 (regardless of the shape of the coastline), and its reference condition is therefore 0.79 μg Chl-*a*/L (Table 2).

The reference conditions for type II-A and type III-W WBs are similar to those measured in WBs of the Catalan coast that receive less continental pressure, that is, WBs located within a marine and terrestrial natural park, in the NE of Catalonia. Regarding type III-W, the Cap Norfeu WB has a LUSI value of 0.75 and its 90th percentile Chl-*a* concentration is 0.80 μg/L. For type II-A, the Cap de Creus WB has a LUSI value of 1.50 and its 90th percentile of Chl-*a* concentration is 1.09 μg/L.

This agreement between the calculated and real natural minimum values of Chl-*a* supports the validity of both the previously determined linear models and the reference conditions of the methodology.

4.4 Boundaries Between Water-Quality Categories

The final step of the methodology is to establish the boundaries between water-quality categories. As water-quality shifts from high to nonacceptable, the boundaries should reflect an increase in the difference between the Chl-*a* concentration and the reference condition. The WFD proposes five water-quality categories, but for management reasons, only the boundaries between two of them are of practical interest: between high and good (H–G) and between good and moderate (G–M). The latter defines the limit between an acceptable and a nonacceptable water-quality.

The H–G boundaries and G–M boundaries were established taking into account the variability within each WB type in the dataset. Thus, for a type II-A WB, 50% of the reference condition was added to the same reference condition to establish the H–G boundary and 82% of the H–G boundary was added to this boundary to obtain the G–M boundary. For a type III-W WB, the H–G boundary was obtained following the same procedure as for type II-A, but the G–M boundary was obtained by the addition of 60% of the H–G boundary. Once these boundaries in terms of the 90th percentile of the Chl-*a* concentration (μg/L) were established, boundaries in terms of the EQR could be defined by applying Eq. (5):

$$\mathrm{EQR} = \frac{\text{Chl-}a \quad \text{reference}}{\text{Chl-}a \quad \text{WB}} \tag{5}$$

The boundaries set in terms of the 90th percentile of Chl-*a* (μg/L) and EQR are shown in Table 2.

In summary, to assess the water-quality of a WB based on phytoplankton biomass requires data on the annual mean salinity and on the 90th percentile of Chl-*a* (μg/L). First, the typology of the WB is established according to Table 1. Second, the WB's reference condition is selected depending on its typology, as shown in Table 2. Third, the EQR of the WB is calculated using Eq. (5). And finally, the WB is assigned to a water-quality category with respect to the boundaries, defined in terms of the EQR (Table 2).

Regarding the IC, when a methodology is established, Member States can compare and harmonize boundaries. For the NW Mediterranean, the results show that France and Spain can use the H–G and G–M boundaries to assess the quality of their type III-W and type II-A WBs. In the case of Spain, this assessment can be made directly, without the need for specific correction coefficients. More information on the comparison and harmonization of boundaries between France and Spain can be found in their third phase IC Working Document regarding BQE phytoplankton, presented to the European Commission in 2014.

4.5 Water-Quality Based on Phytoplankton Biomass of the Catalan Coast

The quality of Catalan coastal waters, based on Chl-*a*, was assessed using the above-described methodology and Med-GIG dataset. Concretely, we used the subset corresponding to the Catalan coast since 2007 to 2010. Since with this intercalibrated methodology only type II-A and type III-W WBs can be assessed, the same procedure was applied to the data corresponding to a type I coastal WB of the Catalan coast. First, a linear model was established. Its goodness of fit (R^2) value was 0.81 and its linear equation was described by Eq. (6):

$$\text{Type I}: \quad \text{Chl-}a\ \text{transformed} = 0.03{*}\text{LUSI} + 0.62 \tag{6}$$

The slope and intercept of this linear model were congruent with those of the linear models of type III-W and type II-A WBs (Fig. 5). The slope of the type I linear model was the lowest of the three linear models; thus, as an indicator of the magnitude of the pressure on Chl-*a*, a type I WB is the least sensitive to pressure. Since the value of the intercept of the type I linear model will be the highest of the three linear models, then in type I WBs the theoretical Chl-*a* concentration in the absence of pressures will also be the highest, consistent with this type being the one most influenced by freshwater inputs. Second, type I reference conditions were calculated. For this type, the minimum LUSI value is 3; therefore, the reference condition for this type is 4.13 μg Chl-*a*/L, expressed as a 90th percentile. Finally, boundaries for type I were set at 6.19 μg Chl-*a*/L for the H–G boundary and 13.01 μg Chl-*a*/L for the G–M boundary, taking into account that 110% of the H–G boundary is added to this boundary. For the EQR, the values were 0.67 and 0.32 for the H–G boundary and the G–M boundary, respectively. These boundaries were in agreement with those of type III-W and type II-A WBs.

All WBs from the Catalan coast, including transitional waters, were assessed by assigning each one a typology using Table 1 and a water-quality category following Eq. (5) and by applying the reference conditions and boundaries listed in Table 2. The results are shown in Fig. 6.

Our assessment of all Catalan WBs showed that 89% have an acceptable (high or good) water-quality based on their Chl-*a* concentrations, with 47% having a high water-quality. By typology, the water-quality of 81% of the type III-W WBs is high or good, whereas for type II-A and type I WBs, this percentage is 100%. Only four WBs failed to achieve an acceptable water-quality (Barcelona, El Prat de Llobregat-Castelldefels, Vilanova i la Geltrú, and Tarragona-Vilaseca). Our results are in agreement both with previous assessments of Catalan coastal waters carried

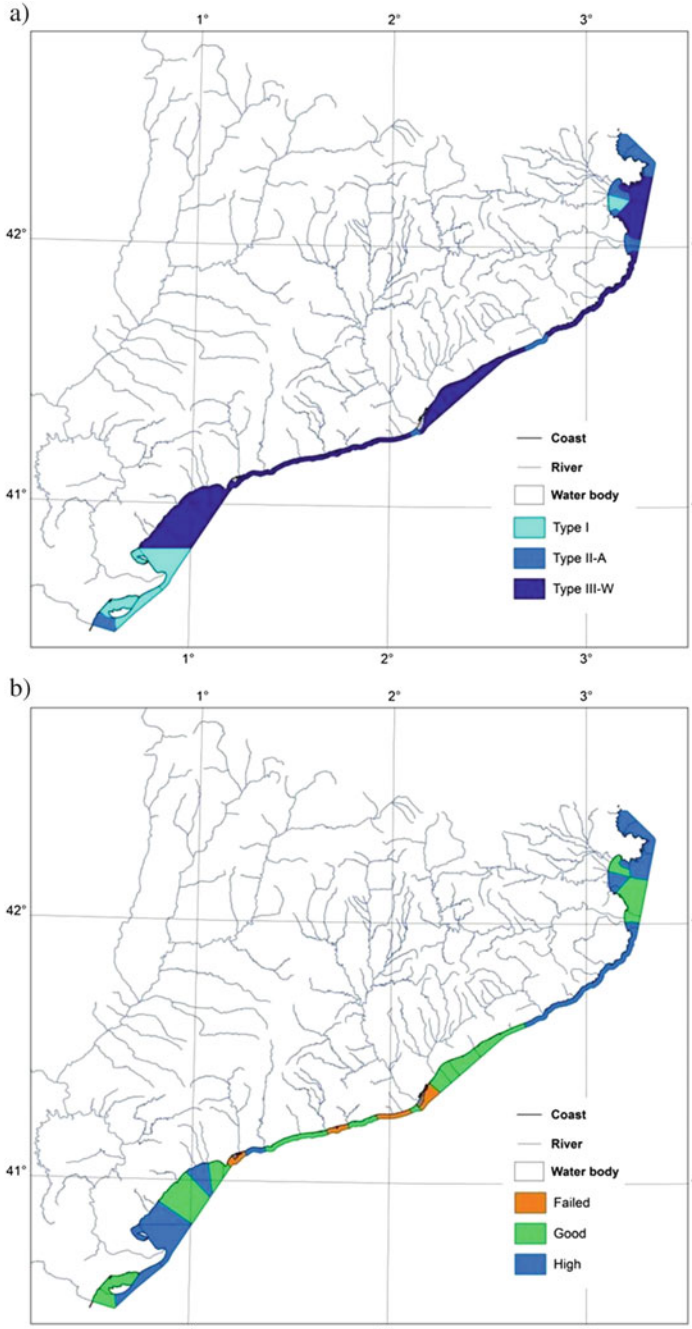

Fig. 6 Maps of the Catalan coast showing the typology of each water body (**a**) and its water-quality based on phytoplankton biomass (Chl-*a*) (**b**). Water bodies that correspond to harbors were not assessed and are indicated in *grey*

out before the implementation of the WFD and with parallel studies. Therefore, our linear models, selected reference conditions, and boundaries are appropriate and the methodology is valid. With this water-quality assessment approach based on BQE phytoplankton, the ecological status of Catalan WBs will be assessed and included within the third River Basin Management Plan.

5 Final Discussion on the Use of Phytoplankton as a BQE

We conclude this chapter by again addressing the general assumptions of the WFD and by considering the knowledge gained by the use of BQE phytoplankton to assess water-quality.

Ecological systems usually exist as a continuum such that their spatial delimitations are difficult, if not impossible, to recognize. This is the first challenge in the implementation of the WFD because its basic management unit is the WB, whose spatial delimitation is an artificial condition that must be fulfilled by Member States. As defined by the WFD, coastal waters are those within "a distance of one nautical mile on the seaward side from the nearest point of the baseline from which the breadth of territorial waters is measured." The absence of clear hydromorphological quality elements makes the definition of a marine WB much more challenging than is the case for other surface waters, such as rivers or lakes. For BQE phytoplankton, the establishment of the offshore limits of the WB is crucial, because phytoplankton communities are present and can be sampled within the whole WB and not only along the coast, as is the case for other BQE such as macroalgae or phanerogams. The results of the first attempt to define Catalan coastal WBs are now available, and the definitions have been used within the first and second River Basin Management Plans; however, a revision of the limits of those WBs should be considered.

Other weaknesses in the implementation of the WFD became apparent during the testing of the phytoplankton-related approaches to evaluate the water-quality here, in the Catalan coast, and elsewhere. These were linked to the temporal and spatial samplings of the WBs. The directive allows Member States to select sampling sites and sampling frequencies within each WB. However, coastal WBs are characterized by spatial heterogeneity and asymmetry, with continental anthropogenic pressures being most obvious near the coastline, especially in the tideless Mediterranean Sea. To adequately characterize Mediterranean WBs, they must be sampled at different distances from the coastline. The failure to include sampling points near the coastline will result in values that are not representative and therefore in large biases regarding spatial heterogeneity. But even before the WFD, water-quality surveys in the Catalan coast included sampling sites at different distances from the coastline [36]. Regarding the time frame for sampling, the directive allows the assessment of water-quality based on BQE phytoplankton with a minimum of two samples per year. Yet, as pointed out in this chapter, because phytoplankton communities are highly dynamic, a higher sampling frequency is necessary to define the temporal heterogeneity of a given WB. Moreover, only a wide dataset will allow the necessary statistical integration needed to reveal changes in parameters of interest, such as Chl-*a*. For the water-quality surveys conducted along the Catalan coast, sampling is carried out weekly, fortnightly, monthly, and quarterly, depending on the degree of variability of the sampling point. As is the case for phytoplankton, the inadequate sampling of Chl-*a* could lead to a misrepresentation of the spatio-temporal heterogeneity of the respective WB and therefore to erroneous quality assessments based on BQE phytoplankton.

The WFD assumes that changes caused by anthropogenic pressures can be distinguished from those resulting from natural causes, which in turn implies known pressure–impact relationships. However, current scientific knowledge, including our own efforts to relate anthropogenic pressures to the parameters proposed for BQE phytoplankton, is too limited to reveal the nature of this relationship; consequently, its translation into management actions is not possible. Nonetheless, Chl-*a* is widely used as a bioindicator of eutrophication in coastal waters. At the management level, some insight has been gained regarding the changes in Chl-*a* caused by anthropogenic pressures (in the form of nutrient inputs), but distinguishing them from changes resulting from natural causes is often difficult. For the purpose of defining and implementing management actions, water-quality assessments based on phytoplankton biomass should be evaluated with respect to the general conditions defined by the WFD. These conditions are defined by physical and chemical quality elements, which in the case of the Catalan coast include dissolved inorganic nutrient concentrations. An evaluation of this type could provide insights into the origin of the detected nutrients, i.e., whether they are due to anthropogenic activity inputs into coastal waters, and thus of Chl-*a*. The results will allow decisions to be made regarding the need for management actions, since naturally high levels of Chl-*a* may not warrant external correction. Along the Catalan coast, these evaluations have already been conducted and the findings taken into account within River Basin Management Plans.

Finally, a much better understanding of nutrient-phytoplankton relationships is needed before the effects of eutrophication based on BQE phytoplankton can be fully understood and the appropriate measures taken. The complexity of the interactions between physical, chemical, and biological factors and phytoplankton hinders the establishment of well-defined impact-pressure relationships, and therefore effective management strategies. Until these challenges are overcome, we recommend the implementation of good practices aimed at nutrient load reduction in coastal areas in order to achieve the GES of all European water bodies, which is the main goal of the WFD.

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Bioindicators, Monitoring, and Management Using Mediterranean Seagrasses: What Have We Learned from the Implementation of the EU Water Framework Directive?

Javier Romero, Teresa Alcoverro, Guillem Roca, and Marta Pérez

Abstract Seagrasses are flowering plants that inhabit coastal and transitional waters. They colonize sedimentary seabeds (and to a lesser extent rocky substrates) and present unique adaptations to the marine environment. Seagrasses are especially sensitive to environmental deterioration and live in a world that is particularly threatened by human activity. The response of the plants and their associated communities to disturbances is relatively well known. This has facilitated the development of a large number of seagrass bioindicators based on biochemical, physiological, morphological, structural, demographic, and community measures, especially after the deployment of the EU Water Framework Directive (WFD) and to a lesser extent the implementation of the Marine Strategy Framework Directive.

Bioindicators are at the interface between science and policy. In order for their use by managers for different purposes (monitoring, water quality assessment, long-term changes, etc.) to be robust and consistent, a clear definition of management goals is needed. The development of bioindicators must also be based on careful

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evaluation together with rigorous and transparent selection processes to ensure their scientific credibility.

Here, we present bioindicator indices based on seagrasses that were developed with the context of the implementation of the WFD in Catalonia, NE Spain, to assess the ecological status of coastal and transitional water bodies. Ecological status includes aspects concerning both the quality of the biological community and the hydrological and chemical characteristics of the environment. For this reason, and to develop a WFD-compliant system for ecological status assessment based on Mediterranean seagrasses, we used multivariate techniques to combine different bioindicators, gathered from different levels within the biological organization, into single biotic indices (POMI and CYMOX, based on the species *Posidonia oceanica* and *Cymodocea nodosa*, respectively). We report how this was achieved and how the robustness and reliability of those indices were assessed through correlation with human pressures, uncertainty analysis, and intercalibration. Finally, besides their applicability, we discuss their shortcomings and what we, as seagrass biologists, have learned overall from responding to the challenges posed by the WFD and specifically by the part dealing with seagrasses.

Keywords Coastal waters, *Cymodocea nodosa*, Ecological status, Human pressures, *Posidonia oceanica*

Contents

Acronyms

ACA Catalan Water Agency
BQEs Biological quality elements
EQR Ecological quality ratio

ICCM Intercalibration Common Metric
WB Water body
WFD Water Framework Directive (2000/60/EC)

1 The Concept of Bioindicator and Its Development at the Interface Between Science and Management

In a broad sense, "indicators" are signals that capture complex information in a simple but effective way. Although indicators are used in very different fields, including social sciences, marketing, economics, and business, they are particularly useful in environmental assessment and planning. Environmental indicators are at the interface between science and policy [1], and thus, to be helpful, both sides of that interface should adapt to, or at least understand, the constraints of the other. Specifically, managers should clearly define the problem under consideration [2], while scientists should make every effort to design indicators that are not only helpful in solving the problem but are also easy to understand and communicate [3].

Biological indicators or "bioindicators" are a subset of environmental indicators that rely on measurements of biological entities. Their use is highly recommended, because organisms provide an integrated response to environmental stress. The measurements on which bioindicators rely can be gathered from several levels of biological organization, from the subcellular to the community level. Measurements at lower levels (from molecular to individual, e.g., biochemical, genetic, or morphological traits) are usually more specific to stressors and respond faster than those at higher levels (from population to community, e.g., abundance, biomass, or taxonomic composition), which are more relevant to concepts such as ecological integrity [4]. Consequently, measurements at the molecular, cellular, or individual level present rapid time responses and great specificity, making them excellent early warning indicators, while measurements at the population or community level present longer response times, tend to be more integrative, and consequently are more relevant to assess conditions at the level of the ecosystem [5].

When bioindicators are selected for use in environmental assessment programs, managers should, first of all, clearly define the problem to be addressed and what kind of information is needed. For example, there could be an interest in assessing the actual condition of a system or in evaluating trends over time, or in evaluating the effects of a given coastal development (harbor construction, beach nourishment, etc.), or in establishing the causes behind an observed deterioration of the ecosystems [6]. In any of these scenarios, scientists should face the challenge of choosing from the literature or specifically designing robust and reliable bioindicators that are suitable for each specific goal. In all cases, the development of sets of bioindicators implies a step-by-step process in which the selection of adequate measurements and eventually their aggregation in the form of simple and readable indices are crucial milestones. The development of bioindicator sets must be based in a rigorous and transparent selection process to ensure scientific credibility and adequacy with respect to the needs of managers [7].

Success in the use of biological indicators to solve environmental problems relies on the identification of an effective set of variables[1] to be measured. Some criteria these variables should meet are [6] easy to understand, simple to measure and cost effective, sensitive to stresses, predictable response to stress, low natural variability, and the provision of relevant information on environmental issues. Similarly, some additional requirements to be met by variables to be used as bioindicators are [8] relevance to ecological integrity, broad-scale applicability, early-detection capacity, feasibility of implementation, interpretability against reference conditions, and capacity to link ecosystem degradation with its causative stressors. The challenge is, therefore, to choose from among the hundreds of bioindicators proposed so far, or among the thousands of measurements that can be performed on biological entities, those that best fulfill the criteria mentioned above.

Recently, there has been a rapid increase in the development and application of ecological indicators. For instance, governments in the United States, Canada, Europe, and Australia are developing programs for routine reporting based on ecological indicators [3]. In Europe, the European Water Framework Directive establishes a framework for the protection of groundwater, inland surface waters, estuarine waters, and coastal waters. The WFD represents a challenge for water resource management in Europe, because, for the first time, water management is based on biological assessment of ecosystem status or health [9]. In coastal and transitional waters, the biological elements to be considered include phytoplankton, macrophytes (macroalgae and seagrasses), zoobenthos, and fish (only in transitional waters). The WFD and its implementation have generated a considerable amount of research on bioindicators and specifically on indicators based on seagrasses.

2 Seagrasses: Flowering Plants on the Seabed

Seagrasses are flowering plants which, after evolving in the terrestrial environment, have secondarily (and "recently," i.e., about 100 million years ago: the end of the Cretaceous) colonized coastal marine waters. As a result of this somewhat atypical history that parallels that of whales and other marine mammals, seagrasses present unique adaptations to marine conditions, including roots (and modified underground stems, called rhizomes), hydrophilic pollination, an internal gas circulation system (aerenchyma), and basal leaf meristems, among others. Despite their

[1] As explained, indicators are variables measured for biological entities. These variables are sometimes referred to as *descriptors*, *attributes*, or *traits*. In the Water Framework Directive, they are often called *metrics*.

worldwide distribution (except Antarctica), seagrasses constitute a group poorly diversified and includes only ca. 70 species [10].

Unlike algae, seagrasses can colonize sedimentary bottoms, where they can attach (and from which they can take up nutrients), thanks to their roots. This capability to colonize sedimentary bottoms has allowed seagrasses to extend over thousands of square kilometers, constructing a habitat (called "meadow") of high primary production which harbors hundreds of species that find food, substrate, or shelter there, with a global importance. Seagrass meadows are remarkable for the ecosystem services and goods they provide, including, among many others, beneficial effects on fisheries (playing a nursery role), shore protection, providing biodiversity hotspots, nutrient cycling, and constituting carbon sinks [11]. However, seagrasses are especially sensitive to environmental deterioration, and they are threatened by human activity. In recent decades, this has become a matter of concern, as seagrass meadows seem to be suffering worldwide regression [12]. This has generated a considerable amount on research on how seagrasses and their associated communities respond to man-made disturbances [13–16].

In the Mediterranean Sea, we find only five seagrass species (excluding the genus *Ruppia*). Of these five, one is an introduced species (*Halophila stipulacea*, a Lessepsian migrant), for the most part present in the Eastern basin, and two, belonging to the genus *Zostera* (*Z. noltii* and *Z. marina*), are relatively rare, with discontinuous distributions, mostly associated with brackish or extremely calm waters. The other two (*Posidonia oceanica* and *Cymodocea nodosa*) are much more abundant by far, and they extend over large coastal stretches.

P. oceanica and *C. nodosa* are both sensitive to environmental deterioration, just as other seagrasses are [17], although the former is more sensitive than the later, and their responses to specific disturbances (e.g., hypersalinity, trawling, eutrophication, coastal works, fish farming, etc.) have repeatedly been studied [18–23]. All these results, along with extensive knowledge of their biology and ecology [24], represent an excellent starting point for the identification of variables or descriptors whose association with given disturbances is well known and unequivocal, thereby providing a solid base for defining reliable bioindicators.

3 Digging Through Seagrasses in Search of Bioindicators

A number of variables (biological traits, attributes, etc.) associated with seagrasses and seagrass ecosystems have been reported in the literature to respond to environmental alterations. As outlined in Sect. 1, sensitivity to environmental stress is a necessary but not a sufficient condition for a variable to be used as a reliable bioindicator. Perhaps most critically, indicator responses must match the spatial and temporal scales that are appropriate for the specific management needs. In addition, a useful indicator would help identify the specific pressures (eutrophication, coastal development, fish farming, etc.) affecting the system, to allow managers to remedy the problem at its source. Consequently, before a bioindicator is employed, it is essential to understand how it behaves and to determine if it fits the specific management goals. This may often require a specific validation process which, although time-consuming, will serve to ensure that the bioindicators chosen are reliable, sensitive, and managerially effective.

During the implementation of the WFD along the coast of Catalonia in NE Spain, the behavior of measurements based on both *P. oceanica* and *C. nodosa* was carefully assessed [25, 26]. For both species, the process followed included (1) screening for variables, (2) validation over an environmental gradient of stress, and (3) detection of potential redundancies among variables.

3.1 Screening for Candidate Variables

The most relevant variables (or metrics, following the WFD) were selected from a suite of measurements obtained from in-depth screening of the literature. The entire suite of candidate variables showed, in accordance with the published evidence, a clear response to stressors, although through a great variety of methods and spatial and temporal scales. A total of 59 and 54 candidate seagrass variables were selected for *P. oceanica* and *C. nodosa*, respectively, following the criteria given above. The complete lists of variables selected are in Table 1 of Martínez-Crego et al. [8] for *P. oceanica* and Oliva et al. [26] for *C. nodosa*.

3.2 Validation Over an Environmental Gradient

We assessed the behavior of the candidate variables over environmental quality gradients at spatial scales appropriate for the management objectives, i.e., the deployment of the WFD along the coast of Catalonia (approximately 700 km; see Fig. 1) for *P. oceanica* and within the transitional waters (approximately 100 km^2; see Fig. 2) for *C. nodosa*.

For *P. oceanica*, we chose nine sites encompassing the maximum range of environmental quality in the area. A similar design was used for *C. nodosa* within the transitional waters. In both cases, every effort was made to capture the maximum spread of random spatial variability, performing nested sampling (scales of replication, 10 and 100 m). Seagrass samples were taken at a single depth (15 m for *P. oceanica*, 1 m for *C. nodosa*; see more on potential confounding effects of depth in Sect. 5) and within the shortest possible time (to avoid confounding effects of seasonality). In parallel, the environmental gradients were assessed using water column information (chlorophyll a, water transparency, salinity, and ammonium concentration in the water), sediment data (total phosphorus, ammonium concentration in pore water, and Hg and Pb content), and information obtained independently from other bioindicators (macroalgae [28]). Based on these environmental data, we attributed an environmental quality category (healthy, intermediate,

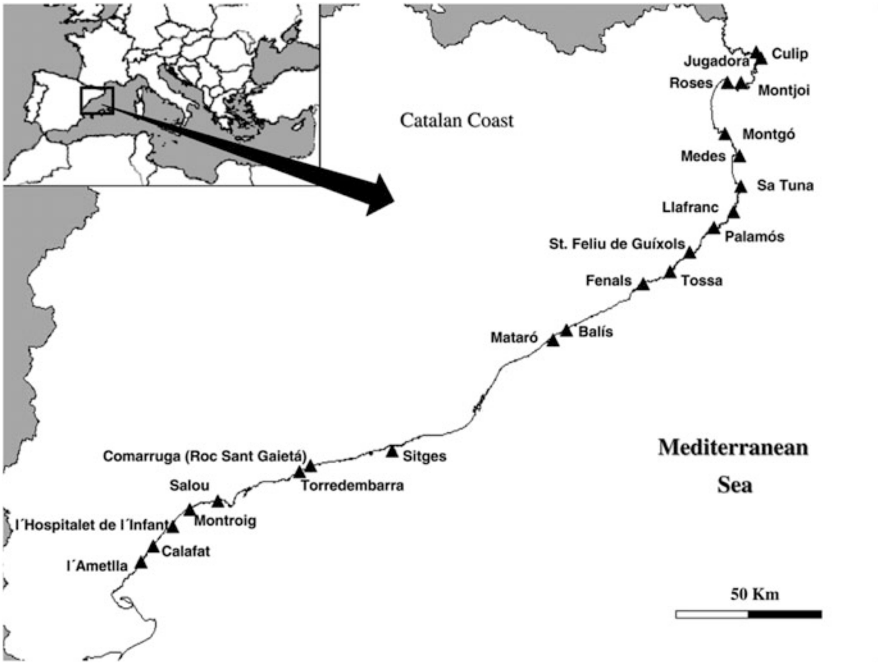

Fig. 1 Map of the Catalan coast including sampling sites for the POMI index and the seagrass (*Posidonia oceanica*) monitoring network (from Romero et al. [27])

unhealthy) to each of the sites, and we performed nested ANOVA (fixed factor, environmental quality; random factor, sites) for each of the candidate variables. We retained only those variables that displayed significant differences between the different environmental qualities. An initial remarkable fact was that only 22–24 (depending on the depth) of the potential 59 variables selected showed significant variability in accordance with the environmental status (in the case of *P. oceanica*) and 37 (out of 54) for *C. nodosa*. This indicates that a field evaluation at spatial scales that match those that are relevant for management is an unavoidable step in the selection of metrics.

3.3 Detecting Redundancies and Selection of Variables

Once the field validation was completed, we dropped the nonresponsive variables and performed a principal components analysis (PCA [29]) with the rest to identify common trends of variability within them, potential redundancies, and their correlation with environmental status. Based on this, and taking other aspects into account (cost, expertise needed, etc.), we selected a set of variables as biological indicators for WFD implementation (metrics). The variables selected for

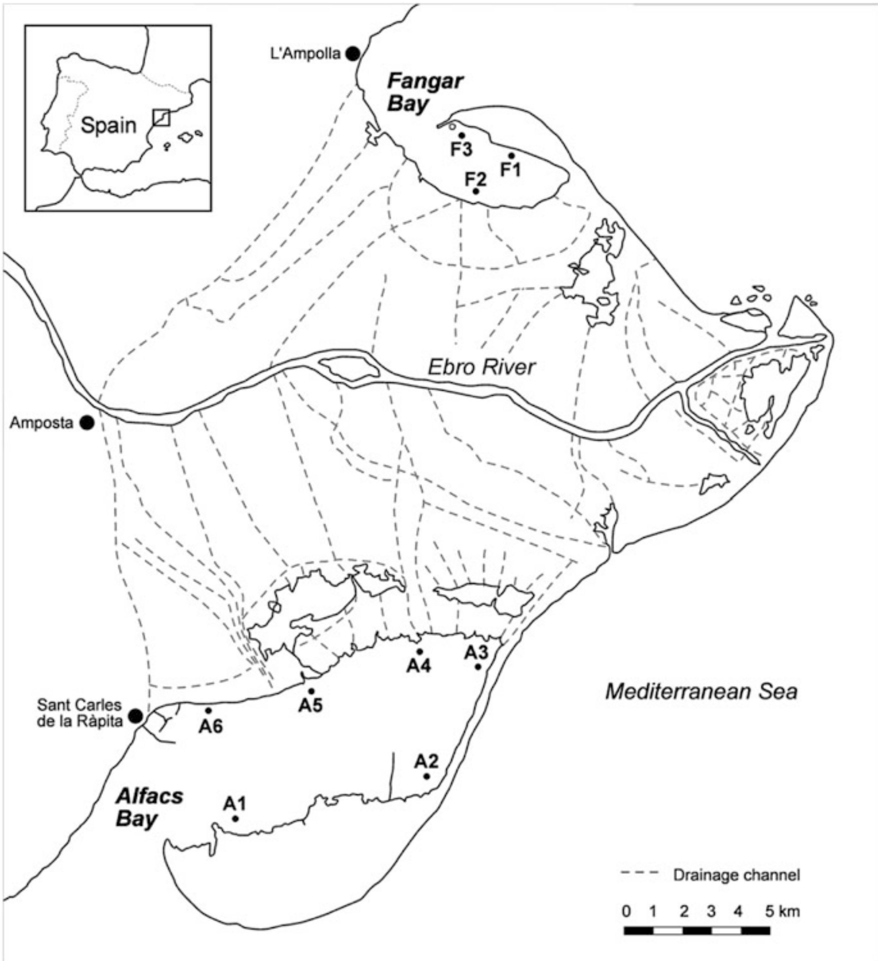

Fig. 2 Map of the study area and sampling sites where the CYMOX index based on the seagrass *Cymodocea nodosa* was applied (from Oliva et al. [26])

P. oceanica were phosphorus, nitrogen, and sucrose content in rhizomes, $\delta^{15}N$ and $\delta^{34}S$ isotopic ratios in rhizomes, percentage of leaves with necrosis, shoot size, meadow cover, shoot density, percentage of plagiotropic rhizomes, nitrogen content of epiphytes, and copper, lead, and zinc content of rhizomes. For *C. nodosa*, they were root weight ratio (ratio between root and roots plus leaves weight), shoot size, epiphyte load, and N, $\delta^{15}N$, P, $\delta^{34}S$, Cd, Cu, and Zn content of rhizomes (Table 1).

Table 1 List of selected metrics to construct the POMI and CYMOX indices (from Oliva et al. [26] and Romero et al. [27])

Biological organization level	POMI metrics	CYMOX metrics
Sub-individual	P, N, and sucrose content in rhizomes	N and P content in rhizomes
	$\delta^{15}N$ and $\delta^{34}S$ isotopic ratios in rhizomes	$\delta^{15}N$ and $\delta^{34}S$ isotopic ratios in rhizomes
	Pb, Cu, and Zn content in rhizomes	Cd, Cu, and Zn content in rhizomes
Individual	Percentage of leaves with necrosis	Shoot size
	Shoot size	
Population	Meadow cover	Root weight ratio (ratio between root and roots plus leaves biomass)
	Shoot density	
	Percent of plagiotropic rhizomes	
Community	N content in epiphytes	Epiphyte load

4 Integrating Time Scales: Multivariate Biotic Indices

In the preceding sections, we have stressed the requirements that a "perfect" bioindicator should meet. Unfortunately, there is no single variable (plant trait, ecosystem attribute) that adequately meets all those requirements. A reasonable alternative, always keeping in mind the management objectives, is the combined use of different variables; this approach has a number of advantages. On the one hand, the results for these variables can be presented independently and their values interpreted individually. If the set has been chosen properly, all the needs regarding sensitivity to and specificity for different environmental stressors will be covered. On the other hand, the set of variables can be numerically aggregated into a single index. Such aggregated indices are usually called multimetric indices or multivariate indices if multivariate statistical techniques are used for the aggregation. For the WFD we developed multivariate indices for both seagrass species by (1) integrating the variables, (2) setting reference conditions, and (3) assessing the relationship between pressures and impacts.

4.1 Integrating Selected Variables

The integration of the different variables was based on PCA. As all the metrics selected were correlated to environmental quality (see preceding sections), there was substantial common variability, which was clearly reflected in the clustering of the variables along the first axis [26, 27] (see Figs. 3 and 4, for *P. oceanica* and *C. nodosa*, respectively). The scores of the sites for the first component were then

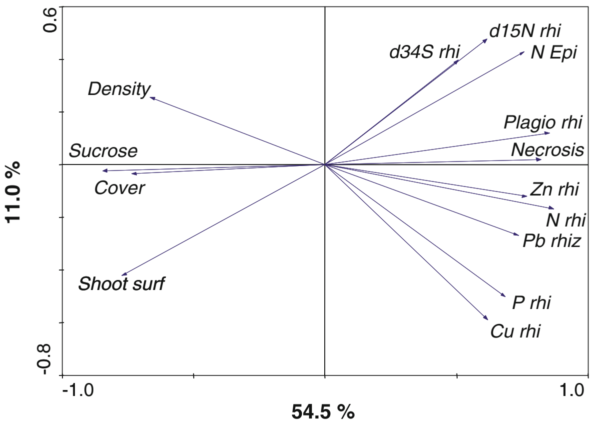

Fig. 3 Factor loading of the different metrics used to construct the *Posidonia oceanica*-based POMI index including the variability explained (%). The factors include shoot surface, percent of necrosis in leaves, nitrogen content in rhizomes, phosphorus content in rhizomes, sucrose in rhizomes, δ^{15}N isotopic ratio in rhizomes, δ^{15}S isotopic ratio in rhizomes, trace metals in rhizomes (zinc, lead, and copper), meadow cover, shoot density, percent of plagiotropic rhizomes, and epiphyte nitrogen content (from Romero et al. [27])

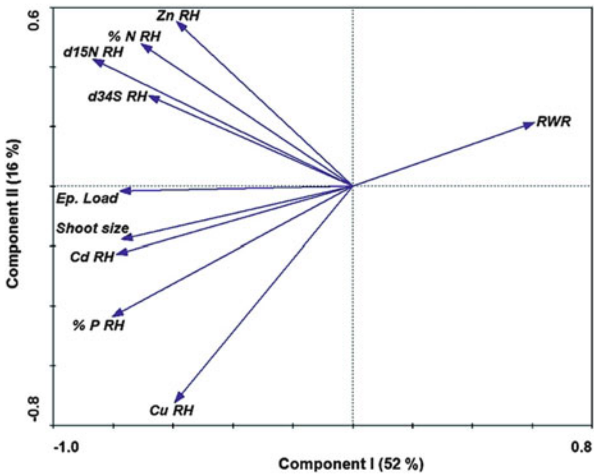

Fig. 4 Factor loading of the different metrics used to construct the *Cymodocea nodosa*-based CYMOX index including the variability explained (%). The factors include root weight ratio, shoot size, nitrogen content in rhizomes, phosphorus content in rhizomes, δ^{15}N isotopic ratio in rhizomes, δ^{15}S isotopic ratio in rhizomes, trace metals in rhizomes (zinc, cadmium, and copper), and epiphyte load (from Oliva et al. [26])

taken as an expression of their ecological status and in order to fulfill the WFD requirements, scaled to the interval 0–1 (ecological quality ratio, EQR) using reference conditions (see below).

4.2 Setting Reference Conditions

Reference conditions are usually understood as those corresponding to undisturbed, near-pristine sites; therefore, they present the optimal value for a given bio-indicator. Reference conditions are commonly used (and specifically in the WFD) as a kind of benchmark against which actual conditions are compared, and the difference (or distance) between reference and actual conditions is used to express the status of the system under analysis. Therefore, defining adequate reference conditions is a critical step and often represents a challenge, because in most coastal regions (at least in Europe), areas that are unambiguously devoid of all anthropogenic impact are extremely scarce.

In the case of the Western Mediterranean, potential reference sites can be found in zones far from human pressures, such as some island areas (e.g., Corsica, Sardinia, and the Balearic archipelago). However, the problem of the lack of comparability between ultraoligotrophic insular waters and the oligo-mesotrophic waters of continental coasts is a serious drawback of this approach. Other possible reference areas are marine reserves [30]; however, communities within marine reserves, although protected from major human impacts, can be subjected to drivers of change beyond the protective regulations (eutrophication, climate change, invasive species, changes in land uses, etc. [31]). To overcome these constraints, and for the seagrass-based indices developed for the coast of Catalonia, a reference frame was constructed using a modeling type of approach. For each variable, data from all the sites were pooled and ranked from best to worse, which were increasing or decreasing values depending of the variable under consideration. The average of the values above the 90th percentile was considered the reference (optimal) value for each specific variable, while the average of the values below the 10th percentile was considered the worst possible value. This strategy agrees with the modeling approach proposed in the WFD [32]. Arguments supporting it are given in Romero et al. [27].

4.3 Defining the Indices

We included the optimal (reference) and worst sites in the PCA described in Sect. 4.1 as passive objects (software used: CANOCO v 4.5 [33]), and their scores on the first axis (Figs. 5 and 6, for *P. oceanica* and *C. nodosa*, respectively) represented the two extreme conditions of the system. We calculated the EQR (following the WFD, comprised between 1 and 0, as mentioned above) as

$$\mathrm{EQR}_x = (\mathrm{CI}_x - \mathrm{CI}_{\mathrm{worst}})/\left(\mathrm{CI}_{\mathrm{optimal}} - \mathrm{CI}_{\mathrm{worst}}\right) \quad (1)$$

where EQR_x is the EQR of the site x and CI_x, $\mathrm{CI}_{\mathrm{optimal}}$, and $\mathrm{CI}_{\mathrm{worst}}$ are the scores on the first axis of the PCA of sites x, optimal and worst, respectively.

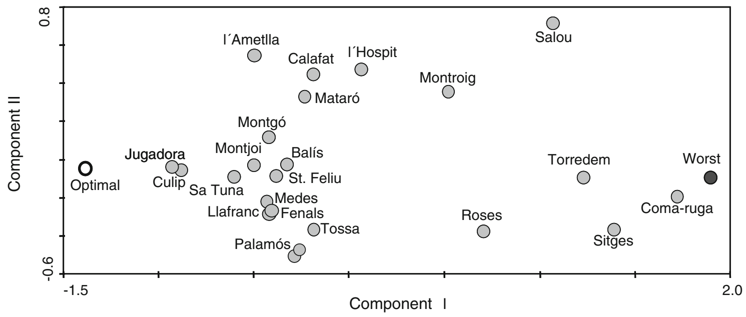

Fig. 5 PCA ordination of the studied sites (factor scores) along the Catalan coast for POMI, showing the optimal and worst sites. The sites are those depicted in Fig. 1 (from Romero et al. [27])

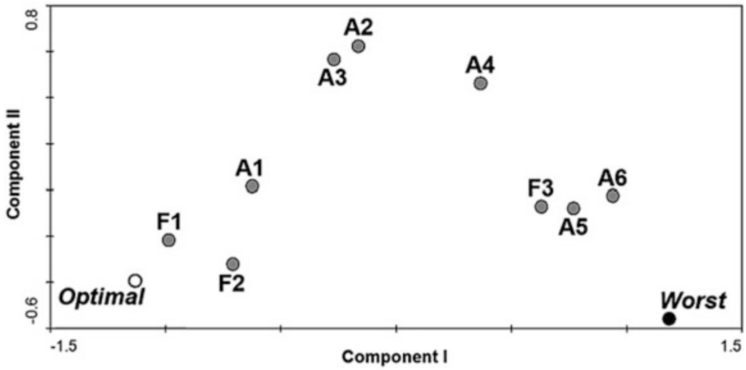

Fig. 6 PCA ordination of the studied sites in transitional waters for CYMOX (factor scores) showing the optimal and worst sites as supplementary objects. The sites are those depicted in Fig. 2 (from Oliva et al. [26])

As the WFD requires classifying the ecological status into one of five classes, from high to bad, the boundaries between classes have to be set within the 1 to 0 EQR scale. Since the relationship between pressures and the EQR was found to be linear, we simply divided the scale 0 to 1 into five equal classes (Table 2A, for *C. nodosa*, the CYMOX index). However, considering that *P. oceanica* is highly sensitive to anthropogenic disturbances, we constructed a slightly different index called POMI. This is based on the assumption that meadow disappearance has been reported in environmental conditions under which other biological assemblages persist [34, 35]. According to that we defined the bad class for the POMI index as the ecological status in which *P. oceanica* cannot survive. In other words, wherever and whenever a *P. oceanica* bed is able to survive, even heavily degraded, the ecological status is above bad. We arbitrarily assigned the range from 0 to 0.099 to this bad ecological status. The other EQR boundaries were obtained by dividing the remaining scale (from 0.1 to 1) into four categories of equal amplitude (0.225 each;

Table 2 Boundaries between the different ecological status classes (from Oliva et al. [26] and Romero et al. [27])

EQR (CYMOX)	Ecological status	Colour code
A: CYMOX		
1.00–0.80	High	Blue
0.79–0.60	Good	Green
0.59–0.40	Moderate	Yellow
0.39–0.20	Poor	Orange
0.19–0.00	Bad	Red
B: POMI		
1.000–0.775	High	Blue
0.774–0.550	Good	Green
0.549–0.325	Moderate	Yellow
0.324–0.100	Poor	Orange
0.099–0.000	Bad	Red

see Table 2B). Therefore, when *P. oceanica* exists, the EQR is computed as follows:

$$\mathrm{EQR}_x^{\prime} = (\mathrm{EQR}_x + 0.11)/(1 + 0.10)$$

being $\mathrm{EQR}_x^{\prime}$ the EQR for site x (where living *P. oceanica* exists) and EQR_x obtained from formula (1).

4.4 Assessing Pressure-Impact Relationships

Human activities (or drivers of change) that can potentially and adversely affect the status of ecosystems are considered pressures. When such an adverse effect actually occurs and produces a change in the status of the ecosystem, it is considered that an impact has taken place. This causal chain, known as DPSIR (Driver-Pressure-Status-Impact-Response) approach [36], has been established as an analytical framework for the determination of pressures and impacts under the WFD. In the context of European coasts, population density, tourism, industries, shipping activities, agriculture, fishing, and aquaculture are highlighted as the main drivers causing pressures on coastal ecosystems [37]. As a part of the WFD deployment, biotic indices used to assess ecological status under the WFD should be able to reflect the pressures acting on the water bodies. Therefore, we tested the sensitivity of the two seagrass-based indices (POMI and CYMOX) to pressures, in order to ensure their suitability for monitoring programs. For the analysis of the POMI index, pressures on coastal waters were estimated based on the document [38]. The main pressures considered were urban sewage discharge (kg/day/km coast), urban soil surface (ha/km coast), tourism pressure (rooms/km of coast), and harbor pressure (number of moorings/km of coast). All the pressures were normalized and reduced and then summed to estimate an aggregate pressure for each water

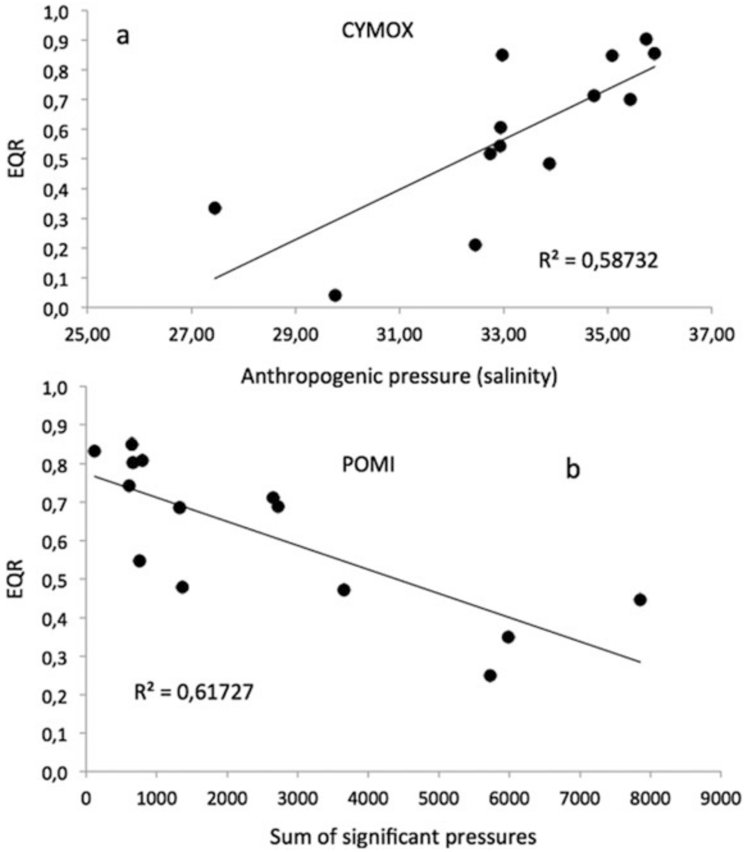

Fig. 7 Relationship between the CYMOX index (**a**) and the POMI index (**b**) and anthropogenic pressures. For the CYMOX index, anthropogenic pressure is represented by the salinity gradient, which decreases with increasing pressure. For the POMI index anthropogenic pressures are represented by a sum of significant pressures including sewage, urban use, tourism, and the presence of harbors

body where *P. oceanica* is currently present ($n = 17$, covering >80% of the coastline). POMI and the aggregate pressure were significant and negatively associated (Fig. 7b, $R^2 = 0.617$, $p < 0.05$). In the case of CYMOX, we considered that the main drivers of pressures affecting transitional waters were freshwater loadings (carrying nutrients, organic matter, and other pollutants). Accordingly, to test the CYMOX index, we assessed the correlation between the index values and salinity, which showed a positive and significant correlation (Fig. 7a, $R^2 = 0.588$ and $p < 0.05$).

