Ecotoxicological characterisation of sediments from stormwater retention basins

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ABSTRACT

Retention–detention basins are important structures for managing stormwater. However, their long-term operation raises the problem of managing the sediments they accumulate. Potential uses for such sediments have been envisaged, but each sediment must be characterised beforehand to verify its harmlessness. In this paper we address this issue through the development of a battery of bioassays specifically adapted to such sediments. We tested the method on samples taken from four retention basins in the region of Lyon (France). This battery focuses on the toxic effects linked to both the solid phase (ostracod and Microtox® solid-phase tests) and the liquid-phase (interstitial water) of sediments (rotifer and Microtox® liquid-phase tests). The results obtained permit the sorting of sediments presenting little toxicity, and which could therefore be potentially exploitable, from those from more polluted areas presenting higher toxicity that limits their use.

Key words | ecotoxicity, methodology, retention basin, sediment, stormwater

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INTRODUCTION

In both industrialised and developing countries, urban development leads to larger impervious surface areas and flows of stormwater runoff. Retention–detention basins are essential for managing these effluents. They reduce the risk of flooding and contribute to protecting receiving environments (Marsalek & Chocat 2002).

The works performed on stormwater over the last 20 years have shown that suspended solids often constitute the main vector of pollutants and that settling could be a relatively efficient means of treatment (Marsalek *et al.* 1992; Matthews *et al.* 1997). Thus detention basins are used for the efficient settling of metallic pollution and hydrocarbons mainly bound to suspended materials (Schueler 1987; Persson & Wittgren 2003; Strecker *et al.* 2004).

However, the operation of these basins raises the problem of the fate and sustainable management of the sediments accumulated over time. Different potential uses for these sediments have been envisaged (e.g. filling material in urban environments, use in the formulation of new products), but each one requires prior characterisation of their ecotoxicity to verify that they are not dangerous for human or environmental health.

To achieve this, chemical analysis is an essential but insufficient tool. Indeed, it is now accepted that total chemical content does not systematically permit assessing toxicity to living organisms. It is also acknowledged that combined actions linked to the presence of mixtures of pollutants (e.g. synergetic and/or antagonistic effects) cannot be predicted on the basis of a list of these pollutants (even if detailed). Bioassays (or ecotoxicity tests) can contribute towards solving these problems (Perrodin *et al.* 2010).

In this study, a battery of bioassays specifically adapted to urban sediments has been proposed and implemented. This battery was formulated on the basis of the results of the first works performed on stormwater (Moura *et al.* 2007; Angerville 2010; Becouze-Lareure *et al.* 2012), which showed the need to select organisms highly sensitive to pollution for this type of study, given the lower toxicity of urban sediments in comparison to the matrixes usually studied by ecotoxicologists (wastes, polluted industrial soils, contaminated seaport sediments, etc.). This battery has been tested on four sediments taken from four retention basins in the region of Lyon (France).

The purpose of this paper is therefore twofold: (i) assess the toxicity effects of urban sediments with an adapted

doi: 10.2166/wst.2014.006

battery of bioassays; (ii) improve the knowledge in terms of sediment toxicity from stormwater retention basins.

DESCRIPTION OF SITES

Four retention basins were selected. They are all integrated in structures that comprise downstream stormwater infiltration basins which contribute to groundwater recharge. These structures are located in the eastern part of Lyon (France) in the municipalities of Décines, St Symphorien d'Ozon, Mions and Chassieu. Table 1 shows the main characteristics of these retention basins (ARTELIA 2012).

MATERIALS AND METHODS

Preparation of samples

The sediments of all four basins were sampled, with account being taken of areas with high settling levels (i.e. with thick sediment layers). All samples were collected between April and May 2012 during a wet period for each basin. Regarding physical characteristics, particle density of sediments from stormwater retention basins was reported to be between 1,500 and 2,300 kg/m^3 (Guo 1997; Sébastian *et al.* 2013).

In order to obtain a representative sample, the complete vertical profile of sediment samples was homogenised manually in the field. The samples were subjected to analyses of the solid phase and the aqueous phase:

- the solid phase corresponds to the 'raw' sediment sampled after homogenisation,
- the aqueous phase was extracted from the 'raw' sediment after centrifugation for 40 min at 9,000 rpm.

After the sampling procedure, the solid and aqueous phases were frozen immediately. This protocol was set up in order to highlight (i) the potential ecotoxic effects of the sediments and (ii) the toxic effects of the interstitial water of the sediments containing pollutants that can be easily mobilised on site by the action of stormwater and thus reach different compartments of the natural environment (i.e. soil and groundwater).

