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Review

# Methods for evaluating the pollution impact of urban wet weather discharges on biocenosis: A review

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A B S T R A C T

Rainwater becomes loaded with a large number of pollutants when in contact with the atmosphere and urban surfaces. These pollutants (such as metals, pesticides, PAHs, PCBs) reduce the quality of water bodies. As it is now acknowledged that physico-chemical analyses alone are insufficient for identifying an ecological impact, these analyses are frequently completed or replaced by impact studies communities living in freshwater ecosystems (requiring biological indices), ecotoxicological studies, etc. Thus, different monitoring strategies have been developed over recent decades aimed at evaluating the impact of the pollution brought by urban wet weather discharges on the biocenosis of receiving aquatic ecosystems. The purpose of this review is to establish a synthetic and critical view of these different methods used, to define their advantages and disadvantages, and to provide recommendations for futures researches. Although studies on aquatic communities are used efficiently, notably on benthic macroinvertebrates, they are difficult to interpret. In addition, despite the fact that certain bioassays lack representativeness, the literature at present appears meagre regarding ecotoxicological studies conducted *in situ*. However, new tools for studying urban wet weather discharges have emerged, namely biosensors. The advantages of biosensors are that they allow monitoring the impact of discharges *in situ* and continuously. However, only one study on this subject has been identified so far, making it necessary to perform further research in this direction.



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## 1. Introduction

It is now acknowledged that growing urbanisation has a major environmental impact (Paul and Meyer, 2001), whether on hydrology, physico-chemistry or the biology of aquatic ecosystems (Stearman and Lynch, 2013). One major type of urban pollution which remains relatively ignored by the public authorities is urban wet weather discharges. These stem in particular from the atmosphere (Gunawardena et al., 2013) and water running off impervious surfaces in urban areas (roads, roofs, etc.) (Göbel et al., 2007) which are undergoing constant expansion (Foster, 2001). Thus rainwater is returned to the natural environment with or without still limited treatments (retention ponds, infiltration basins, biofiltration systems, reed-bed systems, etc.), because of the important volume to treat. Furthermore, rainwater is subject to other forms of urban contamination. Chocat et al. (2007) described urban wet weather discharges (UWWD) as all the water discharged by sewerage installations (mixture of treated wastewater and rainwater), by combined sewer overflows (CSO) (mixture of non treated wastewater and rainwater), and stormwater outfalls (generally non treated rainwater), during a rain event and the dry period following it.

Pollution by urban wet weather discharges is therefore complex due to the diversity of catchments and human activities, and significant concentrations of macro and micropollutants. Weibel et al. (1964) and Burm et al. (1968) were the first to focus on the physico-chemical nature of urban wet weather discharges. Many studies have since been devoted to their characterisation and they permitted highlighting the diversity of pollutants they contain (House et al., 1993; Pitt et al., 1995; Mulliss et al., 1997; van Buren et al., 1997; Admiraal et al., 2000; Lee and Bang, 2000; Lee et al., 2002a,b; Chebbo and Gromaire, 2004; Gasperi et al., 2008 or Curren et al., 2011). They can, in many cases affect adversely water utilisation (drinking fishing, bathing, etc.). The presence of molecules featuring in the list of priority organic pollutants established by the European directives was detected in urban wet weather discharges. The presence of Polycyclic Aromatic Hydrocarbons (PAHs) was detected by Hwang and Foster (2006), Terzakis et al. (2008), Zgheib et al. (2011), Kalmykova et al. (2013), diethylexylphtalate by Bjorklund et al. (2009), Zgheib et al. (2011), 4 nonylphenol by Bjorklund et al. (2009), Zgheib et al. (2011), and bisphenol A and alkylphenolethoxylate by Cladière et al. (2012). Deffontis et al. (2013) also detected traces of hormones, the presence of which had already been revealed in river water by Pailler et al. (2009) and Peng et al. (2008). Other families of micropollutants like heavy metals (copper, lead and zinc, etc.), polychlorobiphenols (PCBs), pesticides, and phthalates have been detected too. The inventory of all these micropollutants has led to characterising urban wet weather discharges as highly complex chemical cocktails. This pollution is also subject to considerable variability (Kafi et al., 2008) due in particular to surfaces leached by rain, considerable variations of runoff flows through time and climatic conditions prior to rain events. Thus characterising urban wet weather discharges is complicated due to the intermittent release of micropollutants during rainfall events (Karlavičienė et al., 2009). The pollution transported by urban wet weather discharges is also biological, with the inflow of many bacteria (such as *Escherichia coli*, the enterococcus, faecal coliforms, etc.) (Jeng et al., 2005; Parker et al., 2010). The same problem of variability has been highlighted for bacterial contamination as for chemical contamination (McCarthy et al., 2012). Urban wet weather discharges are therefore the source of substantial sanitary and environmental risks.

In order to monitor the quality of the discharges, different parameters have to been studied. It is important to observe these parameters as they are liable to have a major impact on the living conditions of the organisms living in aquatic receiving ecosystems. Many studies have focused on erosion phenomena in rivers, on the basis of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), pH, and total concentrations of phosphates, nitrates and ammonia (Mulliss et al., 1997; Chebbo and Gromaire, 2004; Kafi et al., 2008; Lee and Bang, 2000; Mallin et al., 2009). The biological component (especially the inflow of faecal bacteria) has also been studied (McLellan, 2004). Massive inflows of ammonia were observed at contaminated sites (Mulliss et al., 1997), as well reductions of the concentration of dissolved oxygen in certain environments. Stormwater is the main vector of underground contamination by nitrogen and phosphorous (Lee et al., 2003; Wakida and Lerner, 2005). Monitoring these different parameters is important as they play an essential role in the survival of aquatic species. Indeed, concentrations of ammonia and dissolved oxygen in water are the leading causes of mortality in fish in environments contaminated by urban wet weather discharges (Magaud et al., 1997). In addition, a reduction of dissolved oxygen leads to an increase in the sensitivity of certain fish to micropollutants such as heavy metals (Lloyd, 1961). It has also been shown that suspended particles play a fundamental role in the toxic impact of urban discharges (Tsui and Chu, 2003; Angerville, 2009). This can be explained by the fact that some of the micropollutants contained in urban wet weather discharges are adsorbed on the surfaces of particles, and predominantly found in this phase. A large amount of studies described this phenomenon, as Gasperi et al. (2008), Zgeib et al. (2011) or Li and Zuo (2013). If this one is particularly true for pollutants such as heavy metals, PAHs, PCBs (100% particle-bound in the study of Zgheib et al. (2011)), or phthalates, it has also been observed pesticide, heavy metals, alkylphenols, organotins or PAHs residues, in the dissolved phase of urban wet weather discharges (Zgheib et al., 2011), generally with weaker concentrations.

However, taken alone, these parameters are insufficient to evaluate the ecological impact of urban wet weather discharges. It is therefore necessary to carry out studies on the biological communities through bioassays *in situ* and in the laboratory in order to evaluate the stress on populations exposed to wet weather discharges (Burton et al., 2000).

In Europe, the chemical analyses performed are often compared to environmental quality standards (EQS) that are threshold values used to determine the good quality of rivers receiving rainwater, as it was done, for example, by Zgheib et al. (2011). However, the ecological impact of urban wet weather discharges cannot be evaluated only in comparison to these standards and a more inclusive view of the impact is needed (Novotny and Witte, 1997).

Due to global urban expansion, urban wet weather discharges could have a deleterious impact on numerous aquatic areas and different water bodies. For example, effects have been observed in lotic aquatic environments (Custer et al., 2006; Scholes et al., 2007; Casadio et al., 2010; Gillis, 2012), underground environments (Foulquier et al., 2009, 2011), lacustrine environments (Hatch and Burton, 1999) and marine environments (Schiff and Bay, 2003; Roberts et al., 2007). Furthermore, all the trophic levels in these aquatic ecosystems (producers, decomposers, primary and secondary consumers) are potentially affected (Miltner et al., 2004; McQueen et al., 2010; Tang et al., 2013). The organisms living in waste water treatment plants (especially in infiltration basins) are also affected (Bartlett et al., 2012a, 2012b). Animal and plant species in the water column are not alone in being contaminated, since the deposits of particles contained in urban runoff, in rivers or in discharge treatment infrastructures leads to severe contamination of benthic species (such as annelids) (Schiff and Bay, 2003). Urban wet weather discharges therefore constitute a complex matrix for study due to their variable composition and the diversity of organisms and sites affected.

In Europe, the Urban Wastewater Directive (91/271/EEC) requires the management of urban wet weather discharges, though the means are relatively limited (mainly retention and/or infiltration basins), notably due to the large volumes that have to be treated. Furthermore, the European Water Framework Directive (WFD, 2000/60/EC) requires maintaining the good ecological status of water bodies exposed to such large volumes of discharges. To achieve this, it is important to implement effective monitoring methods to ensure compliance of the quality of aquatic environments. As expressed before, chemical analyses alone are not sufficient to evaluate water quality (Mankiewicz-Boczek et al., 2008), and thus the impact of urban wet weather discharges on the environment. Laboratory bioassays (algae and daphnia tests, etc.) are often chosen as they require few resources, they are relatively fast and simple to implement (Blaise, 1991), and they are sensitive and sufficiently repeatable (Marsalek et al., 1999b). Methods combining both approaches, the "Toxicity Identification Evaluation" (TIE), have been developed to help understand the action mechanisms of the most prevalent families of pollutants responsible for the toxicity of urban wet weather discharges (Gersberg et al., 2004; Custer et al., 2006). However, analyses that better integrate the variability of discharges, such as *in situ* studies, provide more realistic views of the consequences of exposure to urban wet weather discharges on an aquatic ecosystem (Burton et al., 2000); however, they remain to be developed (caging, biosensors, etc.).

The purpose of this review is therefore to take stock of all the techniques currently used to study the impact of pollution brought by urban wet weather discharges on the biocenosis of receiving aquatic ecosystems. To this end, the following are described and

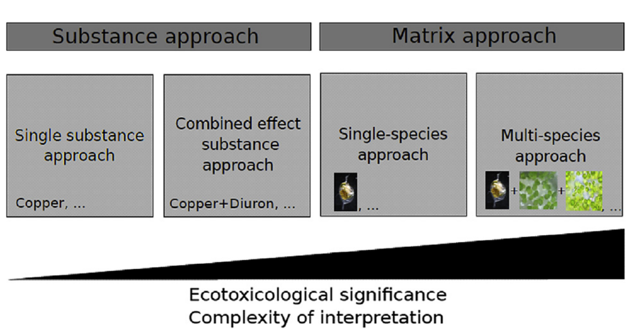


**Fig. 1.** Substance and matrix approaches for studying the biocenotic impact of urban wet weather discharges (modified, according to Angerville (2009)).

discussed: (i) the different biological indices, ecotoxicological bioassays and *a priori* approaches used to evaluate the impact of discharges on contaminated aquatic organisms; (ii) a critical appraisal of these often used methods; and lastly, (iii) the need to turn to new methods that take better account of the variation of the quality of receiving environments and which permit the rapid acquisition of information on the impacts of discharges, such as prediction/prevention tools that can be used before the damage caused does not jeopardise the survival of ecosystems.

## 2. Evaluation of the pollution impact of urban wet weather discharges on biocenosis

This study considers contamination by combined sewer networks and by separate sewer systems, that in particular drain urban and road flows in urban and outer-urban areas, during wet weather events only. Moreover, all the potential receiving environments are considered (whether freshwater, seawater or artificial environments such as infiltration basins with their own biocenoses).