To further assess the sensitivity of our indices to pressures, we explored the relationship between land uses and the POMI index [39]. Land uses were quantified from public databases in SIG format (*Mapa de Cobertes del Sòl de Catalunya*, http://www.creaf.uab.es/mcsc/) for coastal stretches corresponding to each water body and extending 1.5 km inland. The three main land use categories considered were urban, natural, or nonirrigated agricultural and irrigated agricultural. Significant correlations between land use categories and ecological status, as estimated by POMI, were found. The category "natural or nonirrigated agricultural" was positively correlated with POMI values ($R^2 = 0.668$, $p < 0.01$). The surface occupied by the categories "urban" and "agricultural irrigated" correlated negatively with POMI ($R^2 = 0.679$ and $R^2 = 0.446$, respectively; p from 0.01 to 0.05). The direct relationship between land uses and coastal water status has profound implications for management and stresses the fact that to a large degree coastal water quality depends on human activity on land.

5 Evaluating Uncertainty Associated with Ecological Status Classification

A common concern of those involved in the use and design of bioindicators is the natural variability (i.e., not related to human impacts) of the variables measured. For example, most seagrass attributes exhibit marked seasonality and/or strong bathymetric dependence, which can potentially confound the interpretation of values. In these cases, the problem can be easily fixed, sampling at a specific time of year and at a fixed depth. However, seagrass attributes (such as those included in the POMI and CYMOX indices) also show variability at different spatial scales (e.g., clonal integration, microhabitat, sediment patchiness, etc.), which is not always obvious to the researcher. This variability can result in unintentionally misclassifying water bodies and wrong management decisions, both in terms of action and inaction, which may in turn have high associated social and economic costs. Obviously, as in any sampling, this (random) variability should be taken into account through adequate replication. However, it is not always straightforward how to deal with this variability. Indices aggregating different attributes are common in most WFD approaches, including the examples reported here (i.e., CYMOX and POMI). In these cases, while the variability of each individual indicator (metric) can be easily assessed through replication, and expressed using the usual statistics means (standard deviation, confidence interval, etc.), the variability of the composite index cannot be directly evaluated. To address this problem, a number of techniques can be applied (bootstrapping, uncertainty assessment, error propagation techniques, etc.). Here we briefly describe one particular exercise that we conducted with POMI in order to evaluate different sources that contribute to the variability of the final index, as reported in Bennett et al. [40]. We identified relevant factors that could introduce uncertainty into the ecological status classification: small-scale (tens of meters) spatial variability, medium-scale (hundreds of meters) spatial variability, large-scale (tens of kilometers, but always within the same water body, i.e., a zone considered homogeneous from the point of view of the ecological status) spatial variability, depth (5 and 15 m), interannual variability

(variability between consecutive years within the 4-year period over which samples can be measured according to the WFD), and surveyor error (variability between surveyors, as some of the attributes such as density and cover are estimated directly in situ by divers). The main source of variability was, as expected, depth, which accounted for more than 60% of variability, leading to the conclusion that depth should remain fixed or be controlled in monitoring programs based on *P. oceanica*. In contrast, the variability in POMI scores between different surveyors was extremely low, explaining less than 1% of total variability, while interannual variability accounted for approximately 5% of the total. Additionally, variability at medium and large spatial scales did not differ from variability at the small spatial scale, which suggest that replication within scales of tens of meters is enough to capture most spatial variability.

Based on the results of this variability assessment, we evaluated the probability of misclassification of a given water body, as a function of the value of the POMI index obtained (EQR). Without entering into excessive detail (see [40, 41]), it has to be noted that the risk of misclassification, provided the design is controlled (e.g., fixed depth, fixed season, etc.), is low (as low as <0.1%) for values far from the thresholds between classes, but increases significantly (up to 50%) for values within an interval of ±0.1 EQR from the threshold value or with uncontrolled designs (Fig. 8). The issue of how to deal with this risk of misclassification remains an open question. It would seem reasonable, when this risk is high and before assigning a

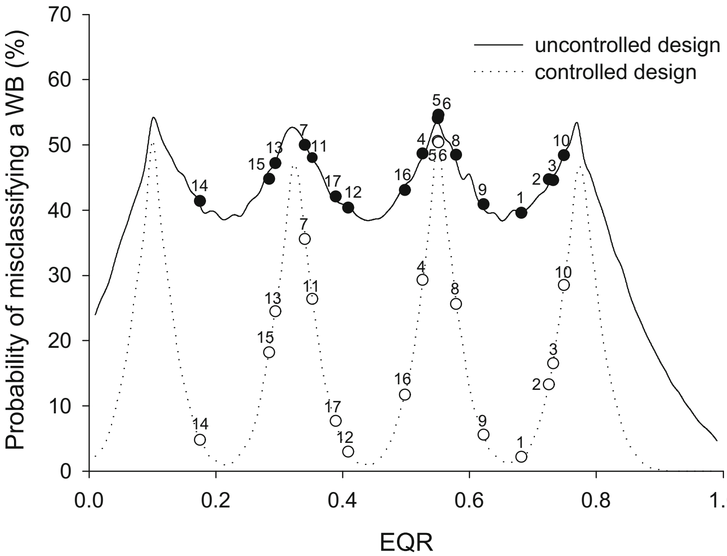

Fig. 8 Uncertainty analyses applied to establish the probability of misclassifying the ecological status class among EQR values calculated within a water body (WB) with a controlled design (fixed depth, fixed sites, fixed years, fixed zones, fixed surveyors) and an uncontrolled design where these variables are not fixed. *Full* and *open circles* represent the actual probability of misclassification for 17 Catalonian WB (in *numbers*) (from Bennett et al. [40])

water body status (and especially if the uncertainty is between the moderate and good classes), to obtain additional data on environmental conditions from independent sources (additional seagrass variables, physicochemical variables, etc.). The same kind of analysis was performed with CYMOX, with quite similar results [42].

6 Harmonizing Indices: Making Sure the Indices of Different Member States Compare Across the EU

A critical component of the WFD is the aim of ensuring the methods employed by different member states are intrinsically comparable, so that ecological status categories have the same meaning across the EU. This has required a process of careful intercalibration between assessment methods, which has proven to be extremely hard, as the first choice for intercalibration (applying different methods to the same set of sites) was ruled out due to budgetary constraints (however, see below). The intercalibration exercise was led by the EU and its main aim was to guarantee that the five classes required by the WFD represented equal levels of environmental health/deterioration across all EU member states, independently of the assessment method used. When complete, this will eventually result in the harmonization of all the different methods used by member states so that the definition of ecological status will not vary across water bodies and will be independent of the method used for its assessment. In the case of Mediterranean seagrasses, several assessment methods based on different indices (all related to the seagrass *P. oceanica*) were compared against each other. Specifically three methods were presented and intercalibration was successfully completed on them: POMI (Spain—Catalonia, Balearic Islands, Murcia, Andalusia, and Croatia [27]), Valencian CS (Spain, Valencia [43]), and PREI (France, Italy, Cyprus [44]). The methods were all multimetric, but differed in two aspects: (1) the individual indicators (i.e., metrics) used and (2) how the individual indicators were aggregated or combined to produce values on a unique EQR 0 to 1 scale. The more the two aspects differ, the more difficult it is to compare the indices, especially in the absence of a common experimental approach. However, as most of the methods included, as a metric or at least as complementary observations, values on leaf length, shoot density, and lower limit typology [45], an ICCM (intercalibration common metric) was constructed based on these three variables. Then, the relationship of the ICCM to (1) human pressures and (2) each one of the different methods assessed was evaluated using linear regression. The fitted linear models were used to detect whether the boundaries good/moderate and high/good corresponded, for all the methods, to the same level of human pressure. Although some methods needed some fine tuning, finally all of them were successfully intercalibrated, despite their differences.

This comparability between methods was confirmed by the experimental exercise performed by Spanish, French, and Italian teams and reported in Lopez y Royo

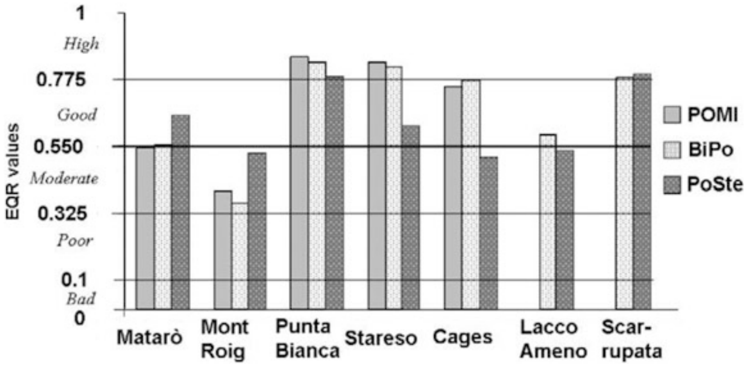

Fig. 9 EQR values and classification of sites according to the POMI index, BiPo index, and PoSte index, all three based on the seagrass *Posidonia oceanica* and applied in Catalonia (Spain), Corsica (France), and Campania (Italy) (from Lopez y Royo et al. [46])

et al. [46]. Joint sampling was performed in different areas of the Western Mediterranean at sites that encompass a gradient of human pressure. From the simultaneous data collection, three indices (POMI, BiPo, and PoSte; see references in [46]) were computed for each site, and then each one was compared to the others. Two of the indices, POMI and BiPo, showed a very good relationship with human pressures and were in almost complete agreement ($R^2 = 0.987$). In contrast, one of the methods, the PoSte index, clearly diverged from the other two as it was based on a different rationale for defining reference conditions and used a different response scale (Fig. 9). Taken altogether, this intercalibration approach highlights two main points: (1) indices with very different metrics can still provide completely reliable and comparable results; (2) indices that are based on different conceptual approaches (e.g., in their definition of what is near-pristine status) may diverge considerably.

7 Conclusions: What Have We Learned and Questions That Remain Open

The implementation of the WFD for the coastal waters of Catalonia stimulated a considerable amount of work and specifically a huge effort in building, validating, and analyzing seagrass-based bioindicators. From all this effort, summarized in the present document, a few basic conclusions emerge: (1) it is necessary to validate the response of individual metrics to human pressures at spatial and temporal scales suitable for the monitoring programs; (2) it can be extremely useful to use different metrics simultaneously, to cover different requirements of monitoring programs (early detection, specificity to stressors, relevance to ecosystem integrity, etc.); (3) it is a crucial careful assessment of the variability of such metrics and, based on this, a careful sampling design; (4) in-depth knowledge of the behavior of each individual indicator (e.g., response time, potential hysteretic properties, etc.) is necessary; and (5) PCA has a great potential as an optimal method not only to aggregate different metrics into a single index objectively but also to reveal redundancy among metrics.

The indices based on seagrasses reported here (POMI and CYMOX) have a number of advantages as detailed below:

1. The individual metrics selected to construct the indices are gathered from several levels of biological organization and encompass different time responses to stress and different specificity to stressors. Thus, they represent a good integrated measure of ecosystem status. Specifically, biochemical and physiological measurements are typically not influenced by hysteretic properties, making them much better candidate bioindicators to detect recovery in environmental conditions over time scales relevant for management.
2. Under the WFD, individual metrics are aggregated to construct a single indicator expressing the distance between the present status and an ideal status of the system. In our indices, we use multivariate statistical techniques based on PCA to extract a common variability (associated with ecosystem status). This not only takes into account redundancy among individual metrics, but it probably introduces less bias than expert judgment scoring, a common practice in implementing multimetric indices.
3. Both indices (POMI and CYMOX) show good correlation to human pressures and, therefore, seem to be good indicators as required by the WFD for management purposes.

However, some open questions remain. We would like to stress three of them.

First, the application of multivariate indices that incorporate a large number of metrics, such as CYMOX and POMI, is time and resource consuming and could require some degree of expertise. Of course, this is an unavoidable counterpart to the strengths of the indices reported above. However, and following the needs of managers and the objectives of specific monitoring programs, the multivariate nature of POMI and CYMOX allows for simplification; for example, if there is no need for great precision or robustness, in this respect, we tested the reliability of POMI-9 (using only nine metrics, instead of 14) with good results [43]. Further simplifications, tailored to manager needs and budgets, could be explored in the future.

Second, a crucial aspect of the classification of water bodies based on their ecological status is the reference frame used. Bad or incorrect references can lead to misclassifications with undesired consequences for managers. The issue of how to define reference conditions is not a closed discussion, especially in the case of indices based on *P. oceanica*, for which some of the metrics used show very slow recovery. More research is needed, with inputs from different fields (paleoecology, modeling, etc.) to refine this crucial aspect.

Third, we have shown how large the uncertainty is in close to the boundary values between classes. Once this uncertainty has been recognized and properly quantified, how it should be incorporated into the decision-making process associated with environmental monitoring remains an open question.

Overall, bioindicators have proved to be stimulating and fertile ground for research, at the interface between science and management, where research into ecology finds considerable social utility.

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Environmental Quality of Catalan Coastal Waters Based on Macroalgae: The Interannual Variability of CARLIT Index and Its Ability to Detect Changes in Anthropogenic Pressures over Time

Xavier Torras, Susana Pinedo, María García, Boris Weitzmann, and Enric Ballesteros

Abstract CARLIT is a Water Framework Directive-compliant methodology permitting a rapid assessment of water quality using rocky-shore macroalgae as biological quality elements. Here we present the water quality assessment of 32 coastal water bodies of Catalonia (Northwestern Mediterranean) during a period of 14 years (1999–2012) applying CARLIT. The averaged ecological status of the water bodies ranges between high and poor and the Ecological Quality Ratio shows a significant negative relationship with a modified LUSI index, thus providing further evidence on the utility of CARLIT to detect anthropogenic pressures. The lowest interannual variability in water quality was found in water bodies having most of their shore covered by natural rocks, while the highest variability was observed in water bodies situated in semi-confined environments or located close to freshwater discharges. In spite of the multiple advantages of CARLIT as a monitoring methodology, it can show strong disagreements in water quality assessment with other methodologies using other biological quality elements (i.e., macroinvertebrates). These discrepancies mainly occur in water bodies with reduced extension of rocky shores, questioning the use of CARLIT in these situations.

Keywords CARLIT, Ecological status (ES), Macroalgae, Rocky-shore assemblages, Water Framework Directive (WFD)

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Contents

Abbreviations

BQE	Biological quality elements
CARLIT	Cartografia Litoral (Littoral Cartography)
CFR	Quality of Rocky Bottoms
EEIc	Ecological Evaluation Index continuous formula
EQ	Environmental quality
ES	Ecological status
LUSI	Land Uses Simplified Index
MA-LUSI-WB	Modified LUSI index
MEDOCC	MEDiterranean OCCidental
RSL	Reduced species list
WB	Water body
WFD	Water Framework Directive

1 Introduction

The main objective of the Water Framework Directive (WFD; European Commission 2000/60/EC) is to achieve the good ecological status (ES) in all the surface water bodies (WBs) by 2015 and to prevent its deterioration in the subsequent years. Different biological quality elements (BQE) have been proposed to assess the ecological status in coastal water bodies: phytoplankton, macrophytes (macroalgae and seagrasses), and macroinvertebrates.

Macroalgae have been frequently used to assess the environmental quality in the implementation of the WFD [1–11]. Species of the brown algal genus *Cystoseira* (Fucales, Cystoseiraceae) usually dominate the upper infralittoral levels from non-polluted environments in the Mediterranean Sea [12–18] but they are replaced by other species when nutrient or heavy metal concentrations increase [3, 4, 11, 16, 19–22]. In non-polluted Mediterranean environments, the upper infralittoral algal beds thriving in moderately exposed to highly exposed rocky shores are dominated whether by *Cystoseira mediterranea* or by *Cystoseira stricta* (*C. amentacea* v. *stricta*) [3, 4, 12, 23–26]. When pollution or the frequency of any other kind of disturbance increases, *Cystoseira* spp. populations are first replaced by beds of the red alga *Corallina elongata* and the mussel *Mytilus galloprovincialis* [11, 13, 21, 23, 25, 27, 28]. Green ephemeral algae (*Ulva* spp., *Cladophora* spp.) and cyanobacteria replace *Corallina* and *Mytilus* in highly disturbed environments and near freshwater discharges [11, 13, 25, 29–32].

Several methodologies based on macroalgae (EEIc [2], CARLIT [3], RSL [5], CFR [7]) have been developed to assess the ES of water bodies. CARLIT is based in the cartography of littoral and upper infralittoral rocky-shore assemblages and was developed to assess the water quality in the coast of Catalonia (Northwestern Mediterranean). Afterward, it has been officially used for the implementation of the WFD in most of the Mediterranean coasts of Spain, France, and Italy [33, 34]. CARLIT has been applied to the coast of Catalonia (Northwestern Mediterranean) during 14 years, continually from 1999 to 2012, and has provided enough data to explore its interannual variability and its possible utility to detect long-term changes in the environmental quality of coastal WBs.

2 Material and Methods

The sampling surveys consisted in a run of the entire coast with a small boat kept as close as possible to the shoreline [3]. Littoral and upper sublittoral assemblages were identified (Table 1) and directly annotated in a graphic display (aerial photographs, nautical charts, or ortho-photographs at a scale of 1:5000). Highly human-modified WBs such as the inner part of harbors and marinas were not sampled, as they do not reflect the environmental quality of the adjacent coast. The final result of each survey was a partition of the rocky shoreline in several sectors – at least 50 m long – characterized by an assemblage category (corresponding to a single assemblage or a combination of assemblages). The sectors harboring *Cystoseira* species were assigned to *Cystoseira* categories taking into account also the coverage in a semiquantitative scale [3]. When more than one species were present in a sector (i.e., *Corallina elongata* and *Mytilus galloprovincialis*), the assignment was given to the most visually abundant one, but scoring the presence of both species (i.e., Coelo+Mgal or Mgal+Coelo; Table 1). Each category was assigned to a sensitivity level (SL) regarding their vulnerability to any environmental stress based on literature and expert judgment (Table 1; [3]).

The environmental quality (EQ) assessment of a WB was calculated as

$$\mathrm{EQ} = \frac{\sum \mathrm{l}_i * \mathrm{SL}_i}{\sum \mathrm{l}_i} \quad (1)$$

where l_i is the length of the coastline occupied by the community category i and SL_i is the sensitivity level of the assemblage category i.

The Ecological Quality Ratio (EQR) is defined as the ratio between the EQ calculated in the study region and the EQ calculated at reference sites [35]. Reference sites were located in Corsica and in the Balearic Islands [3]. In the reference sites the abundance and relative cover of each category of assemblages depend on

Table 1 Summarized description and sensitivity levels of the main assemblage categories distinguished in the Catalan coast

Category	Description	Sensitivity level
Cymed continuous	Continuous belt of *C. mediterranea*	19–20
Cymed dense	Abundant patches of dense stands of *C. mediterranea*	15
Cymed rare	Abundant scattered plants of *C. mediterranea*	10–12
Other Cy spp.	Populations of *Cystoseira* spp. in sheltered environments	15
Cymed+Cycom	*C. compressa* and *C. mediterranea* populations in exposed or sheltered environments	12–15
Cycom	*C. compressa* populations in exposed or sheltered environments	12
Coelo	Belt of *Corallina elongata*, devoid of *Cystoseira*	8
T	Buildups of *Lithophyllum byssoides* (Trottoir)	20
Mgal	Mussel (*Mytilus galloprovincialis*) beds, without *Cystoseira*	6
Green algae	Belts of *Ulva* and *Cladophora* species	3
Cyano	Communities dominated by cyanobacteria and *Derbesia tenuissima*	1
Photophilic algae	Communities dominated by *Padina/Dictyota/Dictyopteris/Halopteris*	10

Table 2 Environmental quality (EQ) values calculated for the six geomorphological relevant situations in reference sites

Geomorphological relevant situation (i)	Coastal morphology	N/A	EQ_i
1	Decimetric blocks	Artificial	12.1
2	Low coast	Artificial	11.9
3	High coast	Artificial	8.0
4	Decimetric blocks	Natural	12.2
5	Low coast	Natural	16.6
6	High coast	Natural	15.3

coastline geomorphology and on the kind of substrate (artificial versus natural) [3]. Thus, different geomorphological situations potentially influencing the establishment and the abundance of the littoral and upper sublittoral assemblages were defined (Table 2).

The EQR of a sector of coast is calculated as

$$\mathrm{EQR} = \frac{\sum \frac{\mathrm{EQss}_i * \mathrm{l}_i}{\mathrm{EQrs}_i}}{\sum \mathrm{l}_i} \tag{2}$$

where i is the situation, EQss_i is the EQ in the study site for the situation i, EQrs_i is the EQ in the reference sites for the situation i, and l_i is the coastal length in the study coast for the situation i. The EQR values range from 0 (bad ES) to 1 (high ES). Five ESs (high, good, moderate, poor, and bad) are considered. According to

Table 3 Correspondence between ecological quality ratio (EQR) intervals and ecological status (ES)

EQR	ES
>0.75–1	High
>0.60–0.75	Good
>0.40–0.60	Moderate
>0.25–0.40	Poor
0–0.25	Bad

the normative definitions of the ecological classes in the WFD and the expert judgment, a correspondence between the EQRs and ESs was obtained ([3] Table 3).

CARLIT methodology was applied to the whole Catalan coast, which extends for more than 400 lineal km, yearly from 1999 to 2012. Sampling was always performed during spring (from May to June) in coincidence with the highest development of littoral assemblages [14]. Although the Catalan coast is divided into 34 coastal WBs defined according to anthropogenic pressures and typology, the CARLIT index was calculated for 32 WBs since two WBs (C33 and C34) are completely devoid of rocky shores.

The information obtained on the surveys was transcribed into a GIS system and the yearly ES for the different WBs was calculated. There is not a minimum percentage of rocky coast or natural rocky coast to be considered for the calculation of the CARLIT index on a WB [3], and therefore, despite the existence of several WBs with a low percentage of natural rocky coasts (Fig. 1), EQRs were calculated for all WBs where some sector of rocky shore was present.

A Nonmetric Multidimensional Scaling (NMDS) ordination was performed to visualize the similarities of the 32 WBs according to the EQR values. A modified version of the Land Uses Simplified Index (LUSI) [36] was used to analyze the relationship of anthropogenic pressures and CARLIT results. LUSI is based on a combination of factors that reflect the continental influence in the coastal WBs: (1) land uses (urban, industrial, and agricultural), (2) the vicinity and the typology of a river, and finally (3) the shape of the coast (concave, convex, or straight). The scores were calculated taking into account 1.5 km inland between the limits of each WB on a Corine Land Cover map based on 2006 data. Two new factors were added to the original LUSI based on (1) the density of population and (2) the artificialization of the coastline at each WB. The density of population was estimated as the logarithm of the total population in the littoral municipalities divided by the length of each WB. The artificialization of the coastline was calculated as the relative length, from 0 to 1, of artificial structures in the total rocky coastline sampled at each WB. The modified LUSI index (MA-LUSI-WB) was thus calculated as

$$\mathrm{MA-LUSI-WB} = \mathrm{LUSI} + \log\left(\frac{\text{Inhabitants}}{\text{Coastline length}}\right) + \left(\frac{\text{Length artificial}}{\text{Total rocky length}}\right) \quad (3)$$

The relationship between the scores of MA-LUSI-WB and the mean EQR value at each WB was obtained by means of Pearson correlation analysis.

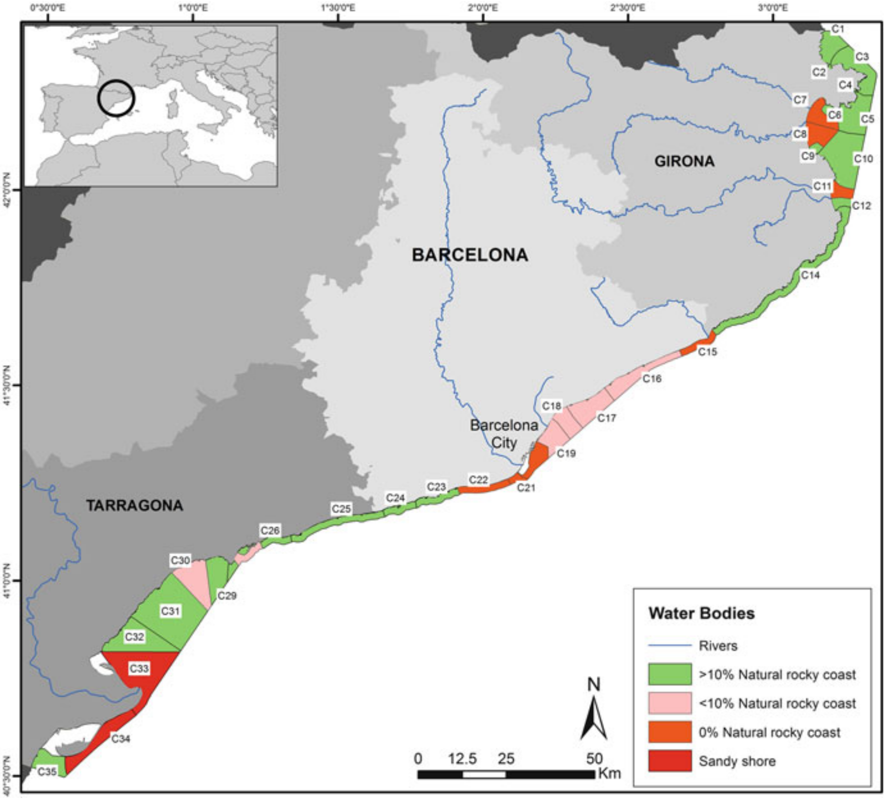

Fig. 1 The 34 coastal water bodies of Catalonia (Northwestern Mediterranean) delimited according to its typology and anthropogenic pressures. Completely sandy shores, water bodies without natural rocky coast or with a percentage lower than 10% of natural rocky coast are shown on a red scale

3 Results

Water bodies are ordinated in the MDS plot following a gradient of water quality, with the WBs having the highest EQR (C14, C10) situated at the right side of the plot and those having the worst water quality situated at the left side (C21, C22) (Fig. 2). The EQR values and the ES at each WB change over time (Table 4). The lowest variability is found in WB C14 with only 5% of variability in the 14-year period. The highest variability is found in WB C21 with 82%. The SD oscillates between 0.01 (C14) and 0.13 (C02 and C21) (Table 5). The values of EQR usually range between rather restricted intervals in most water bodies, as summarized in Fig. 3. The highest values are obtained in the northern shores, medium values in the southern shores, and the lowest values in the central sector of the coast. Seven WBs

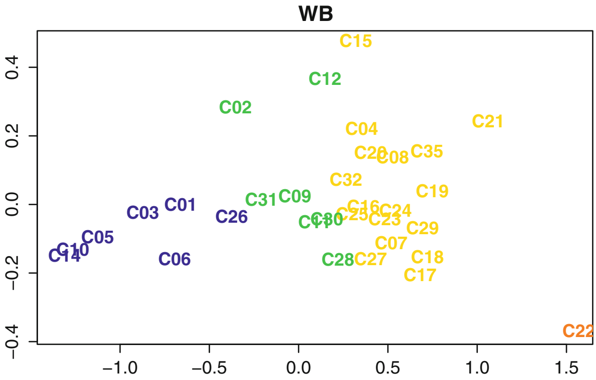

Fig. 2 Nonmetric Multidimensional Scaling (NMDS) ordination of water bodies based on Kruskal-Wallis mapping for yearly EQR data

register a high averaged ES; of these, both high and good ES have been assessed for WBs C01, C03, C06, and C26, while WBs C05, C10, and C14 have always been rated high. Good averaged ES has been assessed at seven WBs. Moderate averaged ES has been obtained at seventeen WBs, mainly in Barcelona coastline, where good or poor ES has also been obtained during some surveys. Finally, one WB (C22) sampled only from 2005 to 2010 has been evaluated with a poor averaged ES. A significant negative relationship between MA-LUSI-WB scores and EQR mean values was obtained ($R^2 = 0.5033$; $p<0.001$) (Fig. 4).

4 Discussion

After sampling the Catalan coast using CARLIT for 14 years, we feel confident to reinforce some its advantages when compared with other WFD-compliant methodologies based on macroalgae [1, 2, 5–8]. First, CARLIT is a nondestructive methodology. Second, there is almost no laboratory work, therefore saving time and money. Third, sampling is fast, being possible to survey a coast length of 400 km in no more than 6 weeks, minimizing the seasonal variability on the composition and development of the assemblages. And, fourth, it takes into account the totality of the coast, avoiding the misclassification of a WB due to special features of the sampling stations chosen to evaluate the WB. Moreover, the ES assessment of the WBs agrees with the intensity of the anthropogenic pressures as previously reported [18, 37–39]. The good negative relationship between the averaged EQR values and the modified LUSI index used here (Fig. 4) provides new evidence on the utility of CARLIT to detect anthropogenic pressures.

Our data shows a low to high interannual variability in the values of the CARLIT index for the same WB. One of the factors that may explain this variability is the subjectivity of the observers as reported in other studies [40, 41]. This is especially critical in CARLIT where the assignment of a category to a sector of coastline depends on the identification skills of the observer. However, this is not a cause of

Table 4 EQRs and ESs for the 32 water bodies assessed in Catalan coastal waters from 1999 to 2012 with CARLIT methodology

	WB	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
EQR															
Girona	C01	0.82	0.68	0.75	0.81	0.93	0.83	0.85	0.75	0.70	0.74	0.77	0.90	0.89	0.95
	C02	0.73	0.50	0.50	0.70	0.75	0.92	0.71	0.84	0.60	0.69	0.82	0.85	0.53	0.73
	C03	0.81	0.72	0.76	0.82	0.87	0.88	0.68	0.87	0.83	0.89	0.95	0.96	0.96	0.99
	C04	0.51	0.49	0.59	0.55	0.40	0.68	0.43	0.55	0.54	0.57	0.64	0.73	0.59	0.72
	C05	0.94	0.80	0.91	0.86	0.94	0.95	0.88	0.98	0.91	0.92	0.98	0.98	0.96	0.98
	C06	0.68	0.64	0.78	0.70	0.80	0.82	0.90	0.95	0.71	0.75	0.98	0.86	0.85	0.88
	C07*	0.43	0.50	0.62	0.65	0.58	0.38	0.55	0.55	0.49	0.56	0.54	0.55	0.52	0.50
	C08*	0.43	0.36	0.59	0.51	0.67	0.59	0.59	0.45	0.67	0.67	0.56	0.47	0.48	0.59
	C09	0.57	0.55	0.68	0.56	0.64	0.66	0.65	0.67	0.65	0.71	0.75	0.66	0.80	0.74
	C10	0.99	0.94	0.96	0.93	0.97	0.96	0.86	1.00	0.97	0.97	0.99	0.98	0.96	1.00
	C11*	0.67	0.67	0.67	0.67	0.60	0.58	0.51	0.67	0.67	0.67	0.67	0.63	0.65	0.65
	C12	0.56	0.55	0.60	0.47		0.58	0.27	0.68	0.58	0.61	0.61	0.77	0.75	0.69
	C14	0.99	0.94	0.98	0.98	0.98	0.98	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.98
Barcelona	C15*	0.22	0.31	0.36	0.49	0.51	0.46	0.60	0.46	0.53	0.47	0.41	0.64	0.54	0.63
	C16**	0.54	0.56	0.48	0.55	0.56	0.56	0.59	0.58	0.64	0.54	0.57	0.60	0.55	0.59
	C17**	0.45	0.49	0.50	0.54	0.66	0.48	0.54	0.41	0.51	0.49	0.46	0.38	0.55	0.53
	C18**	0.56	0.51	0.47	0.45	0.51	0.57	0.48	0.45	0.46	0.59	0.31	0.46	0.45	0.47
	C19**	0.46	0.49	0.52	0.41	0.33	0.57	0.46	0.41	0.52	0.47	0.49	0.50	0.52	0.52
	C20*	0.34	0.50	0.56	0.44	0.52	0.63	0.57	0.62	0.59	0.59	0.63	0.55	0.58	0.56
	C21*					0.10	0.40	0.39	0.51	0.55	0.39	0.50	0.40	0.51	0.49
	C22*							0.25	0.24	0.23	0.26	0.33	0.31		
	C23	0.54	0.52	0.54	0.53	0.57	0.49	0.53	0.51	0.52	0.58	0.58	0.55	0.50	0.51
	C24	0.53	0.49	0.50	0.48	0.52	0.51	0.49	0.47	0.52	0.56	0.57	0.51	0.51	0.52
Tarragona	C25	0.61	0.57	0.57	0.54	0.63	0.59	0.57	0.55	0.58	0.63	0.62	0.58	0.59	0.57
	C26	0.72	0.59	0.72	0.73	0.70	0.75	0.72	0.68	0.78	0.78	0.75	0.85	0.81	0.93
	C27**	0.64	0.57	0.61	0.64	0.65	0.66	0.46	0.47	0.57	0.55	0.59	0.51	0.56	0.55

	C28	0.59	0.62	0.62	0.62	0.69	0.63	0.62	0.44	0.62	0.59	0.71	0.60	0.66	0.68
	C29	0.47	0.45	0.52	0.53	0.53	0.51	0.49	0.40	0.50	0.49	0.52	0.47	0.50	0.43
	C30**	0.67	0.60	0.63	0.60	0.64	0.62	0.66	0.62	0.60	0.67	0.63	0.64	0.58	0.61
	C31	0.63	0.59	0.57	0.60	0.74	0.73	0.74	0.76	0.75	0.71	0.69	0.79	0.74	0.74
	C32	0.52	0.47	0.47	0.52	0.58	0.58	0.63	0.60	0.63	0.57	0.59	0.70	0.67	0.61
	C35	0.54	0.30	0.59	0.63	0.54	0.49	0.53	0.44	0.52	0.51	0.54	0.54	0.55	0.57
ES															
Girona	C01	H	G	H	H	H	H	H	H	G	G	H	H	H	H
	C02	G	M	M	G	H	H	G	H	G	G	H	H	M	G
	C03	H	G	H	H	H	H	G	H	H	H	H	H	H	H
	C04	M	M	M	M	M	G	M	M	M	M	G	G	M	G
	C05	H	H	H	H	H	H	H	H	H	H	H	H	H	H
	C06	G	G	H	G	H	H	H	H	G	H	H	H	H	H
	C07*	M	M	G	G	M	P	M	M	M	M	M	M	M	M
	C08*	M	P	M	M	G	M	M	M	G	G	M	M	M	M
	C09	M	M	G	M	G	G	G	G	G	G	H	G	H	G
	C10	H	H	H	H	H	H	H	H	H	H	H	H	H	H
	C11*	G	G	G	G	G	M	M	G	G	G	G	G	G	G
	C12	M	M	G	M		M	P	G	M	G	G	H	H	G
	C14	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Barcelona	C15*	B	P	P	M	M	M	G	M	M	M	M	G	M	G
	C16**	M	M	M	M	M	M	M	M	G	M	M	G	M	M
	C17**	M	M	M	M	G	M	M	M	M	M	M	P	M	M
	C18**	M	M	M	M	M	M	M	M	M	M	P	M	M	M
	C19**	M	M	M	M	P	M	M	M	M	M	M	M	M	M
	C20*	P	M	M	M	M	G	M	G	M	M	G	M	M	M
	C21*					B	M	P	M	M	P	M	M	M	M
	C22*							P	B	B	P	P	P		
	C23	M	M	M	M	M	M	M	M	M	M	M	M	M	M
	C24	M	M	M	M	M	M	M	M	M	M	M	M	M	M

(continued)

Table 4 (continued)

	WB	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Tarragona	C25	G	M	M	M	G	M	M	M	M	G	G	M	M	M
	C26	G	M	G	G	G	H	G	G	H	H	H	H	H	H
	C27**	G	M	G	G	G	G	M	M	M	M	M	M	M	M
	C28	M	G	G	G	G	G	G	M	G	M	G	G	G	G
	C29	M	M	M	M	M	M	M	M	M	M	M	M	M	M
	C30**	G	G	G	G	G	G	G	G	G	G	G	G	M	G
	C31	G	M	M	G	G	G	G	H	H	G	G	H	G	G
	C32	M	M	M	M	M	M	G	G	G	M	M	G	G	G
	C35	M	P	M	G	M	M	M	M	M	M	M	M	M	M

Water bodies without natural rocky sectors are indicated with a single asterisk, while those with less than 10% of natural rocky coast are indicated with a double asterisk

H high ES, *G* good ES, *M* moderate ES, *P* poor ES, *B* bad ES

Table 5 Mean and standard deviation (SD) of EQRs assessed with CARLIT methodology from 1999 to 2012, ES (mean value), and MA-LUSI-WB scores for the different water bodies

	WB	Total length (Km)	Evaluated (%)	Artificial (%)	Natural (%)	Mean (EQR)	SD (EQR)	Mean (ES)	MA-LUSI-WB
Girona									
	C01	48.9	92	5	95	0.81	0.08	H	2.55
	C02	4.0	78	9	91	0.71	0.13	G	2.59
	C03	89.4	98		100	0.86	0.09	H	1.50
	C04	2.7	75	28	72	0.57	0.10	M	2.80
	C05	32.4	89		100	0.93	0.05	H	0.75
	C06	10.6	89	4	96	0.81	0.10	H	2.04
	C07	13.5	17	100		0.53	0.07	M	7.48
	C08	7.9	6	100		0.55	0.10	M	7.25
	C09	11.1	79	34	66	0.66	0.07	G	4.09
	C10	31.5	98	1	99	0.96	0.04	H	1.01
	C11	6.0	12	100		0.64	0.05	G	6.06
	C12	5.2	29	6	94	0.59	0.13	G	5.06
	C14	179.8	88	4	96	0.98	0.01	H	1.04
Barcelona									
	C15	11.6	2	100		0.47	0.12	M	4.51
	C16	27.0	22	91	9	0.57	0.04	M	4.60
	C17	13.4	18	94	6	0.50	0.07	M	4.47
	C18	5.9	8	72	28	0.48	0.07	M	5.03
	C19	14.7	67	97	3	0.48	0.06	M	5.52
	C20	13.4	86	100		0.55	0.08	M	5.57
	C21	4.0	15	100		0.42	0.13	M	7.61
	C22	15.1	2	100		0.27	0.04	P	6.14
	C23	19.4	77	39	61	0.53	0.03	M	2.39
	C24	15.7	56	60	40	0.51	0.03	M	3.19
Tarragona									
	C25	35.5	28	83	17	0.59	0.03	M	3.18
	C26	12.2	56		100	0.75	0.08	H	2.74
	C27	14.2	68	94	6	0.57	0.06	M	5.48
	C28	3.0	96		100	0.62	0.06	G	1.50
	C29	10.0	35	26	74	0.49	0.04	M	4.59
	C30	15.5	28	99	1	0.63	0.03	G	2.56
	C31	23.2	49	38	62	0.70	0.07	G	1.38
	C32	26.4	90	7	93	0.58	0.07	M	1.07
	C35	9.4	54	44	56	0.52	0.08	M	4.44

H high ES, *G* good ES, *M* moderate ES, *P* poor ES, *B* bad ES

great concern here because all surveys have been performed by the same pool of observers. Another factor that can account for interannual differences is the period of the year when the survey is made, but as stated above, surveys were always performed in May to June, dismissing this possibility.

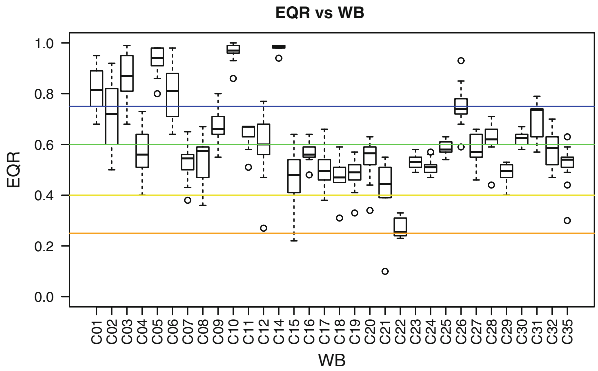

Fig. 3 Box plot showing the average (*black line*), SE value (*box*), SD value (*vertical line*), and outlier values (*circles*) of the EQRs at the Catalan water bodies sampled during a period of 14 years (1999–2012). *Horizontal lines* show boundaries between the different ecological statuses (ESs). *Blue line*, limit between high and good ESs; *green line*, limit between good and moderate ESs; *yellow line*, limit between moderate and poor ESs; *orange line*: limit between poor and bad ESs

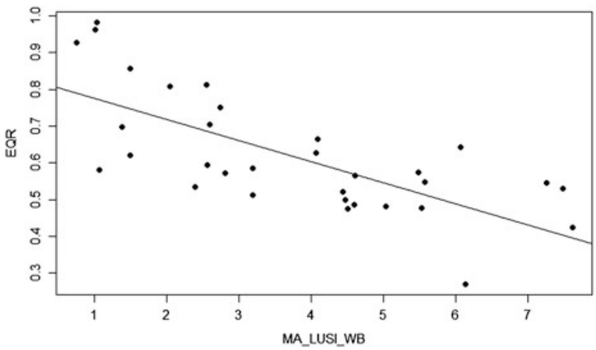

Fig. 4 Relationship between the EQR mean values obtained by CARLIT and the anthropogenic pressures measured according to MA-LUSI-WB index (see Table 2)

Another factor that can account for an interannual shift in the EQR could be a sudden change in coastal geomorphology due to a natural disaster or to a human-induced coastal modification. It is not usual that a human-induced coastal modification encompasses a sector big enough to have an incidence on a WB as a whole. However, we report here one case that fits into this situation. The coastline of the sector C21 was completely modified due to an enlargement of the Barcelona harbor and a deviation of the Llobregat river mouth. The building up of new rocky substrata (breakwaters and quays) changed a big sector of the coast from sandy to rocky and from assemblages dominated by microbes (2003) – typical of bad ecological quality – to assemblages dominated by ephemeral algae (2004) and later to a turf of *Corallina elongata* accompanied by populations of *Mytilus* *galloprovincialis* (year 2005 and beyond), assemblages that indicate a moderate water quality [3].

Other factors that can lead to changes in ES assessment are natural variabilities or changes in human pressures. Natural variability is inevitably associated to ecological systems and we contend here that this should be the major cause of variability in our data, mainly in those WBs where there is not a significant trend of change during the surveyed period. The highest interannual variability corresponds to WBs situated inside little bays (C02, C04) or to WBs located southward of river mouths (C08, C12, C15, and C21). Differences in river discharges associated to rainy periods [42–44] can modify the assemblages close to the inflows. Unexpected strong storms [45–47], differences in nutrients by upwelling processes and river discharges [43, 48, 49], salinity changes [50, 51], temperature variability [50, 52], and hydroclimatic variability [53–55], among other factors, have also been reported as major drivers for Mediterranean marine ecosystems and may be also affecting the interannual variability on the composition and development of upper infralittoral assemblages on rocky shores, and thus, they may be shaping the assessment of the water quality using CARLIT.

Changes in human pressures should revert into changes on indexes based on biological indicators if these indexes are appropriate, irrespectively of the natural variability intrinsically associated to natural assemblages. We have observed an increase in the ES of three WBs that are probably related to an improvement in the water quality at the level of the whole basin. In C09 and C12 the ES has shifted from moderate to good or high, and in C15 it has changed from bad-poor to moderate-high. These three WBs are placed just south of the mouth of three rivers (Fluvià, Ter, and Tordera) whose water quality may have improved [56]. Because the general coastal water circulation in Catalonia is moving southward [57], an improvement on the water quality from these rivers may also imply an increase on the CARLIT values of the coastal water body situated south of the water mouth.

Another example that could be related to water improvement is the shifting from bad to moderate in WB C21. In this WB the water treatment was highly improved, and from 2006 there is a special treatment to reduce nutrient loading [58]. However, and as stated above, the improvement from bad to moderate ES seems to be related to the succession patterns of the assemblages since hard bottoms evaluated in this WB were build up in 2003.

The values of ES obtained with the CARLIT methodology show sometimes striking differences with the ES obtained using other BQEs such as macro-invertebrate assemblages thriving in fine sands (MEDOCC; [56]). For instance, most WBs from Barcelona are rated as moderate and most WBs from Tarragona are rated between moderate and good, using CARLIT; however, they are rated from good to high using the MEDOCC methodology [56]. These differences mainly occur in shores where rocky substrates at the littoral level are mainly artificial and are surrounded by large sandy beaches. Assemblages developing in these artificial substrates are usually dominated by stress-resistant species (*Corallina elongata* and *Mytilus galloprovincialis*) and in some cases by opportunists (green algae and cyanobacteria). *Cystoseira mediterranea* is never encountered in these littoral rocks surrounded by sand. In fact, *C. mediterranea* has been observed growing in artificial breakwaters (authors' pers. obs.) but only when placed around rocky shores with widespread populations of *C. mediterranea* nearby. This is partially to be explained by the low dispersion of *C. mediterranea* zygotes [59], which impairs colonization on sites that are far away from well-developed populations. Another factor that seems to account for the lack of colonization by *C. mediterranea* in these environments is the inhibition of *Cystoseira* recruits by *C. elongata* turfs and *M. galloprovincialis* populations. There is no chance for *Cystoseira* colonization if these stress-resistance space occupiers are not removed [60], a problem that is not only faced by artificial structures but also by degraded natural rocks. Moreover artificial structures are always close to zones with a high human pressure like harbors, whose environmental conditions and water quality do not represent WBs as a whole. For instance, *C. mediterranea* is present on natural rocks in WB C16, but it is completely absent on breakwaters that make up the 90% of the rocky shore in this WB. Thus, results obtained using CARLIT methodology in WBs with a low percentage of rocky shores, mainly when they are not natural, have to be questioned.