Chemical analyses

The chemical characterisation of the sediments accumulated in the four retention basins was performed in the framework of research carried out by Sébastian *et al.* (2013) and the engineering office ARTELIA (2012). The chemical characterisation of the sediments provides information on the potentially toxic concentrations of the pollutants present in the samples.

Chemical characterisation was performed on the pH and the total organic carbon (TOC) content of the soil (ISO 10694 1995). The determination of the hydrocarbon (TCH) index C10-C40 was carried out as per standard ISO 9377-2 (2000). Sixteen polycyclic aromatic hydrocarbons (PAHs) classified by the US Environmental Protection Agency (US EPA) as priority pollutants were determined as per standard ISO 18287 (2006). The 20 volatile halogenated organic compounds (VHOC) (vinyl chloride; 1,1,1-trichloroethane; 1,1,2-trichloroethane; 1,1-dichloroethane; 1,2-dichloroethene; 1,1-dichloroethylene; 1,2-dichloropropane; bromochloromethane; bromoform; chloroform; cis-1,2-dichloroethylene; trans-1,2-dichloroethylene; 1,3-dichloropropylene; dibromochloromethane; dichlorobromomethane; dichloromethane; hexachlorobutadiene; tetrachloroethylene; tetrachloromethane and trichloroethylene) were determined as per standard ISO 22155 (2011). Benzene, toluene, ethylbenzene and xylenes (BTEX) were evaluated using the method based on simplified QuEChERS extraction (Quick, Easy, Cheap, Effective, Rugged and Safe), followed by a large-injection volume–fast gas chromatography and mass spectrometry detection (García Pinto *et al.* 2011).

Table 1 | Retention basin characteristics

Name of basin	Chemin de Feyzin	Grange Blanche	Zac de Pivolles	Django Reinhardt
Municipality	Mions	St Symphorien d'zon	Décines	Chassieu
Date implemented	1988	1997	1992	1975
Catchment type	Residential & agricultural	Residential & agricultural	Commercial	Industrial
Catchment surface (ha)	315	300	40	185
Tank retention surface (m^2)	7,360	6,130	3,112	11,000
Mean sediment thickness (cm)	2	2	2	3.6
Date of last clean	2009 & 2006	Before 2006	Before 2006	2006

The contents of seven polychlorobiphenyl congeners (PCB: 101, 118, 138, 153, 180, 28, 52) were determined as per standard ISO 10382 (2002). The analysis of metals (i.e. Cd, Cu, Pb, Ni, Zn) was performed by inductively coupled plasma – mass emission spectroscopy (ISO 17294-1/2 2004), while chlorine pesticides, phosphorous pesticides and nitrogenous pesticides were assessed following the US EPA method 1699 (US EPA 2007) and the method described in detail by Lazartigues *et al.* (2011).

Ecotoxicity tests

Four complementary tests were performed on the four sediments studied.

Liquid-phase Microtox® test (*Vibrio fischeri*)

This acute toxicity test was performed as per standard ISO 11348 (2009). The protocol of the latter permits evaluation of the inhibition of the luminescence of a suspension of the bacteria *Vibrio fischeri* in comparison to a control sample, following their contact with a range of dilutions of an aqueous sample. The initial luminescence of the bacteria was recorded first before they were brought into contact with the sample, then after incubation periods of 15 and 30 min following contact.

The bioassay with the bacteria *Vibrio fischeri*, marketed at the beginning of the 1980s under the name Microtox® (developed by Azur Environmental), then Lumistox® (developed by Dr Lange), met with rapid success for detecting the toxic effects of effluents of domestic and industrial wastewater treatment plants. The device used in the framework of our study was the Microtox® M500 luminometer.

The standard recommends performing the test on the filtrate with 0.45 μm of the sample to be tested. The dilution medium used was distilled water with salt at a concentration of 20 g/L. The range of dilutions generally comprised eight dilutions of the filtrate to be tested. We used the dilution medium for the control sample (two tubes). This range was employed directly in the test tubes, which were adapted to the luminometer used.

The results are presented in the form of EC50 (effective concentration inhibiting 50% of luminescence of the bacteria suspension following the test period). In this paper the results are presented as percentage of stimulation or inhibition of the luminosity.

Solid-phase Microtox® test

This test permits detection of acute toxicity linked to the particle fraction of the sediment. The material and the biological reagent (strain *Vibrio fischeri*) were the same as those used for the test on the liquid matrix. The protocol applied for the test is that of standard ISO 11348 (2009), adapted by AZUR Environmental for the solid phase (AZUR Environmental 1998).