The evaluation of the ecological impact on the biocenose requires studying the effect of pollutant matrixes on the organisms present in contaminated aquatic environments. Evaluating the impact of urban wet weather discharges, and more particularly ecotoxicological impacts, can be approached in two different ways:

- The first is known as the "substance approach" (Fig. 1). It is often associated with a chemical analysis of a sample studied to determine the most prevalent pollutants, and as a function of the ecological pertinence, their risk, bioavailability, and whether or not they can be chosen as risk tracers. It then entails studying the impact of these risk tracers on key organisms. The impact of these pollutants is often assessed by laboratory tests that expose these organisms to a range of pollutant concentrations, or by acquiring data from international databases (BDI). There are several sub-types of substance approach: simple substance approaches, where only one pollutant is taken into account, and combined effect substance approaches where several pollutants are tested in mixtures (Perrodin et al., 2011). Although this approach is often used only for relatively well-known matrixes in which only a few toxic substances predominate, urban wet weather discharges have already been the subject of such studies. Mention can be made of the approach used by Oulton et al. (2014) who studied the impact of zinc present in urban wet weather discharges on the foraging behaviour of *Paratya australiensis*. One of the main criticisms levelled at the substance approach is the lack of representativeness of the tests performed in comparison to reality in the field. Indeed, since the matrixes discharged represent complex mixtures of all families of molecules, synergetic, antagonistic or additive effects may occur between them. This was shown in particular by Angerville (2009).
- The second is the "matrix approach" (Fig. 1), which is a much more realistic approach in relation to the matrix and the scenarios studied. The tests are then carried out on the whole matrix to be studied and not on one or several selected molecules, by exposing organisms to a range of effluent concentrations (Perrodin et al., 2011; Orias and Perrodin, 2013). In this case, the impact observed takes into account the synergetic, antagonistic and additive phenomena of the pollutants present. However, it is then much more difficult to determine which pollutants are responsible for the impact observed and their share of responsibility in the effects observed (Orias and Perrodin, 2013). It is nonetheless recommended to perform chemical analyses to determine the pollutants most present in order to determine the causes of the toxicity observed. As with

**Table 1**
Identification of the studies using biological indices for urban wet weather discharges ecological impact assessment.

| Approach | Toxic matrix | Contaminated water | Studied community | Indices used | Author |
|---|---|---|---|---|---|
| Matrix | Combined sewer overflows | Coastal marine water | Macro-invertebrates (subtidal fauna) | Species relative abundance; Polychaeta, Mollusca and Arthropoda richness, abundance, and biomass; Dissimilarity; Polychaete feeding type proportion | Armstrong et al., 1980 |
| Matrix | Urban wet weather discharges (combined sewer overflows and urban stormwater runoff) | Freshwater (river) | Macro-invertebrates (benthic fauna) | Species diversity (Brillouin index); Hierarchical diversity; Major taxa composition; Dissimilarity | Pratt et al., 1981 |
| Matrix | Urban stormwater runoff | Freshwater (river) | Macro-invertebrates (benthic fauna) | Species diversity (Brillouin index); Population density | Medeiros et al., 1983 |
| Matrix | Urban stormwater runoff | Coastal marine water | Macro-invertebrates (intertidal mudflat fauna) | Species abundance | Roper et al., 1988 |
| Matrix | Urban wet weather discharges (combined sewer overflows and urban stormwater runoff) | Receiving water (river, ponds, etc.) | (zoo- and phyto-) Plankton | Biomass (chlorophyll-a concentration); Species diversity; Saprobic level | Gast et al., 1990 |
| Matrix | Urban wet weather discharges (combined sewer overflows and urban stormwater runoff) | Receiving water (river, ponds, etc.) | Macro-invertebrates (benthic fauna) | Taxa richness; Relative abundance of taxa | Willemsen et al., 1990 |
| | | | Algae (diatoms) | Relative abundance of taxa; Trophic level; Saprobic level | |
| Matrix | Urban stormwater runoff | Freshwater (creeks and rivers) | Macro-invertebrates (benthic fauna) | Hilsenhoff family-level biotic index; EPT (Ephemeroptera, Plecoptera, Trichoptera) index; EPT/Chironomidae abundance; Taxa richness | Masterson and Bannerman, 1994 |
| Matrix | Urban motorway runoff | Freshwater (streams) | Macro-invertebrates (benthic fauna) | Relative abundance; Diversity (log series index and modified Sorenson index); Biological Monitoring Working Party (BMWP) score; Average score per taxon (ASPT); Family richness | Maltby et al., 1995a |
| | | | Fungi (aquatic hyphomycetes) | Species Relative importance values (RIV); Diversity (log series index and modified Sorenson index); Similarity index | |
| | | | Algae | Sampled genera; Diversity (log series index and modified Sorenson index); Biomass (Chlorophyll-a concentration) | |
| Matrix | Combined sewer overflows | Coastal marine water and freshwater (river) | Macro-invertebrates (benthic fauna) | Organisms abundance; Species richness | Hall et al., 1998 |
| Matrix | Urban land use (associated to urban impervious surfaces and urban stormwater runoff) | Freshwater (little streams) | Algae (Epilithic and epiphytic benthic diatom) | Genus and species richness; Relative abundance | Sonneman et al., 2001 |
| Matrix | Urban land use (associated to urban impervious surfaces and urban stormwater runoff) | Freshwater (little streams) | Macro-invertebrates (benthic fauna) | Ephemeroptera, Plecoptera and Trichoptera (EPT) richness | Walsh et al., 2001 |
| Matrix | Urban land use (associated to urban stormwater runoff) | Freshwater (streams) | Fishes | Fish abundance; Species richness; Diversity (Shannon index); Index of biotic integrity (IBI) | Wang et al., 2001 |
| Matrix | Urban land use (associated to urban stormwater runoff) | Freshwater (streams) | Macro-invertebrates (benthic fauna) | Generic and species richness; Diversity (Shannon Wiener index); Biotic index (BI); Ephemeroptera, Plecoptera, and Trichoptera pollution intolerant individuals; Feeding groups proportion (Filterers, Scrapers, Shredders, Collectors, Gatherers); Pollution tolerance (Hilsenhoff biotic index) | Stepenuck et al., 2002 |
| Matrix | Urban stormwater runoff | Coastal marine water (estuaries) | Macro-invertebrates (benthic fauna) | Number of taxa; Total and relative abundance of taxa | Morrisey et al., 2003 |
| Matrix | Urban stormwater runoff | Coastal marine water | Macro-invertebrates (benthic fauna) | Total and mean taxa abundance and biomass; species richness; Diversity (Shannon–Wiener index); Evenness (Pielou's index); Benthic Response Index (BRI) | Schiff and Bay, 2003 |

(*continued on next page*)

**Table 1** (*continued* )

| Approach | Toxic matrix | Contaminated water | Studied community | Indices used | Author |
|---|---|---|---|---|---|
| Matrix | Urban wet weather discharges (combined sewer overflows and urban untreated or treated stormwater) | Freshwater (creeks and river) | Macro-invertebrates (benthic fauna) | Benthic communities abundance and richness; Evenness; Taxonomic composition | Grapentine et al., 2004 |
| Matrix | Urban stormflows | Freshwater (river) | Macro-invertebrates (benthic fauna) | Total abundance and biomass (dry mass); Ephemeroptera, Plecoptera, and Trichoptera (EPT) contribution to the community (EPT taxa, EPT density and EPT biomass); Similarity (Jaccard index); Community tolerance index | Gray, 2004 |
| Matrix | Urban land use (associated to sites impacted directly by discharges from combined or sanitary sewer overflows, sites receiving wastewater treatment plant (WWTP) discharges, sites impacted by instream sewer line placement and construction, etc.) | Freshwater (creeks and run) | Fishes | Index of Biotic Integrity (IBI); Relative abundance of sensitive and high sensitive species | Miltner et al., 2004 |
| Matrix | Combined sewer overflows | Neither (batches and flow chambers) | Periphyton (algae and bacteria) | Total biomass (Dry mass, Ash free dry mass and Organic mass); Algal biomass (chlorophyll-a and phaeopigment concentrations); Bacteria abundance | Parent-Raoult, 2004; Parent-Raoult et al., 2005 |
| Matrix | Urban stormwater runoff | Freshwater (run and branch) | Macro-invertebrates (benthic fauna) | Taxa abundance (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Chironomidae, Tipulidae, Simuliidae, Isopoda, Platyhelminthes) | Gresens et al., 2007 |
| Matrix | Urban stormwater runoff | Coastal marine water | Epifauna (invertebrates inhabiting the alga *Sargassum linearifolium*) | Total and Taxa (Anemones, Copepods, Gammarids, Gastropods, Isopods, Ostracods and Polychaetes) abundance | Roberts et al., 2007, 2008 |
| Substance | Zn, Cu and Pb solutions | Neither (flow chambers) | Bacteria | Bacterial communities composition based on 16S rRNA gene sequences analysis | Ancion et al., 2010 |
| Matrix | Combined sewer overflows | Freshwater (creeks) | Macro-Invertebrates (benthic fauna) | Global Normalised Biological Index (GNBI); Biological Monitoring Working Party (BMWP) score; Family and genus diversity | Rollin et al., 2010 |
| | | | Algae (Diatoms) | Biological Diatom Index (BDI); Specific Pollutant sensitivity Index (SPI) | |
| Matrix | Urban road runoff | Neither (mesocosms) | Algae | Algal taxa cell numbers and biovolumes; Biomass (chlorophyll-a concentration and ash-free dry mass (AFDM)) | Johnson et al., 2011 |
| Matrix | Urban Stormwater Runoff | Freshwater (streams) | Sediment denitrifying Bacteria | Communities structure (Based on terminal restriction fragment length polymorphisms of nosZ genes) | Perryman et al., 2011 |
| Matrix | Urban freeway and residential stormwater runoff | Stormwater Treatment facility ponds | Macro-invertebrates (benthic fauna) | Taxonomic richness; Abundance; Diversity (Shannon Wiener index); Evenness (Simpson's index); Oligochaete Index of Sediment Bioindication (IOBS); Index of sediment biological quality (IOBL) (based on Oligochaete taxonomic richness and abundance) | Tixier et al., 2011, 2012 |
| Matrix | Urban stormwater impoundments | Freshwater (creek) | Fishes | Tolerance index; Species richness; Relative abundance of species | Stearman and Lynch, 2013 |

the substance approach, there are two main types of matrix approach: the single species bioassay approach where a single species is exposed to a matrix (generally in the laboratory), and the multi species approach where a community of species (generally species of different levels of biological organisation) is exposed all together and at the same time to the matrix studied (in microcosms or mesocosms). It is possible to perform these tests *in situ* by exposing the organisms directly in the contaminated environment. One of the drawbacks of this approach is the high variability when setting up the tests (inter-test variability) (Chapman, 2000).

There are two types of assessment to study the impact of urban wet weather discharges on ecosystems: ecotoxicological studies and studies requiring biological indices. Contrary to ecotoxicological studies, assessing the disturbance of aquatic communities using biological indices is mainly done through the matrix approach, since the aim is to study how the structures of the communities present in the receiving aquatic environments are modified. However, studies using the substance approach have been performed, as in the case of Ancion (2010), who studied the modifications occurring in bacterial communities due to exposure to heavy metals such as zinc, copper and lead present in urban wet weather discharges.

Whatever the case, focussing on the total effluent is far more representative of the phenomena induced as it is based on studies that underline the reactivity of the ecosystem, taking into account physico-chemical descriptors, environmental quality criteria, the habitat, the hydraulic regime, autochthonous biological communities and toxicological data.

### 2.1. Biological indices of ecosystem disturbance

Biological indices have been used in view to assessing the impact of urban wet weather discharges on the biodiversity of the communities in place. Most studies are performed on urban and sub-urban rivers and streams as they receive the water discharged from combined sewer overflows. Very few (6 studies identified) have concerned lacustrine environments or coastal areas and only one study has focused on groundwater. The studies performed most usually involve evaluations of abundance, species richness, community diversity (for example, using the Shannon index), and standardised indices for indicator species specific to the environment (invertebrates, fish, diatoms, oligochaetes, macrophytes). Three of them, the Global Normalised Biological Index (GNBI), the Biological Diatom Index (BDI) and the River Fish Index (RFI), have a good status reference framework. This is determined by the statistical exploitation of a large number of data obtained from field studies. However, these indicators are often designed with a single reference that does not take into account the type of watercourse. The different studies using indices to characterise urban wet weather discharges are summarised in Table 1.

#### 2.1.1. Indices on decomposers (bacteria and fungi)

Modifications of bacterial communities in receiving environments mainly stem from the inflow of faecal bacteria from animal faeces (carried by runoff, etc.) and human faeces (combined sewer overflows, etc.). Thus the study by Dwight et al. (2011) on 78 stations on the south coast of California highlighted an imbalance in bacterial composition with the presence of pathogenic species which can therefore have an impact on human health. Bacterial community composition disturbance have relative unclear long-term effects for aquatic ecosystem, but probably leads to a modification of processes as decomposition, nutrient cycling, etc. (Wakelin et al., 2008). Moreover, fecal bacteria would be responsible of the oxygen depletion (linked to biodegradable dissolved organic carbon) often observed with combined sewer overflows, and impacting survival of many organisms, as the fishes (Even et al., 2004).

Modifications can also occur within river communities as has been pointed out by several studies. Ancion et al. (2010) studied the modification of the composition of bacterial communities present in biofilms after exposure to metals mostly found in urban runoff (zinc, copper and lead), by using the polymorphism of DNA sequences located between genes coding for RNA 16S and RNA 23S.

Other authors (Maltby et al., 1995a) studied the impact of highway runoff (located on the outskirts of cities) on other communities of decomposers in rivers and streams, in this case fungi, more specifically hyphomycetes, by comparing their assemblages (with the determination of their species richness and the use of Relative Importance Values (RIV)) upstream and downstream of outfalls of contaminated water. It is important to monitor the composition of fungal communities, and more particularly aquatic hyphomycetes, as they are responsible for decomposing and modifying the structure of leaves (notably via the transformation of indigestible compounds into consumable ones) for their consumption by macro-invertebrates, and thus the distribution of both particulate organic matter and aquatic macro-invertebrates in rivers (Bärlocher, 1985; Maltby et al., 1995a).