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Using MEDOCC (MEDiterranean OCCidental) Index to Evaluate the Ecological Status of Catalan Coastal Waters (Northwestern Mediterranean Sea) Over Time and Depths

Susana Pinedo, Esther Jordana, Marta Manzanera, and Enric Ballesteros

Abstract The Ecological Status (ES) of littoral coastal waters from Catalonia (Northwestern Mediterranean Sea) has been evaluated between 2002 and 2010 using MEDOCC (MEDiterranean OCCidental) index. Macroinvertebrate assemblages inhabiting both littoral fine sands (<20 m depth) and littoral sandy muds (25–35 m depth) have been sampled. The relative abundance of four Ecological Groups (e.g., sensitive, indifferent, tolerant, and opportunistic taxa) has been studied along a disturbance gradient. Sensitive taxa are often present in disturbed situations, and tolerant species are usually associated with the presence of opportunistic taxa, both in fine sands and muddy sediments. Sensitive species dominate in sandy habitats, while tolerant species dominate in muddy bottoms. The ES of Catalan coastal waters measured with the samples collected in littoral fine sands has improved since 2002, and all locations rated above a good ES in 2010. The ES measured with the samples collected in littoral sandy muds was above a good ES although the Ecological Quality Ratio (EQR) was always lower than in littoral fine sands. Consequently, the ES of all water bodies was rated as good or high ES in 2010, and no water body was at risk of noncompliance of Water Framework Directive (WFD) requirements.

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Keywords Catalonia, Littoral fine sands, Littoral sandy muds, Macroinvertebrates, Mediterranean Sea, MEDOCC, Water bodies (WBs), WFD

Contents

Abbreviations

EG	Ecological Group
EGI	Sensitive taxa
EGII	Indifferent taxa
EGIII	Tolerant taxa
EGIV	Opportunistic taxa
EQR	Ecological Quality Ratio
ES	Ecological Status
LUSI	Land Uses Simplified Index
MEDOCC	MEDiterranean OCCidental
MPD	Median Particle Diameter
OM	Organic Matter
SC	Percentage of Silt-Clay
WB	Water Body
WFD	Water Framework Directive

1 Introduction

The main objective of the European Water Framework Directive (WFD [1]) is to achieve "good ecological and chemical status" for all European water bodies (WBs) by 2015. According to the WFD the Ecological Status (ES) should be calculated taking into account at least one of the biological elements listed in the WFD. Soft-bottom macroinvertebrates from coastal waters respond in a predictable and relatively rapid manner to a wide array of natural and anthropogenic stressors [2–7] and include a variety of species with a wide range of feeding modes, stress tolerances, and abilities to withstand disturbances [8, 9].

Several biotic indices have been proposed to measure the ecological quality in coastal waters using macroinvertebrates [10]. Most of them use indicator species or Ecological Groups (EGs) according to their sensitivity/tolerance to stress and are rooted in the model of Pearson and Rosenberg [2] and Rhoads et al. [11], which predict a succession of species along an organic matter gradient [12]. Some authors [13, 14] accept that a biotic index is unlikely to be universally applicable. Moreover, species requirements, preferred habitat, and ecological significance can differ between eco-regions as they depend on environmental conditions and species interactions [15–19]. Therefore, the use of species-specific sensitivity/tolerance values adopted for one region (i.e., the North Atlantic) is not necessarily valid in other regions (i.e., the Northwestern Mediterranean [20–22]). Thus, it is advised that the used methodologies should allow experts of every region to modify the species assignments according to their criteria [18, 23]. Another common problematic issue is to assume that the abundance patterns of the EGs along the disturbance gradient respond in the same way everywhere. Thus, the thresholds between ecological classes assigned for each index when based on the EG patterns could not be always appropriate [24, 25] and may differ from region to region. Here, we will use the index MEDOCC (MEDiterranean OCCidental [22]), a methodology compliant to WFD requirements and based on soft-bottom macroinvertebrate assemblages, which overcomes the two main drawbacks cited above [22].

Several benthic soft-bottom communities have been described at the lower infralittoral and upper circalittoral zones in the Northwestern Mediterranean Sea. Following Guille [26], five benthic communities can be identified along the Catalan coast: (1) the *Spisula subtruncata* community associated with fine sands, (2) the *Nephtys hombergii* community associated with muddy sands, (3) the *Scoloplos armiger* community associated with sandy muds, (4) the *Nucula sulcata* community associated with pure muds, and (5) the *Venus ovata* community inhabiting heterogeneous muds. However, Picard [27] had already proposed a different classification for the Provençal coast: (1) well-sorted fine sand community associated with fine sands, (2) coastal detritic and muddy detritic communities associated with muddy sands, and (3) terrigenous coastal mud community associated with pure muds. Most recently Labrune et al. [28] accomplished the correspondence between the communities identified by Guille [26] and the ones described by Picard [27], together with the communities found during another study carried out between the French-Spanish border and the mouth of the Rhône River. They concluded that there were three different polychaete assemblages associated with depth and sediment granulometry in the Gulf of Lion (Northwestern Mediterranean): (1) littoral fine sand assemblages at 10–20 m depth, (2) littoral sandy mud assemblages at 30 m depth, and (3) terrigenous coastal mud assemblages at 40–50 m depth. In accordance with the knowledge on assemblages' composition in Catalonia [29–32], the last classification was adopted in the present study.

The Catalan coast is a densely populated area where tourism, agricultural, urban development, and industrial activities are the most important land uses [33, http://www.creaf.uab.es; http://www.gencat.net]. The major sources of pollution currently affecting the Catalan coasts are (1) wastewater effluents derived from the increasing population and urban settlements [34], (2) discharges of nutrients and organic matter coming from agriculture and aquaculture activities [35–38], and (3) pollutants coming from industrial development [39–41] mainly associated with big cities (Barcelona and Tarragona). All of these pollution factors discharge close to the coastline deteriorating water quality and changing assemblages' composition. Although the most affected assemblages should be those found at shallow bottoms (littoral fine sands), because of the hydrodynamic conditions and hydromorphological features, the most confined sediments are located at deeper depths (littoral sandy muds) where fine sediments, organic matter, and pollutants accumulate [42–48].

The main objectives of this study were (1) to assess the ES of Catalan coastal waters using MEDOCC applied to macroinvertebrate assemblages inhabiting both littoral fine sand (shallow) and littoral sandy mud (deep) sediments, (2) to explore the temporal quality changes from 2002 to 2010 in shallow assemblages and from 2007 to 2009 in deepwater assemblages, and (3) to compare the ES results based on shallow and deepwater habitats.

2 Materials and Methods

2.1 Data Series

Sediment samples for biological and sedimentological analysis were collected along the Catalan coast (Fig. 1) in June–July at 57 (2002), 19 (2003), 54 (2007), and 44 (2010) locations as a part of an environmental monitoring program to accomplish WFD requirements. The selection of sites in 2002 was made according to a design devoted to assess the ES of coastal WBs following WFD requirements. Samples collected in 2003 were focused in the most disturbed areas (*hotspots*) of Catalonia such as the mouth of Besòs and Llobregat rivers, the shores of Barcelona and Tarragona, and surrounding areas. The information provided by the samples of these 2 years was used to create a preliminary monitoring network in 2007 and a definitive network of locations composed of 44 sites sampled in 2010. All samples (174) were collected in shallow (less than 20 m depth) soft bottoms corresponding to the littoral fine sand habitat [28]. However, aiming both to assess the ES in areas where littoral fine sands were not present and to compare the ES of a WB at different depths, two supplementary surveys were carried out in 2007 and 2009 in deeper sediments (20–35 m depth), corresponding to the littoral sandy mud habitat [28]: 29 sites in 2007 and 7 sites in 2009. Geographical coordinates and sampling depth were recorded at each site.

Two replicate samples were collected at each location with a van Veen grab (600 cm^2). Sediments were sieved through a 0.5 mm sized mesh and preserved in a 4% buffered formaldehyde solution with Rose Bengal. The fauna was sorted and identified to species level (or higher taxonomic ranks if not possible) at the

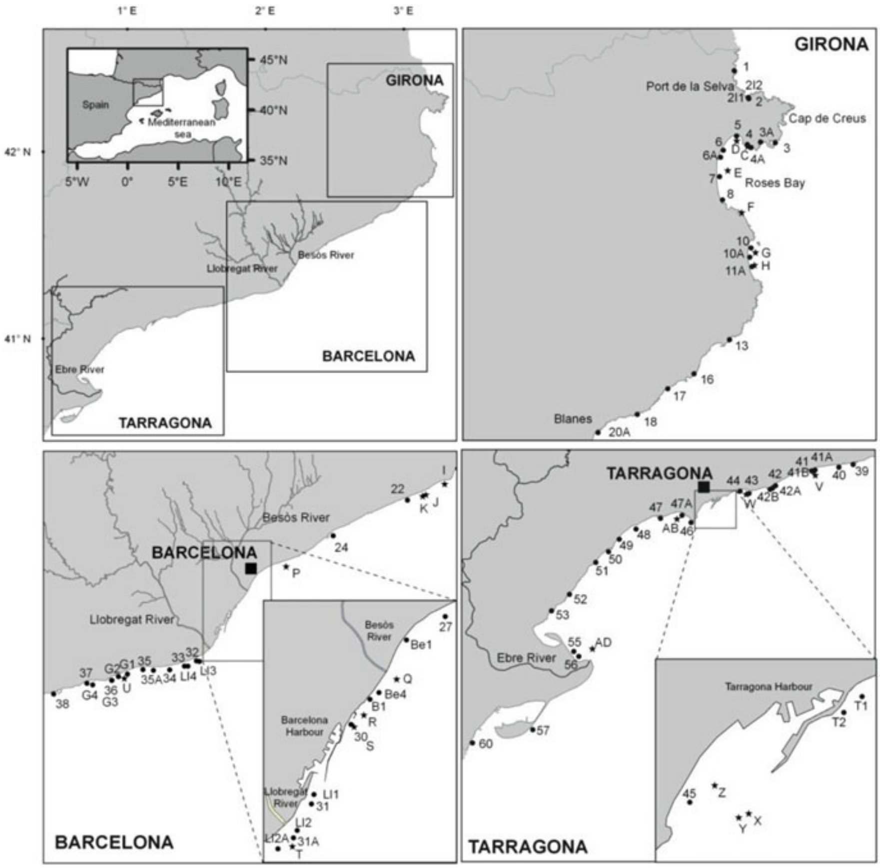

Fig. 1 Locations assessed with MEDOCC index along the Catalan coast (Northwestern Mediterranean) belonging to fine sediments of littoral fine sand (●) and littoral sandy mud (⋆) assemblages

laboratory. Abundances (number of individuals) were estimated for each taxa to characterize the assemblages and to assess the ES. MEDOCC index was applied at each sample, but mean values were used to assess the ES both at each site and WBs in every period.

For each grab, two additional subsamples were obtained: one for determination of grain particles size and organic matter content and the other to analyze heavy metal concentrations. The subsamples were stored at −20°C until processing. Sediment was submitted to the standard dry-sediment procedure for granulometric analysis [49]. Seven classes were considered [50]: mud (<63 μm), very fine sand (63–125 μm), fine sand (125–250 μm), medium sand (250–500 μm), coarse sand (500–750 μm), very coarse sand (750–1,000 μm), and gravel (>1,000 μm). Median Particle Diameter (MPD) and percentage of Silt-Clay (SC, sediment <63 μm) were used to characterize the sediments. Organic Matter (OM) content of sediment was estimated as loss of weight after ashing for 5 h at 450°C. Heavy metals analyzed in both habitats were Al, Cd, Cu, Hg, Pb, V, and Zn. Arsenic, Cr, Fe, Ni, and Se were also analyzed in deep habitats. Under the WFD's requirements [51], Cd, Cu, Hg, Ni, and Pb are considered priority substances. Metals concentrations were determined weighing amounts of about 0.1 g of freeze dried sediment following the methodological procedure described in Pinedo et al. [41].

2.2 MEDOCC (MEDiterranean OCCidental) Index

MEDOCC index [22] was developed for Western Mediterranean soft-bottom macroinvertebrate assemblages aiming at avoiding the drawbacks of other indices. Although MEDOCC was inspired by AMBI [52], discrepancies with this index arose in the number of EGs, the assignment of species to an EG and the different abundance patterns of the EGs along the disturbance gradient in the study area. MEDOCC is based on the degree of sensitivity/tolerance of species to organic enrichment and was summarized into four categories or EGs: sensitive, indifferent, tolerant, and opportunistic. Assignments of every taxa to an EG is based on knowledge available in literature [e.g., 30, 53–56] and expert judgment. Assignments of taxa to EGs provided in AMBI (http://ambi.azti.es) and Bentix (http://www.hcmr.gr) indices have been also taken into account and incorporated or changed according to expert judgment.

MEDOCC index is calculated as following:

$$\mathrm{MEDOCC} = (0 \times \%\mathrm{EGI} + 2 \times \%\mathrm{EGII} + 4 \times \%\mathrm{EGIII} + 6 \times \%\mathrm{EGIV})/100 \quad (1)$$

where EGI, EGII, EGIII, and EGIV correspond to sensitive, indifferent, tolerant, and opportunistic taxa, respectively.

Index values can vary between 0 (only sensitive taxa are present) and 6 (only opportunistic taxa are present).

2.2.1 Boundaries

Boundaries between ES are not predefined in MEDOCC as their selection depends on the distribution pattern of the four EGs along the increasing values of the index itself [22]. The response of EGs along increasing values of MEDOCC index for littoral fine sands was analyzed with samples from 2002, 2003, 2007, and 2010 (Fig. 2) obtaining a result very similar to that obtained by the authors during the development of the index [22]. Moreover, the comparison between this figure and the pattern described by Borja et al. [52] for Atlantic waters shows the same differences observed in Pinedo et al. [22], being the most remarkable the position of EGIII with respect to the curves of the rest of EGs. Sensitive taxa (EGI) were present throughout all the values of MEDOCC, indifferent taxa (EGII) always

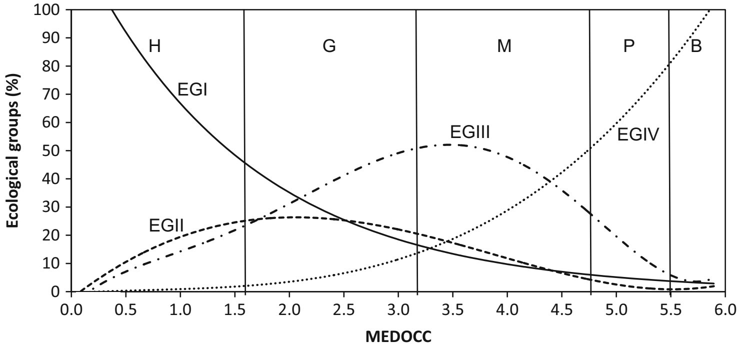

Fig. 2 MEDOCC values according to the percentages of the different Ecological Groups (EGs) for the littoral fine sand assemblages in Catalonia. Data from 2002, 2003, 2007, and 2010 are included. *EGI* sensitive species, *EGII* indifferent species, *EGIII* tolerant species, and *EGIV* opportunistic species. *Vertical lines* show boundaries between the different Ecological Status (ES). *H* high ES, *G* good ES, *M* moderate ES, *P* poor ES, and *B* bad

Table 1 Boundary values delimiting the five ES classes in Catalonia for littoral fine sand and sandy mud habitats

Ecological Status	*Boundaries* (0–6)	*Boundaries* (1–0)
High/good	1.60	0.73
Good/moderate	3.20	0.47
Moderate/poor	4.77	0.20
Poor/bad	5.50	0.08

coexisted with sensitive taxa, and tolerant taxa (EGIII) were more associated with the presence of opportunistic taxa (EGIV). However, in Atlantic waters the EGIII was more associated with the presence of sensitive taxa [52]. Because the obtained patterns are similar to that in Pinedo et al. [22], the same boundaries defining the five levels (high, good, moderate, poor, and bad) of ES required by the WFD were assigned (Table 1) following Pinedo et al. [22].

MEDOCC index was aimed at describing and suggesting the way to establish boundaries for ecological classes based on the composition of the assemblages in terms of relative EG abundances, instead of proposing fixed and rigid values [22]. Thus, to assess the ES of the Catalan coastal waters based on macroinvertebrate assemblages thriving in littoral sandy muds, a new distribution pattern of EGs was represented (Fig. 3). The most relevant difference between Figs. 2 and 3 is the short gradient of MEDOCC values observed for deepwater assemblages. Moreover, while sensitive taxa dominate shallow water assemblages (mean = 55%), tolerant species dominate in deeper assemblages (mean = 39%). However, the shapes of the EG distribution patterns are quite similar, and the boundaries for littoral fine sands also in littoral sandy muds were used.

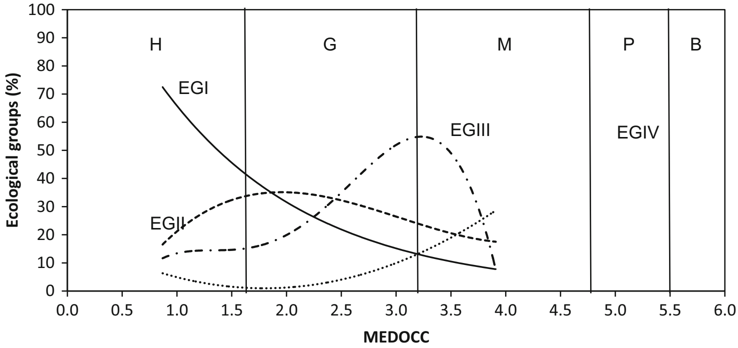

Fig. 3 MEDOCC values according to the percentages of the different Ecological Groups (EGs) for the littoral sandy mud assemblages in Catalonia. Data from 2007 and 2009 are included. *EGI* sensitive species, *EGII* indifferent species, *EGIII* tolerant species, *EGIV* opportunistic species. *Vertical lines* show boundaries between the different Ecological Status (ES). *H* high ES, *G* good ES, *M* moderate ES, *P* poor ES, and *B* bad

2.2.2 Reference Conditions

Reference conditions are places where anthropogenic disturbances are very low or nonexistent. In the absence of pristine areas, the use of "virtual" reference conditions [57] probably is the best approach [58–60]. During the development of MEDOCC index, sites with low anthropogenic pressures were selected; these places had no harbors, no beach regeneration, no urban sewages, no industrial activities, no fish farms, no desalination plants, and no agriculture activities, and they were at least 3 km away from the closer city with more than 1,000 inhabitants.

It is not suitable to oversimplify the approach to assess the values of the biological elements in reference conditions [61] because of the heterogeneity of habitats within the different water bodies [62]. Type-specific reference conditions must summarize the range of possibilities and values for the biological quality element over periods of time and across the geographical extent of the type [63]. In this way, clear and reliable reference conditions for each of the types (even habitat: [24]) should be determined [61]. Thus, habitat-specific reference conditions have been defined for Catalan coastal waters. The best situation (location) was selected to create a "virtual" reference condition both for littoral fine sand (shallow) and sandy mud (deep) habitats. After choosing this "best of all" situation, a new theoretical situation (as reference condition) was created where fauna composition differs depending on the habitat: the fauna is composed by (1) sensitive and indifferent taxa, with 90% EGI and 10% EGII, and a MEDOCC = 0.2 for littoral fine sand assemblages and (2) sensitive, indifferent, and tolerant taxa with 75% EGI, 15% EGII, and 10% EGIII and a MEDOCC = 0.7 for littoral sandy mud habitats.

2.2.3 EQR (Ecological Quality Ratio) Calculation

MEDOCC index as calculated in (1) varies from 0 (high ES) to 6 (bad ES) in a scale reverse to EQR scale. Following WFD requirements, the EQR values have to be calculated by rescaling MEDOCC values between 1 (high) and 0 (bad) and introducing the correction for the reference condition ($MEDOCC_{ref}$). Therefore, the EQR is given by:

$$EQR = 1 - [(MEDOCC_{obs} - MEDOCC_{ref})/(6 - MEDOCC_{ref})] \qquad (2)$$

where $MEDOCC_{obs}$ is the MEDOCC value observed for a WB or a location and $MEDOCC_{ref}$, the MEDOCC value considered as the reference condition.

2.2.4 Response to Pressures

The existence of a good relationship between MEDOCC index and some relevant anthropogenic pressures was demonstrated in Pinedo et al. [22] for littoral fine sands. Organic matter content and LUSI (Land Uses Simplified Index [64]), as a measure of the global pressure, were used to assess the relationship between MEDOCC index and environmental pressures in littoral fine sands. MEDOCC index values increased when both LUSI and organic matter increased [22]. When testing the response of the index to OM content based on littoral sandy mud assemblages, a significant ($\rho = 0.571$; $p < 0.05$) relationship was also observed (Fig. 4). However, when OM > 3%, the index did not respond as expected.

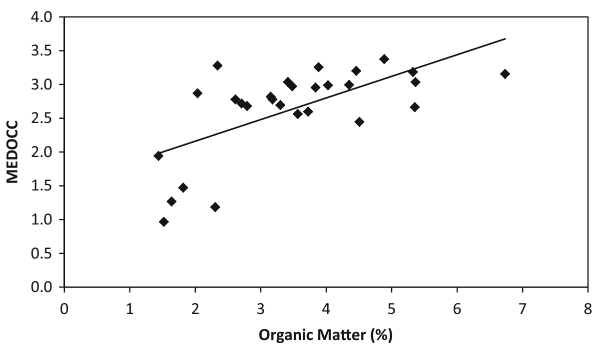

Fig. 4 Response of MEDOCC index to organic matter content in sediments of littoral sandy mud habitats. The linear regression is shown

3 Results

3.1 Sedimentological Variables

The results for sedimentological variables (granulometrical characteristics, organic matter content, and heavy metals concentrations) for shallow sediments are explained in Pinedo et al. [41]. The most remarkable differences compared to deepwater sediments (Table 2) were the finer fraction composition and the organic matter content. The average value of SC was 7% in shallow sediments compared to 28% in deepwater bottoms. Furthermore the MPD was obviously higher in shallow sediments (126 μm average value) than in deeper ones (103 μm). The interannual comparison analyzed by Pinedo et al. [41] for shallow habitats reveals that the SC decreased in 2007 in most sites sampled in 2002–2003. The year 2007 was dry compared to 2010 [41], and therefore, the inputs of freshwater material to the sea were lower. However, this general pattern was not observed for locations sampled in deep waters between 2007 and 2009. The percentage of SC increased substantially from 2007 to 2009 at sites X and Y, while at J and R decreased.

Regarding OM content, the maximum value registered in deepwater sediments was 6.7% at the mouth of Besòs River (site Q) in 2007, while 3.0% was the maximum value observed in shallow sediments during the studied period [41]. A decrease in organic matter content (with the exception of site K) was observed in deepwater sediments during 2009. However, the OM content did not change significantly during the surveys in shallow bottoms [41].

The metal concentrations in deepwater sediments did not overpass the registered values detected in shallow ones [41]. The higher values detected in the Besòs River mouth and Barcelona surrounding areas (sites Q and R) for Zn and Pb matched with those registered in shallow sediments in Pinedo et al. [41]. Mercury concentration in deepwater sediments registered the highest values both in Barcelona and Tarragona coastal sites (R, S, and Y).

3.2 Ecological Status

The ecological status provided by MEDOCC index for each location was calculated for samples with fine to mud sand sediments (Fig. 1). Samples with medium and coarse sands were excluded for ES calculation as MEDOCC index was developed to be only applied in fine sediments [22]. Moreover, samples with more than 20% of the individuals without any EG assignment were also excluded to assess the ES. Thus, from the whole dataset, 44 sites in 2002, 13 in 2003, 53 in 2007, and 39 in 2010 were used representing littoral fine sands (shallow), while 22 sites in 2007 and 6 in 2009 were used representing littoral sandy mud (deepwater) sediments (Fig. 1).

The 71% of shallow sites were rated high in 2002, 25% good, and only one site (2%) was rated moderate and another (2%) bad (Table 3). The moderate classified

Table 2 Physicochemical values (average of two replicates) at the littoral sandy mud studied sites along the Catalan coast: percentage of organic matter content (OM, %), percentage of silt-clay (SC, %), median particle diameter (MPD, μm), and metal concentration are expressed as μg/g except for Al (%). Two or more values into a variable for a site mean that the same site was sampled in different years. Site order in the table follows a north–south geographical position. Values for Se equal to zero were under detection limit of the spectrometer

Site	WB	Year	OM	SC	MPD	Zn	Cu	Pb	V	Cd	Hg	Se	As	Cr	Ni	Fe	Al
Girona																	
C	C06	07	4.51	21.34	176	60.39	11.40	24.74	46.78	0.11	0.070	0.60	20.57	24.01	13.23	1.93	1.57
D	C07	07	2.71	51.78	61	50.49	9.96	15.11	35.24	0.13	0.065	0.00	13.69	21.13	13.80	1.81	1.30
E	C08	07	3.18	27.79	94	49.96	9.80	12.70	32.78	0.14	0.053	0.00	11.00	21.10	15.96	1.94	1.41
F	C10	07	1.44	7.41	90	36.86	5.54	8.52	24.44	0.10	0.018	0.00	12.20	14.28	13.67	1.56	0.89
G	C11	07	1.52	2.47	129	58.12	6.65	16.36	26.50	0.09	0.026	0.00	12.71	16.17	16.40	1.97	1.19
H	C12	07	1.82	1.34	176	69.13	6.77	19.43	28.23	0.09	0.023	0.00	13.10	9.34	14.72	2.03	1.24
Barcelona																	
I	C15	07	3.30	18.77	86	111.12	15.28	29.41	47.23	0.14	0.068	0.12	6.98	23.94	12.54	2.95	2.07
J	C15	07/09	4.46/2.03	37.12/11.54	72/220	114.18/92.29	16.98/12.64	35.70/24.81	48.70/39.27	0.16/0.12	0.101/0.257	1.27/1.74	10.00/8.48	26.39/17.29	14.52/10.74	2.91/2.39	2.16/1.63
K	C15	07/09	3.84/4.35	25.77/26.72	98/72	98.47/49.50	14.68/8.93	27.40/18.30	46.97/33.46	0.11/0.09	0.059/0.043	0.10/1.71	8.44/12.79	21.86/15.30	11.41/10.12	2.62/1.36	1.95/1.13
P	C17	07	2.31	10.74	91	78.58	9.83	17.38	58.61	0.06	0.092	0.10	12.72	16.28	6.61	2.71	1.90
Q	C19	07/09	6.73/5.35	25.56/24.36	86/85	196.63/177.80	63.39/52.48	61.16/56.11	48.39/38.72	0.53/0.51	0.523/0.243	0.49/1.77	11.16/9.91	95.40/89.58	28.25/17.48	2.72/2.14	2.54/1.74
R	C19	07/09	3.88/2.61	28.50/14.14	84/89	242.03/191.09	62.84/50.48	87.10/77.43	44.04/32.12	0.89/0.66	1.128/0.153	0.04/1.68	15.80/16.85	96.32/58.52	16.64/15.42	2.37/1.75	1.79/1.20
S	C20	07	4.03	22.27	91	385.84	83.62	117.22	47.34	1.36	1.524	0.32	21.44	116.17	18.77	2.54	1.77
T	C21	07/09	4.89/2.34	33.12/33.21	203/63	127.07/62.02	41.56/14.29	41.93/21.47	43.26/21.29	0.42/0.21	0.488/0.057	0.38/1.28	14.43/9.06	51.72/22.64	22.88/13.81	2.65/1.41	2.42/0.98
U	C23	07	3.15	28.68	84	62.87	15.27	25.81	29.44	0.19	0.251	0.05	12.75	29.44	11.12	1.66	1.22
Tarragona																	
V	C25	07	2.79	54.96	61	51.61	12.03	22.17	25.11	0.21	0.308	0.23	9.86	24.42	10.60	1.42	1.12
W	C26	07	3.48	45.81	65	47.22	11.27	22.69	25.30	0.18	0.251	0.04	8.65	23.86	8.47	1.27	1.12
X	C27	07/09	5.33/4.17	43.56/61.17	70/46	75.60/72.43	19.40/17.57	31.88/30.63	38.91/33.36	0.19/0.16	0.945/0.441	0.32/1.61	9.35/10.18	33.92/29.27	13.84/17.39	1.78/1.64	1.80/1.56
Y	C27	07/09	5.36/3.57	43.41/54.38	69/53	72.02/71.00	17.26/17.38	29.28/29.77	38.62/32.66	0.23/0.17	1.303/0.610	0.00/1.76	10.08/10.27	32.40/28.51	13.65/16.97	1.71/1.64	1.71/1.50
Z	C27	07	1.64	8.46	128	35.51	5.83	9.95	19.44	0.28	0.938	0.00	7.81	10.47	4.35	0.86	0.63
AB	C29	07	3.42	52.93	61	62.01	12.37	23.55	36.80	0.14	0.431	0.00	10.13	21.45	10.89	1.63	1.47
AD	C33	07	3.72	20.02	109	56.72	11.72	22.08	33.58	0.26	0.383	0.00	10.21	23.43	14.32	1.73	1.41

location (site 2) was located in the little bay of Port de la Selva, north of Girona, and the bad evaluated site was situated at the mouth of Llobregat River (site 31). Unfortunately, during the following surveys it was not possible to sample the site 31 as it was replenished of sediment up to the surface by infrastructural works in Barcelona harbor. Regarding the locations sampled in 2003, 46% were rated high, 46% were good, and only one site, located at the mouth of Besòs River (Be4), was moderate (8%). In 2007, 77% of sites were rated high, 19% good, and two sites (4%) moderate: site 5, located at the Roses Bay near the harbor, and site 31A at the mouth of Llobregat River. Finally, 67% of the locations were rated high and 33% good in 2010.

The ES comparison between years (Table 3) for shallow habitats shows that some locations remained with the ES unchanged, although variations in their EQR values were observed. However, other locations displayed remarkable changes. As mentioned above, site 2 increased the ES from moderate to high; Be4 changed progressively from moderate (2002) to good (2010), and 31A switched from moderate in 2007 to high in 2010. Other less relevant changes from high to good and vice versa were common. In general, while ES remained unchanged in the coast of Girona, the other sectors of the coast displayed a high variability.

When the MEDOCC was applied to deepwater assemblages (Table 4), most of the locations rated good in 2007 (77%) and all rated good in 2009. Comparing the ES between years for the six reassessed sites, all of them maintained the ES, but the EQR value mostly increased in 2009.

During 2007 both shallow and deep sediments were monitored. Some locations are close to each other but at different depths (Fig. 1 and Table 5). Deepwater assemblages usually showed lower EQRs than shallow water ones (Fig. 5).

When looking at the ES in WBs at shallow waters, some of them remain unchanged over time, mainly in Girona coastal waters (Table 6). However, there was a general trend to increase the EQR value in 2010 in Girona and Barcelona, while a slight decrease was observed in some WBs at the southern coast of Barcelona and in Tarragona. Only the southern section of Tarragona coast (WBs C33, C34, and C35) increased the EQR value and ES at the last survey. The most relevant shifts were, however, the increasing ES at C02 from moderate in 2002 to high ES both in 2007 and 2010 and, on the contrary, the decreasing ES changing from high (in 2002, 2003, or 2007) to good ES in 2010 at C24, C26, C29, and C32. Water bodies C12, C16, and C20 could not be compared because they were only sampled during one survey. Only two WBs (C03 and C04) were not evaluated based on shallow assemblages over time.

Regarding the assessment of ES of WBs based on deepwater assemblages, no differences were detected between 2007 and 2009 (Table 6). In this case nearly half of the total WBs were not evaluated as they were not sampled twice.

Finally, the comparison of the ES of WBs obtained based both on shallow and deepwater assemblages was possible in 14 WBs (Table 6). Although six WBs obtained the same ES, the EQR obtained in deepwater assemblages tended to be lower than that obtained in the shallowest ones. Moreover, most of the WBs rated high in shallow sediments rated good in deeper habitats. Changes in the ES

Table 3 Ecological Quality Ratio (EQR) and Ecological Quality Status (ES) of littoral fine sand samples in Catalonia (NW Mediterranean Sea) from 2002 to 2010. WB: water body in which the sample is situated. *H* high ES, *G* good ES, *M* moderate ES, *B* bad ES

Site	WB	Year	EQR	ES		
Girona						
1	C01	02/07/10	0.90/0.91/0.96	H	H	H
2	C02	02/07/10	0.44/0.94/0.83	M	H	H
2I1	C02	07	0.85		H	
2I2	C02	07	0.85		H	
3	C05	02	0.84	H		
3A	C05	07	0.75		H	
4A	C06	07/10	0.81/0.80		H	H
4	C06	02/07/10	0.71/0.53/0.75	G	G	H
5	C07	02/07/10	0.71/0.46/0.69	G	M	G
6	C07	02/07/10	0.69/0.75/0.71	G	H	G
6A	C07	07/10	0.75/0.75		H	H
7	C08	02/07	0.79/0.77	H	H	
8	C09	02/07/10	0.79/0.78/0.90	H	H	H
10	C11	02/07/10	0.70/0.96/0.93	G	H	H
10A	C11	07	0.76		H	
11A	C12	07	0.89		H	
13	C14	02/07	0.87/0.88	H	H	
16	C14	02/07/10	0.70/0.74/0.84	G	H	H
17	C14	02/07	0.88/0.90	H	H	
18	C14	02/07/10	0.95/0.92/0.94	H	H	H
20A	C14	07/10	0.89/0.87		H	H
Barcelona						
22	C16	02	0.84	H		
24	C16	02	0.93	H		
27	C18	02	0.87	H		
Be1	C18	03/07/10	0.72/0.70/0.71	G	G	G
Be4	C19	03/07/10	0.29/0.58/0.71	M	G	G
B1	C19	03/07	0.80/0.79	H	H	
30	C20	02	0.78	H		
31	Land	02	0.07	B		
Ll1	Land	03	0.52	G		
Ll2	Land	03	0.71	G		
31A	C21	07/10	0.45/0.86		M	H
Ll2A	C21	07/10	0.74/0.81		H	H
Ll3	C22	03	0.76	H		
32	C22	02	0.88	H		
33	C22	02/07/10	0.84/0.82/0.93	H	H	H
Ll4	C22	03	0.76	H		
34	C22	02	0.81	H		

(continued)

Table 3 (continued)

Site	WB	Year	EQR	ES		
35A	C22	07/10	0.73/0.87		G	H
35	C22	02/07/10	0.59/0.68/0.71	G	G	G
G1	C23	03	0.70	G		
G2	C23	03/07	0.72/0.72	G	G	
36	C23	02	0.88	H		
G3	C23	03	0.82	H		
37	C23	02/07/10	0.87/0.93/0.75	H	H	H
G4	C23	03	0.67	G		
38	C24	02/07/10	0.71/0.94/0.73	G	H	G
Tarragona						
39	C25	02/07/10	0.89/1.02/0.83	H	H	H
40	C25	02	0.75	H		
41	C25	02/07/10	0.80/0.82/0.89	H	H	H
41A	C25	07	0.81		H	
41B	C25	07	0.86		H	
42	C25	02/07/10	0.80/0.88/0.65	H	H	G
42A	C25	07	0.77		H	
42B	C25	07	0.85		H	
43	C26	02	0.89	H		
44	C26	02/07/10	0.87/0.86/0.73	H	H	G
T1	C27	03/07/10	0.87/0.88/0.61	H	H	G
T2	C27	03	0.94	H		
45	C27	02/07/10	0.93/0.92/0.91	H	H	H
46	C28	02/07/10	0.88/0.88/0.75	H	H	H
47A	C29	07/10	0.80/0.58		H	G
47	C30	02/07/10	0.86/0.88/0.81	H	H	H
48	C30	02/07/10	0.85/0.90/0.71	H	H	G
49	C31	02/07/10	0.70/0.84/0.92	G	H	H
50	C31	02	0.84	H		
51	C31	02/07/10	0.82/0.89/0.72	H	H	G
52	C32	02/07/10	0.76/0.89/0.90	H	H	H
53	C32	02/07/10	0.69/0.71/0.50	G	G	G
55	C33	02/07/10	0.72/0.80/0.93	G	H	H
56	C33	02/07/10	0.64/0.59/0.75	G	G	H
57	C34	02/07/10	0.89/0.68/1.00	H	G	H
60	C35	02/07/10	0.76/0.58/0.76	H	G	H

classification usually follow one of these three different patterns: (1) same evaluation in 2007 both in shallow and deepwater sediments (good ES), but increased to high ES in 2010 only for shallow habitats (C21); (2) different ES in 2007 for both habitats (high ES in shallow assemblages and good ES in deeper ones), but in some cases the ES of shallow sediments decreased to good ES in 2010 (C26 and C29),

Table 4 Ecological Quality Ratio (EQR) and Ecological Quality Status (ES) of littoral sandy mud samples in Catalonia (NW Mediterranean Sea) for 2007 and 2009. WB: water body in which the sample is situated. *H* high ES, *G* good ES, *M* moderate ES, *B* bad ES

Site	WB	Year	EQR	ES	
Girona					
C	C06	07	0.67	G	
D	C07	07	0.62	G	
E	C08	07	0.61	G	
F	C10	07	0.77	H	
G	C11	07	0.95	H	
H	C12	07	0.85	H	
Barcelona					
I	C15	07	0.62	G	
J	C15	07/09	0.53/0.59	G	G
K	C15	07/09	0.57/0.57	G	G
P	C17	07	0.91	H	
Q	C19	07/09	0.54/0.63	G	G
R	C19	07/09	0.52/0.61	G	G
S	C20	07	0.57	G	
T	C21	07/09	0.50/0.51	G	G
U	C23	07	0.60	G	
Tarragona					
V	C25	07	0.63	G	
W	C26	07	0.57	G	
X	C27	07	0.53	G	
Y	C27	07/09	0.56/0.65	G	G
Z	C27	07	0.89	H	
AB	C29	07	0.56	G	
AD	C33	07	0.64	G	

reaching the same ES than deepwater assemblages; and (3) the ES of shallow sediments maintained high in 2010 (C23, C25, and C27). The case observed at C27 was the most notable as high ES was always obtained in shallow waters, while good ES was assessed in deeper ones.

4 Discussion

The application of MEDOCC index both in shallow and deepwater soft-bottom assemblages in coastal waters from Catalonia shows that the distribution of EGs frequency is quite similar even though the habitats are different, the shallow ones corresponding to littoral fine sands and the deep ones corresponding to littoral sandy muds. However, although the patterns are similar, the EGII and EGIII reach slightly higher values in deep waters. Conversely, EGI is higher in shallow assemblages than in deeper ones. The dominance of sensitive taxa (mean average value = 55%) in shallow assemblages and of tolerant species (mean average

Table 5 Site comparability between littoral fine sand (shallow) and sandy mud (deep) assemblages in the 2007 survey

Littoral fine sands	Littoral sandy muds
4A, 4	C
5	D
7	E
10, 10A	G
11A	H
Be4	Q
B1	R
Ll2A	T
G2	U
41, 41A, 41B	V
45	X, Y
47A	AB
56	AD

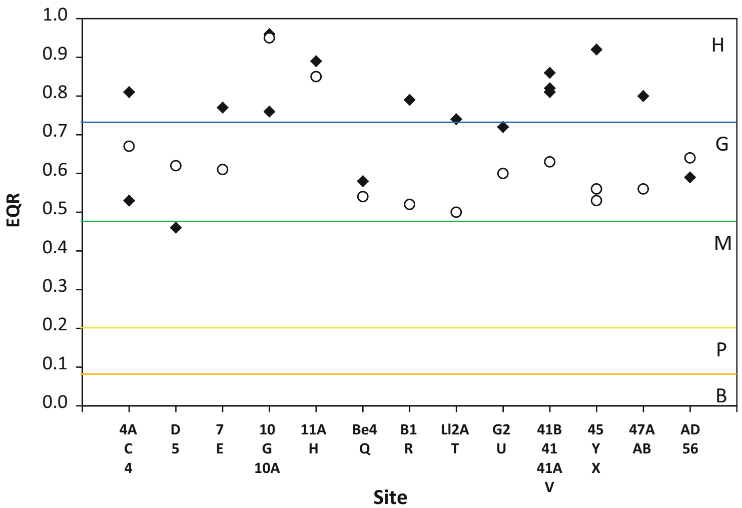

Fig. 5 Comparison of EQR values obtained applying MEDOCC index at littoral fine sand (♦) and sandy mud (○) assemblages sited close to each other during 2007 survey. *Horizontal lines* show boundaries between the different Ecological Status (ES). *H* high ES, *G* good ES, *M* moderate ES, *P* poor ES, and *B* bad

value = 39%) in deep assemblages seems to be related to the organic matter enrichment observed in the littoral sandy muds typical of deep waters. Moreover, opportunistic species contribute considerably in deepwater assemblages (mean average value = 8%), which is again related to the higher OM content. Species can either actively respond to the increase in OM or remain indifferent. Moreover, the presence of tolerant and opportunistic species, deposit-feeder organisms mainly,

Table 6 Ecological Quality Ratio (EQR) and Ecological Quality Status (ES) of Catalan water bodies from 2002 to 2010 for littoral fine sand and sandy mud assemblages. *WB* water body. *H* high ES, *G* good ES, *M* moderate ES, *B* bad ES

	Littoral fine sand						Littoral sandy mud			
WB	Year	EQR	ES				Year	EQR	ES	
Girona										
C01	02/07/10	0.90/0.91/0.96	H		H	H				
C02	02/07/10	0.44/0.88/0.83	M		H	H				
C03										
C04										
C05	02/07	0.84/0.75	H		H					
C06	02/07/10	0.71/0.67/0.78	G		G	H	07	0.67	G	
C07	02/07/10	0.70/0.66/0.72	G		G	G	07	0.62	G	
C08	02/07	0.79/0.77	H		H		07	0.61	G	
C09	02/07/10	0.79/0.78/0.90	H		H	H				
C10							07	0.77	H	
C11	02/07/10	0.70/0.86/0.93	G		H	H	07	0.95	H	
C12	07	0.89			H		07	0.85	H	
C14	02/07/10	0.85/0.87/0.88	H		H	H				
Barcelona										
C15							07/09	0.58/0.58	G	G
C16	02	0.88	H							
C17							07	0.91	H	
C18	02/03/07/10	0.87/0.72/0.70/0.71	H	G	G	G				
C19	03/07/10	0.54/0.68/0.71		G	G	G	07/09	0.53/0.62	G	G
C20	02	0.78	H				07	0.57	G	
C21	02/03/07/10	0.07/0.62/0.60/0.83	B	G	G	H	07/09	0.50/0.51	G	G
C22	02/03/07/10	0.78/0.76/0.74/0.84	H	H	H	H				
C23	02/03/07/10	0.88/0.73/0.83/0.75	H	G	H	H	07	0.60	G	
C24	02/07/10	0.71/0.94/0.73	G		H	G				
Tarragona										
C25	02/07/10	0.81/0.86/0.79	H		H	H	07	0.63	G	
C26	02/07/10	0.88/0.86/0.73	H		H	G	07	0.57	G	
C27	02/03/07/10	0.93/0.90/0.90/0.76	H	H	H	H	07/09	0.66/0.65	G	G
C28	02/07/10	0.88/0.88/0.75	H		H	H				
C29	07/10	0.80/0.58			H	G	07	0.56	G	
C30	02/07/10	0.85/0.89/0.76	H		H	H				
C31	02/07/10	0.79/0.86/0.82	H		H	H				
C32	02/07/10	0.72/0.80/0.70	G		H	G				
C33	02/07/10	0.68/0.70/0.84	G		G	H	07	0.64	G	
C34	02/07/10	0.89/0.68/1.00	H		G	H				
C35	02/07/10	0.76/0.58/0.76	H		G	H				

induces an intense bioturbation [65], which would contribute to the settlement of indifferent species that are always present in low densities.

The different SC between shallow sands and deep sandy muds would determine the amount of OM trapped in the sediment. The maximum value for OM in shallow sediments is 3%, although the majority of sediments did not overcome the 2% [41]; on the contrary, OM content is usually around 4% in deepwater sediments. Both factors, SC and OM content, have been reported to affect directly the species composition of the assemblages [2, 28, 66] and the structure of polychaete assemblages both in the Mediterranean Sea [67, 68] and elsewhere [69, 70]. Thus, the different frequency of EGs depending on the habitats seems to be related to the SC and OM in the sediment.