As with the 'liquid phase' test, the inhibitive effect on the luminescence of the suspension of *Vibrio fischeri* was assessed in comparison to a control, following contact between the bacteria and a range of dilutions of the 'solid phase' kept in suspension in the dilution medium (water with salt at 20 g/L). The luminescence of the bacteria was recorded before they were brought into contact with the sample and then following an incubation period of 30 min.

The results are presented in the form of EC50. In this paper the results are presented as percentage of stimulation or inhibition of the luminosity.

Rotifer test (*Brachionus calyciflorus*)

This test of chronic toxicity was implemented as per the indications of standard ISO 20666 (2007). Its availability in the form of Toxkit, as well as the sensitivity of the organism and its fast reproduction, features among the criteria that led to the choice of this test for studying the toxic effects of our samples. This bioassay is used to determine the inhibition of the growth of a population of the rotifer *Brachionus calyciflorus* in comparison to a control, after the organisms have been brought into contact for 48 h with a range of dilutions of an aqueous sample.

The results are expressed in this paper as percentage of stimulation or inhibition of the growth of a rotifer population with non-diluted effluent (100%), according to the vertical axes of Figure 1(d).

Ostracod test (*Heterocypris incongruens*)

This subchronic toxicity test performed over 6 days was implemented in conformity with the instructions of standard ISO 14371 (2012). The general principle of this bioassay is based on the direct contact of the organism with the sediment or solid matrix to be tested, to which is added fixed volumes of algal suspension and the prepared dilution medium. A control performed with standard sand (i.e. the organisms were exposed to standard sand or reference sediment as a control, according to the standard methods) was used for comparison to assess the