#### 2.1.2. Indices for monitoring primary producers

Since algae are specific species to the environment in which they exist, they are an excellent indicator of the impact of urban discharges on receiving environments. Contrary to certain benthic macro-invertebrates and fish, most of them are unable to migrate (drifting, burrowing, etc.) to habitats with more favourable conditions and less subject to the impact of discharges (Parent-Raoult and Boisson, 2007). Algae, along with detritus, are also the main source of energy in small watercourses (Maltby et al., 1995a), and the largest (macro-algae) shelter macro-fauna. Thus they play an essential role in watercourses.

Regarding algae, diatoms have been relatively well used for studying urban wet weather discharges and have been the subject of several studies (Gast et al., 1990; Willemsen et al., 1990; Sonneman et al., 2001; Rollin et al., 2010). In particular, diatoms are subject to a standardised index often used in the works cited previously, namely the Biological Diatom Index (BDI) based on the presence of species, their abundance and their sensitivity to pollutants, among other things. The determination of species and their abundance is often done by microscope on the basis of a minimum of 300–400 algae per sample taken randomly.

Assessing the impact of urban wet weather discharges on algae is not necessarily expressed by a reduction of abundance, biomass or the number of species (Maltby et al., 1995a; Rollin et al., 2010). For example, Maltby et al. (1995a) noted that neither abundance in epilithic algae, nor their diversity was affected by runoff discharges from roads. However, several authors have observed changes in the composition of algal communities. Sonneman et al. (2001) studied communities of epilithic and epithetic algae in watercourses located around Melbourne and observed changes in the diatom communities exposed. However, contrary to the macro-invertebrates also affected, the impact on algae in this case was mainly explained by the high inflow of nutriments in the discharges, making these algae good indicators of the enrichment of the environment by urban activities. Rollin et al. (2010) studied colonisation by diatoms of media placed directly downstream of stormwater outflows using the BDI and a second index, the SPI (Specific Pollutant sensitivity Index) also based on the abundance of the species observed (400 individuals observed per sample recovered) and the sensitivity of species. They nonetheless observed that the impact on diatoms was less marked than for other compartments (in this case macro-invertebrates). This could be due to a greater capacity for renewal in algae. Algae communities therefore present several drawbacks for monitoring the impact of urban wet weather discharges.

Although the study of communities has often been performed *in situ*, several authors have carried out experiments in the laboratory, namely on periphytons (algae and bacteria) subjected to simulated stormwater outflow discharges in artificial canals (Parent-Raoult, 2004; Parent-Raoult et al., 2005) and on communities studied in 1300 L mesocosms (Johnson et al., 2011).

### 2.1.3. Indices for monitoring benthic macro-invertebrates

The aqueous phase of urban wet weather discharges is not the only phase containing pollutants. Studies have shown that the particulate phase is the most pollutant discharge since most of the priority substances are found adsorbed to the surfaces of the particles (Parent-Raoult and Boisson, 2007; Zgheib et al., 2011). What is more, during the discharge of urban effluents, pollutants and particles fall progressively in the water column and deposit on sediments on the beds of watercourses (in the benthic zone). It has also been shown that the pollutants from discharges are also liable to accumulate in estuary sediments at concentrations likely to cause adverse biological effects (Morrisey et al., 2003). Therefore it is important to monitor the composition of macro-invertebrate communities since the compartment in which they live is highly exposed to dissolved and particulate fractions of urban wet weather discharges. Furthermore, since they colonise these sediments, they are heavily exposed to cumulative effects which makes them excellent bio-indicators (Rochfort et al., 2000; Komínková et al., 2005). This is why benthic macro-organisms are the indicators most often used to monitor the impact of urban wet weather discharges on aquatic ecosystems (see Table 3). The studies performed involved organisms of both the benthic and hyporheic zones.

Several authors have emphasised that the type of catchment cannot be the only parameter to be taken into account when evaluating the impact of urban wet weather discharges. Rainfall intensity and the sampling site play a major role. This is why, regarding the choice of sector to be prospected, Angerville (2009) hypothesised on the existence of a preferential zone governed by suitable conditions (gentle longitudinal slope, slow flow rate, laminar flow, etc.) favouring the settling of particles contained in urban wet weather discharges. Therefore interstitial organisms are exposed to a larger share of the particulate pollution contributed by urban wet weather discharges. This choice seems consistent to observe a strong impact on communities. Indeed, as shown in Borchardt et al. (1990), studying the loss of Gammarus populations, low flow rates cause a stronger effect. This may be due to of the sedimented particles containing adsorbed pollutants but also to a preferential particles deposition due to low flow (Brunke, 1999). This can leads to a siltation of interstitial zones hindering macro-invertebrates to protect themselves inside, and therefore leads to greater exposure to discharges (Borchardt et al., 1990).

The first studies of this type applied to urban wet weather discharges emerged as early as the beginning of the 1980s, notably those of Armstrong et al. (1980), Pratt et al. (1981) and Medeiros et al. (1983), with observations of a marked disturbance of communities by discharges. Contrary to other types of community (algae, decomposer and fish), benthic macro-invertebrates have been studied in most aquatic environments: marine (Schiff and Bay, 2003), estuarine (place of transition) (Morrisey et al., 2003), freshwater (Gray, 2004; Gresens et al., 2007) and artificial (treatment basins) (Tixier et al., 2011, 2012). Three orders of macro-invertebrates (Ephemeroptera, Plecoptera and Trichoptera (EPT)) were studied in particular by Walsh et al. (2001), Stepenuck et al. (2002), and Gresens et al. (2007). One the reasons for this is that organisms of these three orders are relatively sensitive to pollution (Walsh et al., 2001) and their density and relative abundance declines rapidly as pollution increases.

There are two types of study dedicated to benthic macro-invertebrates for assessing the impact of urban wet weather discharges: direct sampling of the communities present on site, with the constraints of the sampling site mentioned above (meaning most studies), and studies based on the installation of an artificial substrate colonised by the organisms. But, in the two cases, an important limit is to find a reference site with no pollution, which is now very difficult.

Although most authors have sought to assess the direct impact of urban wet weather discharges (from separate or combined sewer networks) on benthic communities, others, on the contrary have sought to find a link between impervious urban area (in direct connection with quality and quantity of rainwater runoff) and the impacts on these communities. However, the use of these proxies is not always relevant. As explained by Walsh et al. (2001) in their study, alterations of the structure of macroinvertebrate communities between low and highly urbanised areas are not always explained by the imperviousness rate only, because some natural parameters can interact, as climate, habitat and catchment geology. Moreover, as they wrote, sites having the same rate of imperviousness don't always show the same impact on communities. It can be explained by: on one hand, the presence of systems of drainage infrastructure, and on another hand, the difficulty to make a direct link between imperviousness and pollutants concentrations in discharges, which depends on the watershed studied.

Measures of the species richness of benthic communities have often made it possible to assess the impact of pollution on the benthic communities. Generally, biological indices provide information in accordance with the decrease of richness. However, several studies have shown that if no change of species richness is observed, it is not possible to use biotic indices to draw conclusions as they often depend on this parameter (Cao et al., 1996). In addition, changes to some benthic macroinvertebrate communities can occur over different timescales (from a few months to a few years) following contamination (Damasio et al., 2008). These indices are used to show the effects of pollution already present for a long time. So, it is not advisable to use them to assess the impact of a one-time event, but rather a more diffuse pollution in time. Bioassays remain preferable in the other case.

### 2.1.4. Indices for monitoring fishes

In general, the impact of urbanisation on fishes has been given far less attention than to invertebrates (Paul and Meyer, 2001); likewise for urban wet weather discharges. Indeed, it is much more difficult to monitor their impact on fish communities as they are not confined to local receiving environments. Besides, due to their mobility, it is difficult to assess the time in which fish are really in contact with urban wet weather discharges (La Point and Waller, 2000). However, it is interesting to observe modifications of fish communities, as they have relatively long lifetimes and thus good indicators of long term disturbances. Indeed, several studies have sought to demonstrate the correlation between the imperviousness of soils due to the urbanisation of catchments (responsible for rainwater runoff which picks up all the molecules described previously from these surfaces) and the disturbance of fish communities in urban and sub-urban watercourses (Wang et al., 2001; Miltner et al., 2004). But, this method has the same limits than those for benthic communities. In these two studies, a common good health index of fish communities and watercourses was used: the IBI (Index of Biotic Integrity), calculated following the capture of

**Table 2**
Identification of the studies using a substance approach for the ecotoxicity assessment of urban wet weather discharges.

| Pollutant | Approach | Organism type | Organism | Bioassay type | Exposure time | Endpoint | Author |
|---|---|---|---|---|---|---|---|
| Diazinon, Chlorpyrifos | Single and combined substance | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 24 h; 48 h; 72 h; 96 h | Mortality | Bailey et al., 1997 |
| Zn, Cu, Pb | Single substance | Phytoplankton | x | Biomarker | 6 h | Respiration/ Photosynthesis | Seidl et al., 1998 |
| Zn | Single substance | Vertebrate | *Oncorhynchus mykiss* | Monospecific bioassay | 96 h | Mortality | Bailey et al., 1999 |
| Paraquat, Norflurazon, Atrazine, Flazasulfuron | Single substance | Plant | *Lemna minor* | Monospecific bioassay | 48 h | Growth | Frankart et al., 2003 |
| | | | | Biomarker | 48 h | Photosynthesis | |
| Zn | Single substance | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 48 h | Mortality | Kszos et al., 2004 |
| Atrazine, Diazinon | Single and combined substance | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 48 h | Mortality | Banks et al., 2005 |
| Cu | Single substance | Vertebrate | *Oncorhynchus kisutch* | Biomarker | 3 h | Neurotoxicity (olfactiv system olfactory sensitivity) and predator avoidance behaviours | Sandahl et al., 2007 |
| Cd, Cu, Pb, Zn, NH4 | Single and combined substance | Invertebrate | *Daphnia magna* | Monospecific bioassay | 24 h | Mortality | Angerville, 2009 |
| | | Algae | *Pseudokirchneriella subcapitata* | Monospecific bioassay | 72 h | Growth | |
| NaCl | Single substance | Invertebrate | *Hyalella azteca* | Monospecific bioassay | 4 weeks | Growth | Bartlett et al., 2012a |
| | | | | Monospecific bioassay | 1 week; 2 weeks; 3 weeks; 4 weeks | Mortality | |
| Galaxolide, Cd | Single and combined substance | Vertebrate | *Carassius auratus* | Biomarker | 7 days; 14 days; 21 days | Oxidative stress | Chen et al., 2012 |
| Zn | Single substance | Invertebrate | *Paratya australiensis* | Biomarker | 72 h | Foraging behaviour | Oulton et al., 2014 |

fish by electrofishing in sampling zones. This index is especially easy to assess as fish are easy to collect and identify. Stearman and Lynch (2013) sought to establish a link between urbanisation, urban fragmentation, and rainwater management practices (especially those of reservoirs and basins), rather than the rainwater itself, and the disturbance of stream fish communities. Their study relied on the analysis of two indices: total species richness and a tolerance index based on the proportions of fish classed into categories of tolerance to habitat degradation and environment quality.

### 2.2. Ecotoxicological bioassays

The main studies and methods found for ecotoxicological bioassays carried out on urban wet weather discharges using substance and matrix approaches are summarised in Tables 2 and 3.

There are two possibilities for classing ecotoxicological tests: (1) classification into three categories: genotoxicity tests, acute tests and chronic tests; (2) classification with respect to the scale of the study. We have chosen the second solution for the sake of clarity. The methods used are classed into four groups: biomarker tests (genotoxicity, enzymatic activity, etc.), single species tests (organism scale), multi species bioassays (several species studied simultaneously) and *in situ* tests.

#### 2.2.1. Biomarker tests

Several authors, including Tang et al. (2013), have observed that in the case of contamination by urban wet weather discharges, parameters such as photosynthesis (a toxicity biomarker) responded with greater sensitivity than more global parameters such as algae population growth (in particular due to the presence of herbicides). This is why biomarkers are especially important when studying the impact of urban wet weather discharges. The biomarkers are early pollution detectors. They enable both to identify the presence of toxic effects of pollutants faster than global parameters, but also to detect effects at concentrations below those that product irreversible effects (van Gestel and van Brummelen, 1996; Burton et al., 2000). However, Marsalek et al. (1999a) observed that some biomarkers could be less sensitive than single-species assays (e.g. SOS-Chromotest less sensitive than testing Daphnia magna mortality to 48 h). One explanation mentioned is the difference in sensitivity to particles of discharges.

Several families of biomarker have been studied such as exposure and effect biomarkers, genotoxicity, cytotoxicity and biomarkers of enzymatic activity or more global metabolic activity.

Regarding genotoxicity and cytotoxicity biomarkers, tests such as the SOS-chromotest, which uses strains of the bacteria *E. coli* and measures beta-galactosidase activity to determine the level of induction of the DNA repair mechanism, and the umuC test, which is similar but uses a strain of *Salmonella typhimurium*, have already been the subject of several studies (Marsalek et al., 1999a, 1999b; Tang et al., 2013). Cytotoxicity tests based on the inhibition of mitochondrial electron transfer (Sub-mitochondrial Particle bioassays) have also been used to assess the impact of urban wet weather discharges (Tang et al., 2013).