When regarding the dominance of EGs in the three coastal sectors (Girona, Barcelona, and Tarragona), different compositions were also observed depending on the habitats. In shallow waters the higher dominance of sensitive taxa in Girona and Tarragona could be related to less disturbed habitats than those from Barcelona, where a decrease in sensitive taxa in behalf of increasing opportunistic species is observed. In deepwater habitats tolerant taxa dominate both in Barcelona and Tarragona, and the abundance of opportunistic species represents the 11% and 7%, respectively. In general, the EG composition indicates that the most structured assemblages are observed in Girona, while the most fluctuating ones related to disturbed habitats are found in Barcelona. This observation is reflected in the ES results as the minimal interannual variation of the ES was observed in Girona, while it was higher in Barcelona and Tarragona.

The MEDOCC index can be applied to littoral sandy muds, but we found a weak, although significant, response to OM enrichment as already noted by Pinedo et al. [22]. MEDOCC indices never exceed 3.5 (Fig. 4), even at OM content higher than 3%. However, similar or worse responses are observed with other indices such as AMBI ($\rho = 0.374$; $p < 0.05$) or BOPA ([71]; $\rho = 0.349$; n.s.) when they are applied to our data and related to the OM gradient.

Outputs from rivers, such as Besòs, Llobregat, and Ebre, result in an increase in the SC and OM of superficial sediments in Catalan coasts [41, 44, 72, 73]. Disturbed shallow water assemblages have been observed close to the mouths of the Besòs (site Be4 in 2003) and Llobregat rivers (31 in 2002 and 31A in 2007). The SC at these locations is high enough to harbor high levels of OM although it does not reach the 2%. The worst classified site located in the nearshore area of Llobregat River (site 31) registered only 1% of OM content in the sediments, but the abundance of the opportunistic polychaete *Capitella capitata* was extremely high (2,783 individuals in 600 cm^2). The elevated abundance of this species is responsible for the bad ES assessment. Kinoshita et al. [74] observed that in a process of rapid population growth of *Capitella*, the decomposition of organic matter in the sediment was enhanced. During 2003 the area was monitored by sampling the location Ll1 that rated good, with an EQR value equal to 0.52. The shift from an EQR of 0.07 in 2002 to 0.52 in 2003 shows a significant progress in the ES in only 1 year. The improvement of wastewater systems and the construction of a sewage effluent offshore since 2002–2003 in Llobregat River (http://www.gencat.net [75]) should be responsible for the improvement. Unfortunately it was not possible to resample neither location 31 nor Ll1 during the following surveys because of the infrastructural works in the Barcelona harbor and surrounding areas and the deviation of the Llobregat River mouth in 2004. The increasing EQR observed in the mouth of Besòs River (Be4) over time, matching with an improvement of the ES since 2003, seems to be related to the implementation of a new wastewater system in 2005 [75]. Thus, MEDOCC index detects highly polluted environments when they are present.

Other factors, such as exceptional raining periods and storms, also increase the inputs of SC and OM. The SC at site 2 changed from 23% in 2002 to less than 1% in 2007, and OM content decreased also from 2002 to 2007 [41], which points to a period of heavy rain registered in 2002 (http://www.gencat.net). Thus, natural factors can also change the composition of the assemblages and consequently EG frequencies and ES.

Both the morphology of the continental shelves and the energy of the hydrodynamic processes in the Northwestern Mediterranean allow the accumulation of sedimentary deposits around the mouths of most rivers [40, 76] where not only fine sediments and OM are deposited but also heavy metals. Some of the locations are subjected to heavy metals pollution [41], which also affect the biota [77–80], increasing the abundance of tolerant and opportunistic species at the most disturbed locations. The moderate ES observed in site 5 in 2007, although with an EQR value close to the boundary between moderate and good, demonstrates that the index detects the increase not only in OM but also in Zn and Cu concentrations registered in 2007 [41].

Although the 2003 survey was designed to assess the ES at potentially highly disturbed areas, only one location was classified as moderate at the mouth of Besòs River (Be4). This result indicates that many pressures related to the industrial activity acting in this area [40] do not origin a high impact on macroinvertebrate assemblages on the shallow soft-bottom habitats. Neither in 2007 nor in 2010, the shallow samples were significantly disturbed as only two locations – site 5 mentioned above and site 31A sited at the southern area of the new Llobregat River mouth – were rated moderate in 2007. Moreover, in 2010 the ES increased again changing from moderate to good at the first location and to high at the second. The conclusion is that there is an improvement in the ES since 2002 in shallow coastal sediments, although many sites get worse from high ES to good ES in 2010. Future monitoring programs are needed to explore this decline in ES.

Our data also suggests that the ecological quality of macroinvertebrate assemblages in deep waters is always worse than that observed for the shallowest sediments, but also less variable over time. The accumulation of fine sediments and OM in deepwater bottoms enhances the presence of tolerant and opportunistic species. Likewise, deepwater habitats are more stable environments than the shallowest ones, and assemblages suffer few changes in species abundance and composition determining less variability in the ES.

Another factor that can affect MEDOCC values is recruitment. As the index is calculated based on the abundance (number of individuals) of the four EGs, very high abundances of recruits or juveniles of one species belonging to a particular EG affects to the frequency of the rest of the groups. Thus, natural and intrinsic events of the populations themselves are able to change the ES results as they have to the dataset the same effect of a perturbation when recruits belong to tolerant or opportunistic species. Thus, it is possible to find erroneous disturbed situations during recruitment periods although they quickly recover in the post-recruitment period [3, 30]. On the contrary, better ES would be assessed when recruits belong to sensitive organisms. In fact, the seasonal dynamics of shallow soft-bottom macroinfaunal assemblages in the southern coast of Girona (Blanes Bay) exhibited a very predictive annual cycle, with abundances rising sharply during spring, followed by a striking drop through summer and showing lower values during autumn and winter [29, 30, 32]. Our sampling surveys were performed in June–July to avoid recruitment events [29].

It is relevant that there is no location/water body in default risk of WFD requirements in 2010, at least regarding soft-bottom macroinvertebrates as biological quality elements, both in shallow and deepwater habitats. Only two WBs (C03 and C04) located in the Cap de Creus (northern coast of Girona) have not been evaluated in this study as the habitats (littoral fine sands or sandy muds) are lacking [81]. Other locations have been sampled only occasionally, but in our opinion this does not affect the big picture.

In conclusion, regarding macroinvertebrate soft-bottom assemblages inhabiting both littoral fine sand and sandy mud habitats, there is no water body in default risk of WFD requirements in 2010 in the Catalan coast. It seems that the wastewater management is effective enough to maintain at least a good ES for the WBs and thus Catalonia being compliant with the WFD objectives by 2010. However, future monitoring programs are needed to explore if the ES obtained in 2010 is preserved at present.

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Assessing the Environmental Quality in Heavily Modified Transitional Waters: The Application of MEDOCC (MEDiterranean OCCidental) Index in Ebre Delta Bays

Esther Jordana, Susana Pinedo, and Enric Ballesteros

Abstract The assessment of ecological quality in the frame of the Water Framework Directive has been performed in Ebre Delta bays (Alfacs and Fangar) using MEDOCC (MEDiterranean OCCidental) index. Results demonstrate the applicability of the index in heavily modified transitional water bodies and its response to pressures (mainly agricultural activities) affecting macroinvertebrate assemblages inhabiting soft bottoms. According to previous studies and the organic matter enrichment and heavy metal gradients inside the bays, three different Transitional Waters (TW) have been distinguished, but only two of them are assigned to Heavily Modified Water Bodies (HMWB). Two different reference conditions have been considered depending on the acting pressures: a Maximum Ecological Potential (MEP) for the areas highly affected by the pressures (i.e., HMWB) and a Reference Condition (RC) for non-modified waters from the southern shelf of Alfacs Bay where the effect of agricultural activity is scarce. A good Ecological Potential/Status (EP/ES) was always achieved in the surveys.

Keywords Ebre Delta bays, Heavily Modified Water Bodies (HMWB), Macroinvertebrates, Maximum Ecological Potential (MEP), Mediterranean sea, MEDOCC (MEDiterranean OCCidental), Transitional Waters (TW)

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Contents

Abbreviations

EG	Ecological Group
EP	Ecological Potential
EQR	Ecological Quality Ratio
ES	Ecological Status
GES	Good Ecological Status
HMWB	Heavily Modified Water Body
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
MEDOCC	MEDiterranean OCCidental
MEP	Maximum Ecological Potential
RC	Reference Condition
TW	Transitional Water
WFD	Water Framework Directive

1 Introduction

Estuarine environments and coastal lagoons (Transitional Waters (TW) following Water Framework Directive (WFD) [1] definitions) are among the most productive ecosystems on earth, providing important socioeconomical resources [2–4]. In addition to human activities, these ecosystems are exposed to a wide variation of physicochemical parameters due to their particular hydrogeographic conditions (confinement, shallowness, marine influence, freshwater inputs, etc.). Sometimes, the response of communities to natural and anthropogenic stress is similar, making it difficult to discern between natural and human-induced changes [5–7]. This feature has implications for the implementation of environmental management actions and requires an accurate interpretation of the data.

Among several legislative instruments for the environmental management of water bodies, the WFD establishes a common basis to protect and improve the ecological quality of European waters, which must reach at least the Good Ecological Status (GES) by 2015. Many water bodies have been heavily modified in their physical structure or hydrological dynamics in order to serve various human uses including navigation, flood protection, hydropower, water regulation, land drainage, and agriculture [8]. When removing such uses and entailed modifications for achieving GES is technically unfeasible from a socioeconomic perspective, or the changes would have significant adverse effects on the water body or at a wider scale, the water body is designed as Heavily Modified Water Body (HMWB). In fact, a large number of European transitional water bodies (26% in number and 47% of surface) are considered as HMWBs [8]. Environmental managers are required to assess the status of HMWBs in terms of achieving at least a Good Ecological Potential (GEP), a less stringent quality target than GES. According to the WFD, a HMWB shows a GEP when there are slight differences in the values of the relevant biological quality elements as compared to the values found at Maximum Ecological Potential (MEP), which is considered as the Reference Condition (RC) for HMWB [8–11]. At present only minimal advancement has taken place, in terms of understanding the meaning of GEP and how to define MEP, especially within an ecological context [12–14]. The meaning of the Ecological Potential (EP) concept comprises several difficulties [15], and the WFD gives little guidance as to the definition of these “potentials” but indicates that they should be water body specified and as close as possible to the most comparable natural situation.

The Ecological Status (ES) and Ecological Potential (EP) assessment and the subsequent environmental management actions should be based upon the status of biological, hydromorphological, and physicochemical quality elements. Among the biological indicators considered in the WFD, soft-bottom macroinvertebrates constitute one of the most used, both in coastal and transitional waters, because of their many advantages [16]. In the last decade, a certain number of indices have been proposed (AMBI [17], Bentix [18], BQI [19], BOPA [20], BITS [21] and MEDOCC [22], among others) to assess the environmental quality using macroinvertebrates as biological element. MEDOCC (MEDiterranean OCCidental) index was developed for the establishment of the ES in Northwestern Mediterranean coastal waters and has been widely used in several countries from the Mediterranean eco-region [22–28]. Nonetheless, this index has not yet been applied in TW or in HMWBs.

The present work has been performed in Alfacs and Fangar bays, two semi-enclosed embayments of the Ebre River delta complex (Northwestern Mediterranean Sea). The rice cultivation developed on the emerged delta since the second half of the nineteenth century and resulted in a substantial modification of the natural hydrological regime [29, 30]. Because of the physical alterations by human use, these bays are considered as transitional HMWBs [31]. Nevertheless, the two bays are renowned for its naturalistic value being included in the Natura 2000 network following the EU Habitat Directive: full bay for Fangar Bay and half of the Alfacs Bay. In 2013 the delta and the lower watershed of the Ebre River (total area of 367,769 ha) were awarded as a UNESCO Biosphere Reserve (http://www.unesco.org). Moreover, the delta plain is also considered an important bird area [32].

Here we examine data relative to sediment characteristics, organic matter content, heavy metal concentrations, and composition of the benthic macroinvertebrate assemblages in the two bays. Our aims were (1) to check the response of MEDOCC index to the pressures in TW and HMWBs, (2) to demonstrate the suitability of MEDOCC index in the ecological assessment in modified and transitional waters at the assemblage level, (3) to set the best reference conditions and MEP considering areas with different degree of pressures, and finally, (4) to determine the EP/ES at water management units level.

2 Materials and Methods

2.1 Study Area

The study area is located in two bays connected to the open sea, on the Ebre Delta plain (Fig. 1). Fangar, the northern bay, covers an area of 12 km^2 with a capacity of 16×10^6 m^3 and 2 m mean depth, with a mouth 1 km wide. The residence time of water ranges between 1 and 2 days depending on freshwater inputs [33]. The largest, Alfacs Bay, is located at the southern part of the delta. It has a surface of about 50 km^2 and a volume of 150×10^6 m^3 with a mean depth about 4 m and a mouth 2 km wide. The average water turnover is 10 days, depending on freshwater inputs [33]. In both bays, the major source of pollution is related to agricultural activity. Nutrients, organic matter, heavy metals, and pesticides deriving from freshwater discharges [34–39] make up the major disturbances affecting the water quality of the two bays [31]. However, other activities such as salt refinery, fisheries, aquaculture, and tourism can cause a potential impact on both bays.

2.2 Sampling and Laboratory Procedures

Samples were collected in June 2006 and June 2011 (18 and 16 sites, respectively) in Alfacs and Fangar bays (Fig. 1). Sampled stations were homogeneously distributed throughout both bays according to different hydrogeomorphological areas, distribution of different microhabitats, and anthropogenic pressures [38, 40, 41]. Two replicate samples of sediment were collected at each station using a van Veen grab (600 cm^2). Samples were filtered through a 500 μm mesh and stored in 4% formaldehyde solution stained with Rose Bengal. Samples were sorted at the laboratory, and faunal composition was identified to species level (when possible).

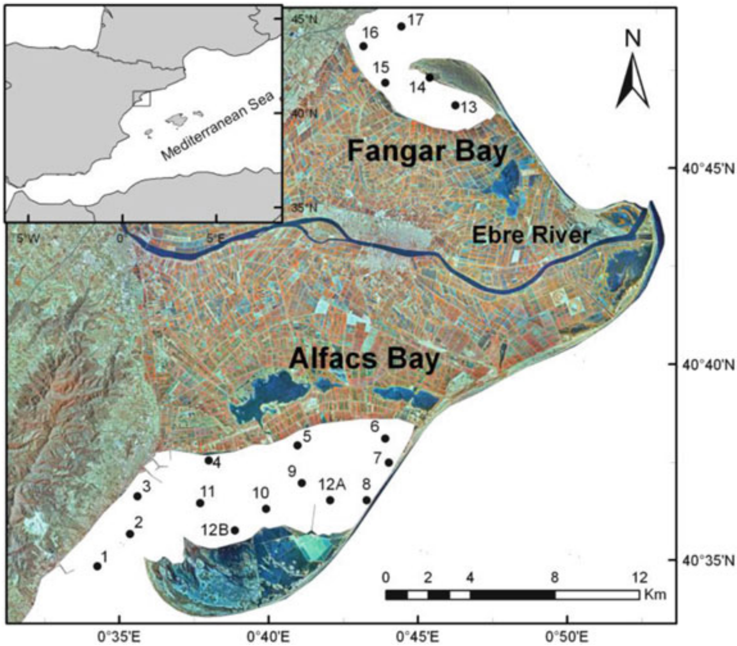

Fig. 1 Location of sampling stations in Alfacs and Fangar bays (Northwestern Mediterranean)

Sediment subsamples were taken for analysis of physicochemical parameters at each grab: sediment characteristics, organic matter content, and heavy metal concentration. Granulometrical analysis was conducted by standard dry-sediment procedure [42]. Median particle diameter and percentage of silt-clay (<63 μm) were used as variables to identify sediment characteristics. Organic matter content in sediments was estimated as ash-free dry weight (5 h at 450°C). Heavy metals analyzed were Zn, Cu, Pb, V, Cd, and Hg. Their concentrations were determined weighting an amount of about 0.1 g of freeze-dried sediment. The latter was weighted and digested in a concentrated HNO_3 and hydrogen peroxide solution (Suprapur Merck® reagents) in an oven at 90°C for 24 h. Digested solution was diluted with Milli-Q purified water, stored at 3°C, and analyzed by Inductively Coupled Plasma Mass Spectrometer (ICP-MS, PE Elan-6000). Blanks solutions were done to detect eventual contamination during analytical procedure. Certified reference material was also analyzed using the same procedure to check the accuracy of the measurements [43]. All metal concentrations, expressed in micrograms of metals per gram (μg g^{-1}) of dry sediment, were determined at the Scientific-Technical Services of the University of Barcelona.

2.3 Computation of MEDOCC

MEDOCC index [22] is based on the relative abundances of Ecological Groups (EGs) made according to the degree of sensitivity/tolerance of species to organic enrichment. MEDOCC was calculated at each site (average value of two replicates) using the following formula:

$$\mathrm{MEDOCC} = (0 \times \%\mathrm{EGI} + 2 \times \%\mathrm{EGII} + 4 \times \%\mathrm{EGIII} + 6 \times \%\mathrm{EGIV})/100 \quad (1)$$

where EGI, EGII, EGIII, and EGIV correspond to sensitive, indifferent, tolerant, and opportunistic taxa, respectively. MEDOCC values range from 0 to 6, low values indicating high environmental quality.

2.3.1 Boundaries

The definition of boundaries between ecological classes defined by WFD is an intrinsic computation of the MEDOCC index [22]. This index, rather than providing fixed values between ES, suggests to establish new boundaries on an ecological theory basis (i.e., the response of EGs along the disturbance gradients) for each group of data, eco-region, etc. Thresholds based on the distribution of the frequency of the four EGs along an increasing gradient of the MEDOCC values for the dataset obtained in Alfacs and Fangar bays were explored.

2.3.2 Definition of Reference Conditions

The MEP is considered as the reference condition for HMWB and is intended to describe the best approximation to a natural aquatic ecosystem that could be achieved given the hydromorphological characteristics. In this context, these features cannot be changed without significant adverse effects on the specified use or the wider environment [10, 11].

In the case of Ebre Delta bays, the southern platform of Alfacs Bay was the less disturbed area by human activities. Moreover, results of the characterization of Alfacs and Fangar bays in terms on environmental variables and macrobenthic composition of assemblages [44] demonstrated that the effects of the pressures affecting these bays (mainly rice cultivation) did not reach the southern coast of Alfacs Bay. A recent study on the distribution population of *Pinna nobilis* in Alfacs Bay [45] demonstrates high abundance of this species at the southern platform, while it is practically absent at the rest of the bay. Thus, although communities sampled in both bays were included within two different water bodies (each one corresponding to each bay), two groups of stations are considered to define RCs: one including stations sampled in the southern platform of Alfacs Bay (8, 10, 12A, and 12B) and the other including the rest of the stations (1, 2, 3, 4, 5, 6, 7, 9, and

11, in Alfacs Bay and the overall stations in Fangar Bay). Stations located at the southern shelf of Alfacs Bay, with minor effects of freshwater inputs from drainage channels of rice fields, must have a more restrictive reference condition (i.e., with a lower value of MEDOCC) compared with the rest of stations. Moreover, this sector of Alfacs Bay should be considered as non-modified TW, following WFD definitions [8]. Conversely, stations at the northern shelf, directly affected by freshwater inputs from rice cultivation discharges, and the stations situated in central areas of Alfacs Bay where the contents of these inputs accumulate, are considered as HMWB. Due to the smaller size and the degree of confinement of Fangar Bay, together with the pressures affecting the bay, it is also defined as a HMWB. Thus, the optimal environmental quality that can achieve these two HMWBs should be different to that for the southern platform of Alfacs Bay. A MEP will be defined for the former, while RC will be assigned to the latter.

Both MEP and RC are based in samples reflecting minor disturbance. For each group of stations, the sample with the lower MEDOCC value was improved, creating a new "virtual" reference condition [46, 47], corresponding to the option 4 (expert judgment) from the four options proposed by WFD to identify reference conditions [48]. The new theoretical situation based for the southern platform of Alfacs Bay is defined by a macrofaunal community composed by sensitive, indifferent, and tolerant species with a frequency of 80%, 10%, and 10%, respectively (MEDOCC value = 0.6). In the other areas of both bays, affected by natural and human-induced disturbances, the MEP is defined by a community composed by a low percentage of sensitive taxa (40%), but higher percentages of indifferent and tolerant species (30%). The MEDOCC value of this less restricted reference condition is 1.8.

2.4 Assessment of EP/ES

Following WFD requirements, the Ecological Quality Ratio (EQR) at each site was calculated by dividing the MEDOCC values by RC/MEP value and by rescaling the results from 0 to 1. To determine the EP/ES at Ebre Delta bays, the mean EQR value was calculated for each management unit: (1) the nonimpacted southern platform in Alfacs Bay (TW), (2) the area affected by the human pressures in Alfacs Bay (HMWB), and (3) the whole Fangar Bay (HMWB).

2.5 Data Analysis

The response of the index to the anthropogenic pressures was analyzed by Spearman's rank correlation coefficients between MEDOCC values and organic matter content and heavy metal concentrations in sediment. SPSS v21 software was used to run the analysis. Non-metric Multidimensional Scaling (nMDS) ordinations were conducted to visualize the distribution pattern of samples based on faunal composition by means of the Bray-Curtis similarity coefficient and data pretreatment using a square root transformation. Species assemblages were identified by cluster analysis and similarity levels. The macrofaunal data were analyzed using the PRIMER v6 software.

3 Results

The distribution pattern of the four EGs along increasing values of MEDOCC index obtained for the samples from Alfacs and Fangar bays (Fig. 2) shows an exponential decrease of sensitive taxa (EGI), while the percentage of opportunistic species (EGIV) increases also exponentially. EGII increases to a maximum value of MEDOCC near 2 and then decreases nearly linearly, while EGIII reaches the maximum percentage for MEDOCC values close to 3.75.

The process for setting the boundaries between different levels of EP/ES has been the same to the process followed in coastal waters [22]. Once the distribution of the EGs has been plotted, the boundaries have been established (Fig. 2) following quality status definitions proposed by the WFD [1, 22]. As the changes of EGs abundances across the gradient are very similar to those obtained for coastal waters [22, 28], the selected boundaries between EP/ES are identical to those selected for coastal waters (Table 1).

In Alfacs Bay, high values of silt-clay (around 80%) have been observed in the northern platform, the central basin, and the outer part of the bay (Fig. 3a), and low values of silt-clay content have been measured in the southern shelf. In Fangar Bay, higher values have been recorded in the external area and to a lesser extent in the platform where the freshwater discharge occurs and also on the opposite shore. The distribution pattern of organic matter content in sediments is quite similar (Fig. 3b). The values increase progressively from the northern platform to the central basin in

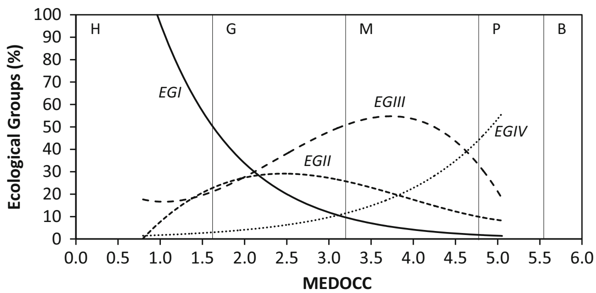

Fig. 2 Distribution of the percentage of Ecological Groups (EGs) along the increasing gradient of MEDOCC values in Alfacs and Fangar bays. *EGI* sensitive taxa, *EGII* indifferent taxa, *EGIII* tolerant taxa, *EGIV* opportunistic taxa. *Vertical lines* show the boundaries between the different Ecological Potential (EP). *H* high, *G* good, *M* moderate, *P* poor, *B* bad

Table 1 Threshold values between the four ES/EP using MEDOCC index for high modified transitional waters in Northwestern Mediterranean sea

EP/ES	MEDOCC	EQR
High/good	1.60	0.73
Good/moderate	3.20	0.47
Moderate/poor	4.77	0.20
Poor/bad	5.50	0.08

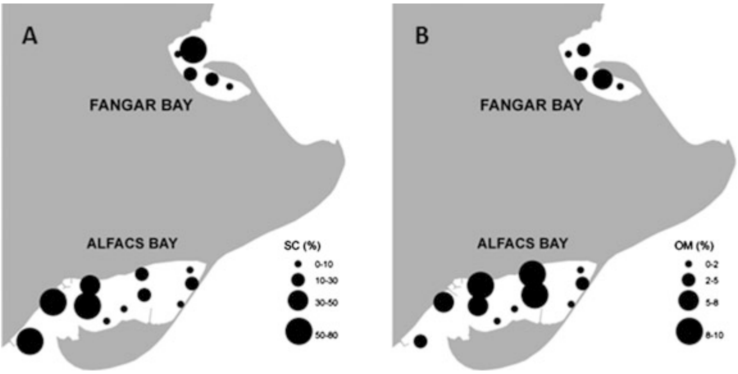

Fig. 3 Spatial distribution of silt-clay (**a**) and organic matter (**b**) contents based on 2011 survey data

Alfacs Bay, but they decrease again in the southern platform. In outer areas of the two bays, organic matter content is slightly lower than in areas directly affected by freshwater inflows, even if the percentage of silt-clay is high. The distribution pattern of the metals measured in sediments (Fig. 4) is similar to the silt-clay content and is comparable for all considered metals, with higher values observed in the northern platform and central basin of Alfacs Bay and also at station 14 in Fangar Bay. Lowest values have been measured in the southern platform of Alfacs Bay, in the most confined areas of both bays and in the most external platform of Fangar Bay. Accumulation of metals in sediments is lower in the outer part of the two bays despite the high percentage of silt-clay, in a similar pattern to organic matter content.

The nMDS ordination plot of sampling sites regarding species composition (Fig. 5) clearly illustrates the segregation of three main groups, based on a degree of similarity of approximately 30%. The group 1 includes samples of the outer area of both bays and the central basin of Alfacs Bay, characterized by macrofaunal assemblages highly influenced by a marine regime (Fig. 6). The group 2 contains samples from the shallow northern and eastern platform of Alfacs Bay and from both shallow platforms in Fangar Bay (stations 14 and 15) characterized by macrofaunal assemblages with a significant presence of tolerant and opportunistic species. Finally, the most compacted set of stations is located at the southern platform of Alfacs Bay and locations 13 and 16 of Fangar Bay (group 3), distant from the main anthropogenic pressures. Although this group of stations is dominated mainly by sensitive and indifferent species, location 13 shows a particular

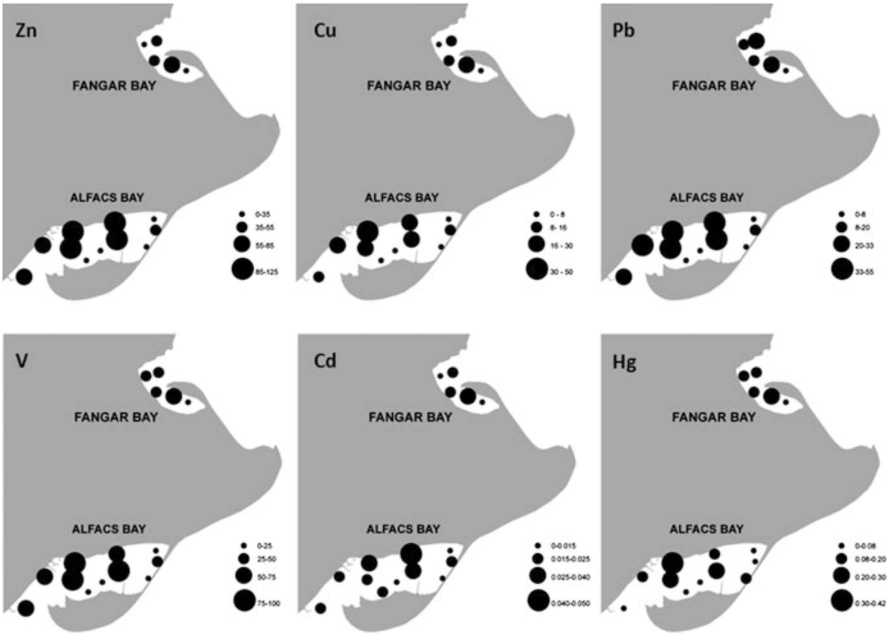

Fig. 4 Spatial distribution of metal concentrations (Zn, Cu, Pb, V, Cd, and Hg; μg g^{-1}) based on 2011 survey data

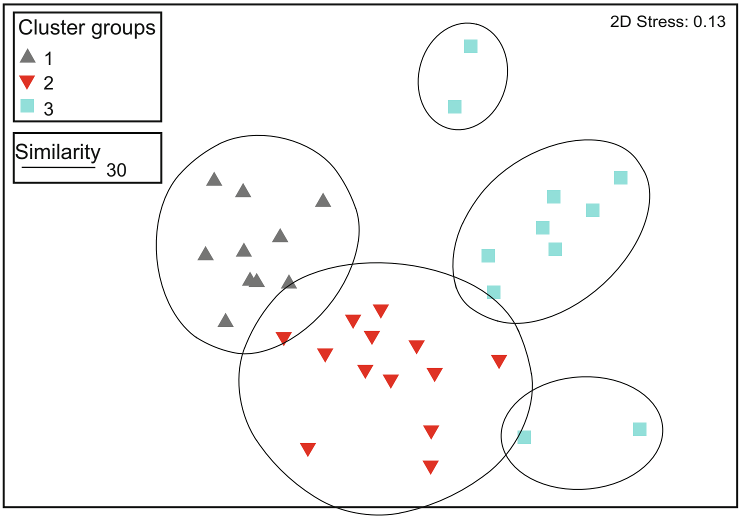

Fig. 5 n-MDS ordination based on faunal composition. *Lines surround groups* identified at 30% similarity levels in the cluster analysis

Fig. 6 Location of three main assemblages defined by cluster analysis

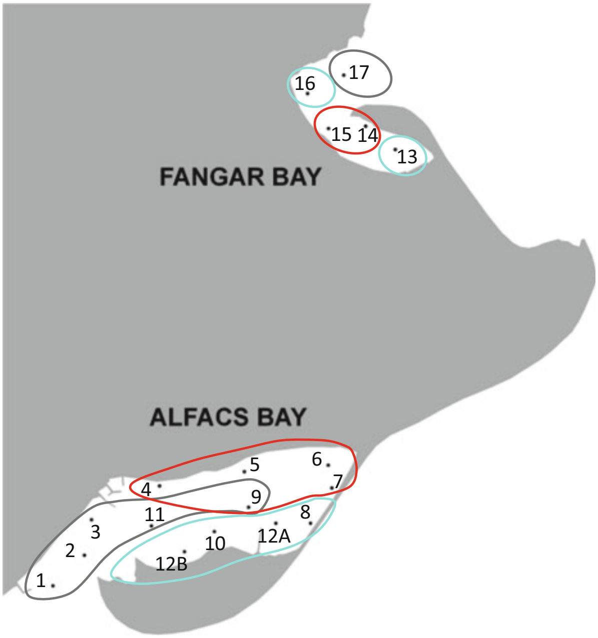

Table 2 Results of Spearman's rank correlation coefficients between MEDOCC values and variables measured in sediments: silt-clay content (SC; %), mean particle diameter (MPD; μm), organic matter content (OM; %), and metal concentrations (Zn, Cu, Pb, V, Cd, and Hg; μg g^{-1}). Value ranges are given

Variables	Range	MEDOCC
SC	0–94	0.415**
MPD	48–316	−0.398*
OM	0.7–12.7	0.528**
Zn	14–124	0.591**
Cu	2–45	0.614**
Pb	4–52	0.551**
V	14–93	0.504**
Cd	0.07–0.47	0.422*
Hg	0.00–0.40	0.516**

*$p < 0.05$; **$p < 0.01$

species composition with important abundances of opportunistic and tolerant species.

Significant correlations between MEDOCC index and sediment variables have been obtained (Table 2), with heavy metal concentrations and organic matter content showing highest correlations.

Samples from the southern platform of Alfacs Bay (stations 8, 10, 12A, and 12B) show high and good ES both in 2006 and 2011 (Fig. 7 and Table 3). Samples from the outer areas of both bays (stations 1, 2, and 17) are classified as good EP in both surveys. The most confined areas of Alfacs Bay reach also good EP, with the exception of station 7 in 2011, which rates moderate. Sites 3 and 5, located at

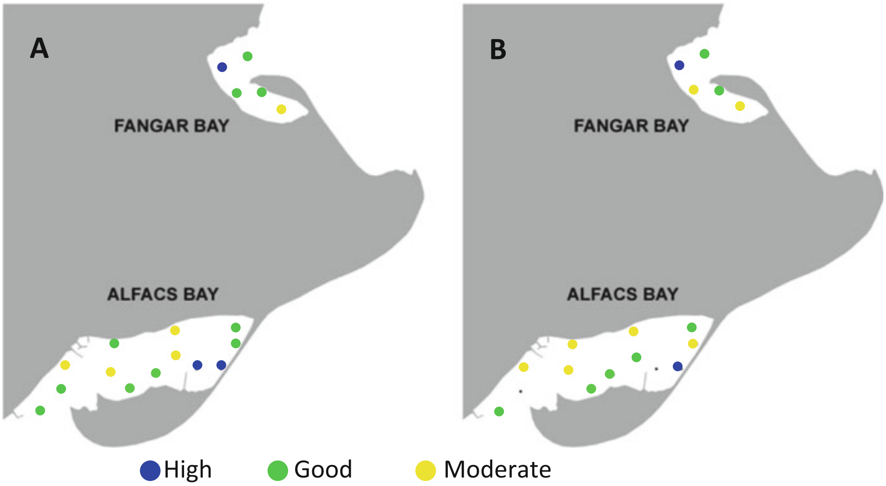

Fig. 7 Assessment of EP/ES using MEDOCC index in soft-bottom assemblages sampled in Ebre Delta bays in 2006 (**a**) and 2011 (**b**)

Table 3 Assessment of EP/ES with MEDOCC index in Ebre Delta stations sampled in 2006 and 2011. *EQR* ecological quality ratio, *H* high EP, *G* good EP, *M* moderate EP, *B* bad EP

Station	Year	MEP/RC	MEDOCC	EQR	EP/ES	
1	06–11	1.8	3.60–3.67	0.57–0.56	G	G
2	06	1.8	3.47	0.60	G	
3	06–11	1.8	4.30–4.21	0.40–0.43	M	M
4	06–11	1.8	3.90–4.01	0.50–0.47	G	M
5	06–11	1.8	4.55–4.58	0.34–0.34	M	M
6	06–11	1.8	3.32–3.52	0.64–0.59	G	G
7	06–11	1.8	3.59–4.00	0.57–0.48	G	M
8	06–11	0.6	0.80–1.21	0.96–0.89	H	H
9	06–11	1.8	4.34–3.74	0.40–0.54	M	G
10	06–11	0.6	2.39–2.75	0.67–0.60	G	G
11	06–11	1.8	4.27–3.95	0.41–0.49	M	M
12A	06	0.6	1.72	0.79	G	
12B	06–11	0.6	2.25–2.73	0.69–0.61	G	G
13	06–11	1.8	5.03–4.59	0.23–0.34	M	M
14	06–11	1.8	3.78–3.87	0.53–0.51	G	G
15	06–11	1.8	3.54–4.17	0.58–0.44	G	M
16	06–11	1.8	2.54–2.48	0.82–0.84	H	H
17	06–11	1.8	2.95–3.63	0.73–0.56	G	G

shallow areas in the northern platform of Alfacs Bay directly influenced by the discharge of freshwater from irrigation channels, remain with a moderate EP during

Table 4 Assessment of EP/ES with MEDOCC index for each management unit. *EQR* ecological quality ratio

	Year	EQR	EP/ES	
Alfacs Bay				
Southern platform	06–11	0.78–0.70	G	G
Disturbed areas	06–11	0.49–0.49	G	G
Fangar Bay	06–11	0.58–0.54	G	G

the two surveys. In the same area, station 4 is rated good in 2006, while in 2011 it shifts to moderate EP. Samples located in the central basin of Alfacs Bay (9 and 11) are classified as moderate in 2006, increasing their EQR value in 2011, but only station 11 improves, achieving a good EP. EPs in Fangar Bay do not show big changes in the 2 years of study. Only station 15 shifts from good to moderate EP between the two sampling periods. The station 13 of Fangar Bay is rated moderate during both surveys. Results obtained at the defined management units level (Table 4) indicated that the good EP/ES was always achieved in all surveys.

4 Discussion

4.1 Response of MEDOCC Index to Pressures

MEDOCC index is based on the response of macrofaunal assemblages to organic enrichment gradients as described by the model of Pearson and Rosenberg [49]. Relationships between MEDOCC and environmental variables (e.g., organic matter content, metal concentrations) that increase in areas with a high percentage of land dedicated to agriculture makes this index a reliable statistical descriptor of the anthropogenic pressures concerning Alfacs and Fangar bays. However, organic enrichment is also enhanced in nonhuman-stressed estuarine environments, natural stress being an intrinsic feature of these systems [5]. In Alfacs and Fangar bays, factors such as continental inputs, confinement, and shallowness can naturally result in the accumulation of organic matter in the sediments, complicating the interpretation of data obtained with the biotic indices and hindering the distinction between human-related and natural stress [5, 6]. For this reason, several authors consider the use of indices based on tolerance/sensitivity, originally developed in coastal waters, inappropriate to be applied in TW [21, 50–52], and suggest the creation of new and specific methodologies to assess the ecological assessment of TW [50] or adapt the indices to such environments [52].

Many studies have focused in the comparison of indices, readjusting boundaries to improve agreement between methods when assessing ES [53–55]. The need to reexamine and adapt the different index thresholds for the estuarine environments is also highlighted by several authors [5, 56–59]. Indeed, the application of MEDOCC index itself includes how to define boundaries between ES categories based on the distribution pattern of the curves of different EGs [22]. In the current study, the pattern of EGs frequency distribution obtained in the samples collected in Ebre Delta bays is very similar to the one obtained from coastal waters, being unnecessary to make any change in the boundaries. The good relationships of MEDOCC index with environmental variables, the definition of boundaries based on EGs distribution patterns, and the selection of suitable reference conditions in Ebre Delta bays indicates that it is not necessary to change the assignment of the degree of tolerance of species in TW because of differences in species' ecological behaviors [51, 60–62].

4.2 Reference Condition (RC) and Maximum Ecological Potential (MEP)

Appropriate selection of RCs is a crucial step in the application of indices [63]. Setting RCs become even more difficult in TW, due to its natural great variability [47, 64, 65]. In HMWB, RCs are replaced by MEPs, the best possible conditions attainable if hydromorphological causes of change are removed. Several assemblages have been identified in Alfacs and Fangar bays [40, 41, 44], being the southern shelf of Alfacs Bay the less affected by human pressures, mainly agriculture activity [38, 39]. Differences observed between the assemblages of the southern platform in Alfacs Bay with other stations sampled in both bays indicate that the conditions in these areas lead to the development of communities that could be found in other stations if they were not affected by agricultural activity and other pressures. Taking into account that pressures acting in both different groups of stations are not comparable, three management units should be considered: (1) the nondisturbed southern platform in Alfacs Bay, which could be considered as non-modified transitional water body, (2) the areas affected by the human pressures in Alfacs Bay, and (3) the whole water body in Fangar Bay, the last two considered as HMWBs. Therefore, the ecological potential of the communities reached in these areas cannot be equivalent to that achieved in the most distant areas to the origin of the disturbance. Thus, the RCs should be also different, since environmental conditions found at the communities sampled in the southern platform of Alfacs Bay will never be present in the other sampled areas.

The Ebre Delta holds 21,600 ha of rice fields, covering 72% of the total area and producing 113,500 t of rice per year [39], whose management results on a modification of the natural hydrological regime of both bays [29]. Despite this, the Ebre Delta plain is one of the most important wetland areas in the Northwestern Mediterranean and is valuable both economically and ecologically. In Alfacs and Fangar bays, management measurements related to the reduction of agricultural activity that could be implemented to improve environmental quality could affect adversely the general environment of the Ebre Delta plain. According to the compilation document [8], the impact of the management actions on the entire ecological and socioeconomic system could occur at different levels: (1) reducing the area of protected habitats, endangering the favorable conservation status in the Natura 2000 site, (2) endangering environmental status of RAMSAR site or natural park, (3) loss of wetlands, (4) jobs reduction, and (5) loss of agricultural lands and agricultural production reduction. The hydromorphological modification resulting from agricultural activity cannot be reversed even in the long-term because it would compromise the continuation of the uses for which these water bodies were altered, with high economic and social costs [9, 10]. Under this assumption, the sense of determining the EP and MEP by comparison with natural conditions must be questioned as by definition, being HMWBs, they are physically, structurally, and ecologically unattainable.

Even considering the overall Alfacs Bay as a unique transitional HMWB, it is well known that species assemblages in estuarine environments display a high spatial variability [66], and thus, using a single RC, as recommended by the WFD, can be subjected to criticism [58, 64, 65, 67, 68]. Although recent studies on the implementation of the EP in HMWBs have considered a single MEP to assess the EP [15, 69, 70], Borja et al. [14] proposed several values of MEP in estuarine HMWB according to different salinity stretches, being salinity an explanatory variable of anthropogenic-driven hydromorphological changes.

4.3 EP/ES at Sampling Site Level

As expected, the stations with the highest EP/ES are those with the lowest human influence, located on the southern platform in Alfacs Bay. The improvement of the EP (from moderate to good) at the central area of Alfacs Bay (station 9) from 2006 to 2011 could be explained by the replacement of several opportunistic and tolerant species of polychaetes (*Heteromastus filiformis*, *Notomastus latericius*, *Prionospio cirrifera*, and *Prionospio fallax*) by the ophiuroid *Amphiura chiajei*. This brittle star is considered an indifferent species to organic enrichment, and it is mainly responsible for the increase of EQR and, consequently, for the EP improvement. Station 11 remains with a moderate EP in both surveys, although the EQR value obtained in 2011 coincides with the boundary between good and moderate EP. The almost lack in 2011 of the opportunistic species *Fabriciola tonerella*, abundant in 2006 (densities up to 5,000 ind m^{-2}), explains the increase of EQR value. At this point, we do not know whether this improvement is real or it is the consequence of a random inter-annual variability due to the high variability of hydrological conditions [71, 72], which may change if the assemblage is under marine or freshwater influence [44]. Further monitoring is needed to properly address this doubt.

Stations at the northern platform of Alfacs Bay, those directly affected by human pressures, had lower values of EQR and worse EP. Different results were also obtained at the station 4 located at the northern platform near the freshwater discharge and very close to a sewage outfall, which was classified as good EP in 2006 and as moderate EP in 2011. However, the EQR values are similar and close to the boundary between good and moderate. Both organic matter content and heavy metal concentrations measured in sediments enhance the development of more opportunistic and tolerant species [49, 73]. Despite the presence of this kind of species (*Aora spinicornis*, *Corophium acutum*, *Erichtonius punctatus*, and *Prionospio multibranchiata* in 2006 *and Galathowenia oculata* and *Pseudomastus deltaicus* in 2011), the contribution of sensitive and indifferent species is also important, with values up to 16.0% and 20.5%, respectively. In this location, the EPs obtained do not fit the expected ecological assessments (lower ratings), considering the variables measured in sediments.

Station 14 in Fangar Bay shows the same response than station 4 at Alfacs Bay. Despite the high organic matter content and metal concentration, faunal composition indicates a good EP. Changes in the EP between the two surveys (from good to moderate) are observed at station 15 in Fangar Bay. The increase of *Galathowenia oculata* (tolerant species) and *Pseudomastus deltaicus* (opportunistic species) together with the decrease of *Microdeutopus algicola* (sensitive species) in 2011 explains the change. Macrofaunal assemblages from shallow areas influenced by fluctuating freshwater inputs or subjected to a high evaporation are submitted to high temporal physicochemical variability resulting in a coexistence of sensitive and indifferent and tolerant species, together with opportunistic species, which are favored during acute disturbance events [74–76]. The lowest values of EQR (0.23 and 0.34, in 2006 and 2011, respectively) reached in the most confined area of Alfacs Bay (station 13) are explained by the dominance of polychaetes such as *Capitella capitata*, *Neanthes caudata*, *Pseudopolydora* sp., and *Heteromastus filiformis*, opportunistic and tolerant species to organic enrichment, whose abundance could be enhanced by the accumulation of detritus coming from *Cymodocea nodosa* meadows, a sea grass colonizing the sediments in the area [77] but also from filamentous algae (i.e., *Chaetomorpha* sp., personal observation). We contend that the assessment of ecological status in areas organically enriched by a natural factor should be reexamined as suggested by Pinedo et al. [23, 27].

4.4 EP/ES at Management Unit Level

Each of Alfacs and Fangar bays was defined as a single water body in the IMPRESS document [31]. However, the spatial heterogeneity present in the two bays due to hydromorphological conditions (i.e., freshwater inputs, marine regime, and confinement) and a disturbance gradient (i.e., the organic matter and heavy metals mainly discharged from channels of rice fields) points to the division of Alfacs Bay into two management units. The southern platform of Alfacs Bay does not receive the pressures for which it has been considered as HMWB. Moreover, according to the definition of the WFD, each water body is defined also in terms of its pressures, making the situation in Alfacs Bay inconsistent with the WFD. This situation is similar to that found in a recent study conducted at the port of Santander where the water body is divided into smaller sub-domains (water management units) to assess the ecological potential of a HMWB [70]. Thus, we consider that three management units have to be considered in Ebre Delta bays to correctly address suitable management measures: the southern platform of Alfacs Bay on one side and the area of Alfacs Bay affected by the freshwater discharges and Fangar Bay on the other side. We suggest that the recovery of green filters in re-naturalized rice fields could be an effective mitigation strategy without having significant adverse impacts on the delta plain uses. The implementation of this and other management actions could improve the water quality flowing into two bays and consequently the EP/ES.

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First Report on the Distribution and Impact of Marine Alien Species in Coastal Benthic Assemblages Along the Catalan Coast

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Abstract The Mediterranean Sea is especially prone to the introduction of alien species due to an intense marine traffic, the connection with the Red Sea through the Suez Canal and intensive aquaculture. Catalonia, a region in the Northwestern Mediterranean, began an extensive study on the presence, distribution and impact of invasive macroalgae in 1992, which was extended to all macrobenthic alien species by 2007. Gathering all presence and abundance data of introduced species from the monitoring, we also calculated a Biopollution Level (BPL) index to assess the magnitude of the effects of introduced species on the marine biota at a local level (water body) as required by Marine Strategy Framework Directive (MSFD). Seventeen alien species have been identified although only three can be considered so far as threatening in non-modified environments: the green alga *Caulerpa cylindracea* and the red algae *Womersleyella setacea* and *Asparagopsis armata*. These species show an uneven distribution along the coast but sometimes coexist in the same water body. The impact of alien species on native communities was never severe as shown by the low values obtained using the BPL. The only species triggering a moderate to strong impact was *Caulerpa cylindracea* but it only affected a single water body. However, *C. cylindracea* exhibited a great temporal

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variability on its abundance, with sudden collapses of its populations, which also caused a great variability in the BPL. Future monitoring of the coasts of Catalonia is advised as there is an increase in the number of water bodies affected by alien species and an increase in their abundances from 2007 to 2012.

Keywords Alien species, BPL (biopollution level) index, Catalan coast, Invasive species, Littoral rocky shores, Mediterranean Sea, MSFD, Water bodies (WBs)

Contents

1 Introduction

Although much less studied than terrestrial ecosystems, marine habitats are known to hold a great number of alien (=introduced) species [1]. The spread of alien species is considered one of the main threats to biodiversity both in the terrestrial and in the marine realm [2–4]. Inventories of alien species are being published in reports and in the scientific literature as they are useful both for public administrations and for the scientific community. Of special interest are those alien species that behave as invasives, i.e. aliens that undergo a rapid spread and conquer novel areas within recipient ecosystems in which they become dominant [5] because they become major drivers of environmental change and they are the main targets to be monitored and controlled [6].

The Mediterranean Sea is heavily affected by the introduction of alien species [7–9], with almost 1,000 taxa reported in 2012 [10]. Inventories of alien species are only present for certain Mediterranean countries like Turkey [11], Israel [12], Cyprus [13], Italy [14], Greece [15–17] and Libya [18], although they are critical for managing marine ecosystems and providing data for the requirements of major objectives at the European Union level such as the Marine Strategy Framework Directive (MSFD) [19]. In fact, alien species are one of the eleven qualitative descriptors for the assessment of the environmental status of the water bodies according to the MSFD.

Moreover, the descriptor D2 for the assessment of the environmental status according to MSFD requirements states that introduced species have to be "at levels that do not adversely alter the ecosystems" to attain a Good Environmental

Status. This means that presence/absence data has to be available not only on alien species but also on the abundance and impacts of the alien species to the marine habitats. Mediterranean experts know which are the species that are potentially invasive [20–22] and in some cases they also know their spreading rate [16, 23–28]. Moreover, there is plenty of scientific literature dealing with the abundance and effects of invasive species on native Mediterranean assemblages [e.g. 29–43], but they only consider one (or two) species in one or a limited number of habitats. However, as far as we know, there has been no Mediterranean attempt to assess the number of alien species and their effects on the marine biota at a local level, i.e. the level of a water body, as required by MSFD.

Olenin et al. [44] described a methodology specifically focused on the assessment of the magnitude of alien species impacts on native community structure, habitat traits and ecosystem functioning. This methodology uses basic information on abundance and distribution of alien species within a water body (or some other geographical unit) to obtain a score from 0 to 4 at different levels of impact, fitting with the schemes for water quality assessment in the frame of the Water Framework Directive (WFD) [45]. This index was called Biopollution Level (BPL) since, according to Olenin et al. [44], the introduction of alien species is a factor of disturbance that can be viewed as a pollution agent.

The BPL has been first tested in well-studied areas within the Baltic Sea, both in open waters and coastal lagoons, considering also different periods in the same areas to look for changes in the index over time [44, 46]. However the BPL has not been used extensively in other areas although it is easy to apply and compliant with MSFD.

Catalonia is a region situated in the Northwestern Mediterranean with a coastline extending for more than 400 lineal km, whose water bodies are subjected to different pressures and impacts [47]. The regional government from Catalonia initiated in 1992 a monitoring programme to detect the arrival of *Caulerpa taxifolia*, an alien alga that became invasive in southern France [48, 49]. Surveys were carried out by SCUBA diving in 126 stations that were selected to cover the environments where *Caulerpa taxifolia* usually settles at the first stages of colonization. However, in 2006, the monitoring stations were modified in order to cover all kinds of environments, since the programme broadened its aims to make an early detection of other potentially invasive species that were spreading very fast in nearby Mediterranean areas (*Caulerpa cylindracea*, *Lophocladia lallemandii*, *Womersleyella setacea* and *Oculina patagonica*) [28, 37, 38, 40, 42, 50–55]. The methodology was also slightly improved to allow the calculation of BPL because this index was in line with other biotic indexes used in Catalonia for the implementation of the WFD.

The objectives of this work are twofold: (1) to provide a checklist of the main alien species in the coastal waters of Catalonia at the end of 2014, with references to their extension ranges and relative abundances, if available, and (2) to calculate the BPL index at each coastal water body of Catalonia as an indicator of the additive impacts of invasive species during the period 2007–2012.

2 Material and Methods

2.1 Field Procedures

The study area covers the coastal waters of Catalonia (Northwestern Mediterranean) from Punta de l'Ocell (42° 51′ 45,97″N) to Platja de Sòl de Riu (40° 31′ 27,56″N). These coastal waters have been divided into 34 sectors or water bodies according to their geomorphological features, water basins and anthropogenic pressures, as required by the WFD (Fig. 1). The monitoring was exclusively focused on rocky bottoms and was initiated in 1992. The number of sampled stations has been increasing until 2007, when a total of 188 locations were monitored in a 2-year basis, with the sampling dates concentrated between May and October, the period of the year when the sea conditions are more suitable for diving operations.

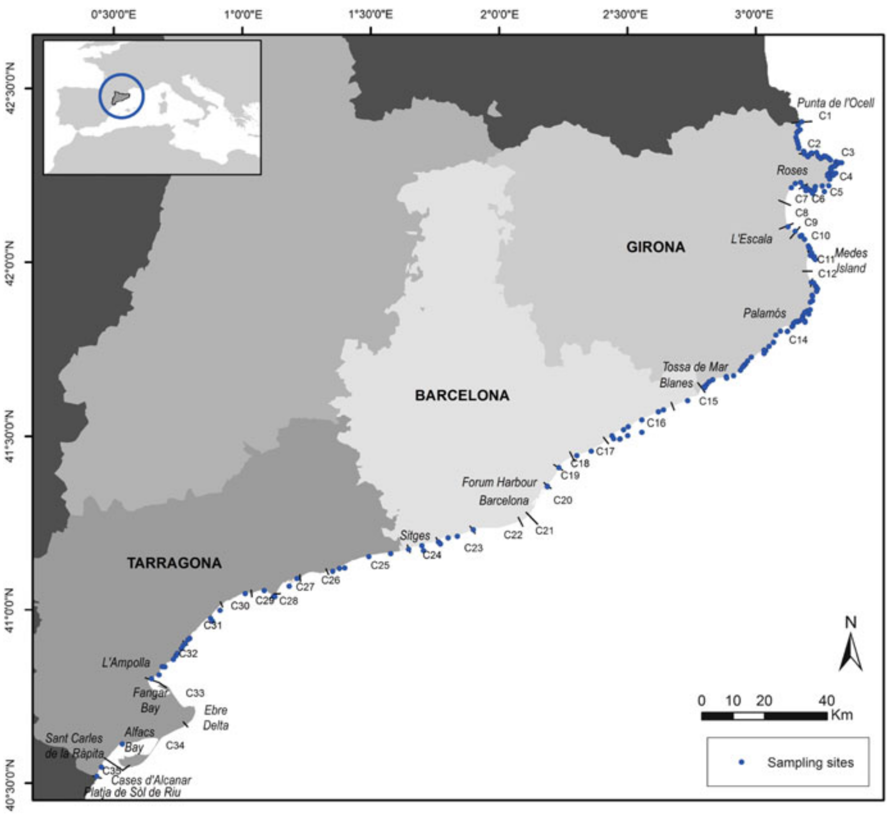

Fig. 1 Coastline of Catalonia with indication of the three provinces, the different water bodies and the sampling stations

Sampling locations were selected to include the geographical range of Catalonia and the environmental diversity of its seabeds, covering all kinds of rocky bottoms and ranges of wave exposure, water quality, depth and orientation. However, water bodies C08, C19, C21, C26 and C34 were not surveyed since they were completely devoid of rocky bottoms. Transects of different lengths according to the bathymetric profile of each station were surveyed by two divers. Surveys covered a width of 10 m (5 m at each side of the transect line) of seabed and crossed different habitats. Main habitats were characterized by native species abundances using a semi-quantitative index widely used in phytosociology (Braun-Blanquet index) [56–58]. Every habitat was checked for over 10 min to make the list of the native species but also to record the abundances of the alien species using the same semi-quantitative index. Moreover, accurate data on alien species' distribution along the bathymetric range, physical appearance, degree of epiphytism and other relevant aspects were recorded. Qualitative samples of unidentified or doubtful species were collected for accurate identification in the laboratory.

There are two alien species that have been detected only outside the monitoring stations. We report these species but we do not provide any indication of its progression.

2.2 Data Analyses

The BPL index [44] was calculated for each water body taking into account the data collected at all the stations situated inside each water body. The assessment was obtained for every 2 years (2007–2008, 2009–2010 and 2011–2012) and was performed using the criteria stated in the BPL calculation. The presence (presence/absence), distribution range (one, several, many, all localities) and abundance (low, moderate, high) of each alien species were analysed at each water body, and all the species were considered to evaluate the impacts. The BPL at each water body was set according to the greatest impact level of alien species found in the water body [44]. BPL ranges from 0 to 4, with five categories indicating no biopollution (BPL = 0) or different levels of biopollution: weak (BPL = 1), moderate (BPL = 2), strong (BPL = 3) and massive (BPL = 4) [44].

3 Results

3.1 Alien Species

A total of 17 alien species, including two green algae, one brown alga, three red algae, one sponge, two cnidarians, two molluscs, one crustacean, four tunicates and one fish, have been recorded in the Catalan coast during this study (1992–2012). Their first record and their invasive potential are summarized in Table 1. Species

Table 1 Main introduced species reported from 1992 to 2012, with indications on the year of first record and their invasive behaviour in Catalonia during the period 2007–2012. Further explanations on invasive behaviours included in the main text

Species	Group	Year	Reference	Invasiveness
Caulerpa cylindracea	Chlorophyta	2008	Ballesteros et al. [51]	Invasive
Codium fragile	Chlorophyta	1981	Ballesteros [59]	Opportunistic
Acrothamnion preissii	Rhodophyta	2006	Ballesteros et al. [60]	Non-invasive
Asparagopsis armata	Rhodophyta	1955	Thomas [61]	Invasive
Womersleyella setacea	Rhodophyta	2006	Ballesteros et al. [60]	Invasive
Dictyota cyanoloma	Ochrophyta	2004	Rull et al. [62]	Non-invasive
Paraleucilla magna	Porifera	2006	Frotscher and Uriz [63]	Non-invasive
Pennaria disticha	Cnidaria	1986	Gili [64]	Non-invasive
Oculina patagonica	Cnidaria	1992	Ballesteros et al. [65]	Opportunistic
Percnon gibbesi	Crustacea	2003	Abelló et al. [66]	Non-invasive
Crassostrea gigas	Mollusca	2010	Ballesteros et al. [67]	Non-invasive
Bursatella leachii	Mollusca	2007	Weitzmann et al. [68]	Non-invasive
Polyandrocarpa zorritensis	Tunicata	1987	Turon and Perera [69]	Opportunistic
Ciona intestinalis	Tunicata	1916	Maluquer [70]	Non-invasive
Microcosmus squamiger	Tunicata	1978	Turon [71]	Opportunistic
Styela plicata	Tunicata	1905	Harant [72]	Non-invasive
Fistularia commersonii	Vertebrata	2007	Pontes (unpublished)	Non-invasive

have been designated as “invasives” if they show an invasive behaviour in natural environments, as “opportunistic invasives” if they behave as invasives only on artificial substrates and anthropogenic environments or as non-invasives if they do not behave as invasives at all. Comments on the impacts and distribution of each species are reported below.

Codium fragile (Suringar) Hariot is common across Catalonia, where it mostly grows on sheltered shallow rocky environments like the entrance of the harbours and some coves with an enhanced nutrient input. It was first reported in Tossa de Mar [as ssp. *tomentosoides* (van Goor) P. C. Silva; [59]]. It was probably present before 1981 since it has been largely misidentified with *Codium tomentosum*, a species that was widely reported before (see Ballesteros and Romero, 1982 [73]). *Codium fragile* can make dense populations in reduced areas, where it covers big patches of the sea bottom but this behaviour is not widespread.

Caulerpa cylindracea Sonder was first reported in 2008 growing between 20 and 50 m in lower infralittoral to circalittoral bottoms from the central coast [51], where it was first detected by artisanal fishermen (Andreu Núñez, personal communication). It invades both rocky and sedimentary bottoms and it even grows over dead *Posidonia oceanica* rhizomes. The species quickly spread and increased its density making dense carpets around 20 m depth offshore Sitges. However, the population collapsed in February 2012 for unknown reasons and recolonized the bottom again after August 2012 (author’s unpublished data). At present (September 2014), the species has spread to 5 m depth (Eduard Llorente, personal communication) and has been found at other distant localities (Blanes, 2013; Aurora Martínez-Ricart and Bernat Hereu, personal communication).

The presence of *Acrothamnion preissii* (Sonder) E. M. Wollaston is anecdotal since only two small thalli have been reported in 2010 (Palamós, Conxi Rodriguez-Prieto, personal communication; l'Escala, Marc Terradas, personal communication). It has never been recorded again.

Both the gametophyte and the tetrasporophyte [*Falkenbergia rufolanosa* (Harvey) F. Schmitz] of *Asparagopsis armata* Harvey were already reported by Thomas (1955) [61]. The distribution of the gametophyte seems to be restricted from the northern coast southward till Blanes, always on moderately exposed shallow waters. It attains large coverages from late winter to early spring (March to May) on rocky bottoms north of Medes Islands [74]. The tetrasporophyte is present everywhere but never abundant. The gametophyte, however, shows an invasive behaviour only in spring and in the northernmost part of the Catalan coast.

Womersleyella setacea (Hollenberg) R. E. Norris was first found in 2006 from at Palamós [60] and has expanded northwards and southwards. Its distribution is always restricted to the northern part of Catalonia, where it thrives on coralligenous outcrops. It shows an invasive behaviour everywhere.

Dictyota cyanoloma Tronholm, De Clerck, Gómez Garreta and Rull Lluch was first collected in 2004 and misidentified with *D. ciliolata* [62] before it was described as a new species [75]. It has been found all along the coast and prefers shallow, slightly polluted waters, growing on rocky bottoms and artificial substrates, mainly in small harbours and nearby areas.

The calcareous sponge *Paraleucilla magna* Klautau, Monteiro and Borojevic, 2004, was first reported in 2006 [63] and is currently found all along the coast on shallow water rocky bottoms. It usually grows as an epiphyte of different seaweeds.

Pennaria disticha Goldfuss, 1820, was first reported as *Halocordyle disticha* at Tossa and Sant Carles de la Ràpita [64] and remained unnoticed until the year 2011, when it appeared again in several areas of the central and southern coasts. It colonizes shallow water environments, always on rocky bottoms in moderately exposed conditions.

A single colony of the zooxanthellate coral *Oculina patagonica* de Angelis, 1908, was found in the breakwater of the southernmost harbour in Catalonia (Cases d'Alcanar) in 1992 [65]. Its distribution has expanded northwards since then [28] and at present is common in the southern and central coasts. Some scattered colonies have been also detected in the northern coast. It grows on shallow rocky bottoms and has a special preference for breakwaters and other artificial habitats.

The crab *Percnon gibbesi* was first reported in the coast of Barcelona [66]. It has been found intermittently in our surveys, always in low abundances, on shallow rocky bottoms and artificial substrates.

The Japanese oyster, *Crassostrea gigas*, has been cultured for a long time in the bays of the Ebre Delta, southern Catalonia. We have reported it growing attached to rocks on the breakwaters of the harbours of l'Ampolla and Cases d'Alcanar, situated at the entrances of Ebre Delta bays [67]. *Crassostrea gigas* seems to be restricted to closed bays and lagoonal environments, not expanding abroad.

The sea slug *Bursatella leachii* (de Blainville, 1817) was detected in Alfacs Bay (Ebre Delta) in 2007 growing on muddy bottoms with *Caulerpa prolifera* at 2 m depth [68]. Besides this station, *Bursatella leachii* has been found intermittently at the Fòrum harbour, close to Barcelona.

One colony of the sea squirt *Polyandrocarpa zorritensis* (Van Name, 1931) was first reported in Fangar Bay in 1986 [69]. It is now common in Alfacs Bay where it grows abundantly on artificial substrates and upper infralittoral rocky bottoms.

Ciona intestinalis is a solitary sea squirt that has been found exclusively in harbours and closed bays, where it grows mainly on artificial substrates. It is common in Ebre Delta bays [71].

Microcosmus squamiger Michaelsen, 1927, is a solitary sea squirt present all along the coast, always growing on rocky and artificial substrates from harbours, marinas and enclosed bays. It can colonize all available substrate in harbours where it behaves as invasive but always restricted to these environments. First reported by Turon (1987) [71] from several localities as *Microcosmus exasperatus*, a closely related lessepsian migrant with which it has been usually misidentified [76, 77].

Styela plicata (Lesueur, 1823) is a solitary sea squirt found all along the coast in enclosed bays (Alfacs, Fangar), harbours and nearby areas, growing particularly on artificial substrates. Already reported from Catalonia by Harant (1927) [72].

Fistularia commersonii Rüppell, 1838, is the first lessepsian fish that has established an enduring population in Catalonia. It was first detected in Palamós in 2007 (Miguel Ponte, personal communication) and its sight by SCUBA and free divers is becoming not exceptional.

3.2 BPL Application

Here we present the results of the application of BPL index along the coast of Catalonia during the periods 2007–2008, 2009–2010 and 2011–2012. Some water bodies have not been evaluated since they have not been surveyed (Table 2).

During the period 2007–2008 (Fig. 2, Table 2), eight alien species were recorded (*A. armata*, *B. leachii*, *C. cylindracea*, *C. fragile*, *D. cyanoloma*, *O. patagonica*, *P. gibbesi* and *W. setacea*) but only *W. setacea* and *C. cylindracea* showed an invasive behaviour in natural environments during our surveys. *Womersleyella setacea* partially covered coralligenous assemblages on the water body C14 but with a weak impact. *Caulerpa cylindracea* colonized coastal detritic bottoms in water body C24 but without causing major impacts. *Oculina patagonica* also displayed weak impacts on artificial substrates of water bodies C27, C32, C33 and C35.

During the period 2009–2010 (Fig. 3, Table 2), thirteen alien species were recorded (*A. preissii*, *A. armata*, *B. leachii*, *C. cylindracea*, *C. fragile*, *C. gigas*, *D. cyanoloma*, *M. squamiger*, *O. patagonica*, *P. magna*, *P. gibbesi*, *S. plicata* and

Table 2 Presence (+) of the main introduced species (A.a., *Asparagopsis armata*; A.p., *Acrothamnion preissii*; B.l., *Bursatella leachii*; C.c., *Caulerpa cylindracea*; C.f., *Codium fragile*; C.g., *Crassostrea gigas*; D.c., *Dictyota cyanoloma*; M.s., *Microcosmus squamiger*; O.p., *Oculina patagonica*; P.m.: *Paraleucilla magna*, P.d., *Pennaria disticha*; P.g., *Percnon gibbesi*; P.z., *Polyandrocarpa zorritensis*; S.p., *Styela plicata*; W.s., *Womersleyella setacea*) recorded during the three studied periods (1, 2007–2008; 2, 2009–2010; 3, 2011–2012) in each water body. Water bodies shaded in grey have never been evaluated

	WB	A.a.*			A.p.			B.l.			C.c.			C.f.			C.g.			D.c.			M.s.			O.p.			P.m.			P.d.			P.g.			P.z.			S.p.			W.s.		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
GIRONA	C01			+										+	+	+				+		+																								
	C02													+	+	+																														
	C03													+	+	+																												+	+	+
	C04													+	+	+																														
	C05	+																																											+	
	C06	+												+	+	+																														+
	C07			+										+	+	+									+			+		+	+															
	C08																																													
	C09			+										+	+	+											+				+															
	C10	+	+	+		+								+	+	+														+	+															+
	C11		+	+										+	+	+																										+	+			
	C12													+	+	+														+	+															
	C14	+	+	+		+								+	+	+				+						+	+	+		+	+													+	+	+
BARCELONA	C15																			+	+	+						+			+															
	C16													+	+	+				+	+	+				+	+	+		+	+			+												
	C17																																													
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	C20									+										+	+	+									+															
	C21																																													
	C22																							+	+		+	+						+									+			
	C23													+	+	+				+	+	+								+	+			+												
	C24										+	+	+	+	+	+				+	+			+	+					+																
TARRAGONA	C25													+	+	+											+	+		+	+															
	C26																																													
	C27													+	+	+										+	+	+						+												
	C28																			+	+	+			+	+	+	+		+	+				+											
	C29																									+	+	+			+												+			
	C30																							+	+	+	+	+						+												
	C31													+	+	+										+	+	+			+															
	C32													+	+	+										+	+	+			+			+												
	C33													+	+	+		+	+					+	+	+	+	+							+											
	C34																																													
	C35							+	+					+	+	+		+	+					+	+	+	+	+		+	+									+		+	+			

*Records of the gametophyte only

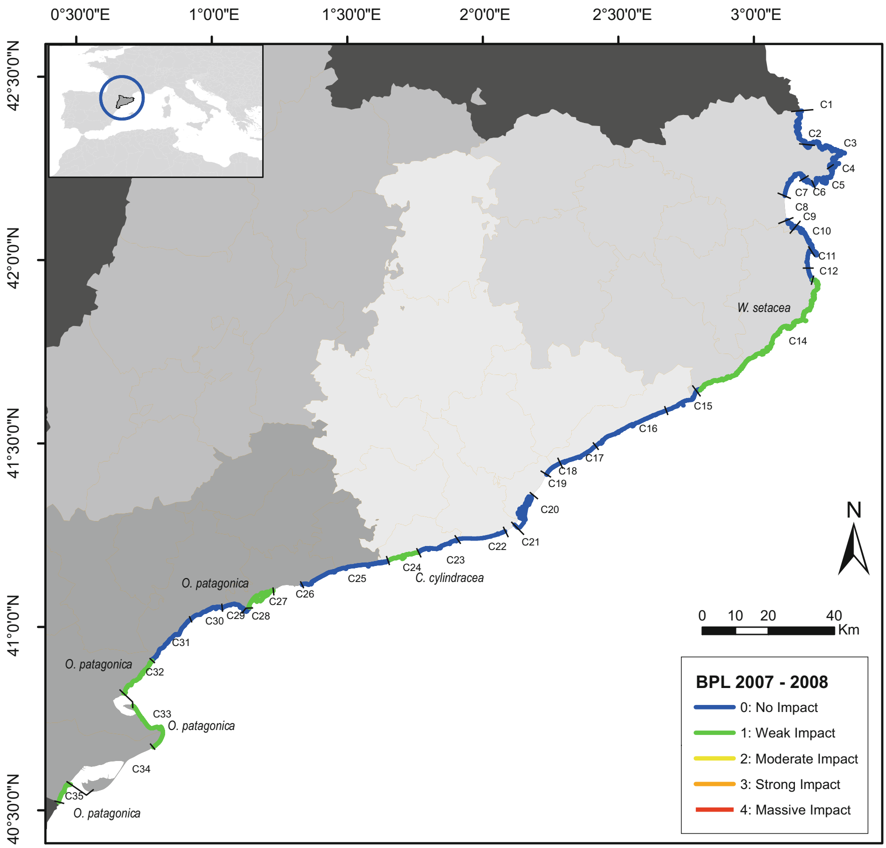

Fig. 2 BPL index for the different water bodies during the period 2007–2008

W. setacea). BPL values ranged between no impact and moderate impact. A moderate impact was produced by *C. cylindracea* in water body C24 since *C. cylindracea* covered large areas of detritical coastal bottoms with densities around 4,500 fronds m^{-2} during fall. *W. setacea* also became more abundant at infected locations and increased its distribution depth; it also colonized some nearby areas in water body C14 and spread to some distant locations (water body C03) but always showed a weak impact on colonized environments. *O. patagonica* generated a weak impact on artificial substrates of water bodies C27, C31, C32, C33 and C35.

Fourteen alien species were recorded during the period 2011–2012 (Fig. 4, Table 2) (*A. armata*, *B. leachii*, *C. cylindracea*, *C. fragile*, *C. gigas*, *D. cyanoloma*, *M. squamiger*, *O. patagonica*, *P. magna*, *P. disticha*, *P. gibbesi*, *P. zorritensis*, *S. plicata* and *W. setacea*), but only *C. cylindracea* and *W. setacea* showed invasive

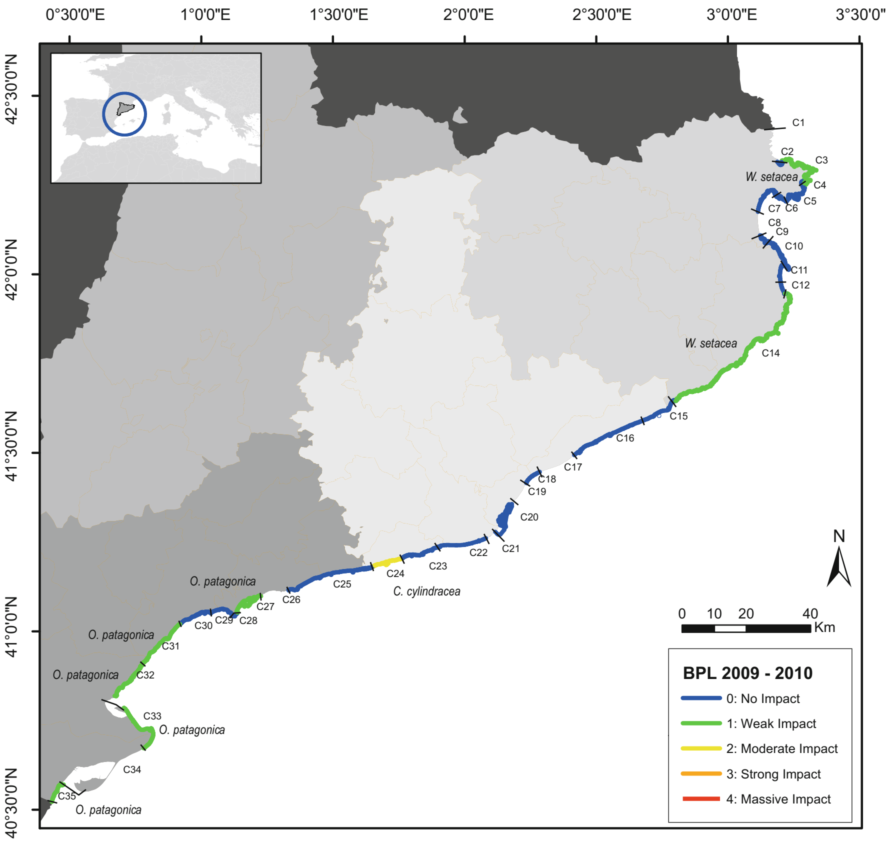

Fig. 3 BPL index for the different water bodies during the period 2009–2010

behaviour on natural environments during the surveying period. *Caulerpa cylindracea* attained frond densities up to 10,000 fronds m^{-2} in the water body C24 during 2011 and displayed a strong impact before totally collapsing in late winter 2012. *Womersleyella setacea* expanded its distribution range to water body C06 but was always showing a weak impact on coralligenous outcrops. *Microcosmus squamiger* constituted monospecific beds in the harbours of Roses (water body C07) and Sitges (water body C22) but its distribution was restricted to the breakwaters and thus having a weak impact at the level of the water body. *Oculina patagonica* expanded its distribution to four new water bodies (C27, C28, C29, C30) but its impact was always weak and restricted to artificial substrates. The sponge *Paraleucilla magna* was detected in 15 water bodies but never showed an invasive behaviour.

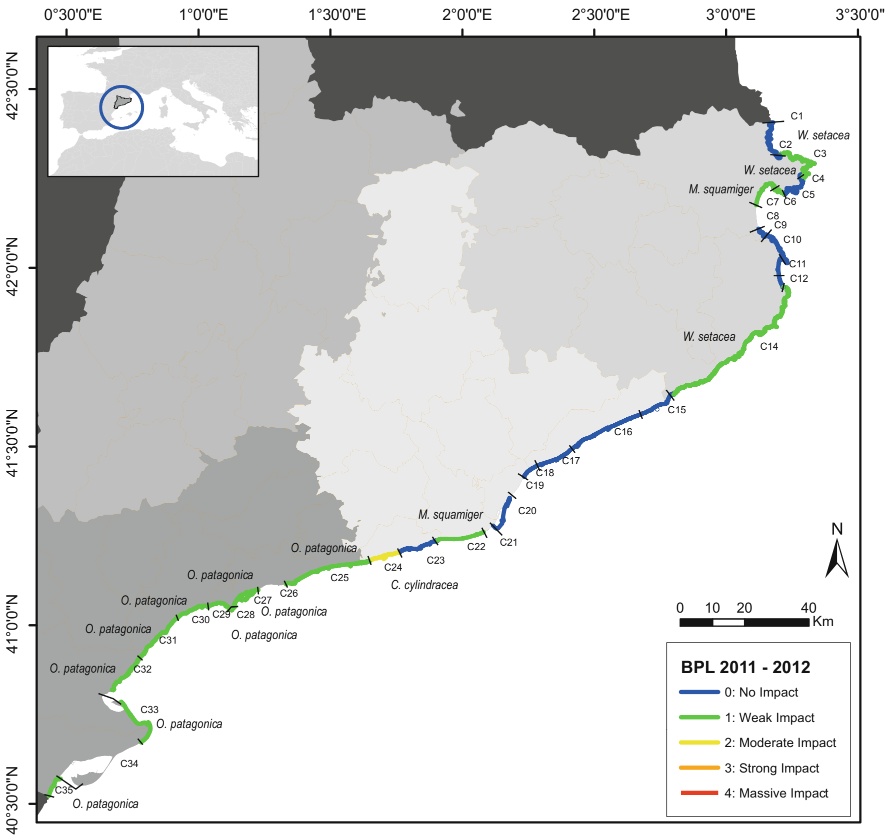

Fig. 4 BPL index for the different water bodies during the period 2011–2012

4 Discussion

Even we have not intensively surveyed areas with high propagule supply in the coasts of Catalonia, the number of detected alien species is still rather low when compared to other Mediterranean areas. The high geographical distance between Catalonia and the Suez Canal, by far the main vector of species introduction in the Mediterranean Sea [7], may explain, at least in large part, the big difference in the number of alien species between Catalonia and Eastern Mediterranean countries [11–13, 16, 17]. The relatively low temperature of the coastal waters of Catalonia [78, 79] should act also as a protection against the colonization of warm-water lessepsian immigrants, as suggested by the data provided by Galil [7] and Zenetos et al. [10]. On the other hand there is a striking difference in the number of alien species present in the neighbouring French Mediterranean coast, where the total number of aliens is enhanced by the large amount of exotic macroalgae arrived by oyster aquaculture [80, 81]. Shellfish farming is also important in the bays of the

Ebre Delta [82], but they are not colonized by the exotic macroalgae that are so common in French Mediterranean lagoons. Globally, the situation in the coasts of Catalonia is much more similar to that found in western Sardinia or the Ligurian Sea coast, where 13 and 38 alien species have been reported, respectively [14]. Comparisons within other Spanish regions are not available since there is no published literature on this issue.

All the alien species reported here were already known from other Mediterranean areas. The species with the lowest number of reports were *Polyandrocarpa zorritensis*, which had been found in two Italian harbours [83], and *Dictyota cyanoloma*, a species that is suspected to be an introduction but that the region of origin is still unknown. Other Mediterranean localities for *D. cyanoloma* outside Catalonia are Izmir (Turkey) [84] and Split (Zuljevic, personal communication).

Tunicates are the taxonomic group holding the highest number of alien species. However, the species reported here are always confined to artificial substrates in highly modified environments, such as harbours, marinas and aquaculture facilities, or to estuaries and bays, as it is usually the case for invasive tunicates [85, 86]. They do not seem to be of great concern outside these environments, although they can generate economic problems in shellfish farms and in harbours as fouling organisms. Breakwaters and aquaculture facilities in the Ebre Delta bays host great numbers of *Crassostrea gigas*. *Bursatella leachii* is exclusively found in enclosed bays and highly modified environments. *Oculina patagonica* also prefers man-made structures such as breakwaters [28, 87, 88] although in the long term may become also abundant in natural environments [40].

Preference for natural environments is evident for the three seaweeds that show an invasive behaviour in Catalonia: *Womersleyella setacea*, *Caulerpa cylindracea* and *Asparagopsis armata*. *Womersleyella setacea* thrives in well-developed coralligenous bottoms [89–93] and also on macroalgal beds and seagrass meadows [93–97]. It has deleterious effects on the suspension feeders living in coralligenous assemblages [53, 98] and on the crustose calcareous macroalgae that build up the outcrops [92].

Caulerpa cylindracea probably is the most aggressive invasive macrophyte in the Mediterranean as it has spread very fast both at a regional level and at the local level across different depths and because its colonization changes the species composition and the structure of the assemblages [25, 26, 34, 54, 99–102]. Consumption of *C. cylindracea* reduces the performances of the herbivores [103–106] but, in any case, herbivory does not affect the ability of *C. cylindracea* to invade [107, 108]. Moreover, *C. cylindracea* is known to show large temporal, not necessarily seasonal, changes in its abundance [37, 109], which explains the sudden collapse and subsequent recovery of *C. cylindracea* in the water body C24.

Asparagopsis armata, a species whose gametophytic stage invades shallow rocky environments in northern Catalonia [75], has not been considered in the calculation of the BPL index because its abundance strongly decays in spring and it has never been found abundant during the surveys. However, even if its abundance only blooms during a short period of time, we agree with Boudouresque and Verlaque [20] in considering it invasive. Further studies have to be performed to account for the real impact of *A. armata* in shallow water assemblages from northern Catalonia.

In our opinion, both *C. cylindracea* and *W. setacea* are the two invasive species that will be most probably threaten the species composition and functioning of benthic assemblages in Catalonia in the long term and at a wide extent. Aside from these two species, *Lophocladia lallemandii* is another candidate to threaten Catalonia's benthic marine assemblages in the near future. It has already been reported to be invading the rocky reefs in the Columbretes Islands [54, 55], an archipelago that is only 35 nautical miles southeast from the southernmost sector of the Catalan coast. *Lophocladia lallemandii* shows a highly invasive behaviour [20, 38, 52, 110–113] with severe affectations on several key Mediterranean ecosystems and species [32, 52, 114, 115].

The application of the BPL index to benthic Mediterranean assemblages has been performed easily. Each station was surveyed by a team of two divers and all the information required by the index could be obtained in a single dive lasting up to 1 h. The assessment of overall water body information obviously depends on the number of dives required to have a good representation and a proper station replication. However, in any case, the time needed is not longer than the time used for other assessments of environmental quality that require SCUBA diving [e.g. 116–121].

There is a clear increase on the alien species impact on shallow benthic assemblages in Catalonia during the last 6 years, with higher number of alien species present and higher coverages for some species. This implies a worsening on the BPL index, with 15 of the 29 water bodies surveyed having a weak impact by alien species and one water body having a moderate impact during the period 2011–2012, versus only six water bodies with a weak impact during the period 2007–2008. The spread of *W. setacea* is driving this worsening in northern shores, while the spread and increasing abundances of *C. cylindracea* and to a lesser extent *O. patagonica* are driving the deterioration in southern shores.

It is hard to compare these BPL values and the deterioration trend with other Mediterranean regions as this is the first time that the BPL has been applied in the Mediterranean. However, available data on the literature related to the current abundances of invasive species and its spread suggests that Catalonia has a lower affectation compared with other countries. We have already pointed out the extended colonization by alien macroalgae in bays and lagoons from the Gulf of Lions (France) [82] and the high level of colonization by *C. cylindracea*, *L. lallemandii* and *W. setacea* in the Balearic Islands [37, 38, 52, 91], which would probably give BPL values ranging from moderate to strong impact in most water bodies of these areas. The spread of *C. cylindracea* [122, 123] and *O. patagonica* [28, 89] in Spanish regions south of Catalonia also suggests weak to moderate values of BPL in these areas. And for sure the situation deteriorates when we move towards the Southeastern Mediterranean where the impacts of invasive species completely transform the whole ecosystem structure and dynamics [e.g. 39, 43, 124–128].

The conjunction of a relatively low water temperature that hinders the settlement and growth of warm-water aliens, the long distance to the Suez Canal and the existence of natural barriers between the lagoons from southern France and those from Ebre Delta bays seem to account for the present generalized weak impact of invasive species in most of the coastal water bodies from Catalonia. However, predicted sea warming following the current scenarios of temperature increase in the Mediterranean region [129], the expected expansion of the Suez Canal [130] and the current spread rates of species such as *C. cylindracea*, *W. setacea*, *O. patagonica* and *L. lallemandii* reported here do not provide any hope for this situation to be maintained or improved, neither in the short or in the long term. Thus, future monitoring for marine species introductions and invasions in the Catalan coast is strongly advised.

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Evolution of Chemical Pollution in Catalan Coastal Sediments

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Abstract In 2000 and from 2006 to 2011, a monitoring program was followed along the Catalan inner shelf, using homogeneous analytical methods and sampling strategies and focusing on the main sites of river sediment accumulation at present. Adjacent areas that are potentially vulnerable to pollution were also selected. Trace metals and organic pollutants were analyzed in surface sediment samples. Mean concentrations in each area show a distribution of organic and inorganic pollutants along the Catalan inner continental shelf. The highest concentrations are located on and around the coast of Barcelona city for most pollutants and locally on the coast of Tarragona city and the Ebre Delta for some of them. The concentrations tend to decrease gradually southward and sharply northward of Barcelona. The trace metal that shows the highest anomalies is Hg (max. enrichment factor, 34), whereas Cd, Zn, Cr, Pb, and Cu show more moderate anomalies. Sediment polycyclic aromatic hydrocarbons, 4-nonylphenols (metabolites of nonylphenol polyethoxylated, non-ionic surfactant), polybrominated diphenyl ethers, polychlorinated biphenyls, and dichlorodiphenyltrichloroethane are also significant. The time evolution of most trace metals shows a decreasing trend mainly between 2000 and 2006, whereas between 2006 and 2011 trends of trace metals and organic pollutants are not clear, as some of them increased and others decreased and many of them peaked in 2007 and 2009. The greatest decreases in trace metals were in the most polluted areas.

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Contents

Abbreviations

DDT	Dichlorodiphenyltrichloroethane (or dichlorodiphenyltrichloroethanes (DDTs) and metabolites DDE and DDD)
HCBz	Hexachlorobenzene
NPs	4-Nonylphenols (metabolites of nonylphenol polyethoxylated, nonionic surfactant)
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated biphenyl ethers
PCBs	Polychlorinated biphenyls

1 Introduction

Contaminants discharged into rivers are transported downstream to the marine environment. However, they can also be trapped in dams or during periods of low water discharge, and they can be partially trapped on the inner side of meanders, in river banks, or in estuaries before reaching the sea. In the Mediterranean, the irregular regime of rivers and streams (most of them small) and the local nature of many discharges mean that pollutants reach the sea in sporadic pulses that are difficult to predict. Once discharged into the sea, pollutants are scavenged when flocculation occurs along the freshwater-saltwater interface [1], where they may also be affected by dilution, desorption, absorption, aggregation, and precipitation [2–5]. In addition, offshore dispersion of pollutants is controlled by dynamic marine processes (mainly currents and waves) that induce transport, accumulation, and/or resuspension of pollutants and determine their final fate [6–9]. In many zones of the oceans, energetic processes transport and dilute the marine pollution load in the transit to the deep sea. However, in the Mediterranean Sea, the transport capacity of the dynamic processes is lower than in large oceans, so a large part of the particulate pollutant load can quickly settle on the seabed near the coast, in some cases before being sufficiently diluted, thus generating persistent and anomalous concentrations in the bottom coastal sediments. Only extreme hydrodynamic events such as strong wave storms or strong wind-induced currents can resuspend coastal sediment and transport it again [10–13], and these events are not too frequent in the Mediterranean.

Trace metals are among the most common pollutants discharged into the marine environment and are indicators of domestic and industrial pollution. In most advanced countries of the European Community, the distribution of trace metals in the marine environment is being studied systematically and extensively in order to estimate the environmental impact and the economic and social effects of pollutants and to take preventive and corrective measures.

During the 1980s and 1990s, studies of trace metals in sediments of specific areas of the Catalan coast including Barcelona, the Llobregat River mouth, and the Ebre Delta coast [14–17] showed significant trace metal pollution at some sites. However, these studies were not continuous in time and did not always follow the same sampling strategies or methods of analysis. Organic pollutants were also studied on the Catalan coast, and it was found that the contamination of PCBs and PAHs was greater near the sites of urban and industrial impact [18, 19].

Because the Water Framework Directive (WFD, DIR 2000/60/EC) is not specific with respect to sediments, the monitoring of organic pollutants in them has been at the discretion of experts. In this context, to protect the environment, the Catalan Water Agency (ACA) has also been analyzing organic pollutants in different matrices. The WFD describes the monitoring of priority substances (PS) and other pollutants in fresh waters and coastal waters. The daughter directive 2008/105/EC defined environmental quality standards (EQS) for priority substances in water, with the aim of protecting the aquatic environment from their adverse effects. This directive includes 33 PS, mainly organic pollutants. In the recent revision of the list of PS (DIR 2013/39/EU), 12 new ones were added, the EQS of some existing PS was changed, and values for biota were introduced. Additionally, compliance monitoring for PS in the WFD requires the achievement of a limit of quantification (LOQ) equal to or below 30% of the relevant EQS. The formula for calculating the LOQ is therefore $0.3 \times$ EQS. The WFD establishes the obligation to carry out programs to monitor and control the quality of water bodies and to establish management measures for them to achieve a good ecological and chemical status by 2015.

In 2000 the Marine Water Unit of the ACA started a surveillance program of heavy metal pollution in marine sediments of the Catalan inner shelf and established a monitoring network that prioritized the control of sediments in the vicinity of river mouths, where these elements tend to be more accumulated. This program provided homogeneity in the analytical methods and sampling strategies.

As a continuation of this work, the ACA started a second program to determine the levels of trace metals and organic pollutants in sediments of the Catalan coast in

2006, giving priority to the most affected areas of control according to the results of 2000 and also to water bodies that had not been evaluated at that time but were at risk of breaching the WFD [20]. Also, the monitoring of organic pollutants in sediment started at the discretion of experts. In the ACA program, which lasted until 2011, trace metals of some areas were analyzed annually, and organic pollutants were sampled at varying frequencies. In 2006, 2007, and 2009, all the sampling points of the monitoring network were analyzed, while in 2008 and 2010, only sampling points of the high-risk areas were analyzed.

1.1 Study Area

The fine sediment and pollutants that are present on the Catalan coast are mainly provided by the rivers, which develop mud belt prodeltas, particularly along the inner and mid-continental shelf [14, 15, 18, 21–24]. Outside these prodeltaic mud deposits, surface sediment can be relict from hundreds and thousands of years ago, when the sea level was lower than today [22, 25, 26]. Therefore, in this paper, we study the evolution of pollutants at selected sites of the present prodeltaic deposits of the rivers discharging on the Catalan coast (Fig. 1). The basic characteristics of the Catalan rivers are shown in Table 1. There are great differences between the Ebre River and the other eight rivers, which form the system known as the Inner Catalan River Basins. These eight rivers show a typical Mediterranean behavior, with short length, low discharges (extremely low in summer) and sporadic major floods typically in autumn and spring.