Recent works on the primary producer compartment have studied chlorophyll fluorescence, which indicates photosynthetic efficiency, and enzymatic activity inhibition (esterase and alkaline phosphatase) underlying the metabolism of nutrients. These tests are used to assess the impact of diffuse pollution in runoff in urbanised coastal areas (Durrieu et al., 2011), as well as the quality of combined sewer overflow discharges and water collected at the inlets of infiltration basins (Ferro, 2013). Substance approach type tests focussing on the inhibition of several fluorescence parameters linked to photosynthesis due to pesticides found in urban wet weather discharges were also developed by Frankart et al. (2003). In addition, measures of metabolic activity (net primary productivity and respiration) by Seidl et al. (1998) and Boisson and Perrodin (2006) on the behaviour of primary producers have also been carried out to study discharges from CSO and road runoff.

What is more, Tang et al. (2013) also used measures of

**Table 3**
Identification of the studies using in matrix approach for the ecotoxicity assessment of urban wet weather discharges.

| Toxic matrix | Samples used for assays | Organism type | Studied organism | Bioassay type | Exposure time | Endpoint | Author |
|---|---|---|---|---|---|---|---|
| Urban road stormwater runoff | Downstream contaminated sediments (fractioned extracts) | Bacteria | *Photobacterium phosphoreum* | Monospecific bioassay | 15 min | Luminescence inhibition | Boxall and Maltby, 1995 |
| | | Invertebrate | *Gammarus pulex* | Monospecific bioassay | 14 days | Mortality | |
| Airport stormwater runoff | Discharges | Vertebrate | *Pimephales promelas* | Monospecific bioassay | 48 h | Mortality | Fisher et al., 1995 |
| | | Invertebrate | *Daphnia magna* | Monospecific bioassay | 48 h | Mortality | |
| Urban stormwater runoff | Downstream contaminated freshwaters and treatment pond discharges | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 7 days | Mortality | Katznelson et al., 1995 |
| Urban motorway stormwater runoff | Artificial pond waters and contaminated waters/sediments/sediments extracts | Invertebrate | *Gammarus pulex* | Monospecific bioassay | 14/15/17 days | Mortality | Maltby et al., 1995b |
| Urban stormwater runoff | Urban stormwater pond sediment porewaters | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 48 h | Mortality | Wenholz and Crunkilton, 1995 |
| Combined sewer outfalls and larger stormwater outfalls | Downstream contaminated freshwaters | Invertebrate | *Gammarus pulex* | *In situ* assay (caging) | 36 days (daily assessment) | Mortality | Mulliss et al., 1997 |
| Urban stormwater runoff | Urban stormwater pond sediments and discharges | Invertebrate | *Hyalella azteca* | Monospecific bioassay | 10 days | Mortality | Karouna-Renier and Sparling, 1997 |
| | | | | Monospecific bioassay | 10 days | Growth | |
| Sawmills stormwater runoff | Discharges | Vertebrate | *Oncorhynchus mykiss* | Monospecific bioassay | 96 h | Mortality | Bailey et al., 1999 |
| Urban stormwater runoff | Discharges and marine contaminated waters | Invertebrate | *Shongyiocennofus purpuratus* | Monospecific bioassay | 20 min | Egg fertilisation by spermatozoa | Jirik et al., 1998 |
| Combined sewer overflows | Downstream contaminated sediments | Invertebrate | *Rhepoxynius abronius* | Monospecific bioassay | 10 days | Mortality | Hall et al., 1998 |
| | | Invertebrate | *Mytilus edulis* | Monospecific bioassay | 48 h | Mortality | |
| Combined sewer overflows | Discharges (filtered and centrifuged) | Phytoplankton | x | Biomarker | 6 h | Respiration and Photosynthesis (Oxygen production) | Seidl et al., 1998 |
| Urban stormwater events | Discharges | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | Exposition: 15 min – 6 h (very toxic samples)/12 h – 48 h (low toxic samples) Post exposition: 48 h | Mortality | Brent and Herricks, 1999 |
| Urban stormwater runoff | Downstream contaminated freshwaters and sediments | Invertebrate | *Hyalella azteca* | Monospecific bioassay/*In situ* assay (caging) | 48 h; 10 days/48 h; 7 days; 10 days | Mortality | Hatch and Burton, 1999 |
| | | | | Monospecific bioassay/*In situ* assay (caging) | 10 days/7 days; 10 days | Feeding behaviour | |
| | | | | Biomarker | 20 min | Enzymatic activities (IQ toxicity assay) | |
| | | Invertebrate | *Daphnia magna* | Monospecific bioassay/*In situ* assay (caging) | 48 h/48 h | Mortality | |
| | | | | Biomarker | 15 min | Enzymatic activities (IQ toxicity assay) | |
| | | Vertebrate | *Pimephales promelas* | Monospecific bioassay/*In situ* assay (caging) | 48 h/48 h | Mortality | |
| Urban stormwater runoff | Inflows and outflows from ponds | Invertebrate | *Daphnia magna* | Monospecific bioassay | 48 h | Mortality | Marsalek et al., 1999a |

**Table 3** (*continued* )

| Toxic matrix | Samples used for assays | Organism type | Studied organism | Bioassay type | Exposure time | Endpoint | Author |
|---|---|---|---|---|---|---|---|
| | Inflows and outflows from ponds (concentrated 10 times) | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 15 min | Luminescence inhibition | |
| | | Bacteria | *Escherichia coli* | Biomarker | ? | Genotoxicity (SOS chromotest) | |
| | | x | *x* | Biomarker | x | Mitochondrial toxicity (Sub-mitochondrial Particle bioassay) | |
| Urban wet weather discharges (combined sewer overflows and urban stormwater runoff) | Inflows and outflows from ponds | Invertebrate | *Daphnia magna* | Monospecific bioassay | 48 h | Mortality | Marsalek et al., 1999b |
| | Inflows and outflows from ponds (concentrated 10 times) | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 15 min | Luminescence inhibition | |
| | | Bacteria | *Escherichia coli* | Biomarker | x | Genotoxicity (SOS chromotest) | |
| | | x | *x* | Biomarker | x | Mitochondrial toxicity (Sub-mitochondrial Particle bioassay) | |
| | Inflows and outflows from ponds and downstream contaminated sediments | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 20 min | Luminescence | |
| | | Bacteria | *Escherichia coli* | Biomarker | 4 h | Genotoxicity (SOS chromotest assay) | |
| | | Invertebrate | *Panagrellus redivivus* | Monospecific bioassay | 96 h | Mortality | |
| | | | | Monospecific bioassay | 96 h | Growth | |
| Urban stormwater runoff | Discharges | Vertebrate | *Oryzias latipes* | Monospecific bioassay | 9–12 days | Embryotoxicity (teratogenesis, hatching and mortality of eggs) | Skinner et al., 1999 |
| | | Vertebrate | *Menidia beryllina* | Monospecific bioassay | 7–9 days | Embryotoxicity (teratogenesis, hatching and mortality of eggs) | |
| Urban stormwater runoff | Downstream contaminated freshwaters (after outlet of a detention pond) | Invertebrate | *Gammarus minus* | *In situ* assay (caging) | 42 days | Mortality | Lieb and Carline, 2000 |
| Urban stormwater runoff | Discharges | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 96 h | Mortality | Schiff et al., 2002 |
| | | Invertebrate | *Mysidopsis bahia* | Monospecific bioassay | 96 h | Mortality | |
| | | Invertebrate | *Strongylocentrotus purpuratus* | Monospecific bioassay | 20 min | Egg fertilisation by spermatozoa | |
| Urban stormwater runoff | Discharges, downstream contaminated freshwater, marine contaminated sediments and interstitial waters from sediments | Invertebrate | *Strongylocentrotus purpuratus* | Monospecific bioassay | 20 min | Egg fertilisation by spermatozoa | Bay et al., 2003 |
| | Marine contaminated sediments | Invertebrate | *Rhepoxynius abronius* | Monospecific bioassay | 10 days | Mortality | |
| Urban stormwater runoff | Downstream contaminated freshwaters | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 48 h; 96 h | Mortality | Gersberg et al., 2004 |
| Urban wet weather discharges (combined sewer overflows and urban stormwater runoff) | Stream sediments contaminated by untreated or treated runoffs/Contaminated downstream waters column by untreated runoffs | Invertebrate | *Hyalella azteca* | Monospecific bioassay/*In situ* assay (caging) | 28 days/7 weeks | Mortality | Grapentine et al., 2004 |

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**Table 3** (*continued* )

| Toxic matrix | Samples used for assays | Organism type | Studied organism | Bioassay type | Exposure time | Endpoint | Author |
|---|---|---|---|---|---|---|---|
| | | | | Monospecific bioassay/*In situ* assay (caging) | 28 days/7 weeks | Growth | |
| | Stream sediments contaminated by untreated or treated runoffs | Invertebrate | *Chironomus riparius* | Monospecific bioassay | 10 days | Mortality | |
| | | | | Monospecific bioassay | 10 days | Growth | |
| | | Invertebrate | *Hexagenia spp.* | Monospecific bioassay | 21 days | Mortality | |
| | | | | Monospecific bioassay | 21 days | Growth | |
| | | Invertebrate | *Tubifex tubifex* | Monospecific bioassay | 28 days | Mortality | |
| | | | | Monospecific bioassay | 28 days | Reproduction | |
| Simulated urban parking lot runoffs | Discharges | Invertebrate | *Shongyiocennofus purpuratus* | Monospecific bioassay | 20 min | Egg fertilisation by spermatozoa | Greenstein et al., 2004 |
| Urban stormwater runoff | Discharges | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 48 h | Mortality | Kszos et al., 2004 |
| Urban motorway stormwater runoff | Discharges | Periphyton | x | Plurispecific bioassay (artificial river)/Biomarker | 1 h or 4 h, three time a week over 30 days | Biomass | Boisson and Perrodin, 2006 |
| | | | | Plurispecific bioassay (artificial river)/Biomarker | 1 h or 4 h, three time a week over 30 days | Metabolic activity (net primary production and respiration) | |
| Urban and motorway stormwater runoff | Downstream contaminated freshwaters (whole and SPE concentrated), sediment porewaters and suspensions | Algae | *Pseudokirchneriella subcapitata* | Monospecific bioassay | 48 h | Growth | Christensen et al., 2006 |
| | Downstream contaminated freshwaters | Invertebrate | *Daphnia magna* | Monospecific bioassay | 48 h | Mortality | |
| Urban and agricultural stormwater runoff and treated wastewaters | Downstream contaminated freshwaters and substrates (cobbles) | Invertebrate | *Isonychia* spp. | *In situ* assay (exposure chamber) | 96 h | Mortality | Custer et al., 2006 |
| | | Invertebrate | *Hydropsyche* spp. | *In situ* assay (exposure chamber) | 96 h | Mortality | |
| | | Invertebrate | *Psephenus herricki* | *In situ* assay (exposure chamber) | 96 h | Mortality | |
| Urban and motorway stormwater runoff | Discharges | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 15 min | Luminescence inhibition | Scholes et al., 2007 |
| | | Algae | *Pseudokirchneriella subcapitata* | Monospecific bioassay | 48 h | Growth | |
| | | Invertebrate | *Brachionus calyciflorus* | Monospecific bioassay | 48 h | Reproduction | |
| Urban stormwater highway runoff | Discharges | Algae | *Pseudokirchneriella subcapitata* | Monospecific bioassay | 96 h | Growth | Kayhanian et al., 2008 |
| | | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 7 days | Mortality | |
| | | | | Monospecific bioassay | 7 days | Reproduction | |
| | | Vertebrate | *Pimephales promelas* | Monospecific bioassay | 7 days | Mortality | |
| | | | | Monospecific bioassay | 7 days | Growth | |
| | | Bacteria | *Photobacterium phosphoreum* | Monospecific bioassay | 22 h | Luminescence inhibition | |
| | | Invertebrate | *Strongylocentrotus purpuratus* | Monospecific bioassay | 20 min | Egg fertilisation by spermatozoa | |
| Urban stormwater runoff | Sediment porewaters from stormwater pond | Invertebrate | *Hyalella azteca* | Monospecific bioassay | 7 days | Mortality | Mayer et al., 2008 |