The system of the Inner Catalan River Basins occupies 16,600 km^2 (52% of the territory of Catalonia) and is densely populated, with about six million people accounting for 85% of the Catalan population. The most populated areas are the Barcelona Metropolitan Area and the Tarragona Bay. Heavily industrialized since the nineteenth century, the Barcelona Metropolitan Area is the sixth most populous urban area in the European Union (4.4 million inhabitants [2011]) and the second urban area on the Mediterranean Coast (Eurostat 2012). Tarragona Bay contains Tarragona city and one of the most important petrochemical clusters in the Mediterranean. The value of the production is equivalent to 0.75% of worldwide production, and the area produces 25% of the petrochemical products and 44% of the plastics for the Spanish market. Stockbreeding and agriculture are also important in the Inner Catalan River Basins. Stockbreeding is more important in the northern basins, while agriculture is more important in the southern ones. Finally, the Ebre River Basin occupies 85,362 km^2 in the north of the Iberian Peninsula, with a population of about three million people. The river flows through a major city, Zaragoza, but far away from the river mouth. In the lower Ebre River, although there is some local industrial activity, such as the Flix complex [27, 28], the main human activity is agriculture and stockbreeding.

2 Materials and Methods

2.1 Sampling

Surface sediment samples were taken from present-day prodelta deposits of the following rivers: Roses, Muga, Fluvià, Ter, Tordera, Besòs, Llobregat, Foix, Francolí, and Ebre. In addition, adjacent areas potentially vulnerable to pollution were also selected: Mataró, Masnou, Barceloneta, Castelldefels, La Falconera, Sitges, El Vendrell, Cambrils, Fangar Bay, and Alfacs Bay (Fig. 1).

In 2000, four transects of sampling stations were established in front of the mouth of each river perpendicular to the bathymetry. Considering that the main

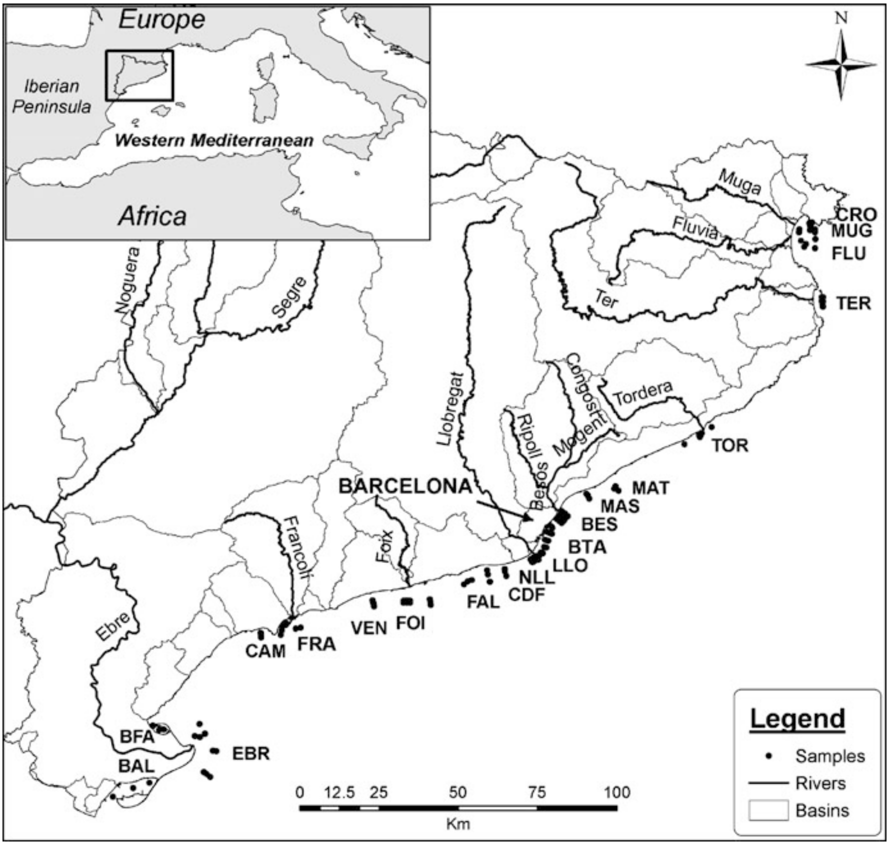


Fig. 1 Location of the study areas and samples in the context of the Catalan coast and the western Mediterranean (*CRO* Roses, *MUG* Muga prodelta, *FLU* Fluvià prodelta, *TER* Ter prodelta, *TOR* Tordera prodelta, *MAT* Mataró, *MAS* Masnou, *BTA* Barceloneta, *BCN* Barcelona city, *LLO* Llobregat prodelta, *NLL* new Llobregat, *CDF* Castelldefels, *FAL* La Falconera, *FOI* Foix prodelta, *VEN* El Vendrell, *FOI* Foix, *CAM* Cambrils, *EBR* Ebre prodelta, *BFA* Fangar Bay, *BAL* Alfacs Bay)

Table 1 Basic characteristics of the rivers flowing into the Catalan coast

River	Length (km)	Basin area (km^2)	Mean water discharge ($m^3\ s^{-1}$, 2005–2011)
Muga	65	854	2.18
Fluvià	97	1,125	3.98
Ter	208	3,010	12.57
Tordera	54	894	0.39
Besòs	58	1,039	4.33
Llobregat	170	4,948	13.64
Foix	49	312	0.25
Francolí	85	838	0.83
Ebre	910	85,534	247.83

Data source: Statistical Institute of Catalonia (IDESCAT) and Ebre Hydrographic Confederation (CHE)

Table 2 Sampling areas and corresponding water bodies following IMPRESS 2005

Area	Water body
Roses (CRO)	C6
Muga prodelta (MUG)	C6, C7
Fluvià prodelta (FLU)	C7
Ter prodelta (TER)	C11, C12
Tordera prodelta (TOR)	C15
Mataró (MAT)	C17
Masnou (MAS)	C17
Besòs prodelta (BES)	C19, C20
Barceloneta (BTA)	C20
Llobregat prodelta (LLO)	C21
New Llobregat (NLL)	C21
Castelldefels (CDF)	C22
La Falconera (FAL)	C23
Foix prodelta (FOI)	C25
El Vendrell (VEN)	C25
Francolí prodelta (FRA)	C27
Cambrils (CAM)	C29
Fangar Bay (BFA)	T1
Ebre prodelta (EBR)	C33
Alfacs Bay (BAL)	T3

current of the Catalan coast has a dominant southwestward component [29], we established one transect north of the river mouth, one just in front of the mouth, and the other two south of the river mouth. In each transect, sediment samples were collected at 10, 20, 30, 40, and 50 m depth. In the following years, only the samples that showed significant pollution or were representative of an area were taken.

In this paper, we selected the stations in which sampling was carried out for 3 or more years. The correspondence between these areas and the coastal water bodies defined in [20] is presented in Table 2. Mean concentrations for all the samples from the same area were calculated for each sampling year and for the whole study period.

Sediment samples were taken with a grab collecting the first cm of surface sediment. Samples for metals were stored at 4°C and later lyophilized and ground. Samples for organics were air-dried and manually ground (2 mm) before extraction.

2.2 Analytical Procedures

2.2.1 Grain Size

The grain size analysis was performed using a SediGraph 5100 (<50 μm fraction) and a settling tube (>50 μm fraction). The results for both fractions were joined in order to obtain the total grain size distribution and the textural statistical parameters for each sample [30]

2.2.2 Trace Metal Analytical Procedures

A total digestion technique was carried out according to Querol et al. [31], using HNO_3, HF, and HCl suprapure acids. For every 18 samples, a blank, a PACS-2 reference material (National Research Council Canada), and a random-replicated sample were used for analytical quality control. Trace elements were analyzed (except Hg) by ICP-MS. Al was analyzed by ICP-AES. The overall analytical uncertainty was below 15% and typically between 5% and 10%.

For Hg analysis, a LECO AMA254 Mercury Analyzer complying with US EPA Method 7473 [32] was used. PACS-2 reference material from the National Research Council Canada, random-replicated for every ten samples and blank samples to avoid memory effects, was used for analytical quality control. The overall analytical uncertainty was below 2%.

Background trace metal levels of sediment samples from several areas were determined in previous studies [14, 16, 23]. Surface samples of the present study with these background trace metal levels were used to estimate the enrichment factor (EF). With these samples, aluminum-trace metal regression curves were established for every trace element in order to find the background levels in terms of aluminum content (Al normalization). The ratio between metal content and background level (Al normalized) for each sample was defined as the EF, which is a dimensionless value. To evaluate toxicity risk, we also used reference values following Long et al. [33], who defined two values for each trace metal, effect range low (ERL) and effect range median (ERM), which defined three ranges whose effects to the environment are predicted to be minimal (value < ERL), occasional (ERL < value < ERM), and frequent (value > ERM).

2.2.3 Organic Pollutants

About 5 g of dried samples were spiked with isotopic labeled standards. Five grams of copper powder was added to the sample to remove elemental sulfur. The extraction was carried out by sonication with hexane/dichloromethane (1:1, v/v). The extract was separated by centrifugation and the extraction process was repeated once again. The extracts were joined, and, after addition of isooctane, the whole extract was concentrated to a volume of 4 mL.

The extract was passed through a glass column containing 5 g of silica gel deactivated with 5% Milli-Q water, and the column was eluted with 100 mL of 95:5 hexane/dichloromethane at atmospheric pressure. The extract was concentrated to a volume of 500 μL and transferred to an amber glass vial. Finally, recovery standards were added. The extract volume was adjusted to 250 μL with gentle nitrogen flow.

The analyses of different families of organic compounds are based on the isotope dilution quantitation method and were performed by gas chromatography coupled to mass spectrometry of low and high resolution (HRGC-LRMS & HRGC-HRMS). These methods are based mainly on United States Environmental Protection Agency (USEPA) methods (e.g., 8270C, 1614, 1668) [19, 24, 34–36].

3 Results

3.1 Distribution of Pollutants

Distribution of mean trace metal concentrations (and EF) and mean organic pollutants in each sampling area during the whole monitoring period shows three main sectors of pollutant levels along the Catalan coast: the northern Catalan coast, where mean pollutant levels were mainly low or absent; the Barcelona city area, where mean pollutant levels were high; and the southern Catalan coast, where mean pollutant levels were mostly medium-high decreasing to low southward (Figs. 2, 3, and 4).

3.1.1 Northern Catalan Coast

The northern Catalan coast sector extends from the French border to Barcelona city area. This sector comprises the Roses, Muga, Fluvià, Ter, Tordera, Mataró, and Masnou areas, including the fluvial basins of the Muga, Fluvià, Ter, and Tordera Rivers (Fig. 1). The samples from the Roses, Muga, and Fluvià were mainly mud, with a fine fraction (silt plus clay) content higher than 60%, whereas the samples from the other areas were mainly muddy sand with a fine fraction of only 30%.

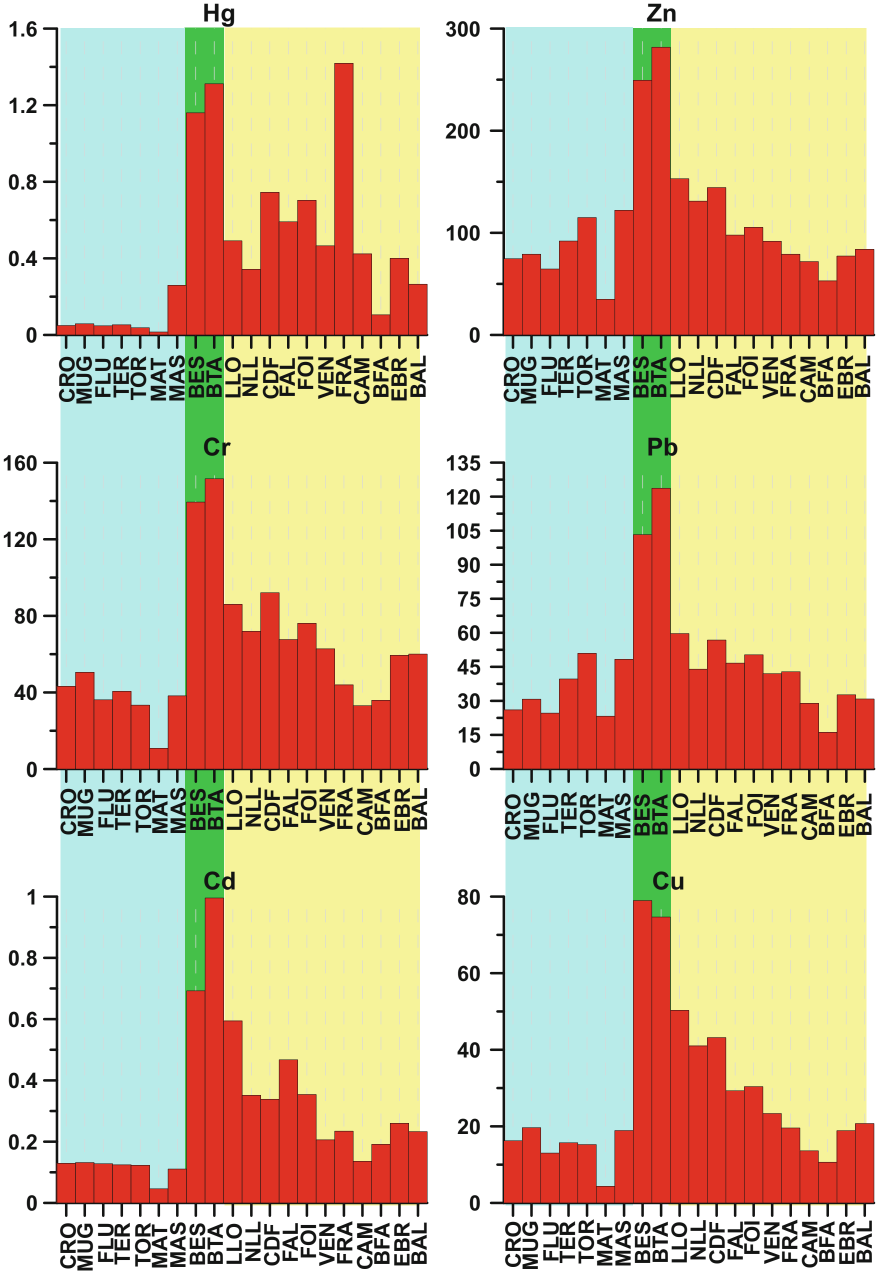


Fig. 2 Mean concentrations of trace metals (mg kg^{-1}) in the study areas during the whole monitoring period. Areas from the northern Catalan coast over *blue*. Areas from the Barcelona city coast over *green*. Areas from the Southern Catalan coast over *yellow*

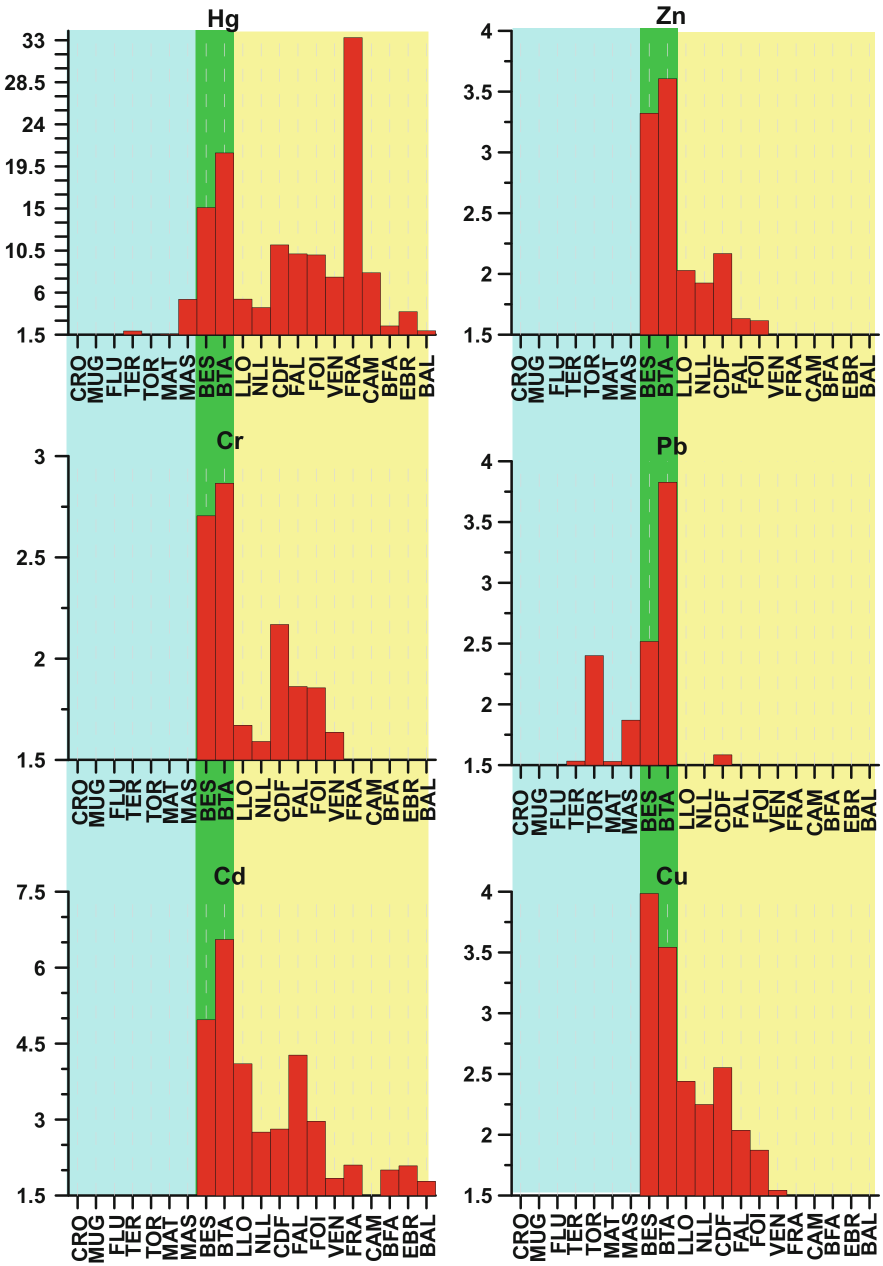


Fig. 3 Mean enrichment factors (EFs) of trace metals in the study areas during the whole monitoring period. Only EFs > 1.5 are considered significant and are represented. Areas from the northern Catalan coast over *blue*. Areas from the Barcelona city coast over *green*. Areas from the Southern Catalan coast over *yellow*

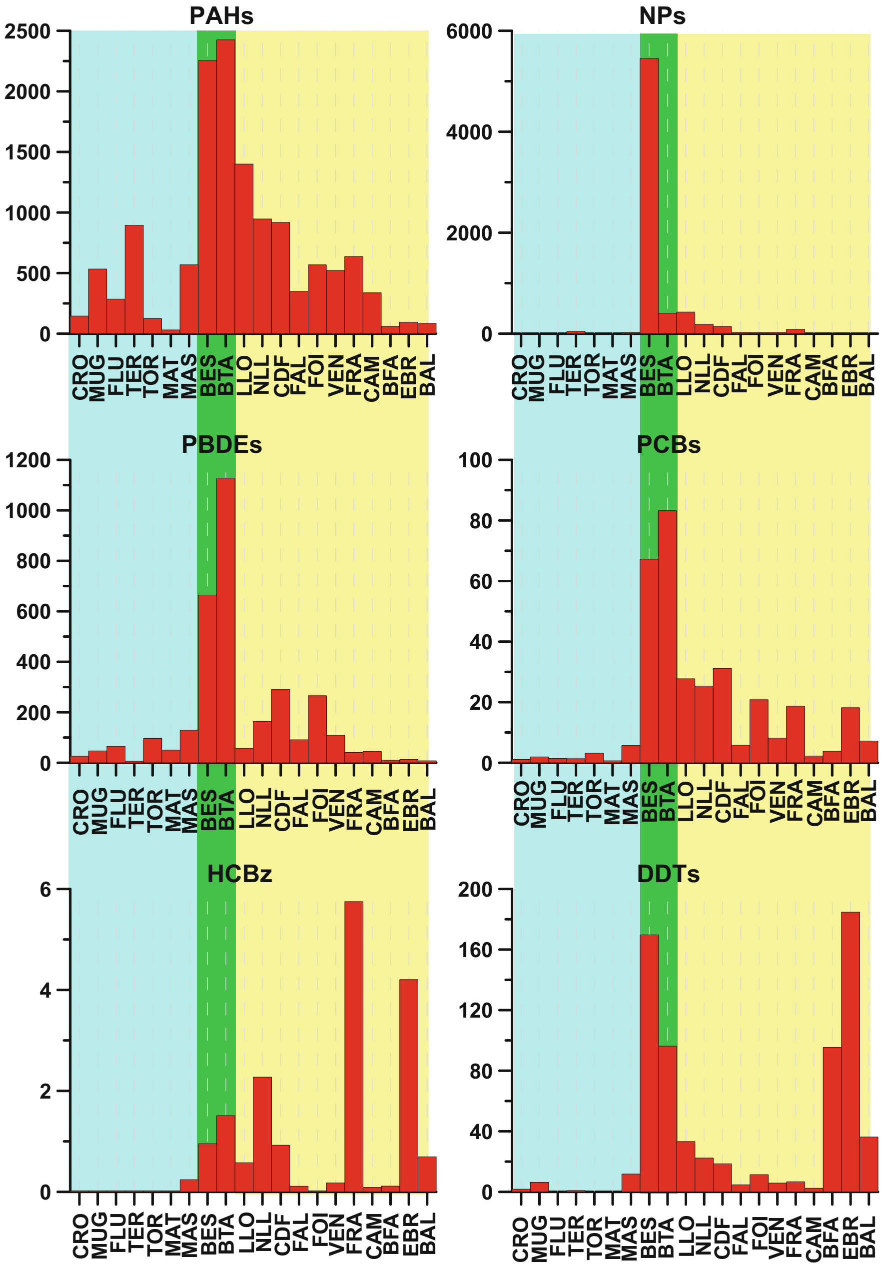


Fig. 4 Mean concentrations of organic pollutants (μg kg^{-1}) in the study areas during the whole monitoring period. Areas from the northern Catalan coast over *blue*. Areas from the Barcelona city coast over *green*. Areas from the Southern Catalan coast over *yellow*

Mean trace metal and organic pollutant concentrations in the areas of this sector during each monitored year are shown in Figs. 5 and 6.

In general, sediment samples taken in this sector showed natural or low levels of trace metal pollution, except in the case of Hg at Masnou where it showed a mean EF of 5.3 and concentrations between ERL and ERM values. Hg also reached a mean EF of 1.5 at Ter and Mataró (Fig. 3), where concentrations were below ERL values. Mean EFs for Pb of 2.4 were found at Tordera and of between 1.5 and 1.8 at Ter, Mataró, and Masnou, but only at Tordera did concentrations exceed ERL values without reaching ERM levels in 2000. Samples from all the other areas of this sector showed concentrations within the natural values below ERL for all the studied trace elements. Only Zn exceeded the ERL values slightly in 2009.

Regarding the organic pollutants, PAHs showed significant concentrations at Muga, Ter, and Masnou. PBDEs were identified at all the sites of this sector, increasing to the south, although significant values were only detected in 2007. The other families of organic compounds showed low concentrations, with only isolated increases in some areas (Figs. 4 and 6).

3.1.2 Barcelona City Coast

The coast of the Barcelona Metropolitan Area comprises the Besòs prodelta and Barceloneta and includes the fluvial basin of the Besòs River (Fig. 1). Most samples from this coast consisted of mud with more than 70% of fine fraction. This sector is where the Catalan coast showed the highest levels of most trace metals in sediments (Figs. 2 and 7). Hg is the element that showed the highest mean EFs (15 at Besòs and 21 at Barceloneta) in the area (Fig. 3). All the mean Hg concentrations on the Barcelona coast were above the ERM values during all the sampling years (Fig. 7). Anomalies of Cu, Zn, Cr, and Pb were lower (mean EF between 2.5 and 4) but relatively high for the marine environment, with concentrations between ERL and ERM values. Cd showed important anomalies in all the samples (mean EF 6.5) but only exceeded the ERL value in one sample (Fig. 7). In general, Besòs and Barceloneta showed the highest mean and absolute trace metal concentrations on the Catalan coast except for Hg, which was higher at Francolí (Fig. 2). However, these higher concentrations at Francolí were measured in only a few samples and mainly in 2000.

Mean organic pollutant levels were high in this sector, reaching maximum values for the PAH, NP, PBDE, and PCB families, which were significantly higher than those from other sectors. PAHs, PBDEs, and PCBs showed large increases in 2007. NPs increased strongly in 2009 at Besòs, which was the only area on the Catalan coast affected by high values of this family. DDT concentrations were also high in this sector, similar to those detected in the Ebre Delta area and increasing sharply in 2008 at Besòs. HCBz values were high but lower than the maximum values detected at Francolí and Ebre on the southern Catalan coast. A major increase in HCBz took place in 2010 at Barceloneta (Figs. 4 and 8).

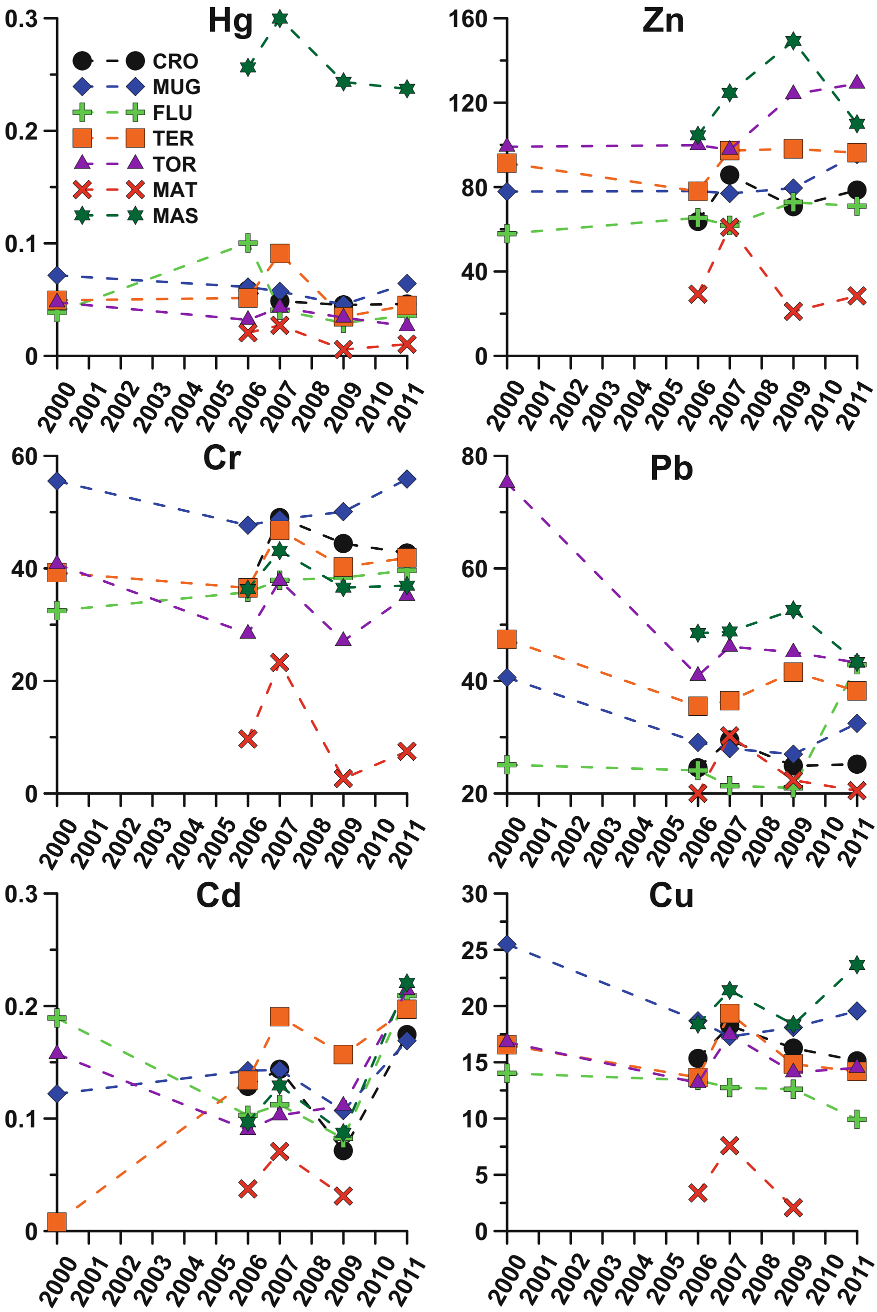


Fig. 5 Mean concentration of trace metals (mg kg^{-1}) in the study areas of the northern Catalan coast during each of the sampling years

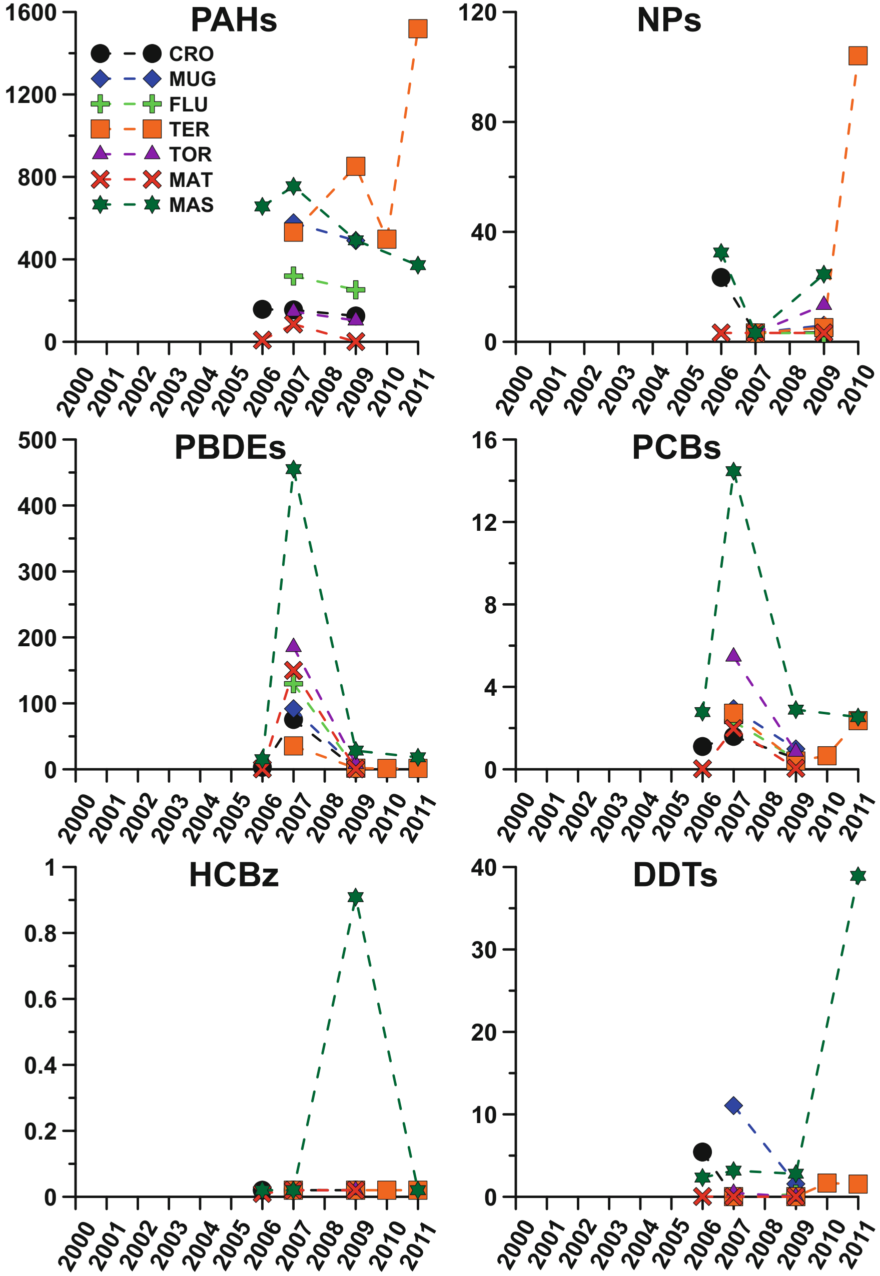


Fig. 6 Mean concentration of organic pollutants (μg kg^{-1}) in the study areas of the northern Catalan coast during each of the sampling years

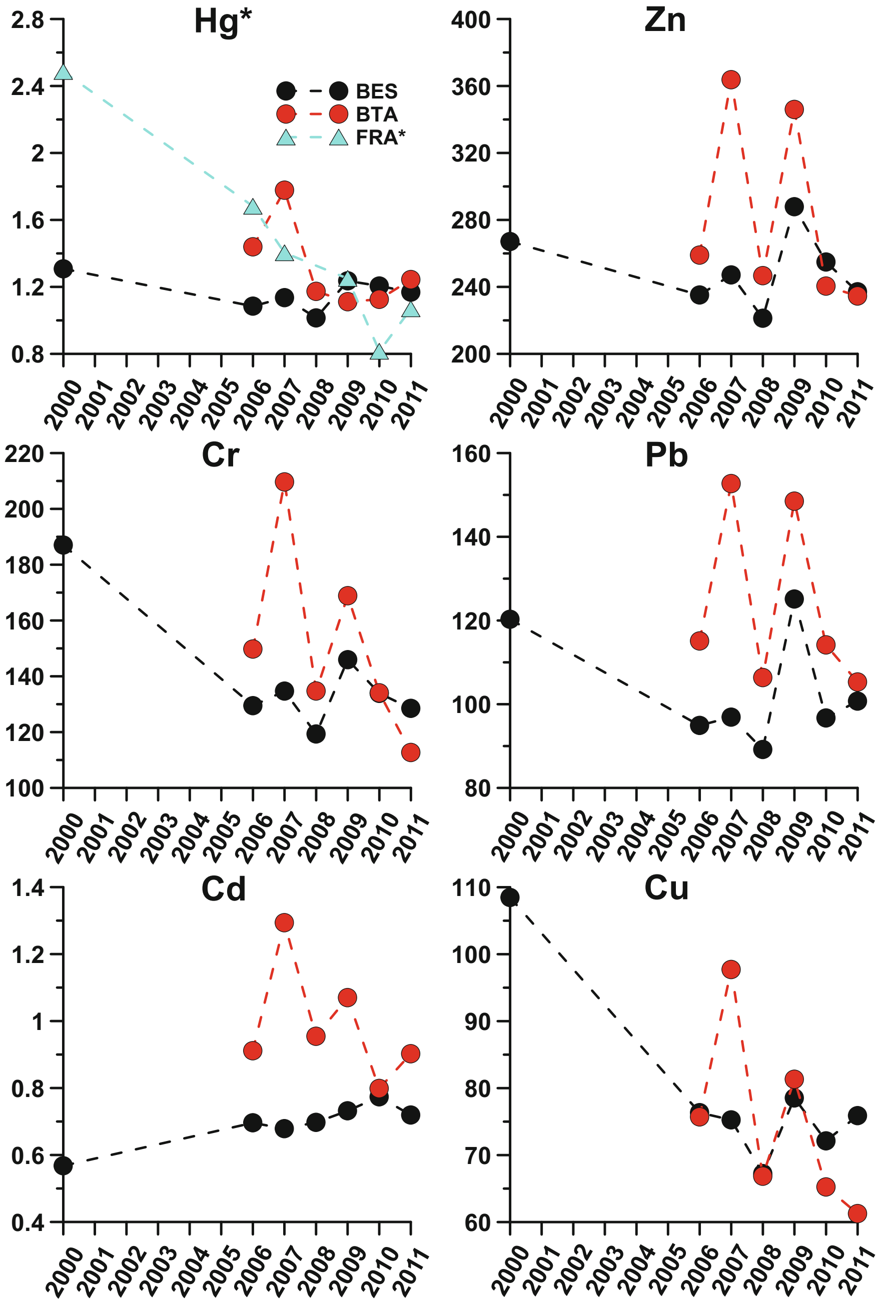


Fig. 7 Mean concentration of trace metals (mg kg^{-1}) in the study areas of the Barcelona city coast during each of the sampling years. *Hg of Francolí from the southern Catalan coast is represented in this figure because its concentration is of the same order of magnitude as those of the Barcelona city coast

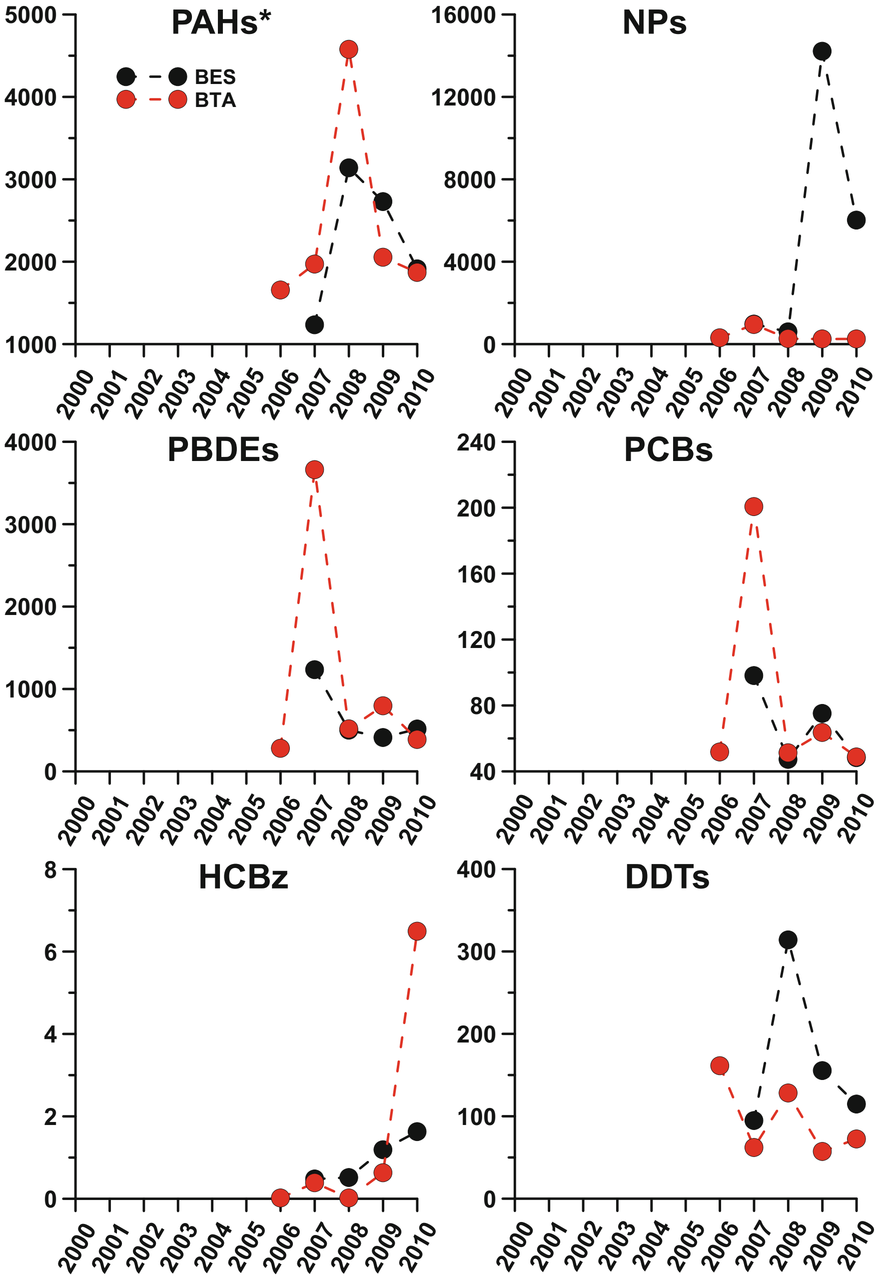


Fig. 8 Mean concentration of organic pollutants (μg kg^{-1}) in the study areas of the Barcelona city coast during each of the sampling years

3.1.3 Southern Catalan Coast

The areas of the southern Catalan coast are Llobregat, New Llobregat, Castelldefels, La Falconera, Foix, El Vendrell, Francolí, Cambrils, Fangar Bay, Ebre, and Alfacs Bay (Fig. 1). They include the basins of the Llobregat, Foix, Francoli, and Ebre Rivers. New Llobregat corresponds to the new Llobregat River mouth, which was moved 2 km southward artificially in 2004 to enlarge the Barcelona harbor. At La Falconera, there is a large dumpsite. The Francoli River mouth is in the harbor of the city of Tarragona. Most samples from this coast are mud with a fine fraction higher than 70%.

The southern Catalan coast was characterized by a gradual southward decrease of most trace metals, from significant pollution to near-natural levels and from general polluted areas to areas with only a few isolated points of pollution. The highest mean concentrations on the southern Catalan coast were at Llobregat and Castelldefels for Zn, Pb, and Cu; at Castelldefels for Cr; at Llobregat and La Falconera for Cd; and at Francolí for Hg. (Figs. 2 and 3). The highest mean Hg concentrations at Francolí were higher than those at Besòs and Barceloneta, with an EF of 34 and concentrations exceeding the ERM. However, the higher levels at Francolí only corresponded to a few samples with a maximum in those taken in 2000, whereas the high levels in the Barcelona city area (Besòs and Barceloneta) extended over a wider area than at Francolí. Hg concentrations of other samples at Francolí were similar to or lower than those at Besòs and Barceloneta. At Castelldefels the mean values and all the samples exceeded the ERM value for Hg (mean EF, 11), and at La Falconera and Foix, concentrations of Hg exceeded ERM values only in 2000. All the other samples from the southern Catalan coast exceeded the ERL value for Hg.

The mean and highest concentrations of Zn, Pb, Cr, and Cu only exceeded the ERL at Llobregat and Castelldefels, and Cd only exceeded the ERL at Llobregat and La Falconera in 2000 with an EF of 4. Toward the south of La Falconera, trace metal concentrations decreased to below the ERL. However, at Ebre trace metal concentrations increased slightly. Fangar Bay and Alfacs Bay are semi-enclosed bays that showed low pollution levels of Hg and Cd (EF around 2) and very low pollution levels of Zn and Cr (EF <1.4).

For the organic compounds, there was also a southward decreasing trend, except for HCBz and DDTs. HCBz showed maximum values at Francolí and Ebre, and DDTs concentrations also showed maximum values at Ebre. These two families of organic compounds are the only organic pollutants that reached the highest values on the southern Catalan coast in addition to the Barcelona city coast. PBDEs and PCBs showed great increases in all the areas of this sector in 2007. HCBz also showed high values at New Llobregat, Francolí, and Ebre in 2007 and at Ebre in 2011. DDTs increased at Ebre in 2007 and 2011 (Figs. 4 and 10). NPs values on the southern Catalan coast are low.