**Table 3** (*continued* )

| Toxic matrix | Samples used for assays | Organism type | Studied organism | Bioassay type | Exposure time | Endpoint | Author |
|---|---|---|---|---|---|---|---|
| Urban stormwater runoff | Downstream contaminated freshwaters | Vertebrate | *Oncorhynchus kisutch* | *In situ* assay | 5 year study | Mortality | McCarthy et al., 2008 |
| Urban stormwater runoff | Stormwater ponds discharges and sediments | Invertebrate | *Hydra hexactinella* | Monospecific bioassay | 96 h (daily assessment) | Mortality | Rosenkrantz et al., 2008 |
| | Stormwater ponds discharges | | | Monospecific bioassay | Exposition: 1 h; 3 h; 5 h or 7 h; Post-exposition: 144 h (measurement each day) | Growth (Pulse toxicity test) | |
| | Stormwater ponds discharges and sediments | | | Monospecific bioassay | 144 h (daily assessment) | Growth | |
| | Stormwater ponds sediments | | | Monospecific bioassay | 144 h | Avoidance behaviour (sediment avoidance) | |
| Urban roadway stormwater runoff | Stormwater pond sediments | Vertebrate | *Bufo americanus* | Monospecific bioassay | 40–48 days | Embryotoxicity (delay of metamorphosis and mortality of embryos) | Snodgrass et al., 2008 |
| | | Vertebrate | *Rana sylvatica* | Monospecific bioassay | 13 days | Mortality (embryos) | |
| Urban highway runoff | Discharges | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 5 min; 15 min; 30 min | Luminescence inhibition | Waara and Färm, 2008 |
| | | Invertebrate | *Daphnia magna* | Monospecific bioassay | 24 h; 48 h | Mortality | |
| | | Invertebrate | *Thamnocephalus platyurus* | Monospecific bioassay | 24 h | Mortality | |
| | | Bacteria | *Lemna minor* | Monospecific bioassay | 7 days | Growth | |
| Urban industrial and road stormwater runoff | Treated or not discharges | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 30 min | Luminescence inhibition | Wium-Andersen et al., 2009 |
| | | Algae | *Scenedesmus subspicatus* | Monospecific bioassay | 72 h | Growth | |
| | | Invertebrate | *Daphnia magna* | Monospecific bioassay | 24 h; 48 h | Mortality | |
| Urban wet weather discharges (combined sewer overflows and urban stormwater runoff) | Discharges, downstream contaminated surface freshwater, benthic and hyporheic zone samples | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 20 min; 30 min | Luminescence inhibition | Angerville (2009) |
| | Discharges | Algae | *Pseudokirchneriella subcapitata* | Monospecific bioassay | 72 h | Growth | |
| | Discharges and downstream contaminated freshwater | Invertebrate | *Daphnia magna* | Monospecific bioassay | 24 h; 48 h | Mortality | |
| | Discharges | | | Monospecific bioassay | 21 days | Reproduction | |
| | Discharges | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 7 days | Reproduction | |
| | Discharges and downstream contaminated freshwater | Invertebrate | *Brachionus calyciflorus* | Monospecific bioassay | 48 h | Reproduction | |
| | Discharges, downstream contaminated benthic and hyporheic zone samples | Invertebrate | *Heterocypris incongruens* | Monospecific bioassay | 6 days | Growth | |
| | | | | Monospecific bioassay | 6 days | Mortality | |
| Urban stormwater runoff | Downstream contaminated sediments | Plant | *Lepidium sativum* | Monospecific bioassay | 48 h | Germination | Karlavičienė et al., 2009 |

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**Table 3** (*continued*)

| Toxic matrix | Samples used for assays | Organism type | Studied organism | Bioassay type | Exposure time | Endpoint | Author |
|---|---|---|---|---|---|---|---|
| | | | | Monospecific bioassay | 48 h | Roots growth | |
| Urban stormwater runoff | Contaminated coastal marine waters and freshwaters | Invertebrate | *Strongylocentrotus purpuratus* | Monospecific bioassay | 20 min | Egg fertilisation by spermatozoa | Reifel et al., 2013 |
| Urban highly travelled roadway stormwater runoff | Stormwater pond sediments | Vertebrate | *Hyla versicolor* | Monospecific bioassay | x | Embryotoxicity (delay to metamorphosis and mortality of embryos) | Brand et al., 2010 |
| Urban combined sewer overflows | Downstream contaminated freshwaters | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 15 min | Luminescence inhibition | Casadio et al., 2010 |
| Highway and urban stormwater runoff | Discharges | Vertebrate | *Bufo viridis* | Monospecific bioassay | 65 days | Embryotoxicity (teratogenesis, delay to metamorphosis and mortality) | Dorchin and Shanas, 2010 |
| Urban stormwater runoff | Urban stormwater ponds and tanks discharges and sediments (inlet and outlet) | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 30 min | Luminescence inhibition | Karlsson et al., 2010 |
| Campus parking lot stormwater runoff | Discharges | Invertebrate | *Ceriodaphnia dubia* | Monospecific bioassay | 7 days | Mortality | McQueen et al., 2010 |
| | | | | Monospecific bioassay | 7 days | Reproduction | |
| | | Vertebrate | *Pimephales promelas* | Monospecific bioassay | 7 days | Mortality | |
| Urban Stormwater runoff | Discharges | Vertebrate | *Oncorhynchus mykiss* | Monospecific bioassay | 21 days | Growth | Milukaite et al., 2010 |
| Urban Stormwater runoff | Urban contaminated streams | Vertebrate | *Oncorhynchus kisutch* | *In situ* assay | x (survey between 2000 and 2009) | Mortality | Feist et al., 2011 |
| Urban highway runoff | Discharges | Algae | Algal community | Plurispecific bioassay (mesocosm (1300 L)) | 28 days | Biomass | Johnson et al., 2011 |
| | | | | Plurispecific bioassay (mesocosm (1300 L))/ Biomarker | 28 days | Metabolic activity | |
| | | Invertebrate | *Physa* sp. | Plurispecific bioassay (mesocosm (1300 L)) | 28 days | Growth | |
| | | | | Plurispecific bioassay (mesocosm (1300 L)) | 28 days | Mortality | |
| | | Vertebrate | *Lepomis gibbosus* | Plurispecific bioassay (mesocosm (1300 L)) | 28 days | Growth | |
| | | | | Plurispecific bioassay (mesocosm (1300 L)) | 28 days | Mortality | |
| Urban Industrial stormwater runoff | Discharges | Algae | *Scenedesmus subspicatus* | Monospecific bioassay | 96 h (daily assessment) | Growth | Kaczala et al., 2011 |
| Urban highway and residential stormwater runoff | Stormwater pond sediments | Invertebrate | *Hyalella azteca* | Monospecific bioassay | 4 weeks | Growth | Bartlett et al., 2012a, 2012b |
| | | | | Monospecific bioassay | 4 weeks | Mortality | |
| Urban Combined Sewer Overflows | Downstream contaminated surface freshwater, benthic and hyporheic zone samples (water phase) | Invertebrate | *Daphnia magna* | Monospecific bioassay | 24 h | Mortality | Becouze-Lareure, 2012 |
| | Downstream contaminated surface freshwater, benthic and hyporheic zone samples (particle and water phase separately) | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 20 min (particles)/ 30 min (water) | Luminescence inhibition | |
| | | Invertebrate | *Brachionus calyciflorus* | Monospecific bioassay | 48 h | Reproduction | |
| | | Invertebrate | *Heterocypris incongruens* | Monospecific bioassay | 6 days | Growth | |
| | | | | Monospecific bioassay | 6 days | Mortality | |

**Table 3** (*continued*)

| Toxic matrix | Samples used for assays | Organism type | Studied organism | Bioassay type | Exposure time | Endpoint | Author |
|---|---|---|---|---|---|---|---|
| Urban wet weather discharges (Urban road stormwater runoff and municipal wastewater) | Downstream contaminated freshwaters | Invertebrate | *Lasmigona costata* | Biomarker/*In situ* assay (sampling) | x | Immune function | Gillis, 2012 |
| | | | | *In situ* assay (sampling) | x | Age | |
| | | | | *In situ* assay (sampling) | x | Condition (physical) | |
| | | | | *In situ* assay (sampling) | x | Reproductive status | |
| Urban highway and residential stormwater runoff | Stormwater pond sediments | Invertebrate | *Hyalella azteca* | Monospecific bioassay | 28 days | Mortality | Tixier et al., 2012 |
| | | | | Monospecific bioassay | 28 days | Growth | |
| | | Invertebrate | *Hexagenia spp.* | Monospecific bioassay | 21 days | Mortality | |
| | | | | Monospecific bioassay | 21 days | Growth | |
| Urban stormwater runoff | Downstream contaminated freshwaters | Invertebrate | *Hyalella azteca* | Monospecific bioassay | 96 h | Mortality | Weston and Lydy, 2012 |
| Urban stormwater (runoff) | Discharges | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 30 min | Luminescence inhibition | Chong et al., 2013 |
| Urban wet weather discharges (combined sewer overflows and urban stormwater runoff) | Discharges | Algae | *Pseudokirchneriella subcapitata*; *Chlorella vulgaris* | Monospecific bioassay | 72 h | Growth | Ferro, 2013 |
| | | Algae | *Chlorella vulgaris*; *Chlamydomonas reinhardtii* | Biomarker | 2 h; 24 h; 48 h | Photosynthesis | |
| | | | | Biomarker | 2 h; 24 h; 48 h | Enzymatic activities (esterase, alkaline phosphatase, catalase) | |
| Urban wet weather discharges (combined sewer overflows and urban stormwater runoff) | Discharges (SPE extracts) | Bacteria | *Vibrio fischeri* | Monospecific bioassay | 30 min | Luminescence inhibition | Tang et al., 2013 |
| | | Algae | *Pseudokirchneriella subcapitata* | Monospecific bioassay | 24 h | Growth | |
| | | | | Biomarker | 2 h | Photosynthesis | |
| | | x | x | Biomarker | x | Endocrine perturbation (E-SCREEN assay) | |
| | | x | x | Biomarker | x | dioxin-like activity (AhR-CAFLUX assay) | |
| | | Bacteria | *Salmonella typhimurium* | Biomarker | x | Génotoxicity (umuC assay) | |
| | | x | x | Biomarker | x | Oxidative stress biomarker (AREc32 assay) | |
| Urban parking lot runoff | Stormwater ponds discharges | Vertebrate | *Oryzias latipes* | Monospecific bioassay | 14 days | Embryotoxicity (teratogenesis, hatching and mortality of eggs) | Colton et al., 2014 |
| | Stormwater ponds discharges and sediments | | | Biomarker | 20 h | Genotoxicity (LA-QPCR assay on embryos) | |
| Urban highway stormwater runoff | Treated and untreated discharges by stormwater pond | Vertebrate | *Danio rerio* | Monospecific bioassay | 48 h; 96 h | Embryotoxicity | McIntyre et al., 2014 |

(*continued on next page*)

**Table 3** (*continued*)

| Toxic matrix | Samples used for assays | Organism type | Studied organism | Bioassay type | Exposure time | Endpoint | Author |
|---|---|---|---|---|---|---|---|
| | | | | Biomarker | 3 h | Neurotoxicity | |
| | | | | Monospecific bioassay | 96 h (daily assessment) | Mortality | |
| Urban highway stormwater runoff | Discharges | Bacteria | *Vibrio qinghaiensis.Q67* | Monospecific bioassay | 15 min | Luminescence inhibition | Wu et al., 2014 |
| | | Vertebrate | *Danio rerio* | Monospecific bioassay | 96 h (daily assessment) | Embryotoxicity (FET-test) (teratogenesis, hatching and mortality of eggs) | |

"Urban stormwater runoff" refers to discharges which may originate from several sources as residential, industrial, road or commercial area runoff.

enzymatic activity, though on recombinant DNA and reporter genes to detect specific classes of pollutants (notably PAHs) (E-screen test, AhR CAFLUX, AREc32; see Table 3).

Lastly, oxidative stress biomarkers were observed in *Carassius auratus* by Chen et al. (2012) following exposure to reconstituted urban runoffs (samples consisting of distilled water and minerals regularly found in urban runoffs, added with the targeted pollutants).

*2.2.2. Single species bioassays*

These bioassays have undergone the most development and are those most often used in the laboratory. Some of them are now the subject of standards that allow comparing results between tests performed in different countries. The main organisms and single species bioassays associated with the study of urban and sub-urban runoff discharges are presented below.

*2.2.2.1. Bacteria bioassays.* It is important to study the decomposer compartment as the organisms of this group permit the transformation and degradation of many types of organic matter from animals and dead plants that decompose, for example, on the beds of watercourses. Generally, two main types of marine bacteria are used for bioassays to assess the toxicity of urban runoff discharges: *Vibrio fischeri* (Marsalek et al., 1999a, 1999b; Scholes et al., 2007; Waara and Färm, 2008; Wium-Andersen et al., 2009; Angerville, 2009; Casadio et al., 2010; Karlsson et al., 2010; Becouze-Lareure, 2012; Chong et al., 2013; Tang et al., 2013) and *Photobacterium phosphoreum* (Boxall and Maltby, 1995; Kayhanian et al., 2008). The assays identified for this purpose (Microtox® bioassays) are exclusively acute toxicity tests based on the inhibition of bioluminescence (caused by the oxidation of luciferin for *V. fischeri* and *P. phosphoreum*), expressing a dysfunction of the energetic pathway. The effects are generally visible after 15–30 min exposure. Microtox® bioassays are known to be relatively sensitive (Parvez et al., 2006) for samples of both the aqueous and sediment phases. In addition, they are fast, easy to perform and inexpensive (Parvez et al., 2006; Karlsson et al., 2010). This was also observed by Scholes et al. (2007), when setting up a battery of bioassays applied to urban and road runoff. However, some studies have shown the contrary, such as that by Wium-Andersen et al. (2009) for whom the Microtox® test presented the least sensitive response to road and industrial runoff in comparison to the other bioassays performed. These discrepancies show that the sensitivity of Microtox® for urban stormwaters toxicity assessment can vary widely depending on their origins, and thus their composition. Indeed, Boxall and Maltby (1995) reported that the Microtox® have a limited sensitivity for substances with a log Kow (coefficient of octanol/water partition) upper to 3 (for example, PAHs with a number of ring between 2 and 5). Moreover, Marsalek et al. (1999a) call into question the use of these marine bacteria to assess the toxicity of chlorine-laden discharges.

*2.2.2.2. Bioassays on primary producers: algae and plants.* Bioassays on single cell algae are highly pertinent for studying urban wet weather discharges, as they represent the primary producer compartment, the first link in the food chain. Disturbances of the functioning of primary producers can precede damage to higher links in the trophic pyramid. Thus, bioassays on primary producers can lead to the early detection of pollution. Single cell planktonic algae (1–20 μm) have been the subject of many studies to assess both the impact of urban runoff from separate and combined networks. Two species have been studied for this purpose: *Pseudokirchneriella subcapitata* (Christensen et al., 2006; Scholes et al., 2007; Kayhanian et al., 2008; Angerville, 2009; Ferro, 2013; Tang et al., 2013) and *Scenedesmus subspicatus* (=*Chlamydomonas reinhardtii*) (Wium-Andersen et al., 2009; Kaczala et al., 2011). They are known to be relatively sensitive to pesticides (Rojíčková and Maršálek, 1999) which are often found in urban wet weather discharges. The main bioassays on algae applied to urban wet weather discharges are based on the exposure of a population for a given time (generally 72 h, though exposures can vary from 48 to 96 h according to the study). Counting the number of cells before and after exposure shows the impact of the discharges studied on algal growth. The rapid growth of algae also facilitates performing chronic toxicity tests. Chronic toxicity is generally expressed by the concentration for which 10, 20 or 50% effect (growth inhibition) is observed (EC10, EC20, EC50), or more simply by the percentage of growth inhibition. The advantage of algae tests is that they require few technical and financial resources and the algae are easy to cultivate in the laboratory (Angerville, 2009). However, they suffer from a lack of reproducibility owing to considerable intra and intertest variations.

Bioassays using macrophytes are also important though less used. Bioassays on aquatic plants such as *Lemna minor* are used to measure the impact of highway runoff (matrix approach) (Waara and Färm, 2008) and micropollutants such as pesticides often present in stormwater (substance approach) (Frankart et al., 2003). The tests applied on the plant are based on an assessment of frond growth inhibition after 7 days' exposure to discharges (generally expressed by percentage inhibition or by EC50). On the contrary, other plants such as *Lepidium sativum* are used to assess the toxicity of sediments (notably river sediments) contaminated by runoff from industrial and residential areas (Karlavičienė et al., 2009). In this case the test is based on the assessment of germination or root development after 48 h exposure to sediments.

As specified before, algal and macrophyte assays with a "matrix approach" are more realistic than "substance approach" assays, because they take into account all the pollutants and molecules in these complex discharges. But, one of the criticisms levelled at these tests is that they are quite sensitive to the presence of nutrients (such as phosphorus, nitrates, etc.) and some trace elements (as metals) in the water (Kaczala et al., 2011), as these can mask the toxicity induced by the pollution of urban wet weather discharges and stimulate their growth in comparison to controls. This problematic phenomenon has already been observed by Kayhanian et al. (2008), Kaczala et al. (2011) or Ferro (2013). But as Kaczala et al. (2011) noted, if these nutrients lead to a stimulation of growth, another important problem can appear and need to be taken into account: a potential eutrophication created by the rejection of urban wet weather discharges in aquatic environments. So, primary producer's growth assays remain important to perform.

#### 2.2.2.3. *Bioassays on invertebrates*

- Bioassays on daphnia and ceriodaphnia:

Tests on daphnia (*Daphnia magna*), organisms belonging to the family *Daphniideae*, small freshwater zooplanktonic crustaceans (<6 mm), are those most often used in ecotoxicology since they are easy to breed in the laboratory, they are ubiquitous, and because of their trophic level (Adema, 1978). The main tests performed to assess the impact of urban wet weather discharges on these organisms concern acute toxicity, for which the mortality of a population is observed for 24–48 h exposure to urban wet weather discharges or to water (river, sea, oceanic, etc.) receiving stormwater (Fisher et al., 1995; Hatch and Burton, 1999; Marsalek et al., 1999a, 1999b; Christensen et al., 2006; Waara and Färm, 2008; Wium-Andersen et al., 2009; Angerville, 2009; Becouze-Lareure, 2012). However, Schiff et al. (2002) extended the exposure time to 96 h. These tests are those used most often as they are very easy to implement and require few material or financial resources for tests using either a substance or matrix approach to assess the impact of urban wet weather discharges. For the most part they involve the calculation of LC50 values expressing the toxicity of the discharges. Chronic toxicity tests have also been used to study the reproduction inhibition of daphnia after 21 days exposure (Angerville, 2009).

Other organisms of the same family, though smaller, namely ceriodaphnia (*Ceriodaphnia dubia*), are also often used for the substance approach (Bailey et al., 1997; Kszos et al., 2004; Banks et al., 2005) and the matrix approach (Katznelson et al., 1995; Wenholz and Crunkilton, 1995; Brent and Herricks, 1999; Gersberg et al., 2004; Kayhanian et al., 2008; Angerville, 2009; McQueen et al., 2010). The principle of using ceriodaphnia for tests to study urban wet weather discharges is similar to that of tests using daphnia except that the exposure times are different. Regarding mortality tests, exposure times vary from 48 h to 7 days according to study, while 7 days is the duration usually used for reproduction inhibition tests.

- Bioassays on rotifers:

Rotifers (*Brachionus calyciflorus*) are freshwater pseudocoelomate metazoans. They play an important role in planktonic communities as they consume algae and are hunted by other invertebrates and fish. Rotifer tests applied to urban wet weather discharges consist in assessing the reproduction inhibition of organisms by counting them after 48 h exposure (Scholes et al., 2007; Angerville, 2009; Becouze-Lareure, 2012). These tests are of great interest for urban wet weather discharges as the effects of the toxicity of PAHs and PCBs on these organisms present sensitive and reproducible results (Dahms et al., 2011).

- Bioassays on ostracods:

Like many environmental matrixes, urban wet weather discharges contain a substantial particulate phase. Zgheib et al. (2011) showed that a large share of the pollution of urban wet weather discharges was found in the particulate phase of these discharges (pollutants such as heavy metals, PCBs and PAHs). Ostracods are bivalve arthropods and consume particles in suspension, on the surface and in sediments. Consequently, bioassays using ostracods are of interest in the presence of a matrix containing a sufficiently large proportion of particles. In addition, these organisms are quite sensitive to pollutants such as hydrocarbons, herbicides, pesticides and heavy metals (Ruiz et al., 2013) which deposit on the bottom of rainwater basins and on the beds of contaminated watercourses. Thus they are useful for assessing the impact of urban wet weather discharges. Tests using these organisms are based on 6 days' exposure to discharges, contaminated water and sediments, after which the survival of the organisms is observed and their size is measured to compare them with the measures taken at the beginning of the assay. The inhibition of their growth is determined and compared to that of controls (Angerville, 2009; Becouze-Lareure, 2012).

- Bioassays on sea urchins:

Urban wet weather discharges from urban areas located on the coast affect marine environments which has led to the use of single species bioassays on marine organisms of which one is used in particular, the sea urchin *Strongylocentrotus purpuratus* (Jirik et al., 1998; Schiff et al., 2002; Bay et al., 2003; Greenstein et al., 2004; Kayhanian et al., 2008; Reifel et al., 2013). The bioassays performed on this organism are based on assessing the inhibition of their reproduction. Thus, they are carried out on eggs and sperm. The sperm is exposed for 20 min to urban wet weather discharges or contaminated media, then brought into contact with the eggs for 20 more minutes to determine the inhibition of egg fertilisation in comparison to controls. Several studies performed to assess the toxicity of urban and road runoff in California liable to affect the coast showed that these organisms are highly sensitive to discharges, and represent the most sensitive of the batteries of bioassays performed (Schiff et al., 2002; Kayhanian et al., 2008). Greenstein et al. (2004) suggested that the toxicity observed on sea urchins caused by road runoff in particular (obtained from a university carpark) was due to the presence of metals (especially zinc) whose presence in these discharges is considerable. Kayhanian et al. (2008), proposed the same hypothesis.

- Bioassays on amphipods and crustaceans:

Crustaceans are organisms found in abundance in both marine environments and in streams and rivers. Therefore ecotoxicological bioassays have been carried out on freshwater organisms such as amphipods: *Gammarus pulex* (Boxall and Maltby, 1995; Maltby et al., 1995b) and *Hyalella azteca* (Karouna-Renier and Sparling, 1997; Hatch and Burton, 1999; Mayer et al., 2008; Bartlett et al., 2012a, 2012b; Tixier et al., 2012; Weston and Lydy, 2012) as well as on marine crustaceans: *Rhepoxynius abronius* (Hall et al., 1998; Bay et al., 2003). These organisms are often used to both assess the toxicity of contaminated discharges, water and sediments as they are detritivores. They are used to represent benthic organisms (notably in stormwater basins and in lentic systems) affected by urban wet weather discharges (Mayer et al., 2008; Bartlett et al., 2012a, 2012b). Generally, the tests performed on these organisms and applied to urban wet weather discharges are lethality tests. The main factor differentiating studies is exposure time, which can range from 48 h to 28 days. Certain authors have studied other sublethal parameters: growth and feeding behaviour (by studying the consumption of leaf discs) (see Tables 2 and 3). Certain studies, such as those by Mayer et al. (2008), Bartlett et al. (2012a, 2012b), appear to show that amphipods are sensitive to salts and metals found in road discharges and stormwater basins and the sources of toxicity found in these species.

- Other organisms:

Many other freshwater and marine invertebrate species have been the subject of laboratory studies of urban wet weather discharges (all the organisms are listed in Tables 2 and 3). Mention can be made of the chironomid (*Chironomus riparius*) and worms (such as *Tubifex tubifex* and *Panagrellus redivivus*) which have been used to assess the toxicity of stormwater basins and that of the sediments of contaminated rivers. Also, other marine crustaceans have been used like *Mysidopsis bahia* to assess the toxicity of urban wet weather discharges on aquatic environments.

*2.2.2.4. Bioassays on vertebrates.* Regarding vertebrates, fish are frequently used for ecotoxicological tests as they are common in aquatic environments and also because they are of great economic value (Milukaite et al., 2010). Many species have been used in studies and the species represented in particular are: *Pimephales promelas* (Hatch and Burton, 1999; Kayhanian et al., 2008; McQueen et al., 2010) and *Oncorhynchus mykiss* (Bailey et al., 1999; Milukaite et al., 2010). The tests performed on fish are generally lethality tests (acute toxicity) with exposure times ranging from 48 h to 12 days according to study. The results of these tests are often expressed in survival percentage or LC50. Other tests have also been developed to assess the impact of urban wet weather discharges: embryotoxicity tests (Skinner et al., 1999) assess the lowest concentrations for which undesirable effects are observed (dysfunctions of eye size, pericardial effusion, etc.) are observed (LOAECs) and growth inhibition after 21 days exposure (Milukaite et al., 2010). Several studies have shown that tests on fish were more sensitive to urban wet weather discharges than classical tests on micro-crustaceans (especially *C. dubia*) (Kayhanian et al., 2008; Milukaite et al., 2010), although they are more cumbersome to set up. This sensitivity is due to the organic contamination of the discharges.

Other vertebrate organisms less known in ecotoxicology have been the subject of single species bioassays. Mention can be made of frogs (like *Bufo americanus*, *Rana sylvatica*, *Hyla versicolor* and *Bufo viridis*), for which the survival of embryos/larvae and delayed metamorphosis have been studied in particular (Snodgrass et al., 2008; Brand et al., 2010; Dorchin and Shanas, 2010) after exposure to discharges (especially from roads), and the sediments of contaminated stormwater basins, since frogs are often found in these basins where they lay their eggs and reproduce (Brand et al., 2010) due to the colonisation of the basins by plants.

*2.2.3. Large scale multi-species bioassays: micro- and mesocosms*

It is possible to change scales to obtain a more representative vision of the impact of urban wet weather discharges on the environment, by studying several trophic levels simultaneously. It is well-known that if a trophic link is affected, the links above it will also be affected. Consequently, multi-species bioassays and micro and mesocosm tests have been developed over the past few decades. However, these tests are more difficult to set up and are more expensive in terms of time, space and equipment. Also, the interpretation of the results is more complex. Few studies of this type have been performed for urban wet weather discharges. We only found one, performed by Johnson et al. (2011). They studied the impact of urban road runoff by using a 1300 L mesocosm composed biologically of an algal community (primary producer), snails of the genus *Physa* (*Physa* spp.) (primary consumers) and the fish *Lepomis gibbosus* (secondary consumers) exposed for 28 days. Parameters such as biomass, metabolic activities, mortality and growth were measured. Only effects on algal populations were observed. They concluded that the quality of the water itself could not explain the impact on algae but supposed that the flow of runoff during renewal of events would be responsible. But, it is only assumptions without evidences. However, other authors such as Villeneuve et al. (2011) already showed the effects of diet flow (exposing periphyton to laminar or turbulent flow) of two pesticides (diuron and azoxystrobin) mixed on the composition of bacterial and algal communities exposed in mesocosms. They concluded that the flow regime had a real impact on the effects of the pesticides on the periphyton, with a higher sensitivity of communities to pesticides.