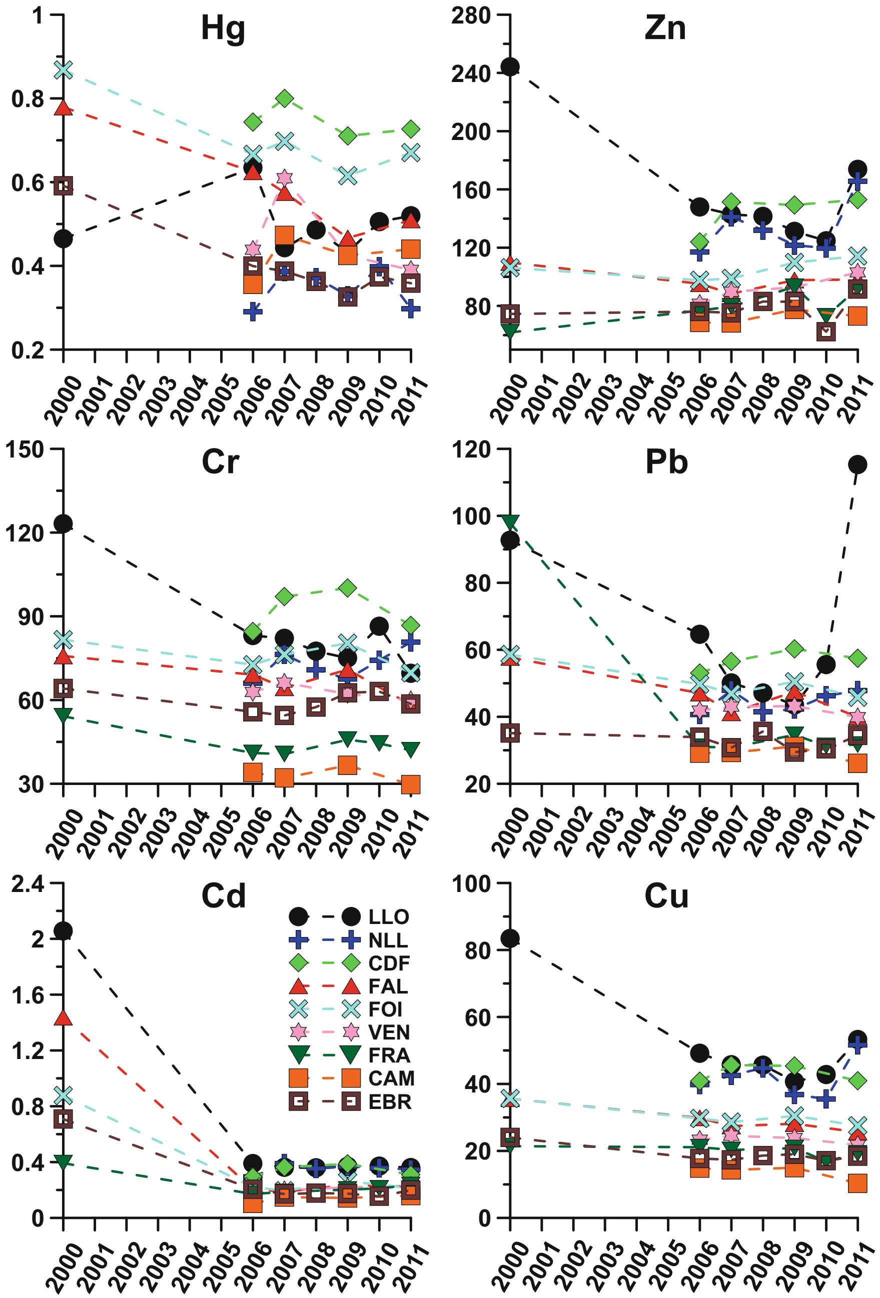


Fig. 9 Mean concentration of trace metals (mg kg^{-1}) in the study areas of the southern Catalan coast during each of the sampling years. Hg of Francolí is represented in Fig. 7 because its concentration is of the same order of magnitude as those of the Barcelona city coast

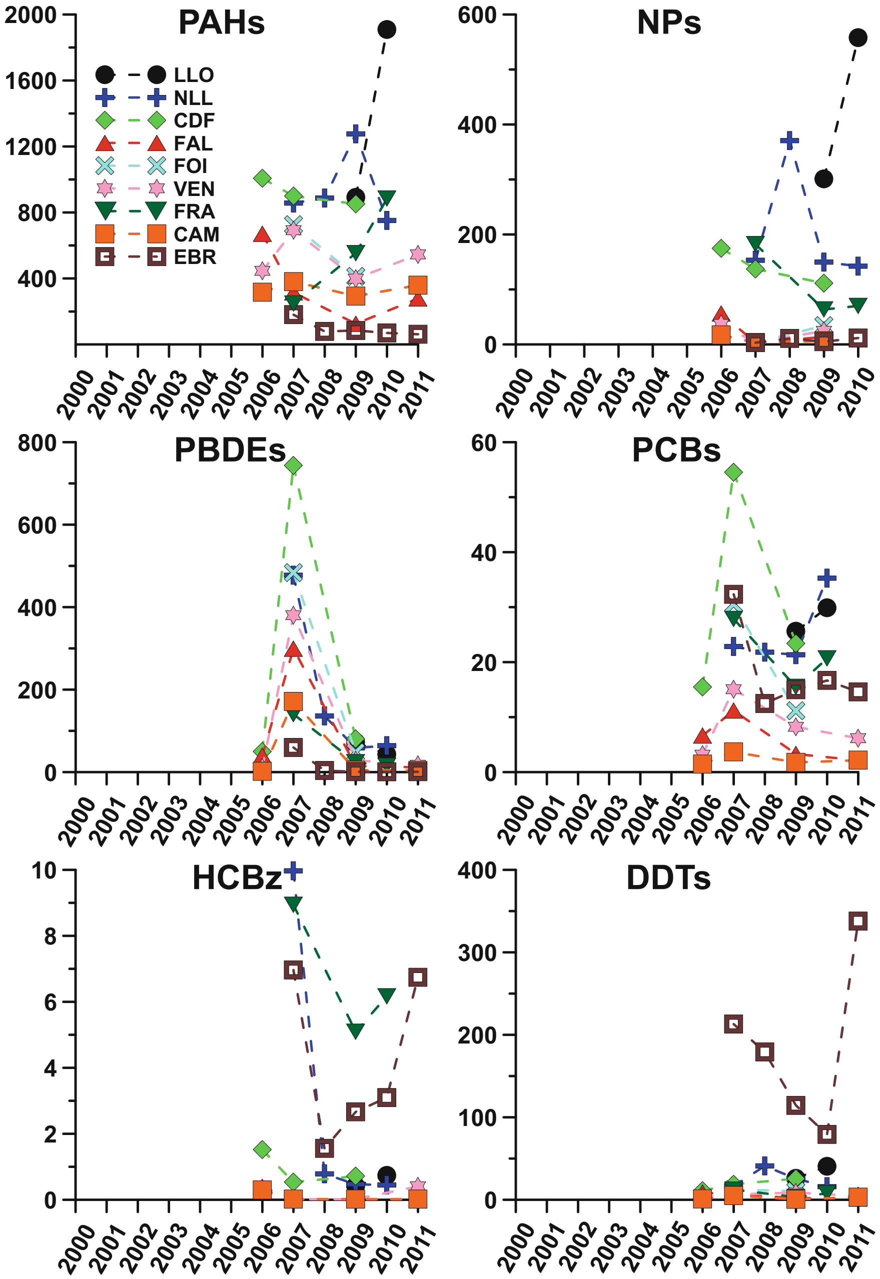


Fig. 10 Mean concentration of organic pollutants (μg kg^{-1}) in the study areas of the southern Catalan coast during each of the sampling years

3.2 Time Trends

Time trends of annual mean concentrations of trace metals in the monitored areas of the three sectors of the Catalan coast can be observed in Figs. 5, 7, and 9. On the northern Catalan coast, there are few clear trends because many samples have near-natural values of trace metals. Observing the difference between the samples taken in 2000 and those taken in 2006, Pb, Cr, and Cu decreased at Muga, Ter, and Tordera; Zn decreased at Ter; and Hg, Zn, and Cr increased slightly at Fluvià. However, comparing the samples taken in 2006 with those taken in 2011, Zn, Cr, Pb, and Cd increased in almost all the areas of this sector except Mataró. Other differences are small (Fig. 5). For organic compounds, there was no sampling in 2000 and in some years of the 2006–2011 period, so there are insufficient samples to define a trend in many areas. From the existing data, it seems that there was a major increase in PAHs and NPs at Ter and of DDTs at Masnou in 2011 and PAHs seem to have decreased at Masnou. Peaks of PBDEs and PCBs occurred in all the areas in 2007 (Fig. 6).

In the Barcelona city area, Hg, Zn, Cr, Pb, and Cu decreased at Besòs from 2000 to 2006 and only Cd increased in this period of time. However, considering only the annual mean concentrations from 2006 to 2011, these decreasing trends were not so clear (Fig. 7). At Barceloneta, there appears to be a decreasing trend for all the studied trace metals between 2006 and 2011, but there were peaks in 2007 and 2009. At Besòs, there were also peaks of some metals in 2009. For organic compounds, there were peaks of PBDEs and PCBs at Besòs and Barceloneta in 2007, of PAHs and DDTs at Besòs and Barceloneta in 2008, of NPs at Besòs in 2009, and of HCBz at BAR in 2011. HCBz seems to have shown an increasing trend at Besòs and Barceloneta, whereas PBDEs and PCBs decreased at Besòs and DDTs decreased at Barceloneta (Fig. 8)

On the southern Catalan coast, the trace metals decreased in all the areas between 2000 and 2006, with the exception of Hg at Llobregat and Zn at Francolí (Fig. 9). However, from 2006 to 2011, Zn increased in all the areas and Cd increased slightly. On the other hand, Hg, Cr, Pb, and Cu decreased slightly in most areas, and Cr, Pb, and Cu increased at New Llobregat and Castelldefels. For organic compounds, there were peaks of PBDEs and PCBs in all the areas in 2007. NPs and HCBz decreased at New Llobregat, Castelldefels, and Ebre (Fig. 10).

4 Discussion

Pollution on the Catalan coast is mainly associated with discharges from urban and industrial areas. The main urban area on the coast is Barcelona and its metropolitan area, and the second is Tarragona. Several small coastal cities and towns can also show local anomalies. The rivers transport trace metal and organic pollution from inland cities and industries. The main sources of industrial pollution in Catalonia

Table 3 Coastal cities with more than 20,000 inhabitants in the three sectors of the Catalan coast

Cities > 20×10^5 inh.	Population
Northern Catalan coast	
Lloret de Mar	40,282
Blanes	39,834
Premià de Mar	28,310
Pineda de Mar	26,040
Palafrugell	22,816
El Masnou	22,595
Mataró	123,868
Sant Feliu de Guíxols	21,814
Total	*325,559*
Barcelona city coast	
Barcelona	1,615,448
Hospitalet	256,065
Badalona	219,786
Santa Coloma de Gramenet	120,824
Sant Adrià de Besòs	341,57
Total	*2,246,280*
Southern Catalan coast	
Tarragona	134,085
Vilanova i la Geltrú	66,905
Viladecans	64,737
Prat de Llobregat	63,499
Castelldefels	63,139
Gavà	46,250
El Vendrell	36,454
Cambrils	33,008
Sitges	28,617
Salou	26,193
Calafell	24,984
Total	*587,871*

are located in the basins of the Besòs, Llobregat, Francolí, and Ebre Rivers [37]. In addition to rivers, other sources are wastewater plants, outfalls, storm sewers, and wind and rain events, which can also discharge significant amounts of pollutants.

On the northern Catalan coast, there are few populated coastal cities and few industrial areas. There are eight cities of more than 20,000 inhabitants which total about 325,000 inhabitants (Table 3). Landward, there are five cities with more than 20,000 inhabitants which total 226,000 inhabitants. Pollution load affecting this part of the coast is lower and is probably more diluted by the rivers discharging on it (the Muga, Fluvià, Ter, and Tordera) than is the case on the southern coast. The sediment discharged by rivers and accumulated on the northern coast often shows near-natural trace metal values and low organic pollution concentrations (Fig. 2). The Ter River has two large dams of 165 and 233 hm^3 along its course, whereas the

Muga River has a small one of 61 hm^3. Pollutants transported by these rivers are partially retained by these dams, but these rivers and the coastal towns produce some local anomalies at sea.

In contrast, in highly populated and industrialized areas, where the pollutant load affecting the rivers is higher, rivers are unable to dilute the concentration sufficiently to reach near-natural values. As a result, they are still discharging significant pollution into the sea and producing anomalies in the bottom sediments. This is the case of the Barcelona city coast, where more than two million inhabitants live in five cities (Table 3). In addition, more than 860,000 inhabitants live in 12 cities of more than 20,000 inhabitants located in the Besòs watershed. The Besòs River, the ancient Besòs wastewater treatment plant, and the sewers from Barceloneta have been generating high trace metal contents in the bottom sediment of the Besòs prodelta for decades [16, 38]. Also, most of the main representative families of organic pollutants are concentrated at Besòs and Barceloneta (Fig. 4). When the Besòs River discharge is low, most of it is treated in the Barcelona-Besòs wastewater treatment plant before being discharged into the sea, but when it is high, the plant is unable to process all of it and the rest goes directly into the sea.

DDT and its metabolites deserve particular consideration as their use was forbidden decades ago. There was an old industrial area of DDT affecting sensitive areas of the Besòs that was studied prior to the appearance of the WFD. However, our data indicate a recent contribution of DDT, apart from its known metabolites DDDs and DDEs.

In the case of NPs, although their use has declined significantly, probably due to their inclusion in the first list of priority pollutants [2455/2001/EC] and their values are low on most of the Catalan coast, a major isolated peak was still detected at Besòs, which should be noted as an exception to this decline.

On the southern Catalan coast, 11 coastal cities have a population of more than 20,000, totaling 580,000 people. A further 20 cities located inland in the watershed of the rivers discharging on this coast have a population of more than 20,000, bringing the grand total to one million people (Table 3). Some of the highest trace metal concentrations and organic pollutants are found around the Llobregat River mouth area, which receives the inputs from several populated cities and industries. The sediments from the Llobregat prodelta, although polluted, have lower trace metal contents than those of the Besòs prodelta. One of the reasons for this is that the Llobregat River has a mean water discharge three times higher than that of the Besòs River (Table 1 and Fig. 11). Therefore, the Llobregat has a higher dilution capacity, which probably helps to produce lower pollutant levels in the Llobregat prodelta sediments than in the Besòs prodelta sediments. The mouth of the Llobregat River was moved about 2 km southward in 2004 to enlarge the Barcelona harbor, and a wastewater treatment plant was built in 2006. However, our data show no clear effect of this move in the pollutants of the sediments of this area. Southward from the Llobregat River, there are eight coastal cities of more than 20,000 inhabitants between Castelldefels and Salou. The largest of these cities is Tarragona (133,000 inhabitants), where the Francolí River discharges into the harbor, causing major local anomalies of Hg and HCBz, as it is affected by the

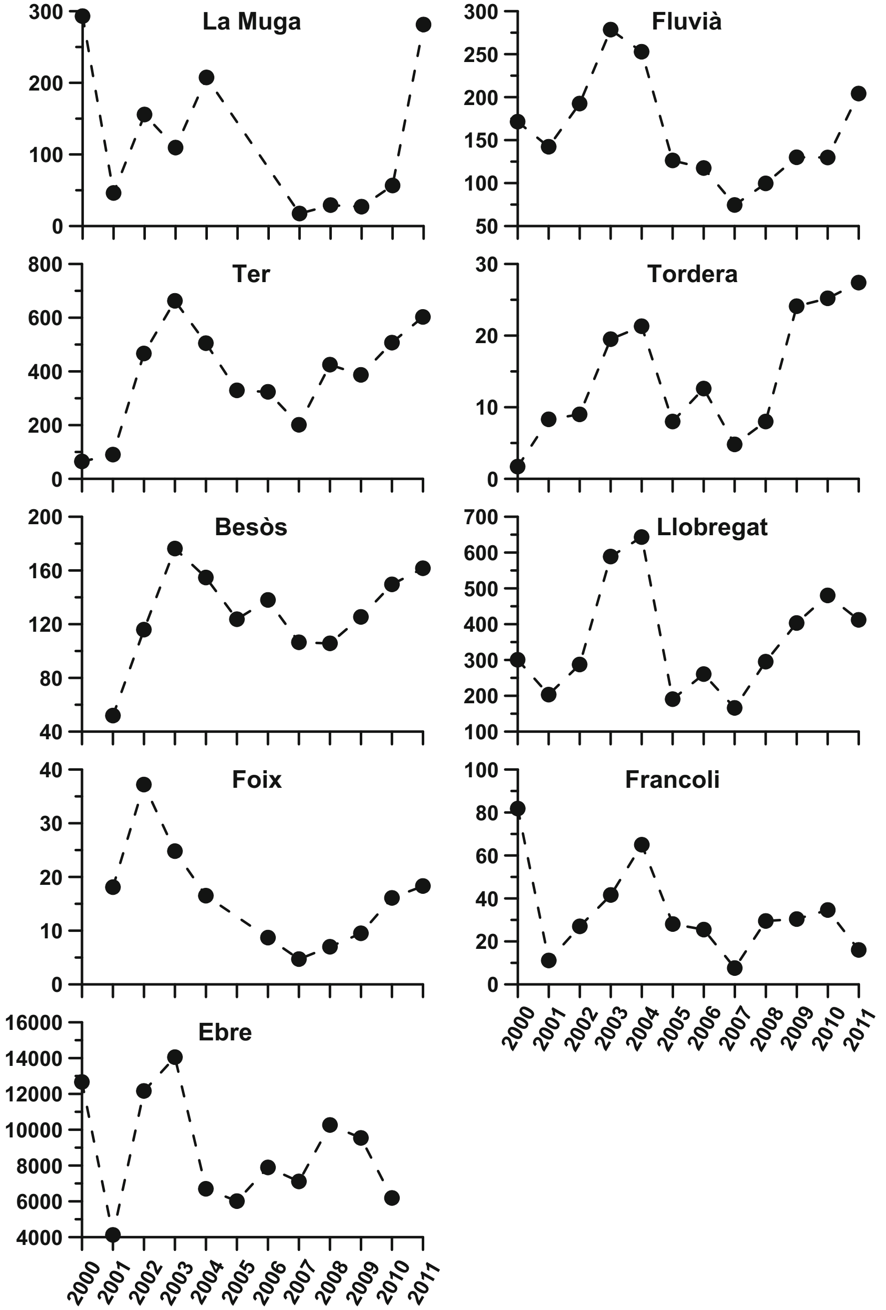


Fig. 11 Annual water discharge (hm^3) of the Catalan rivers from 2000 to 2011 (from idescat.cat)

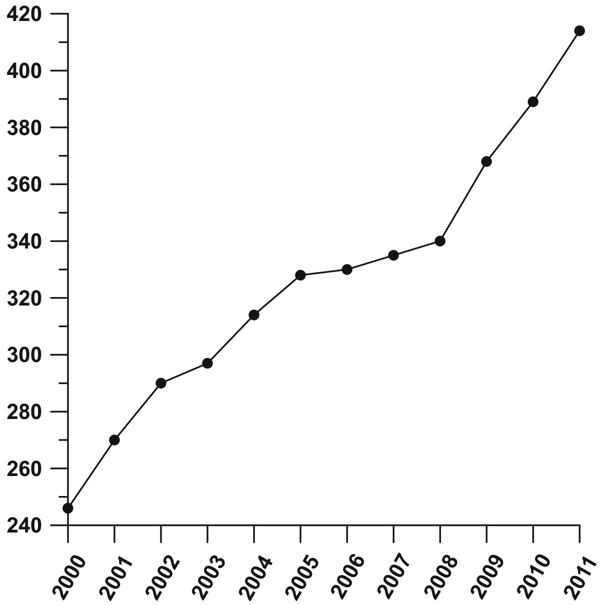


Fig. 12 Annual number of wastewater treatment plants working from 2000 to 2011 in Catalonia

activity of the city and the petrochemical industry. Southward from Salou, there are no populated cities and trace metals and organic pollutants decrease to low values. However, in the south, the Ebre River has a mean water discharge about two orders of magnitude higher than that of the other rivers (Table 1, Fig. 11). The lower part of this river is mainly affected by agriculture and some industry, such as the Flix Erkimia complex [28]. Upstream from the town of Flix, there are several dams along the Ebre River and its tributaries [39], which retain part of the pollutant load of this river [40]. Although this river has a much higher dilution capacity than the other Catalan rivers, there are Hg and Cd enrichments in the sediment accumulated around the Ebre Delta, as well as high values of HCBz and DDTs. As at Besòs, our data indicate a recent input of DDT. These pollutants break the southward decreasing trend. The explanation probably lies in the known contamination of the industrial chemical area of Flix (approx. 50 km from the river mouth), which is currently being treated.

Time trends in trace metals show a fairly general decreasing trend between 2000 and 2006. However, between 2006 and 2011, no clear trend is observed (Figs. 5, 7, and 9). Increasing trends are only observed for Cd at Ter and Besòs and for Zn at Tordera, Castelldefels, and Francolí. One of the reasons for the decreasing trend of trace metals between 2000 and 2006 is the continuous increase in the number of wastewater treatment plants in Catalonia (Fig. 12), including new plants on the Besòs and Llobregat Rivers. However, this increase does not explain why the trace metal levels remained similar from 2006 to 2011. The answer has more to do with the amount of water treated at these plants, which increased significantly from 2000 to 2003 and remained quite similar between 2006 and 2011 (Fig. 13).

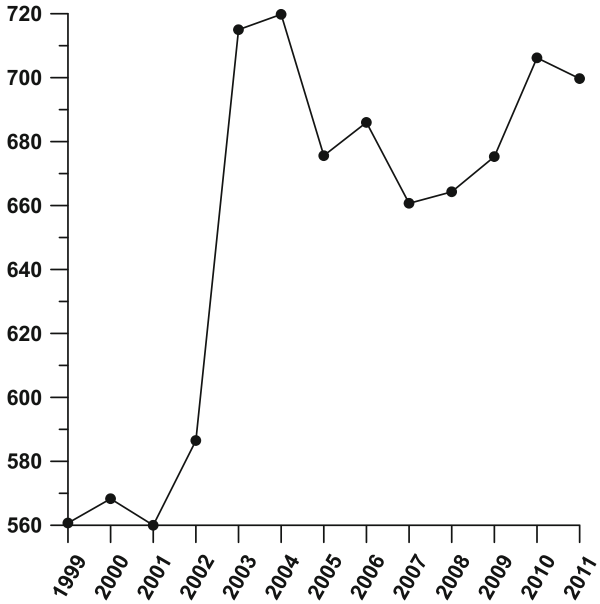


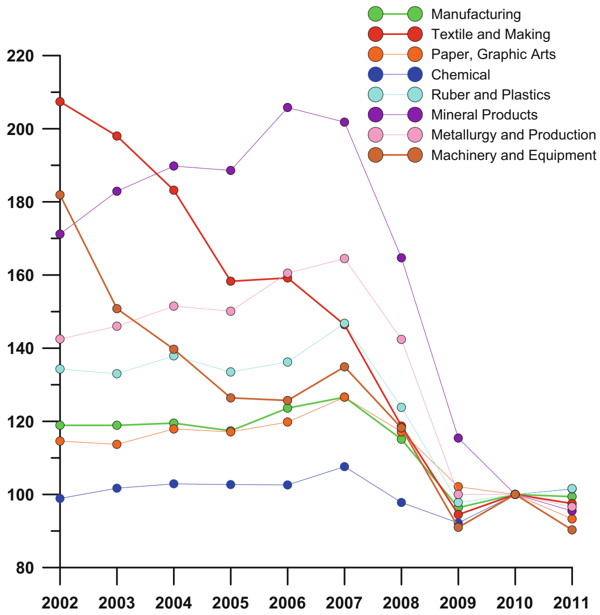
Fig. 13 Annual water outflow treated by wastewater treatment plants in Catalonia (hm^3)

Hydrodynamic processes must also be considered. From 2001 to 2006, 13 storm events with a significant deepwater wave height exceeding 3 m and a minimum duration of 70 h took place on the Barcelona city coast, whereas only six of these events occurred in the 2006–2011 period [41]. In addition, river water discharge also increased between 2002 and 2004 (Fig. 11). These storms and water discharge increases could have led to a decrease in trace metal levels of coastal sediments between 2000 and 2006 by resuspension and dilution.

Another cause of the trace metal decrease could be industrial production (Fig. 14). Textile and machinery and equipment production decreased sharply from 2000 to 2006 and continued to decrease until 2009. However, other industrial activities did not decrease until 2008. A strong decrease in all activities took place from 2008, but trace metal contents did not show this decrease in the monitoring carried out between 2008 and 2011.

In fact, some trace metals and organic pollutants increased in some areas in 2007, 2009, and 2011. These peaks of pollutants may have a variety of causes. The most general one could be the low water discharge of most rivers in 2007 (Fig. 11), which probably led to a lower pollutant dilution in most rivers and to peaks of several pollutants (PBDEs, PCBs, HCBz, Hg, Cr, Pb, Cd, and Cu) in 2007. In addition, the strongest storms of the 2006–2011 period occurred in 2008 and 2010 [41], with higher river discharge than in 2007 (Fig. 11), which could have reduced the levels of some pollutants on the Barcelona coast. The peaks in 2009 only occurred on the Barcelona city coast for NPs, Zn, Cr, Pb, Cd, and Cu and were related to an anomalous discharge of the Besòs wastewater treatment plant.

Fig. 14 Industrial Production Index (IPI). Annual means (Base 2010 = 100)



Finally, other causes could have contributed to these peaks. For example, (a) economic problems in management of wastewater treatment plants due to the crisis could have led to a lower reduction of the pollutant discharge, (b) industry could have invested less in environmental care, (c) the pollution generated during the pre-crisis times and retained in fluvial traps (meanders, small dams, etc.) could have been discharged in the following years, and (d) the pollution accumulated in coastal sediment could have been resuspended and redistributed on the coastal seabed.

If we compare the mean trace metal concentrations of the first and last year of monitoring in each zone, a dominant decreasing trend is observed on the Barcelona city coast and the southern Catalan coast (Figs. 7 and 9). In most cases, the most polluted zones (Besòs, Barceloneta, Llobregat, La Falconera, and Francolí) are the ones that show the highest decrease between the first and the last sampling year. The available data on organic pollutants show no dominant trend.

If we compare the data from this study with those from studies carried out in the 1980s and 1990s [14, 16, 17, 23, 42], in some areas, we observe that at Besòs maximum levels of Pb and Cd decreased by one order of magnitude, maximum levels of Cr and Cu decreased fourfold, and maximum levels of Hg decreased by half. At Llobregat, Cr, Pb, and Cu maximum concentrations decreased by a quarter. These comparisons must be taken with care, as methods and analytical equipment have changed, and the position of the compared samples is different. However, it is evident that this decrease is related to the installation of more efficient water treatment systems and the establishment of new environmental laws that have restricted the use of many pollutants over the last decades, as stated by Pinedo et al. [43], who monitored trace metals in shallow sediments (most of them taken between 6 and 12 m depth) along the Catalan coast in 2002, 2003, 2007, and 2010. Although most of these samples were sands with less than 10% of mud, these authors also detected maximum trace metal concentrations in Barcelona and Tarragona. However, most of the trace metal concentrations detected in this study were lower than those of our study, and pollution levels were not identified in several areas where we detected anthropogenic metal enrichments. Pinedo et al. [43] found increases between 2003 and 2007 and decreases in 2010.

5 Conclusions

This study allows the Catalan coast to be divided into three sectors in terms of trace metal pollution. The northern Catalan coast, from the French border to the town of Mataró, showed natural or near-natural levels, with isolated pollution at some points. The Barcelona city coast showed very high pollution for most of the studied pollutants, especially Hg, Cd, PAHs, PBDEs, and NPs. Finally, the southern Catalan coast showed significant pollution decreasing southward with isolated maximum values of Hg and HCBz at Francolí and maximum values of HCBz and DDTs at Ebre.

The Catalan coast showed considerable pollution of Hg along two-thirds of its length, between Mataró (Barcelona Metropolitan Area) and the Ebre Delta. The main Hg pollution is associated with areas of intense industrial and urban activities (Besòs, Barceloneta, Francolí). Mean Hg concentrations at Besòs, Barceloneta, and Francolí exceeded the ERM value in all years monitored, reaching a maximum mean EF of 33 at Francolí. Mean Hg concentration also exceeded the ERM value at La Falconera and Foix in 2000.

Cd exhibited a similar behavior to Hg but with lower anomalies, reaching maximum EF values of 6.5. It had maximum concentrations on the Barcelona city coast but not at Francolí. Although Cd concentrations were high, they rarely reached the ERL value. The sources were the same as those of Hg.

Cr, Pb, Cu, and Zn also showed levels indicative of pollution, but with a lower EF than Hg and Cd (EF: 3.5-4). They also showed higher values on the Barcelona city coast and decreasing values southward.

Similarly to trace metals, most of the main representative organic families are strongly concentrated on the Barcelona city coast, which receives the organic pollution of the industrial and urban conurbation of Barcelona. This includes the emerging pollution of the PBDEs family and exceptionally the NPs family at Besòs, although their use is declining. Maximum values of DDTs and HCBz at Ebre can be related to the Flix industrial complex and maximum HCBz at Francolí to the Francolí River inputs and the Tarragona harbor.

Rivers generate anomalies in coastal sediment depending on their pollutant load and their capacity to dilute it in their water discharge. In the case of the Besòs River, the pollutant load is too high to be sufficiently diluted by the river water and dispersed by coastal processes. Trace metals showed a dominant decreasing trend between 2000 and 2006 on the Barcelona city and southern Catalan coasts, the most polluted areas being the ones that show the sharpest decrease. This decrease can be due to an increase in the water treated in wastewater treatment plants and to natural causes (dilution by storms and water discharge). The pollutant peaks in 2007 can be associated with dryness and those in 2009 on the Barcelona coast can be associated with malfunctioning of wastewater treatment plants. Economic cuts reducing the observance of environmental policies and the redistribution of ancient pollutants could also have contributed to pollutant increases during some of the crisis years.

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Monitoring Programmes for Bathing Waters Within the Frame of the EU Bathing Water Directive: The Experience of Catalonia

Maria José Figueras, Mariona de Torres, Carolina Silvera, and Maria José Corrales

Abstract The quality of coastal recreational waters is monitored in Europe and other geographical areas through the analysis of faecal indicator bacteria, i.e. total and faecal coliforms and more recently *E. coli* and intestinal enterococci as in the new EU Bathing Water Directive (2006/7/EC). The old and new indicators and their methods of analysis are presented in this chapter underlying the limitations detected for the methods included in the new Directive. The expertise achieved along the ca. 25 years of monitoring the Catalan bathing waters revealed the two factors responsible of sporadically altering the otherwise excellent water quality of our beaches. These factors are rain events and incidences of the sewerage system. The Catalan beaches have been classified on the basis of the impact that rain events may have on altering their water quality. This information is now used to provide almost real-time warnings through the recently launched mobile app. Common alteration of the water aspect such as water discolouration, phytoplankton blooms and presence of foam that may be perceived as contamination by the public showed after investigation to correspond mainly to natural phenomena. The interrelationship established with the municipalities and the developed communication systems enabled to obtain accurate timely information from each bathing area.

Keywords Bathing water profiles, Bathing water quality, Beaches, *E. coli*, Faecal indicator bacteria, Intestinal enterococci, Mobile app, Rain events, Wastewater

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Contents

1 Introduction

Natural waters are subject to important changes in their microbial quality due to discharges of treated or untreated sewage or wastewater as a result of human activity or storm water run-off. Sewage effluents contain a wide range of pathogenic microorganisms that may pose a health hazard to the human population when they are discharged into recreational waters [1]. The density and variety of these pathogens are related to the size of the population, the seasonal incidence of the illness and the dissemination of pathogens [2, 3]. The evaluation of the microbiological quality of bathing water aims to protect the users from illness derived from the potential accidental consumption or contact with open wounds of water that may contain pathogens such as bacteria, viruses and protozoa and thus to prevent water-related outbreaks [4–6]. This has been and still is nowadays an important challenge. For the past century, this evaluation has been performed through the analysis of the bathing water quality using faecal indicator bacteria (FIB), which are expected to predict the potential presence of pathogenic microorganisms in the water [1–7]. However, scientists, engineers, public health officials and water pollution control agencies have faced cases in which the quality of water showed the presence of high levels of indicators when water had already been used for recreation [3]. Outbreaks linked to bathing in a specific recreational area are very difficult to demonstrate, because of the many confounders that can be involved, such as the consumption in parallel of drinking water and food. Nevertheless, a relationship between bathing in faecally contaminated areas and health outcomes has been reported in several studies [2–10].

The World Health Organization (WHO) has been very active in developing guidelines for monitoring bathing waters of universal application (freely accessible through the Internet) and has promoted a more preventive approach based on risk assessment that can anticipate possible contamination events [4–6]. This approach takes into account all factors that can endanger the quality of water in the bathing area and pretend to be able to predict water quality deterioration before it occurs. The European Commission has undertaken an extensive revision of the existing 1976 Directive (76/160/EEC) concerning the quality of bathing water [11], taking into consideration the principles of the WHO guidelines [4–6], that resulted in the publication in February 2006 of a new updated Directive (2006/7/EC) [12]. The first innovation is related with the title of the new Directive that concerns “the management of bathing water quality” and not only the “water quality” as the old Directive (76/160/EEC). The latter will be completely substituted by Directive 2006/7/EC with effect from eight years after its entry into force (i.e. on 31 December 2014). The change in title is very relevant because it is not only mandatory to assess the water quality but also to do interventions in order to increase the quality of bathing water. Despite of that, both Directives shared as a general objective to preserve, protect and improve the quality of the environment and protect human health. The other innovative aspect is the change of the FIB and the requirement for elaborating for each bathing area a risk assessment, the so-called bathing water profiles (BWPs). The latter includes an exhaustive analysis of the potential sources of pollution that can endanger the bathing water quality. In fact, the BWPs, as indicated in the Directive (2006/7/EC), have to rely on or use data obtained from the monitoring and the assessments of the Water Framework Directive (2000/60/EC) [13]. Therefore both Directives are interlinked as both should nourish from each other’s data. The BWPs, which should have been established for the first time in 2011, are meant to be an essential tool for the daily management of bathing water quality during the bathing season [12].

The indicator organisms used to evaluate the microbiological quality of water and their methods of analyses have been extensively reviewed [14–18], as well as the relevance of some of them, e.g. faecal streptococci [16–18], and their relationship with specific pathogenic bacteria, e.g. *Salmonella* [7, 18, 19], *Arcobacter* [20, 21], viruses [22–24] and parasites [25].

In this chapter we will focus on describing the FIB, included in the old and new Directive underlining some of the limitations detected for the microbiological methods included in the new Directive. The factors that alter water quality using the expertise acquired through the monitoring programmes for bathing waters developed in Catalonia (northeast of Spain) since 1990 until now are presented as well as the public information systems including the recently launched mobile app and the interrelationship established with the municipalities and other stakeholders.

2 Faecal Indicators in Bathing Water Control

There are hundreds of different enteric microorganisms that are known to infect humans. Enteric microorganisms are excreted in the faeces of infected individuals or animals and may directly or indirectly contaminate water used for recreational activities [1, 3–5, 7, 22–25]. Some agents of waterborne diseases, such as *Legionella* spp., *Vibrio* spp., *Aeromonas* spp. and *Pseudomonas*, are indigenous of aquatic environments; therefore, infections caused by these microorganisms will depend on the exposure to natural waters [1, 3, 5]. However, most microbial waterborne pathogens of concern originate in the enteric tracts of humans and animals and enter the aquatic environment via faecal contamination [1–7]. The concentration of these pathogens in natural waters will depend on the number of infected persons and/or asymptomatic carriers in the community and the effectiveness of the wastewater treatment before the discharge to reduce their load [1, 3–6]. In addition, the dilution and the natural self-depurating capability of the receiving waters will also reduce the concentration of contaminant microorganisms. The control of microbial pathogens must be carried out by the use of a multi-barrier approach, including protection of the bathing area from receiving direct discharges of water contaminated with sewage or wastewater [4–6].

The presence of enteric pathogens in bathing waters is of great concern, and thus, legislation either in Europe, the USA and other countries requires the analysis of indicators to determine the microbiological quality of these waters [4–6, 11, 12]. Ideally, we would like to analyse the waters for the presence and quantification of specific enteric pathogens. However, many waterborne pathogens are still difficult to detect and/or quantify in waters, and for most of the newly recognised agents, easy methods to detect them in water samples have still to be developed [1, 3]. The introduction of molecular methods has advanced the recognition of these new agents [20–29]. In fact, the US Environmental Protection Agency (USEPA) implemented this methodology for enterococci [29, 30]. The routine application of these methods for the analysis of pathogens is not yet a reality in Europe. Molecular methods are restricted to research studies [20–24, 31, 32], especially those related to tracing the sources of contamination, i.e. microbial source tracking (MST) that pretends to determine the origin of the faecal pollution (human, animal, mix) or to study cases of suspected outbreaks [26, 33].

Nowadays, new approaches based on virulence factor-activity relationships to discover and detect emerging waterborne pathogens are being explored [31, 32]. However, the commonly used approach to determine the potential presence of pathogenic microorganisms in waters is the analysis of FIB or indicators of faecal pollution [1, 3–6, 12, 14–18]. These indicators must fulfil the requirements indicated in a previous study [3]: (1) Indicators should be present in faeces, sewage and sewage-polluted waters whenever the pathogens are also present and absent in waters without faecal pollution; (2) they must not multiply in water; (3) therefore the levels of indicators should have some direct relationship to the density of pathogens in aquatic environments and be proportional to the extent of the pollution; (4) indicator survival rate in water must be at least similar to that of pathogens. In addition, (5) the resistance of indicators to depuration factors and disinfectants should be at least similar to that of the pathogens; (6) indicators must be detectable by a simple, inexpensive, accurate, rapid laboratory methodology; (7) indicators should be nonpathogenic and applicable to all types of water samples that require a monitoring programme.

The most widely used indicators are coliforms (total coliforms), faecal or thermotolerant coliforms, *Escherichia coli* and enterococci (faecal streptococci or intestinal enterococci) [1, 3–6, 11, 12, 14]. Bacteriophages have been used as indicators of viruses [1, 3], but several studies have claimed that the best indicator for viruses is the direct study of the presence of adeno- or noroviruses in the water [22–24]. Cyanobacteria and dinoflagellate toxins have been associated with health outcomes such as contact irritation after bathing in freshwater and marine environments, respectively [4, 5]. Their potential risk in bathing areas is also included in the new Directive and has to be contemplated in the BWPs [12].

2.1 Coliforms

By definition, coliform bacteria are facultative anaerobe, Gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with acid production in 24–48 h at 36°C and are indole negative [1, 3]. Coliforms belong to the family *Enterobacteriaceae* and include *Escherichia*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Kluyvera* and *Leclercia* genera and some members of the genus *Serratia* [1, 3]. The coliform bacteria, traditionally termed the total coliforms, have been the primary standard for bathing and potable water in most of the world and in the European Union. These bacteria were classically used as indicators of faecal contamination of waters because they were considered to be inhabitants of the intestinal tracts of warm-blooded animals [1, 3–5, 11]. However, the ability of some coliforms to grow in natural waters and the lack of correlation between the number of coliforms and those of pathogenic microorganisms have led many regulatory agencies to question its utility as an indicator [1–6, 14, 15]. Furthermore, several studies have demonstrated the presence of coliforms in pristine bathing waters and in sediments in the absence of faecal pollution [34, 35]. For these reasons, this is one of the parameters that have been eliminated in the new Directive (2006/7/EC) [12].

2.2 Faecal Coliforms

These bacteria conform to all the criteria used to define total coliforms plus the requirement that they grow and ferment lactose with the production of acid at 44.5°C [1, 3]. For this reason, thermotolerant coliform would be the scientifically accurate term for this group [1, 3, 6, 14, 15]. Bacteria in this coliform subgroup have been found to have a positive correlation with faecal contamination of warm-blooded animals [3, 14, 19]. However, some thermotolerant coliform bacteria of the genus *Klebsiella* have been isolated from environmental samples in the apparent absence of faecal pollution [14, 15]. Similarly, other members of the thermotolerant coliform group, including *Escherichia coli*, have been detected in some pristine areas [35, 36]. Faecal coliforms display a survival pattern similar to those of bacterial pathogens but their usefulness as indicators of protozoan and viral contamination is limited [1, 3, 6, 14]. Therefore, they tended to be replaced by *E. coli* in several legislations as occurred in the new Directive [12].

2.3 Escherichia coli

This microbe is a member of a faecal coliform group that naturally occurs in the lower part of the gut of warm-blooded animals being therefore a more specific indicator for the presence of faecal contamination as commented above [1, 3, 6, 12, 14]. In addition, *E. coli* is the faecal indicator of choice used in WHO guidelines for bathing water quality [4–6] because it conforms to taxonomic as well as functional identification criteria and is enzymatically distinguished by the lack of urease and presence of β-glucuronidase [1, 3, 14, 37]. The latter reaction is the basis of the ISO 9308-3 method included in the new Directive [12, 38].

Several countries include *E. coli* in their regulations as the primary indicator of faecal pollution (i.e. Europe, USA) [4–6, 12]. As commented, the disadvantage associated with this organism as an indicator is its presence in pristine tropical rainforest aquatic and plant systems as well as soils [34–36]. Additionally, it seems to survive for short periods in aquatic temperate environments [39, 40].

2.4 Faecal Streptococci, Enterococci or Intestinal Enterococci

This group of microorganisms has received widespread acceptance as useful indicators of microbiological water quality, since (1) they show a high and close relationship with health hazards associated with the water use, mainly for gastrointestinal symptoms; (2) they are always present in faeces of warm-blooded animals; (3) they are unable to multiply in sewage-contaminated waters; and (4) their die-off is less rapid than those of coliforms or *E. coli* in water and persistence patterns are similar to those of potential waterborne pathogenic bacteria [1, 3, 8, 9, 14, 16, 29, 41].

Faecal streptococci, enterococci and intestinal enterococci are three synonyms used to refer to species described as members of the genus *Enterococcus*, which also fulfil Sherman's criteria (growth at 10°C and 45°C, resistance at 60°C, growth at pH 9.6 and at 6.5% NaCl and reduction of 0.1% methylene blue) [1, 3, 14, 16, 41]. They comprise species of different sanitary significance and survival characteristics and, in addition, the proportions of the species of this group are not the same in animal and human faeces [1, 3, 14, 16]. *Enterococcus faecalis* and *E. faecium* are the predominant species in human faeces and sewage [16, 18]. In a European study that investigated enterococcal populations in animals, humans and the environment, the most common species detected were *E. faecium* (33%), *E. faecalis* (29%) and *E. hirae* (24%), and the distribution in these hosts has been confirmed using a molecular multiplex PCR technique [1 and references therein].

In 1996, considering the low specificity of the m-*Enterococcus* (m-Ent) medium (31%, i.e. almost 70% of false positives) for the detection and enumeration of faecal streptococci or enterococci, we have described a method for the confirmation of all the colonies grown on m-Ent from different types of water [17]. After incubation at 35 ± 2°C for 48 h of m-Ent, the confirmation technique consisted in the transplantation of the membrane filter from m-Ent to a new Petri dish containing bile-esculin medium for 2–4 h (35 ± 2°C). The intestinal enterococci colonies grow, hydrolyse esculin and were confirmed, because after this incubation the original dark-red colonies change to dark brown, as seen from the reverse of the Petri dish. With some modification, this is the ISO 7899-2 method included now in the new Directive [12, 42].

Despite the definitions provided above for the indicators (total coliforms, faecal coliforms, *E. coli* and enterococci), in practical terms, these are determined on the basis of the biochemical reactions evaluated in culture media that are recognised either by the appearance of characteristic colonies (with a specific colour as response to this reaction in chromogenic substrates) and/or by the emission of fluorescence. Microbiological methods for indicators are far from perfect because they can produce false-positive and false-negative results [6, 14–18]. Despite the comments made about the limitations of total and faecal coliforms, we have to say that they are useful for detecting gross faecal contamination failing normally at the low concentration required by the standards of the new Directive.

3 Analytical Methods Included in the New EU Directive

The new Directive [12] includes standard operating procedures that detail all specific operations linked to the analytical methods developed by the International Organization for Standardization (ISO). Two ISO methods for each indicator (EC and IE), one based on the most probable number (MPN) and the other on membrane filtration (MF), are included in the new Directive [12, 38, 42–44]. Despite that, it is indicated in Article 3.9 that member states may permit the use of other methods if they can demonstrate that the results obtained are equivalent to those obtained using the methods specified in the Directive [12].

The most important limitation of all these methods is the time required to obtain the results, 36–72 h for the MPN methods [12, 38, 43] and shorter for the MF methods ca. 24 h for the EC (with the rapid method) [44] and 48–52 h for the IE [42]. The USEPA has developed an MF method for IE (Method 1600) that provides results in 24 h [45]; in our view, this is more convenient because results of both indicators can be obtained simultaneously in ca. 24 h. We have tested this method in parallel to the MF ISO method [42] in 113 samples and found that the mean results for the MF ISO 7899-2 were lower (245 cfu/100ml) than those obtained with the EPA method (420 cfu/100 ml). Despite of that, results with both methods were <100 cfu/100 ml in 72% of the samples. However, in 11.5% of the samples, the MF ISO method results were <100 cfu (mean 42 cfu/100 ml) while with the EPA method the results were very high (mean 436 cfu/100 ml) indicating that a confirmation should be required for this method. Alternative methods, validated by AFNOR, that provide results for both indicators in 24 h are the Colilert 18® for *E. coli* (http://www.idexx.es/water/products/colilert-18.html) and Enterolert for intestinal enterococci (http://www.idexx.es/water/products/enterolert.html).

In cases of urgency in our laboratory, we have counted MF preliminary results of EC at around ca. 18 h of incubation and those of IE at ca. 40 h, and the classification of the bathing area was within the same category as when waiting for the complete incubation period. Preliminary readings of fluorescent wells can also be done with the MPN methods at ca. 36 h. These preliminary data can be relevant in contamination events to know if the quality of a bathing area has been restored or not so that alerts, warning signs and fences can be removed or not.

3.1 Most Probable Number (MPN)

The MPN analysis is a statistical method based on the random dispersion of microorganisms per volume in a given sample [1, 3, 14]. Classically, this assay has been performed as a multiple-tube (3, 5 or 10 tubes) fermentation test in three sequential phases (presumptive, confirmatory and complete), each phase requiring 1 to 2 days of incubation making therefore this technique very time consuming (5–7 days) [14]. The substantial amount of reagents, tubes, incubation space and cleanup requirements is the principal disadvantage together with the fact that MPN is a probabilistic method in which precision depends upon the number of multiple tubes used. Considering this limitation, the method has been adapted in miniaturised 96-well plates to increase precision and to overcome the other disadvantages. The final results are determined by counting the number of positive (fluorescent) wells at each dilution and transforming this number of positives on a specific number of bacteria (MPN/100 ml) using the MPN tables. This number is not a precise value and confidence intervals, i.e. most probable range, are often published with the MPN result [14]. The 96-well plates are the format used in the MPN ISO methods included in the new Directive (ISO 9308-3 and ISO 7899-1 for EC and IE, respectively) [38, 43].

In both MPN ISO methods, the presence of the indicator is evaluated by enzymatic reactions considered specific for EC and IE. In the case of EC, it detects the expression of the enzyme β-glucuronidase present in most of the strains by the hydrolysis of a highly sensitive fluorogenic substrate (4-methylumbelliferyl-β-D-glucuronide) present in each of the 96 wells of the microplate. For the detection of IE, the method relies on the expression of the β-glucosidase enzyme characteristic of enterococci by the hydrolysis of the substrate 4-methylumbelliferyl-β-D-glucoside. If the enzyme is present, the substrate releases the 4-methylumbelliferyl that emits fluorescence under a UV lamp. The Colilert and Enterolert methods mentioned above are based on the MPN method and on the same fluorogenic enzymatic reactions than the MPN ISO methods and can be used for both drinking water and bathing water. Unlike traditional methods, results require no further confirmation and are not dependent on subjective interpretation. The major advantages of the MPN technique are the following [1, 3, 14]: (1) it can be performed in both clear and turbid samples; (2) it inherently allows the resuscitation and growth of injured bacteria; and (3) the results may be recorded by personnel with minimal skills.