*2.2.4. In situ bioassays*

Contrary to laboratory bioassays, few studies have focused on the impact of urban wet weather discharges on the environment *in situ*. Indeed, we only identified 8 studies. Some authors nonetheless assert that the quality of receiving environments should be performed by *in situ* tests with an evaluation of communities (Marsalek et al., 1999b). These studies permit obtaining much better representativeness of the exposure of organisms to the matrices tested, as they take into account the temporal variations of discharge toxicity. Many of these studies (Mulliss et al., 1997; Hatch and Burton, 1999; Lieb and Carline, 2000; Grapentine et al., 2004; Custer et al., 2006) are based on the “caging” principle, which consists in caging the organisms studied and placing them in the contaminated matrix or in the environment receiving the discharges; the duration of exposure is fixed beforehand. Organisms such as gammarids, *H. azteca* and fish are often used for caging experiments. When setting up *in situ* tests, the exposure time for organisms is often longer than in laboratory tests. Mention can be made of Grapentine et al. (2004), who studied the toxicity of the sediments of basins and streams contaminated by urban wet weather discharges on Hyalella in the laboratory and *in situ* (caging) with exposure times of 28 days versus 7 weeks. This can be explained by the fact that organisms were exposed periodically to urban discharges during rainfall events, in renewed environments, and that the impacts were visible at the end of a longer period.

There are other types of *in situ* tests for evaluating urban wet weather discharges. Some consist in sampling organisms taken directly from contaminated environments and monitoring their physical condition through a reduction of immune system efficiency or monitoring the mean age of the organisms observed (Gillis, 2012). Others consist in monitoring, for example, the mortality over several years of fish species that swim upstream in water contaminated by discharges (McCarthy et al., 2008; Feist et al., 2011).

Several authors (Marsalek et al., 1999b; Bay et al., 2003; Tang et al., 2013) insist that in every case it is vital to multiply the number of bioassays (notion of battery) based on various organisms and toxicity parameters, as the discharges contain many micropollutants with very different modes of action, in view to refining the characterisation of urban wet weather discharges samples. As described above, the different organisms used for bioassays do not all have the same sensitivity to the different families of pollutants present (e.g., sea urchins are sensitive to metals whereas gammarids are more sensitive to PAHs; crustaceans (like ceriodaphnia) are more sensitive to pesticides than are sea urchins).

In addition, although batteries of bioassays permit observing the toxicity of urban wet weather discharges on different families of organisms, it remains vital to undertake chemical and ecotoxicological analyses in parallel (Karlsson et al., 2010).

### *2.3. An a priori method: the assessment of risks linked to urban wet weather discharges*

Several risk assessment methods for urban wet weather discharges have already been used including for the assessment of ecological risks through the calculation of risk factors and the study of discharge pollution by methods like GIS (geographical information system).

The assessment of ecotoxicological risks by calculating risk factors is based on four major standard steps described by Perrodin et al. (2011). These are: 1 – The definition/formulation of the problem in which the study scenario is determined and the different parameters (such as discharge flows, flow rates, etc.) are presented and fixed. 2 – The characterisation of the exposure which permits calculating the Predicted Environmental Concentration (PEC) resulting from the concentrations of substances selected in the substance approach, and the percentage of the pollutant matrix in the environment in the matrix approach. 3 – The characterisation of the effects, which assesses the sensitivity of organisms to pollutants, especially through the utilisation of ecotoxicological databases (substance approach) and the organisation of ecotoxicological bioassays (matrix approach) which permits calculating the Predicted No Effect Concentration (PNEC), by assigning an extrapolation factor to this value. 4 – The characterisation of the risk, involving the calculation of a risk factor (RF = PEC/PNEC). This method has already been used by several authors such as Angerville (2009), Angerville et al. (2013) and Gooré Bi et al. (2015) for urban wet weather discharges. For example, the latter applied this method to combined sewer overflows in a river in a sub-urban district of Lyon, in France. A disadvantage of these methods is the number of organisms used to characterise the effects. Indeed, the smaller the number of organisms, the higher the extrapolation factor and thus also the risk (Angerville et al., 2013). It is therefore necessary to perform a large number of tests (in the case of a matrix approach) to obtain a risk as close as possible to that of the real risk. Eriksson et al. (2007) used a procedure (known as CHIAT: Chemical Hazard Identification and Assessment Tool) derived from the previous one, to calculate the risk due to xenobiotic organic compounds (XOCs) present in urban wet weather discharges into an urban stream in Denmark. Other authors like Hall et al. (1998) and Brix et al. (2010) used the same approach described by Perrodin et al. (2011), by following the principle of the four steps but by using a probabilistic approach considering, according to the authors, the variability of exposures between individuals of a population to determine the risk. Weinstein et al. (2010), used two risk assessment approaches, including one similar to that of Perrodin et al. (2011), but based on calculating two hazard coefficients representing the ecological risk linked to PAHs present in the retention basins studied and based on concentrations below which it is unlikely to observe harmful effects on (TEC: Threshold Effect Concentration), concentrations above which such harmful effects are probable (PEC: Probable Effect Concentration), and on PAHs concentrations observed in sediments.

One of the first objectives of risk assessment is to identify hazard. Thus Baun et al. (2006) created a method (RICH: Ranking and Identification of Chemical Hazards), which, on the basis of identifying different pollutants in urban stormwater, aims at establishing a hazard priority ranking, by taking into account physico-chemical, persistence, bioaccumulation and ecotoxicity data. This method permits, among other things, choosing the molecules to be studied more precisely in procedures like risk assessments based on calculating PEC/PNEC, the CHIAT method, etc.

In the study of ecological risks, the probability of occurrence of ecological impacts is synonymous with risk (Ellis, 2000). Therefore Novotny and Witte (1997) formulated a risk assessment method related to the discharge of urban wet weather discharges in watercourses. It was based only on probabilistic statistical models that took into account parameters such as the probability of a rain event permits obtaining a certain concentration of pollutants in rainwater, and the probability that this contamination will affect species present in the environment (based on substance type bioassays for 96 h on priority pollutants).

Ellis (2000) described the advantages and disadvantages of these two types of risk assessment methods (approach by direct toxicity assessment (DTA) and the probabilistic approach). The latter only concerns acute risks due to urban wet weather discharges, and does not permit taking account of pollutant bioaccumulation phenomena or chronic effects due, for example, to contaminated sediments. Furthermore, this type of method is based only on bioassays most often performed for 48 or 96 h in the laboratory, and not *in situ*. Also it uses inert substrates (sediments) and does not take into account suspended particles. It is possible for risk to be underestimated as the models built fail to take into account the "cocktail" effect of urban wet weather discharges, thus the synergetic effect of pollutants, and the multiple exposures of aquatic organisms to pollutants. However, this was done in the methods employed in particular by Perrodin et al. (2011) and Angerville et al. (2013) and which are widespread. Nonetheless, another limitation of these methods is that they do not take into account the duration of exposure to discharges, since toxicity tests (often performed in the laboratory) do not take into account the transient effects of discharge pollution or seasonal or even diurnal variations (Ellis, 2000). What is more, the bioaccumulation phenomena occurring in trophic networks, and those of eutrophication in aquatic ecosystems, are rarely considered in this type of method (Perrodin et al., 2011). The same problem of ignoring synergy/antagonism also appears in substance type assessments.

Lastly, other authors use a GIS approach to predict the risk of urban wet weather discharges spreading contamination to water bodies. This was, for example, the case of Zandbergen (1998) who used a set of indicators (impervious areas, river habitat, pollutant loads, water quality, sediment quality, fish and human health) to which he assigned a score expressing good or bad status in order to draw a map showing the evolution of these indicators for the entire catchment of the River Brunette in the area of Vancouver. Ellis et al. (2012) also implemented a semi-quantitative method to assess the risk of discharges passing through a sustainable urban drainage system (SUDS) (such as retention basins, porous roads, etc.) and their capacity to reduce the risk of discharges. To do this they used a pollution index and a pollution reduction index based on a GIS that took into account different indicators like sealed surface, runoff concentration/load and the potential treatment performance of individual SUDS. However, these tools still need developing as they consider only the worst cases of pollution by runoff. Nonetheless, they encourage the creation of more refined methods for assessing the risks linked to them (Ellis et al., 2012).

More qualitative methods also exist for characterising the risk linked to pollutants in urban wet weather discharges, like that of Lundy et al. (2012), based on the formulation of numerical values (1–5) for both the probability of occurrence of these pollutants in discharges, and of their level of impact on receiving aquatic environments.

### *2.4. Methods for modelling the pollution impact of urban wet weather discharges*

Other less frequently used methods of studying the impact of urban wet weather discharges have emerged in recent years. Among these, methods of modelling the toxicity of urban wet weather discharges have been developed. The main causes of mortality observed in fish during rainfall events are hypoxia and the presence of ammonia in large quantities. Thus Magaud et al. (1997) developed a model based on laboratory tests performed on *O. mykiss*. They built a model expressing the probability that these organisms would die as a function of two key parameters for their survival: concentrations of ammonia and dissolved oxygen in water. The limitation of their model was that it failed to take into account other parameters crucial for the survival of the fish studied: exposure time and the concentration of the toxic pollutants in the discharges. Indeed, Zhang et al. (2011) developed a time-dependent model of the toxicity of PAHs present in urban road discharges based on a model built by Lee et al. (2002a, 2002b). To do this, they took into account spatial–temporal variations in the composition of discharges, with a pulse-exposure of organisms, to make it more realistic. They coupled the known ecotoxicological data of these compounds with field data (concentrations of PAHs in the discharges and the duration of rainfall events) to calculate the toxicity that these events would cause for different aquatic species. The aim of the model was to take into account parameters that classical laboratory tests cannot, such as the considerable variability of rainfall events. Indeed, it is acknowledged that it is difficult to characterise the impact of these events using aquatic organisms after short and intermittent exposures. However, if some authors tend to say that the flow regime (laminar or turbulent) during rainfall events could also have an effect on the impact of urban discharges during rainy weather on the ecosystem, no study considers the parameter in their models.

In addition, as shown by Magaud et al. (1997), it is important to couple the different parameters studied in models (in this case oxygen and ammonia) rather than simply add the effects of each parameter separately. Indeed, the authors obtained lower fish survival values when the fish were exposed to two combined stresses, rather when the effects obtained for each stress were added. These observations reflect the problem of the synergies and antagonisms between different pollutants and parameters.

## 3. The limits of current methods and the alternative techniques implemented

We saw previously that each of the techniques and organisms used has advantages and disadvantages for studying the impact of urban wet weather discharges. The aim here is to identify the different limits of the methods used classically, and the different solutions that may be proposed.

As explained above, physico-chemical analyses do not allow explaining the impact of discharges on organisms (Ellis, 2000). Furthermore, they often underestimate the severity of contamination in the water column, as this can vary very rapidly (Burton et al., 2000). To achieve this, it is therefore necessary to perform studies on living organisms. Although acute and chronic bioassays in the laboratory have allowed the observation of toxicity on different species, their use for assessing the impact of urban wet weather discharges is limited by several weaknesses:

- There is a lack of representativeness of the exposure of organisms to the discharges studied. Indeed, these studies often consider that organisms are exposed to pollutants continuously. However, it is acknowledged that in reality, due to short though intense rain events, these organisms are exposed episodically to discharges for several hours. This is why certain researchers have sought to develop alternative methods in which organisms are exposed to shorter periods (several hours) before observing the effects of this exposure over several days in a test medium for control. This is the pulse-exposure method implemented in particular by Brent and Herricks (1999) and Rosenkrantz et al. (2008). These exposures often produce higher toxicity than continuous exposures to discharges (Burton et al., 2000). Laboratory tests therefore lead to underestimation of the toxicity of urban wet weather discharges.
- In addition, the samples (whether contaminated water or sediments) used in laboratory bioassays are often conserved by freezing. Thus the organic micropollutants (such as pesticides and PAHs) are liable to be degraded. Although this has been demonstrated with pharmaceutical compounds in wastewater (Fedorova et al., 2014; Negreira et al., 2014), no study has yet focused on this phenomenon in other families of toxic micropollutants. However, it is highly likely that this is the case with the different families of organic micropollutants found in urban wet weather discharges.
- Due to reasons that are often of a technical, human or material nature, the ecotoxicological studies performed on the urban wet weather discharges frequently use punctual samples taken from the sewer systems draining these discharges; temporal variability is not represented in this case regarding drainage installations.
- Lastly, often a single (or a relatively limited number) species is studied, whereas it is necessary to widen the scope (only one mesocosm study has been performed) of studies on the impact of urban wet weather discharges to obtain more results more representative of ecosystem impacts.

Although biological indices can be used to study modifications in the composition of communities exposed *in situ* to rain events, by conforming to the dilution of discharges in the environment and to exposure times, they nonetheless have major disadvantages:

- Species can also react to a stress other than that sought (contamination by pollutants) (Parent-Raoult and Boisson, 2007; Damasio et al., 2008) which makes the interpretation of the results much more difficult than with ecotoxicological tests.
- There is also a problem of natural variability (Roper et al., 1988) in space (notably in estuaries) and time (reproduction cycles during the year, etc.), which makes it difficult to assess the impacts that can be very different over very short distances and the times of the year when communities are analysed.

Risk assessments and impact modelling methods do not allow asserting that an impact will actually occur in an ecosystem, although they remain a good preventive tool for managers of urban wastewater treatment and drainage networks.

Consequently to offset the limits of different methods, most of them are in combination in order to complete and compensate the failings of each one, that is to say that ecotoxicological studies and biological indices are frequently coupled with chemical analyses to better understand the results obtained. The only disadvantage is that physico-chemical analyses do not allow to assess which group of pollutants is responsible in particular for the effects observed (except when only one group of pollutants is present). This is why studies are generalised in the assessment of urban wet weather discharges: the methods used in this case are known as Toxicity Identification Evaluations (TIE). These are initially based on establishing a general profile of the compounds likely to be the source of toxicity, after which the matrixes are studied. For example, Gersberg et al. (2004) used EDTA, a chelating agent, to remove all the divalent metals present in the samples in order to test toxicity once the target group of micropollutants had been eliminated.

Furthermore, other approaches have been developed, such as the triad approach for benthic organisms (in rivers, seas and basins), which consists in performing a physico-chemical analysis,

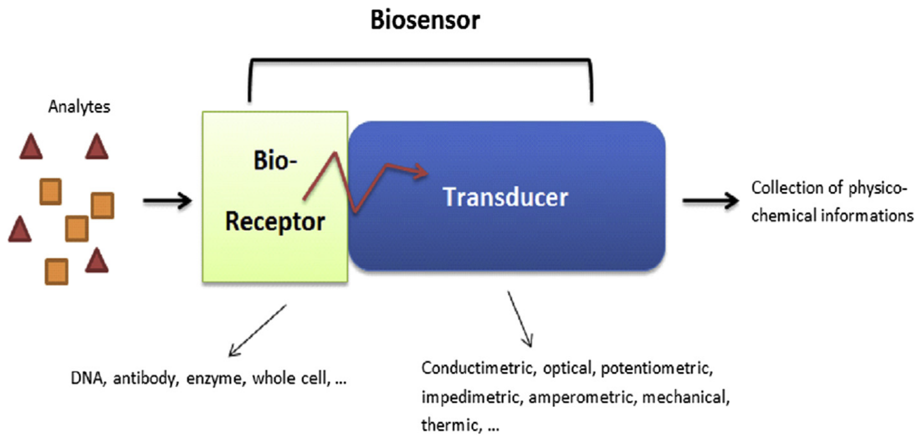


**Fig. 2.** Schematic diagram of how a biosensor functions. The red arrow shows the transmission of the biochemical information received by the bio-receptor to the transducer which changes it into a physico-chemical signal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ecotoxicological bioassays and an *in situ* study at the same time on communities to obtain a global vision of the impact of urban wet weather discharges. This was done, for example, by Tixier et al. (2012). However, these studies are relatively complex to perform. *In situ* bioassays (like caging an organism on site before and after urban wet weather discharges) nonetheless represent a good compromise between laboratory studies and studies of communities.

## 4. Towards new procedures for studying the pollution impact of urban wet weather discharges: biosensors

As seen previously, "classical" impact studies do not appear to fully represent the harm really affecting the environments receiving urban wet weather discharges, and some of them are cumbersome to implement. It is therefore necessary to find new means for monitoring urban wet weather discharges. Certain studies have dealt with the utilisation of sensors capable of continuously monitoring physico-chemical parameters on receiving sites affected by urban wet weather discharges (Ruban, 1995; Zug et al., 2001). However, biological monitoring is required to evaluate the ecological impact of urban wet weather discharges. Biosensors have been developed over the last ten years to solve this shortcoming. They are tools comprising two components: a bio-receptor and a transducer capable of converting biochemical information (like a concentration into analytes) emitted by the receptor into physico-chemical signals (Thévenot et al., 2001) (see Fig. 2). The bio-receptors are generally in contact with a transducer or immobilised on it.

Biosensors have been applied more recently to studying the environment. Their sensitivity has been proven and they allow continuous monitoring of water pollution *in situ* (Rogers, 1995), contrary to laboratory tests. Thus, they are ecologically pertinent tools. They also have the advantage of being far less expensive than chemical analyses and classical ecotoxicological tests, while providing good sensitivity, selectiveness and reproducibility (Dennison and Turner, 1995; Rogers, 2006). Furthermore, certain biosensors are very useful tools as they do not only determine the presence and even the concentrations of chemical pollutants in an aquatic ecosystem, they can also be used to observe a biological response of the organisms or the aquatic environment analysed to the toxicity of a pollutant or discharge (Rodriguez-Mozaz et al., 2005).

The nature of bioreceptors used for monitoring aquatic environments is varied (see Fig. 2). DNA, antibodies or purified enzymes can for example constitute the bioreceptor, with high specificity reaction with the analyte. However, their main drawback is that they are unrepresentative of the real impact of pollutants on the aquatic environment and only to detect the presence of their target compounds. They don't take into account the biodisponibility of pollutant, and the fact that in cells some pollutant's sensitive enzymes can be conserved by native cellular machinery (Pancrazio et al., 1999). This weakness has been improved by development of whole-cell biosensors which often use unicellular microorganisms. They allow to take into account the complex relationships between different cellular compartments and enzymes. They also have the advantage to give informations on both the bioavailability and the presence of pollutants, and give various toxicity informations according to the studied metabolic reactions and transducers used (Hansen and Sorensen, 2001; Lagarde and Jaffrezic-Renault, 2011). Thus, unicellular organisms such as bacteria (Willardson et al., 1998; Kumar et al., 2006; Gavlasova et al., 2008) algae (Chouteau et al., 2005; Tsopela et al., 2014) or yeast (Campanella et al., 1995; Hollis et al., 2000) have been used to measure the toxicity of pollutants. It is therefore possible to monitor the impact of toxic both in prokaryotes and eukaryotes (Lagarde and Jaffrezic-Renault, 2011). Furthermore, these whole-cell biosensors remain, despite the complexity of the organisms, relatively sensitive results in numerous contaminants, as shown Chouteau et al. (2005) who developed conductometric biosensor algal cell, allowing the observation of inhibition of the alkaline phosphatase enzyme activity (APA) to observe effects at concentrations of metals (Zinc, Cadmium) of 10 ppb. Thus, they should be preferred to allow a realistic observation (when they are not genetically modified) of the effects of urban wet weather discharges on the environment. However, whole-cell biosensors also have some drawbacks. For example, the bioreceptor immobilisation step on the transducer can lead to a decrease of the biosensor sensitivity (Eltzov and Marks, 2011). For example, immobilisation using matrix as alginate, albumin or sol–gel which can create a barrier against the diffusion of pollutant to the organisms. It has been shown by Awasthi and Rai (2005), with algae immobilised in alginate and exposed to nickel, zinc, and cadmium. In the case of physical adsorption of cells, as for conductimetric biosensors, a release of cell can appear after few days. It has been demonstrated by Guedri and Durrieu (2008) with algae physically immobilised on self-assembled monolayers (SAMs) of alkanethiolate. Another important point is the conservation of the total integrity and activity of the cells, without any perturbation. Some procedure, as direct sol–gel matrix immobilisation of cells, can impact cell viability (Ferro et al., 2012). Finally, some problem of reproductivity can also appear (Gu et al., 2004). It's why further researches are still necessary to improve these critical points.

A large number of biosensors have been developed according to the families of toxic pollutants to be studied. Thus biosensors have already been used to measure the presence and/or impact of pesticides (Védrine et al., 2003; Chouteau et al., 2005; Touloupakis et al., 2005; Mauriz et al., 2007; Durrieu et al., 2011), heavy metals (Bontidean et al., 2003; Chouteau et al., 2005), PAHs (Alarie et al., 1990; Willardson et al., 1998) and PCBs (Gavlasova et al., 2008), all pollutants found in urban wet weather discharges. However, biosensors are often based on a substance approach, where the pollutant studied is diluted in a solution of pure water. Thus they do not clearly establish the impact of pollutants in whole discharges.

Studies have already been performed to evaluate the hazard of certain urban discharges (*in situ* matrix monitoring). This is the case of wastewater treatment plant effluents, and bacterial biosensors have been developed to study PAHs, phenols, pharmaceutical compounds and pesticides (Farré and Barceló, 2003). However, there is a surprising lack of attention given to the continuous *in situ* monitoring of urban wet weather discharges as a study matrix. Only one study identified, by Ferro (2013), attempted to evaluate the impact of urban wet weather discharges using biosensors based on algal cells. He highlighted the sensitivity for studying discharges of certain enzymatic biomarkers such as Esterase Activity (EA) and Alkaline Phosphatase Activity (APA) in the implementation of conductimetric biosensors, Catalase Activity (CA) coupled with an oximetry transducer, and photosynthetic activity coupled with an optical transducer. Statistical treatments performed in comparison with the compositions of the discharges studied and the disturbances caused by these activities have shown that they are specifically inhibited by certain of the families of pollutants present (e.g., effects of PAHs on EA and effects of metals on APA). But, the specificity of enzymatic or metabolic response to pollutants remains challenging to bring out (Gu et al., 2004).

Although biosensors remain qualitative tools at present, they nonetheless represent an efficient resource for predicting and evaluating the pollution of urban aquatic environments.

## 5. Conclusion and outlook

In this study we attempted to summarise all the different techniques used at present to evaluate the impact of urban wet weather discharges on ecosystems. Despite the growing number of studies on evaluating the impact of urban wet weather discharges, their number still remains relatively low (as shown in Tables 1–3). The important spatial and temporal variation of urban wet weather discharges complicates the assessment of their impact (by substance and matrix approach) on ecosystems (marine and freshwater). It's why a large array of methods/tools has been developed, which generally include chemical and physicochemical analyses, batteries of bioassays and *in situ* tests (whether through the study of biological indices or ecotoxicological studies).

Nonetheless, chemical and physico-chemical analyses alone are not enough to predict the ecological impact of discharges on ecosystems. Ecotoxicological bioassays, most usually carried out in the laboratory (though sometimes *in situ*) only take into account a small number of selected organisms and lack realism (especially for the substance approach). It's why the utilisation of these bioassays is now questioned. They are performed under controlled conditions on samples taken and often stored for periods of varying length. However, in recent years, researchers have tended to show that the conservation of samples, even by freezing, leads to their modification (such a reduction in the concentrations of micropollutants present).

It is now well known that field studies (bioassays and indices) integrate the complexity of phenomena more completely. However, in order to perform in-depth studies of the impact of urban wet weather discharges, many researchers combine ecotoxicological studies, biological indices and chemical analyses to determine which pollutants are responsible for the impact of discharges.

These methods are nonetheless expensive and time-consuming to implement, and they do not permit continuous monitoring of the pollution of urban wet weather discharges. Moreover, the complexity of the results obtained often leads to a limitation of their interpretation. This has led over the past few years to the development of new tools for monitoring them: biosensors. These are designed to evaluate the toxicity of matrixes for organisms exposed *in situ* and continuously. They are good tools that could be useful both for researchers and for managers of urban wet weather discharges. In an embryonic stage at present, they don't allow quantifying pollutants in environments, and chemical analyses remain essential. The aim of these tools is therefore to backup those already available. They are still subject to laboratory studies and only one study (using immobilised algae) has emerged. For all that, the potential of biosensors is strong, which makes it necessary to:

- Continue to develop the utilisation of existing biosensors (with algae) that appear promising for *in situ* and continuous monitoring and develop new biosensors using species from other compartments (primary and secondary consumers, decomposers) and adapted to the field. This would permit monitoring the toxicity of discharges continuously *in situ* at different trophic levels and lead to better understanding of their impact on ecosystems, since the effects observed by monitoring only one species cannot be extrapolated to other organisms.

In addition to developing biosensors:

- Favour more comprehensive *in situ* studies of the variation of pollution in watercourses during rainfall events. These would take into account the whole rainfall event, contrary to studies performed on samples taken punctually.
- Improve and facilitate the development of laboratory techniques to obtain more sensitive results, especially through longer exposures and chronic bioassays that often show toxic impacts not detected by acute toxicity tests, and tests that are more representative of the exposures actually incurred by organisms, something that has already been done, for example, with pulse exposures.

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