3.2 Membrane Filtration (MF)

The MF technique is the most widely used method in microbiological water analyses [1, 3, 14]. The technique is based on the entrapment of the bacterial cells by a membrane filter (pore size of 0.45 μm). After the water is filtered, the membrane is placed on an appropriate medium and incubated. Discrete colonies with typical appearance are counted after 24–48 h, and the population density of the target bacteria is described as colony-forming units (cfu) per 100 ml calculated from the filtered volumes and dilutions used from the original sample. This technique was classically considered more precise than the classical MPN method. The MF test presents the limitation of its use for low-turbidity waters with low concentrations of background microorganisms and does not allow the resuscitation of injured bacteria.

The new Directive includes MF ISO methods for *E. coli* (ISO 9308-1) and intestinal enterococci (ISO 7899-2). The MF (ISO 9308-1) for *E. coli* allows two alternative procedures; the first is the standard test and uses lactose TTC agar with Tergitol-7 and requires a probabilistic confirmation of the colonies (at least 10) and therefore is a relative slow procedure (>48 h). The second is the rapid test that uses a pre-incubation, to recover injured bacteria, in tryptone soya agar (TSA, 4–5 h at $36 \pm 2°C$) after which a transplantation of the filter to tryptone bile agar (TBA, 19–20 h at $44 \pm 0.5°C$) allows confirmation of all the colonies. The ones that turn red, after the addition of drops of the indole reagent (on their top), are *E. coli*. Transplantation can be avoided if both media are included in the same Petri dish and a programmed incubation is used. Since we implemented the new Directive, the rapid test for *E. coli* (ISO 9308-1) and the MF ISO 9308-1 for intestinal enterococci are the methods employed for the majority of the bathing waters of the Catalan monitoring programme. They were also used in the EPIBATHE Project financed by the EU within the VI Framework Research Programme to support the new Directive (2006/7/EC), which in its Article 14 (a) indicated that "The Commission shall, by 2008, submit a report to the European Parliament and to the Council on the results of an appropriate European epidemiological study conducted by the Commission in collaboration with Member States" [12]. In 2014, the ISO 9308-1 for *E. coli* has been modified including now only one culture medium, the chromogenic coliform agar (CCA) [46]. The CCA is a differential agar in which X-glucuronide is used for the identification of β-D-glucuronidase activity after incubation (at 36 ± 2°C) and, in this assay, *E. coli* colonies take on a dark-blue to violet colour. This medium does not contain any antibiotic to eliminate background microbial growth so for its use for bathing water we recommended incubation at 44°C to achieve this goal. This method at this incubation temperature was implemented in the Catalan monitoring programme for bathing areas in 2015.

3.3 Limitations of the Methods Included in the EU Directive

The limitations and contradictions of the old Directive [11] have been broadly discussed by us previously proposing amendments and corrections to be considered when designing the new Directive [47]. Among the limitations pointed out, there was the need for defining, harmonising and updating the methods of analysis and the FIB [47]. A harmonisation of methods has been done, and the new Directive includes well-defined ISO methods [12]. However, these methods can produce such different results for *E. coli* that a very discordant classification (excellent vs. poor) can be obtained for the same bathing area as we describe below. The limitations of the methods are only known when they are tested in parallel with other methods and when results are confirmed independently of the method indicating that it does not require confirmation.

The Catalan experience with the MPN methods included in the new Directive [12] started when we used them in parallel with our conventional methods for total coliforms and faecal coliforms [15], *E. coli* [37] and faecal streptococci [17] in the 2000 summer season within a practical trial promoted by the European Commission that was coordinated by us in Spain. The aim of the trial was to evaluate and compare results of old and new indicators and to test the feasibility of introducing direct management actions in case of unexpected results in view of the introduction of this new approach for managing bathing waters in the new Directive that was under preparation. Within that trial, it was evidenced that while the intestinal enterococci (IE) results between our conventional MF method [17] and the ISO 7899-2 were very similar, for certain bathing areas, the results between the FC and *E. coli* with our methods [15, 37] differed considerably from those of the ISO 9308-3 MPN method [38] that produced much higher results. These higher results were observed again during the development of the EPIBATHE Project entitled "Assessment of Human Health Effects Caused by Bathing Waters" that included

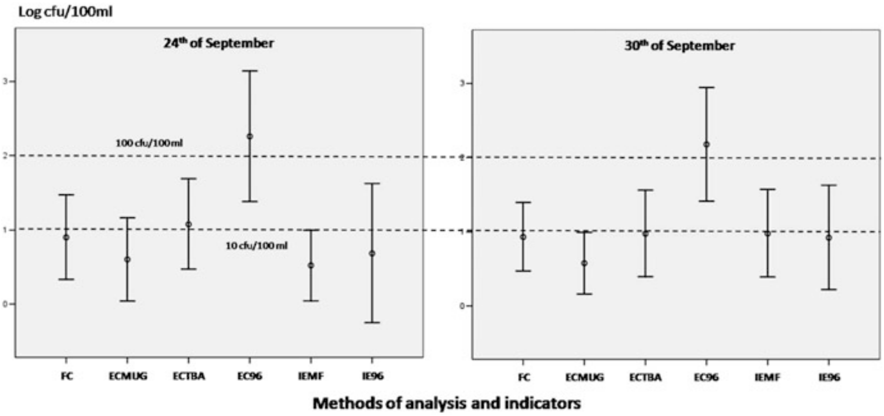

Fig. 1 Mean results and standard deviation obtained from the 54 water samples taken at each of the trials performed in two different dates in 2006 in a bathing area known to be of excellent water quality. *FC*, results of faecal coliforms with MF with m-FC medium; *ECMUG*, results of *E. coli* (EC) on the basis of the glucuronidase reaction; *ECTBA*, results of EC with the MF rapid method of ISO 9308-1; *EC96*, results of EC with the MPN ISO 9308-3; *IEMF*, results of intestinal enterococci (IE) with the MF ISO 7899-2; *IE96*, results of IE with MPN ISO 7899-1. Notice the high discordant results found for *E. coli* with the MPN ISO 9308-3

randomised controlled trials and epidemiological field studies performed in freshwater bathing areas in Hungary and in marine beaches in Catalonia, Spain. The volunteer participants were randomised as bathers and non-bathers, and through interviews and questionnaires, self-reported symptoms both pre-exposure and at one and three weeks postexposure were obtained. In parallel to the exposure, water samples were taken every 20 min during a period of 3 h (12–14 h) at six sampling points separated 20 m and this was repeated in 2 days (see Fig. 1). The analysis performed included the FIB of the new EU Directive (*E. coli*, intestinal enterococci with both MPN and MF ISO methods in parallel) plus faecal coliforms using MF and the m-FC medium. By transferring the membrane to a medium containing 4-methylumbelliferyl-β-D-glucuronide (MUG), we determined which FC colonies were *E. coli*, because they showed fluorescence after cultivation for 2 h at $36 \pm 2°C$ [37]. These methods for FC and EC were the ones employed in the Catalan monitoring programme for many years before implementing the new Directive.

As shown in Fig. 1 and as expected, the concentration of ECMUG was smaller than the one of FC, because the method confirmed which of the FC belonged to EC. Results for FC were relatively similar to those obtained with the MF ISO 9308-1 (ECTBA) rapid test. All these results indicated a very good water quality as also shown by the results (mean <10 cfu/100 ml) obtained with both ISO methods MF (IEMF) and MPN (IE96) for intestinal enterococci. However, the ISO 9308-3 MPN method (EC96) for *E. coli* produced much higher results (mean >100 cfu/100 ml) that would classify the bathing area of poor water quality on the basis of the new Directive when the 95% percentile is calculated [12]. The observed high concentration is due to enzymatic activity from other nontarget bacteria (false positives) at low levels of the targeted bacteria or even by plant extracts and algae including diatoms [6]. However, the MPN methods for intestinal enterococci and *E. coli* had been used for fresh recreational waters in another epidemiological study performed in Germany [10] without finding the false-positive reactions for *E. coli* mentioned above. This is in agreement with our findings also in the Catalan freshwater bathing areas or in marine waters impacted by freshwater where the MPN method of *E. coli* is more reliable than the MF ISO 9308-1 method. The latter was designed for drinking water or treated waters and in our hands provided good results for the bathing waters of excellent water quality of Catalonia as occurred in the bathing area from which the results are shown in Fig. 1.

It has been suggested that intestinal enterococci and *E. coli* may not be useful in tropical waters due to their potential growth in soils/sediments [34, 35]. In fact, molecular methods have proved that *E. coli* can become "naturalised" in the environment and its presence does not necessarily indicate recent faecal pollution [1, 6, 14, 36]. In line with this we have also observed the phenomenon described as "blooms of faecal indicators" that had been attributed to the presence of organic carbon that favours the growth and can be present by preceding rain events, sewage outfalls or green algae. These blooms can also be promoted by the stratification of the water influenced by the wind and water temperatures (18°C) [1, 6, 36]. It has been demonstrated that *E. coli* has the capacity to grow in water and/or sediments at temperatures between 15 and 45°C or that other interfering microbes like *Enterobacter cloacae* and *Citrobacter freundii* can produce these abnormal results [6, 36].

During EPIBATHE, in the two trials performed in 2007 in a totally different beach, we detected important temporal and spatial variations of water quality influenced by the wind speed that changed along the 3 h in which we performed the monitoring in an area impacted by a faecally polluted rivulet. Important temporal and spatial variations have been observed in other studies [48]. In this bathing area we observed that salinity inversely correlated with the indicators of faecal pollution. A similar relationship has been noticed in cases of sewage spills. Salinity is an easy to perform in situ measure that may help to delimit the area impacted by faecal pollution. Salinity has been used with other data to nourish modelling systems to determine the bathing water quality [49].

4 Bathing Water Quality

The microbiological quality of coastal water is obtained through the periodic analysis of seawater during the bathing season, which is carried out in accordance with the criteria established in the new Directive (2006/7/EC) in order to obtain information about the water quality and to protect the health of the bathers [4–6, 12]. Algae, plant debris, changes in water colour or transparency (Fig. 2) and other natural phenomena typical of coastal waters as well as the presence of phytoplankton, sea foam, etc., can cause temporary alterations in the visual appearance of the

Fig. 2 Transparency is evaluated during sampling

Table 1 Bathing water quality categories according to the EU Directive depending on the percentile derived from the results obtained, in the last four bathing seasons, from intestinal enterococci and *E. coli*. (*) Percentile 95; (**) percentile 90; cfu, colony-forming units in 100 ml of water

Indicator	Excellent	Good	Sufficient	Poor
Intestinal enterococci	100 (*)	200 (*)	185 (**)	>185 (**)
E. coli	250 (*)	500 (*)	500 (**)	>500 (**)

water, without changing the microbiological quality of the bathing water. These changes in the appearance of the aspect of bathing water are related with their aesthetical aspect that in most cases does not have an impact on human health with the exception of the presence of harmful toxic algal blooms that occur very rarely at specific sites [4, 5, 50]. However, these aesthetical changes can generate some discomfort to the bathers that can associate these changes not with natural phenomena typical of coastal waters but with contamination (Fig. 2) [4, 5].

Bathing water quality is classified according to the EU Directive into four categories (Table 1), excellent, good, sufficient and poor, depending on the percentile derived from the results obtained, in the last four bathing seasons, from intestinal enterococci and *E. coli* [12].

4.1 The Monitoring Programmes Developed in Catalonia

The microbiological monitoring of the Catalan bathing areas was initiated in the early 1980s when Spain was not a member of the EU yet and depended on the Department of Public Health of the Catalan government. However, this responsibility was assumed in 1990 by the Environmental Department that designed and implemented an integrated sanitary and environmental monitoring programme introducing improvements almost every year from 1990 to 2010. For collecting the samples and performing the inspections (Figs. 2 and 3), we counted with 15–17 ad hoc trained inspectors. The bathing areas studied evolved from ca. 140 in 1990 to ca. 250 in 2010. Currently, the one responsible for the bathing water quality control

Fig. 3 During the sanitary and environmental inspection, data about the aspect of water, sand and potential sources of pollution are collected, originally in paper and when the Internet was available using a PDA (personal digital assistant)

is the Catalan Water Agency (ACA, http://aca-web.gencat.cat/aca/appmanager/aca/aca?_nfpb=true&_pageLabel=P1210054461208200724644) that is carrying out inspections in the bathing areas and analysing the microbiological quality of seawater and continental (lakes, rivers and reservoirs) bathing areas. It is thereby possible to assure that the bathing areas are safe to use. The monitoring programmes developed in Catalonia have been implemented with the aim to provide the public with actualized information about the quality of the bathing areas during the bathing season, avoiding the public to rely on the quality obtained in the previous bathing season. The latter approach is the one used for other existing information systems as the Blue Flag award (http://www.fee-international.org/en/menu/programmes) and by the European Commission (http://www.eea.europa.eu/publications/european-bathing-water-quality-in-2013). Another objective of the programme was to provide information to the public not only about the microbiological water quality but also about the aspect of the water and the sand obtained during the sanitary inspection. The microbiological water quality was monitored once a week (Saturdays to be representative of one of the more crowded days) at a single laboratory (Unit of Microbiology of the Faculty of Medicine of the University Rovira i Virgili, URV), while sanitary inspections were assessed more frequently (four or five times a week). The inspection enabled to recognise the environmental characteristics of the bathing area and also potential sources of pollution (Fig. 3). Data recorded during the inspection include the presence and amount of plastic, sanitary residues, algae, tar, oil and litter, abnormal water colour and anything else that might cause aesthetic revulsion. Any abnormal situation was recorded making photos, which illustrate the problem and its extent. In addition, it was also recorded how thoroughly a beach was machine cleaned and how frequently litter containers were emptied. Municipalities were informed weekly of the results, and this information was simultaneously provided to tourist information offices, NGOs, local newspapers, TV and radio. The information was delivered at first by fax, but once the Internet and e-mail were available, these were the dissemination tools. At that time, the ACA website was developed including information about the monitoring programme and the bathing water quality updated each week (http://aca-web.gencat.cat/aca/appmanager/aca/aca?_nfpb=true&_pageLabel=B1020016795125077548385 9&_nfls=false). In

Fig. 4 Beach warnings in place when a sporadic contamination event occurs

addition, municipalities received each week a report including raw microbial data for each of the evaluated FIB and the results of the visual inspection and at the end of the bathing season a final balance report with suggestions for improvements. This system showed to give confidence to the public that their concerns were being taken seriously and has also encouraged many municipalities to improve the aesthetic aspects of their bathing areas. The approach of including a sanitary inspection and recording aesthetical data perceived by the users was considered so interesting that it is described in the WHO guidelines [5].

The sanitary inspections performed by ACA during the period 1990–2010, with a mean of 70 inspections for bathing area per year (or season), have enabled to obtain a very valuable information from each bathing area (Fig. 3). The latter included: (1) a photographic illustrated inventory of the potential sources of contamination of the bathing areas verified with the microbiological results and (2) a photographic illustrated inventory of the natural phenomena and changes on the aesthetical aspect of each bathing area. All the potential sources of contamination of the Catalan bathing areas are referenced in a map using the geographic information system (GIS). The mean number of water samples investigated per season (year) ranged from 2,500 to 4,500, and the amount of results for the FIB ranged between 10,000 and 15,000. The characterisation of the most relevant factors that affect each bathing area was determined and enabled to implement management actions and strategies for improving the quality of the bathing area. Our approach is totally in line with the requirements now included in the new Directive [12].

In the event of sporadic contamination, ACA informs right away the municipalities and warning notices are always posted. In such cases additional inspections and microbiological analyses are carried out in order to verify if the microbiological quality of the water has been restored. If water quality was not restored, the warning remains in place, and if necessary, the bathing area or a section of the beach is fenced (Fig. 4). The experience demonstrated that there are two main factors that can alter sporadically the microbiological water quality; those are rainfall and incidences of the sewerage system. Both factors will be explained in detail in the next section. These warning signs in place indicate that quality of the bathing water cannot be guaranteed due to rainfall or an incident involving the sewerage system. Point sources of pollution such as rivers and streams have also been recognised, and the implementation of management measures such as introduction of water treatment has led to minimise their impact in the Catalan coast to the extent that most point sources of pollution have been mitigated.

In 2005, ACA created the *Beach Commission* that include representatives from all Catalan municipalities that have bathing areas, administrations with coastal competences or involved in public health protection and beach rescue as well as the scientists of the research centres and experts on marine ecology and pollution control that act as advisers to ACA (URV; Institute of Marine Sciences, ICM; Institute of Advance Sciences of Blanes, CEAB; Institute of Environmental Assessment and Water Research, IDAEA; the last three named all belonging to the Spanish Council for Scientific Research). The aim of this commission is to allow the interrelation between all stakeholders involved in the management of bathing water quality. So to some extent, we anticipated the requirement of the new Directive of disseminating information to stakeholders [12]. In the meetings performed during the bathing season, the common problems are presented and the stakeholders are also invited to present their ideas, worries and expertise.

In 2011, in compliance with the requirements of the new Directive, the ACA elaborated the bathing water profiles (BWPs) that describe the physical, geographical and hydrological characteristics of the bathing area and their potential risk of being affected by sources of pollution following the guidelines described in the Annex III of the new Directive [12]. Also in 2011, the programme has been redesigned reducing the amount of inspections and microbiological controls in agreement with the new Directive.

The ca. 25-year implementation of the monitoring programme has generated a very broad knowledge of the factors that affect the bathing water quality. It also enabled to classify the ca. 300 coastal bathing areas of Catalonia in function of the risk of being sporadically contaminated by rain or by events linked to the sewerage system (sewage leaks) as well as to classify them in relation to their risk of being affected by natural phenomena, as blooms of phytoplankton, that will alter the aspect of the water. All this information has been extremely useful for the elaboration of the BWPs that have also benefited from the fact that ACA is also responsible of the Catalan sewage sanitation system and of the characterisation of the coastal and catchment areas according to the Water Framework Directive (WFD) [13].

In 2012, with the aim of increasing the exchange of information about the bathing areas with the municipalities (local administrations), a “telematic network of beaches” was created to improve the coordination and the management. For the functioning of the network, a dedicated software (with a template) has been designed that enables ACA to receive environmental data from the bathing areas obtained by the local authorities with their own personnel or contracted beach watchers. With the received information from all bathing areas, ACA prepares daily a balance for each specific municipality with a comparative analysis of all Catalan bathing areas and another one at the end of each bathing season. A regular balance about the quality of the Catalan bathing areas is also presented at the web and at the regular meetings of the Beach Commission where the management strategies and the functionality of the telematic network are discussed.

In 2014, a mobile app (Fig. 7) was launched with the idea of making even more readily accessible the information about the water quality of the ca. 300 bathing areas to the public. Information about the water quality is provided by the app daily as are alerts about possible alterations of the water quality (http://aca-web.gencat.cat/aca/platgescat/landing/ca/index.htm).

4.2 Alterations of the Microbiological Quality

The impact of rain in bathing water quality is a well-known phenomenon [4, 5, 48, 49, 51–56]. The microbiological quality of the water in some beaches can be impaired temporarily, when there are fairly strong rains. In those beaches impacted by *rainwater run-off*, there are rainwater outlets, torrents, river mouths, sluices, streams or sewage pumping stations (used to move waste to higher elevations), etc. In the case of high sewage flows in wet weather, pumping stations may be insufficient, or in the case of failure of the pumping station, a backup in the sewer system can occur, leading to a sanitary sewer overflow or a discharge of raw sewage into the environment. Many beaches are not affected when it rains because they do not have pumping stations or natural or artificial course of water reaching the sea. In others it depends on the amount of rain as also determined in studies performed in California [48, 49, 51]. This sanitary quality alteration tends to return to normal 24–48 or 72 h after the rain run-off spell stops [48, 51, 52]. A survey of swimmers in Santa Monica Bay found an increased health risk when swimming within 400 yards (366 m) of a flowing storm drain [52]. Within this area, they separate the classification of the water quality during wet weather and dry weather [53]. In order to effectively discourage use of areas that are of poor quality or discourage use at times of increased risk, signs have been posted permanently in rain drains along the California coast (Fig. 5).

The intensity and regularity of rain events can be influenced by climate change, and this will affect surface water quality, as we have discussed elsewhere [1 and references therein], and therefore the quality of bathing waters. The climate change determinants affecting water quality are mainly the air temperature, the increase of extreme hydrological events, soil drying-rewetting cycles and solar radiation. It is expected that in the temperate zones, climate change will decrease the number of rainy days but increase the average volume of each rainfall event. The higher water temperatures will probably, although there is still no clear evidence, lead to increase the pathogens' survival in the environment, and it has been shown that the increased UV radiation, due to ozone layer depletion, provokes the breaking down of bioavailable organic compounds, minerals and micronutrients, stimulating the bacterial activity in aquatic ecosystems [1 and references therein]. The continuous accumulated knowledge in this field should help to prepare strategies to mitigate the climate change impact on the quality of bathing waters.

The Catalan coastal area embraces 830 km of coastline with 70 coastal municipalities and shows an elevated demographic pressure as it may occur in other

Fig. 5 Different signs posted in California alerting that all what is dump in the ground can drain in the ocean and about the impact of rain events

geographical areas in the Mediterranean Sea. When there is no rain, the Catalan water quality is very stable and usually excellent at the majority of the beaches. However, when strong rains take place, they can impair the bathing water quality temporarily, as the rainwater flowing into the sea may be faecally contaminated. The reason for this is that in Catalonia as in many other geographical regions, the urban sewerage systems are on the whole connected as a unit and these systems are named "combined sewer" (CS). Within this CS, a significant amount of the rainwater run-off enters the municipal sewerage system and then runs into the sea along the beds of rivers, streams or rainwater outlets or as an overflow from the sewerage system. Combined sewer overflows (CSOs) can lead to short-term periods of pollution on the most susceptible beaches. As commented not all beaches are affected when it rains because not all of them receive combined sewage. Storm water discharges that do not come from a combined sewage system or that do not have washed up animal manure may have a limited impact on the microbiological water quality of the bathing water. These beaches tend to be situated in non-urbanised or poorly populated areas.

The fact that the monitoring programme in Catalonia was never discontinued in case of wet weather allowed to record data from all beaches under this condition and to grade the amount of rain required at each bathing site to alter the water quality, and this information has been incorporated in the BWP. The information regarding the risk of the Catalan bathing areas of being impaired by rainfall is recorded, and the beaches are classified as *high/medium/low/nil*. For instance, the excellent water quality obtained from the bathing area (Fig. 6, from which we illustrated the results in Fig. 1) was not altered despite that on the 23rd of September the amount of rain fallen was ca. 23 mm and it did not stop raining all day. Notice in Fig. 1 that the results from the 24th were very similar to those obtained on the 30th when there was no rain. So this bathing area is classified with a risk of *nil* (not being impacted by rain events).

In the app (Fig. 7) developed by the ACA, the water quality classification is changed by an alert about the risk of the bathing water quality of being affected by

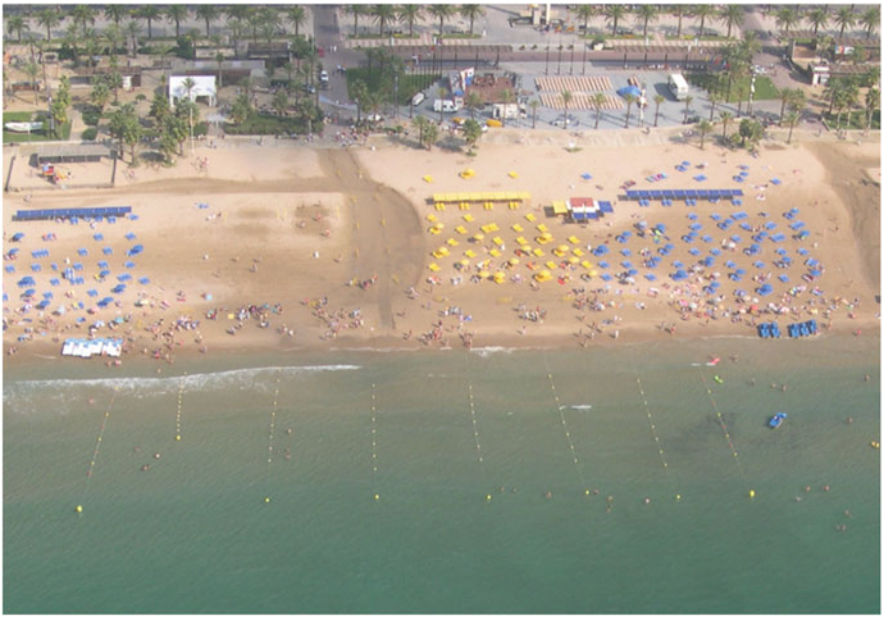

Fig. 6 Image showing the six sampling subareas delimited with buoys from which samples were collected every 20 min during 3 h on the 24th of September. Notice the wet sand that resulted from the constant rain during the day before (23rd of September)

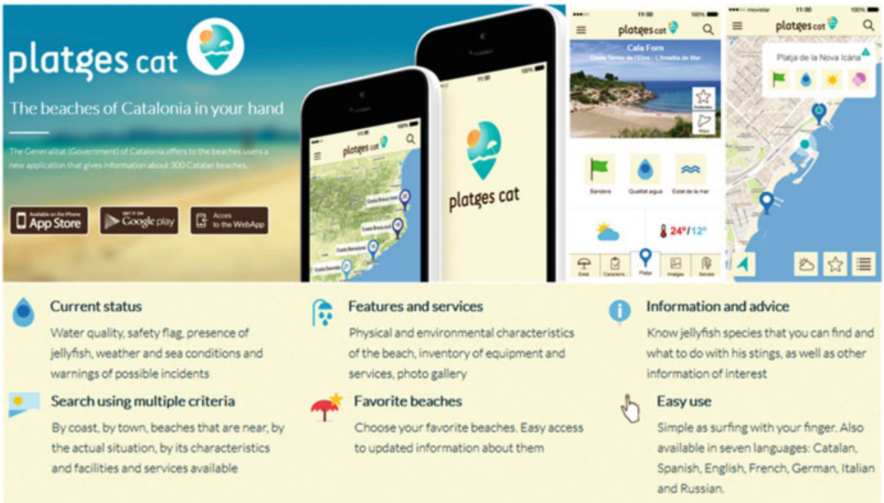

Fig. 7 Free app (PlatgesCat) developed by ACA including information of ca. 300 Catalan beaches

Fig. 8 Images showing the impact of rain events on the bathing area

rain events in a daily and even hourly basis. The rainfall warning notice on the app is reviewed daily throughout the bathing season (1 June to 15 September) using the weather forecast information coming from two sources, a real-time online radar monitoring system developed by ACA (http://aca-web.gencat.cat/aetr) and the information of the Catalan Meteorological Service. Furthermore, to determine the risk, the previous information is complemented with the information of the online monitoring system existing at the pumping stations that record high sewage flows that may lead to sanitary sewage overflows that may affect a given bathing area. Warnings remain until the bathing water quality is restored, something that occurs as commented 24–48 h after the rain has fallen. However, increase in water turbidity due to sediment loads following periods of rainfall may persist for longer periods (Fig. 8), and they may even have a slight impact in the microbiological water quality.

Considering that the variation in water quality that may occur in response to rainfall can be a predictable outcome, this has been used as a tool to develop predictive models on bathing water quality [48, 49, 57]. River flow has also been used to model and predict the microbiological water quality in bathing areas controlled under the frame of the new Directive [57]. Torrential rains wash a large quantity of sediment and terrestrial plant remains onto the beaches, as well as waste from human activity. On some beaches, coastal fronts can form with very murky, brown-coloured water (Fig. 8). This can lead to large amounts of debris accumulating on the sand, which start out in the water and along the shoreline and depending of the quantities may require additional cleaning operations to be implemented by local authorities. The coastal fronts may contain large amounts of reeds, pieces of wood, tree branches and other terrestrial plant remains, as well as all types of waste generated by human activity, which may remain along the coast for a number of days. The responsibility for not littering river areas, urban and interurban land and beaches lies with everyone. People should be aware that every paper, plastic bag or waste thrown on the ground may reach the seawater through rain run-off, and this can be announced as shown in Fig. 5.

Unexpected *discharges of wastewater onto the beach* as a result of isolated incidents involving the wastewater treatment system are rare. When this wastewater reaches the sea at the coastal area or when a pipe of a long sea outfall is accidentally broken or shows a leaking, the microbiological quality of the bathing water can be impaired temporarily. In order to prevent this impact on water quality, all of the sewerage systems in the coastal and pre-coastal areas of Catalonia are regularly inspected to ensure that they are working properly and that all wastewater from urban areas is being directed to the treatment plants. Despite these monitoring activities, isolated incidents may occur which are dealt with as quickly as possible and warning notices are also put up on the beaches (Fig. 4) as a precautionary measure and directly announced at the app.

There are currently 50 sewerage systems operating along the Catalonia coastline made up of collectors, pumping stations and wastewater treatment plants (WWTP) that collect and treat sewage normally with a biological or secondary treatment. The treated wastewater is then discharged into the sea, mainly through long sea outfalls in which the length from the coast depends on the population equivalent of wastewater treated and the characteristics of the coast (form, slope, depth, etc.).

The WHO guidelines for safe recreational water environments classify the sewage discharges, or outfalls, into three principal types [5]:

1. Those where the discharge is directly onto the beach, which will have the greater impact.
2. Those where discharge is through "short" outfalls; in this case, the sewage-polluted water is likely to contaminate the recreational water in the area, but its impact can depend on the distance of the outfall to the bathing area.
3. Those where discharge is through "long" outfalls, where the sewage is diluted and dispersed and the design criteria for the outfall should ensure that sewage does not pollute the bathing areas.

Direct discharges or short outfalls will always produce contamination unless the wastewater has been treated with a procedure that involves the removal of the microbial contamination (UV disinfection).

In our experience, the discharge of primary or secondary treated sewage without disinfection or untreated sewage will have a great impact on the microbiological water quality due to the high loads of faecal coliforms and *E. coli* in these types of water (10^6–10^8 cfu/100 ml). This is very evident at the point of discharge, but it will be less evident at a certain distance (200 m away) due to the dispersion, dilution, sedimentation and inactivation effect that occur in the receiving waters (through sunlight, predation, natural die-off, etc.).

Fig. 9 Images showing the warning sign in five different languages and how the bathers ignore the message

According to WHO, the proper location and design of long outfalls is also important and should include sufficient diffusers and adequate discharge depth to ensure low probability of the sewage reaching the recreational area [5]. Direct discharge of crude, untreated sewage (for instance, through short outfalls or combined sewer overflows, which contain a mixture of raw sewage and storm water) onto beaches presents a serious risk to public health. An imperfect design can generate that sewage which is relatively warm and of low salinity when compared with the receiving water, may mix poorly and form a floating slick [5]. Such slicks should not form where properly designed and operated diffusers are in place on the outfall. Where slicks form, they will be readily influenced by wind and may therefore pollute (even distant) recreational water environments severely [5].

As commented, impairment of water quality due to incidents involving the sewerage system should be identified by warnings (Figs. 4 and 9). The warning will have to remain in place until the authorities have resolved the incident (usually within a few hours), but reparations of pipes may require several days, and in those cases fencing the affected area will be necessary. In all situations it will be necessary to verify if the bathing water quality levels have returned to normal, so that the warning can be removed. However, warning signs are sometimes totally ignored by the bathers as we have experienced (Fig. 9).

4.3 Alterations Associated to Coastal Natural Phenomena

Beaches are natural areas where marine life grows and lives and natural phenomena relevant to the coastal shore ecosystem take place. Among the marine life, there are algae that can be single-celled forms or macroscopic like seaweeds. A massive growth of algae can generate the so-called algal blooms, which although occurred throughout recorded history have increased during recent decades 1, 2, 58–60]. The increased occurrence frequency has accompanied nutrient enrichment of coastal waters on a global scale. As indicated in the WHO guidelines, blooms of non-toxic phytoplankton (i.e. microscopic organisms that live in watery environments) species and mass occurrences of macro-algae can affect the amenity value of recreational waters due to reduced transparency, discoloured water and scum or slime formation. In addition, bloom degradation can be accompanied by unpleasant odours, resulting in aesthetic problems that may impact tourism economy [4, 5, 58].

The accumulation of jellyfish and other gelatinous organisms is an additional natural phenomenon along the coast, and their presence may have an impact in human health through stings that normally result in only a short-lived burning sensation, although some can be dangerous, especially if the swimmer has a severe allergic reaction [4, 5, 59]. As we wrote in the WHO guidelines [4] where we presented the remedial measures to deal with jellyfish in bathing areas in Barcelona (Spain), jellyfish are commonly found in coastal environments, although their normal environment is approximately 50 miles away from the coastlines in oceanic waters and the factors that govern their presence at the coastline are still unclear. However, dry weather conditions (dry years) are considered to contribute to jellyfish along the coastline and their presence usually indicates a flow of oceanic waters towards the coastline and therefore should be considered a natural phenomenon not linked to pollution. Between 2007 and 2010, ACA has financed monitoring research studies developed at the Jellyfish Expertise Centre of the Institute of Marine Sciences (ICM) of Barcelona to try to understand the dynamics of the jellyfish populations [60, 61 among others]. Furthermore, in collaboration with the ICM, extensive public information campaigns dedicated to the recognition of the different species that can be found at our coasts and their associated danger have been developed. A uniform treatment protocol in case of stings was also established as well as a code of good practice for bathers and municipalities in the event of jellyfish stings at the beach. This information is included at the ACA website and at the app.

The BWPs should include an assessment of the potential risk for cyanobacteria, macro-algae and/or phytoplankton proliferation, meaning by proliferation an accumulation in the form of a bloom, mat or scum [12]. In reality, cyanobacteria have a similar size to the unicellular algae but, unlike other bacteria, contain blue-green or green pigments, and they are also termed blue-green algae and are typical members of the freshwater phytoplankton, although they can also be found in brackish waters. In the marine environments, the phytoplankton proliferations are commonly produced by dinoflagellates. As commented, marine plant (macro-algae) remains can also be washed towards our shores from time to time, which may lead to give the bathing area a dirty aspect that can in addition be associated to an increase in water turbidity due to sediment loads following periods of rainfall.

4.3.1 Phytoplankton Blooms

Phytoplankton blooms are occurrences of population explosions related to the suspended microscopic algae that live in the water. This phenomenon can lead to a greenish, yellow or brown colouration of the water which, in the majority of cases, does not constitute any danger to bathers. This abnormal discolouration usually has a negative effect on how the water looks. This is due to the fact that phytoplankton cells contain varying colour pigments and, when in large concentrations, turn the water into different colours (tones of green, yellow, brown or red), the bathing water not being as transparent as a result.

The blooms are most common in summer when the waters are calm and there are intense levels of solar radiation. Along the Catalan coast, like in the rest of the Mediterranean, these are most likely to occur around sheltered beaches, bays and harbours and, in general, in areas with low rates of water renewal. Blooms are also common in areas where river mouths or irrigation channels run into the sea, bringing with them freshwater which is rich in nutrients and suspended matter. In general, when there is a bloom, the seawater change colour and loses its transparency after midday. The blooms may last for a period ranging from a few days to up to 1–3 weeks, depending on the weather and sea conditions.

As indicated above, phytoplankton blooms occur naturally in the marine environment as a manifestation of the life cycle of these organisms and generally do not pose any risk to beachgoers or marine ecosystems. However, exceptionally, some species can produce toxins that can potentially affect the health of bathers [4, 5, 58, 62]. Interestingly, phytoplankton blooms can be recurrent in certain bathing areas. A persistent problem was detected in Catalonia at La Fosca Beach (Costa Brava) characterised by the discolouration of water. Water that appeared to be clean in the early morning became green-brown by late morning and remained so into the evening (Fig. 10). This generated numerous complaints from the public who assumed the problem to be related to wastewater and sewage inputs. An intensive monitoring programme was conducted, and this included:

- Sanitary inspection of the beach and sewage system to search for unauthorised outlets
- Inspection of possible inland water influence
- Study of the temporal and spatial variations of the microbial water quality
- Analysis of physicochemical parameters
- Study of sediments and flora
- An investigation of phytoplankton

The programme (which costs US$35,000 at 1994–1996 prices) unequivocally ruled out wastewater or sewage inputs. The discolouration was attributed to a non-toxic dinoflagellate *Alexandrium taylori* [57]. Once the origin of the problem was identified, a series of press conferences and a local publicity campaign were undertaken to inform the public. The species *A. taylori* had not previously been identified, but since its identification at La Fosca, it has been reported at other Mediterranean locations [62] and later in other Catalan beaches. This incident illustrates that not all water discolouration should be assumed to be due to sewage pollution or to toxic blooms. In this instance, a preliminary investigation to identify dinoflagellate species would have saved both time and money.

Fig. 10 Images showing La Fosca Beach with transparent and brownish water during the phytoplankton bloom

4.3.2 Accumulations or Sea Foam and Lines of Spume and Slime

Algae blooms may also form surface foam/scum/mucus or slime that are mainly linked to the presence and degradation of organic matter in seawater and plant production. Although certain human activities and the accidental discharge of pollutants can provoke the appearance of foam, the lines or patches of spume and slime that can sometimes be observed on the seawater are generally the result of natural processes and do not pose any risk to the health of bathers. The fact that this process is linked to the presence of naturally degraded organic matter means that spume is frequently formed by the waves after the most active hours for plants (from midday onwards), because organic substances are released into the water as a result of plant production. Scum formation is also prevalent following periods of rainfall, due to organic matter being washed into the sea, and also when there are a lot of waves. Patches or lines of spume are created by the movement of the waves; therefore, the evolution and persistence of the scum also varies depending on the sea conditions. However, they usually persist for several hours.

The most commonly observed accumulations or patches of foam or spume in the Catalan bathing waters are caused by the presence of natural tensioactive substances, which result from the degradation of organic matter occurring naturally in the marine environment. This was demonstrated in a study performed by ACA during 2004 in collaboration with several research institutions (URV, ICM and IDAEA). The study involved the analysis of phytoplankton, faecal indicator bacteria and organic and inorganic chemical compounds in 50 samples of foam. The natural origin of the foam was confirmed by the absence of faecal contamination or anthropogenic organic chemical compounds in the foam samples.

Accumulations of slimy substances can also form, less frequently and on a large scale, and are normally associated with the growth of certain phytoplankton genus, such as *Phaeocystis* spp. and *Gonyaulax* spp. (Fig. 11).

Fig. 11 Images showing the formation of foam, lines of spume and floating slime phytoplankton bloom produced by members of the genus *Gonyaulax* (see its microscopic morphology)

Fig. 12 Accumulation of marine plant remains at the sea and at the sand and water. The fibre balls come from a seagrass called *Posidonia*

4.3.3 Deposits of Marine Plant Remains

The huge appearance of seaweed masses that pile on the beaches or on the shore, the so-called seaweed tides, is a phenomenon that has increased worldwide in recent years [63, 64]. In certain areas of the Catalan coast, currents and waves quite commonly wash up remains of marine plants (i.e. seagrass, marine macro-algae or seaweed) that are then accumulated on the water shoreline or deposited on the beach sand (Fig. 12). This phenomenon forms part of the natural cycle of coastal ecosystems. Although these accumulations are generally harmless and are usually temporary, they can generate discomfort or be a nuisance to recreational users or even to nearby residents because when they decompose they may produce unpleasant smells and can attract insects.

The accumulation on the sand or in the shallow water can uncover rocks or broken glass and other anthropogenic litter, and care should be taken to avoid wading among them to avoid injuries or wounds. Furthermore, some studies have indicated that accumulation of seaweed at the sand could provide a protected environment for FIB reducing the impact of UV disinfection and dissection as well as producing a source of nutrients that may facilitate a greater persistence of FIB (like *E. coli*) in the bathing area [64].

4.3.4 Marine Litter

Marine litter has been found to be widespread in marine environments all over the world impacting negatively the marine life [4, 5, 56, 65, 66]. WHO defined marine litter as any waste from human origin that may accumulate in the sand depending on the sea currents that come from three main sources marine, riverine (including torrents and rain drains) and beach user discharge [4, 5]. The impacts of litter in the bathing areas and some possible interventions have been presented in the WHO guidelines [4, 5]. The United Nations Environmental Programme considered this waste to be an important problem and has launched a global initiative on marine litter (http://www.unep.org/regionalseas/marinelitter/initiatives/unepglobal/default.asp) as did the European Commission that studied the problem with the aim of establishing a quantitative reduction targets for marine litter in the coming years (http://ec.europa.eu/environment/marine/good-environmental-status/descriptor-10/pdf/final_report.pdf). Marine litter is also the topic of recent studies performed at the Mediterranean and other geographical areas [56, 65, 66].

The ACA has also paid a lot of attention to the presence of litter at the bathing areas and even launched an educative campaign underlying how dangerous plastics and other human-generated litter may be for the environment (Fig. 13). In fact since 1992, ACA has used pelican boats to remove litter from the Catalan coast during the summer seasons. Along 10 years (2001 to 2010), they performed an intensive and extensive study along all the coast using between 40 and 52 boats (including pelicans and other types of boats) to find out the extent of the problem. The collected marine litter was sorted, weighed and classified as anthropic residues (plastics, wood, glass bottles, hydrocarbons, etc.) and/or residues of natural origin (such as those coming from vegetation, seagrass, seaweed, foam or spume, etc.). These interventions also prevented marine litter from reaching the bathing areas. As indicated the main goal was to quantify the extent of the problem analysing the quantities recovered each season and at each area of the coast during the different years. The mean volume of residues collected by those cleaning boats during each bathing season was around 500 m^3. They corresponded (% of the total) 60% to anthropic residues (33.5% plastics, 16% anthropic wood, 0.5% hydrocarbons and 10% to other origins) and 40% to natural residues (14% land vegetation, 15% marine vegetation and 11% to organic matter). Our results indicated the need for mitigating the problem at the origin that means in the land by reducing the amount of litter and especially the amount of plastics used. Intensive public campaigns have promoted recycling and reducing litter. Plastic bags are not given for free at grocery shops and are almost not used anymore for shopping in our area. One of the recent studies performed in Australia revealed that although the population is motivated

Fig. 13 Poster elaborated by ACA to educate people about the impact that human litter may have in the marine ecosystem and cleaning activities performed by one ACA boat

for preserving marine wildlife, they underestimated the risk that marine litter generated for wildlife [52]. We believe that a similar situation may happen in our population, so probably it would be important that the information campaigns are not punctual educational interventions but are launched at regular intervals, like, for instance, every 2 years.

5 Conclusions and Future Perspectives

In this chapter, we have analysed the indicators and methodologies proposed for them in the new Directive commenting their limitations. The information about the water quality provided to the competent authorities and the public has to be as reliable as possible and not be distorted by false-positive or false-negative results produced by the analytical methods that will over- or underestimate, respectively, the amount of faecal contamination. It is essential to know the limitations of the microbiological methods in order to select the most reliable to undertake management actions with guarantees. Unreliable information in the form of the false-positive results obtained for the *E. coli* ISO 9308-3 method could lead to spend unnecessary time and money directed at understanding sources of pollution that do not exist. In our experience there is not an ideal method that can be applied to all types of waters (seawater, brackish water or freshwater) with different degrees of faecal contamination. For polluted waters (seawater, brackish water or freshwater), the MPN methods of the Directive are the ones that perform better, while for our clean seawaters, the MF ISO methods were reliable and provided results in shorter time. We hope that in the near future we can have access to continuous measures of water quality. Such systems do already exist measuring similar enzymatic reactions as the ones of the MPN ISO methods and therefore may show the same limitations, and in addition, they are too expensive to be used in routing extensive monitoring programmes.

The sanitary and environmental inspections introduced in the Catalan monitoring programmes (ca. 25 years ago) have been a key element for recognising and studying all the factors that affect/alter the quality and aspect of the water and the sand at each bathing area. The follow-up research enabled to distinguish which ones corresponded to natural phenomena or to faecal pollution. Once a good knowledge of the factors that endanger each beach is established, management actions can also be designed in accordance. The amount of information generated for bathing areas within the Catalan monitoring programmes recording those potentially impacted by urban sewage has been extremely useful for quantifying the pressures received by each water body defined in line with the WFD.

Rain events typical of the Mediterranean climate characterised by few rain episodes of a high intensity are one of the most important factors that can alter sporadically the excellent water quality of our Catalan beaches. Each bathing area has been classified in function of the impact that rain may produce in its quality. An online monitoring of the rain events, with different approaches, allows to give fast alerts or warnings about the potential change of the water quality. This information is integrated in the mobile app on the Catalan bathing areas (ca. 300 beaches) developed by ACA and provides alerts in a timely and friendly manner to the public. Modelling is another approach taken to give warnings about the water quality, but independently of the approach taken, the important thing is that information is proactive and not reactive, i.e. bathers should be alerted before the water quality has deteriorated.

We should bear in mind the possible needed adaptations of the monitoring programmes and the risk assessments to face the possible changes on water quality that may occur in response to climate change.

Education campaigns indicating that the seawater aspect may change as result of natural phenomena not linked to pollution are necessary. In the same line, it is important that the public is aware that during and after (48–72 h) a rain event, the quality of water cannot be guaranteed. Furthermore, it is essential to involve the population on reducing marine litter in order to preserve the marine ecosystem by being more responsible and reducing the litter that they produce.

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Index

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