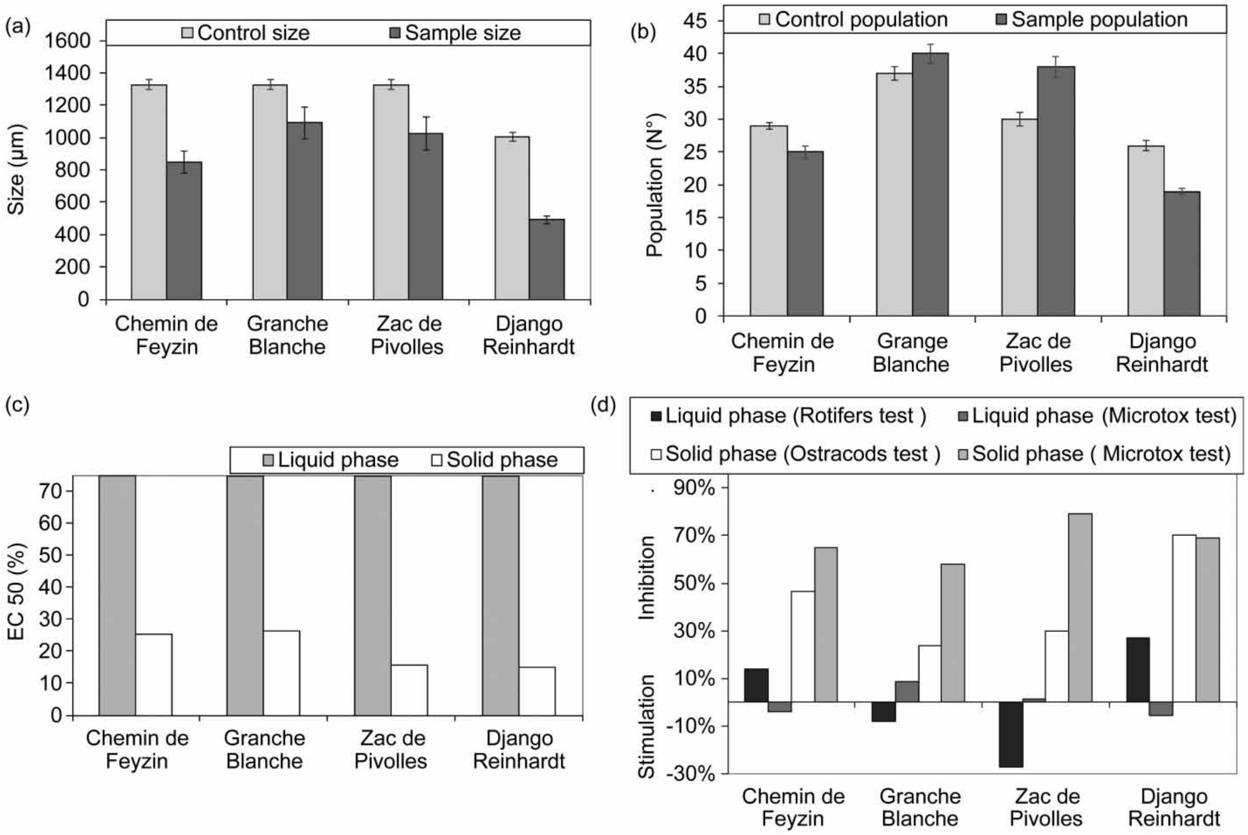

Figure 1 | (a) Size of organisms: Ostracod (solid phase). (b) Organism population: Rotifer (liquid phase). (c) *Vibrio fischeri* EC50 in solid phase (raw sediment diluted at 50%) and in aqueous phase (interstitial water). (d) Effects of inhibition or stimulation on the four sediments studied.

effect of the sample on the organisms. The test was carried out on six well microplates using one microplate per sample and a microplate for the control. The criteria of the effects were the mortality of the organisms and their growth in comparison to their initial size.

At the start of the test, the size of a set of 10 organisms representative of the batch used for the test was measured. Contact was ensured with 10 organisms per well for each microplate. The microplates were then incubated for 6 days according to the instructions of the standard. At this stage, a population of 60 organisms was used for the control and 60 organisms were used for each sample tested. At the end of the test, the number of live organisms per well was counted and their size measured. Lastly, we evaluated the increase in size of the organisms of each well, in comparison to their average size at the beginning of the test

As this test was not performed using a range of dilutions, we did not determine the EC50 value for the samples tested. The results are therefore only expressed as percentage of effects observed for the non-diluted sediment.

Significance of the bioassay results

The significance thresholds of *Vibrio fischeri* and *Brachionus calyciflorus* specified in standard ISO 17616 (2008) were chosen for these two organisms.

Since the significance of the biological effects observed during the ostracod test was not standardised, a statistical analysis of the results was performed with the Wilcoxon test. This permitted comparison of two populations in the light of one criterion (e.g. the growth of the size of the control population and the sample population). In the framework of this study, we considered that the biological effect was significant at a threshold of 5% ($\alpha < 0.05$). It is noteworthy that this test uses the statistical ranks obtained and not the values reported by the observations; nor does it make any hypothesis on the shapes of the distributions.

RESULTS

Chemical analysis

The pH values measured on the solid phase (between 6.7 and 7.9) and on the liquid phase (between 7.4 and 7.8) of the sediments are fairly homogeneous. The results of the other chemical analyses are summarised in Table 2. The highest concentrations of heavy metals (e.g. Pb, Ni, Zn, Cu) and PCB were recorded in the 'Django Reinhardt' basin. This retention basin is supplied by an industrial catchment area (stormwater and water from clean industrial processes like cooling).

The PAH contents of the retention basins studied were similar to each other. All the samples analyzed presented BTEX and VHOC values lower than the quantification thresholds. The higher TCH values were measured in the basins 'Django Reinhardt' (industrial catchment) and 'Zac de Pivolles' (commercial catchment). The presence of pesticides varied from one basin to another basin: certain chlorine pesticides were detected in the samples from the 'Grange Blanche' basin (urban catchment), phosphorous and nitrogenous pesticides were detected in the 'Chemin de Feyzin' basin (urban catchment), and the herbicide Diuron was detected in the 'Django Reinhardt' basin (industrial catchment). The contents detected were nonetheless close to quantification thresholds in all these cases.

Characterisation of ecotoxicological effects

The toxicity effects were analysed according to the percentage of inhibition or stimulation in terms of: (i) size (*Heterocypris incongruens*), (ii) population (*Brachionus calyciflorus*) or (iii) luminescence (*Vibrio fischeri*).

Ostracod chronic toxicity tests performed on the solid phase (Figures 1(a) and 1(d)) showed that the size of the organisms measured in the sediments studied is significantly smaller than that of the organisms measured in the control sample (with a threshold of $\alpha < 0.05$; Wilcoxon statistical test). The ecotoxic effects are greater for the 'Django Reinhardt' retention basin, which collects the stormwater of an industrial catchment area.

Rotifer chronic toxicity tests performed on liquid phase showed a low inhibitory effect with the sediments of 'Chemin de Feysin' and 'Django Reinhardt' retention basins (Figures 1(b) and 1(d)). The tests with the sediments of the 'Grange Blanche' and 'Zac de Pivolles' retention basins, on the contrary, showed a low stimulation of organism population growth. This stimulation could be due to the presence of nutritive elements in their interstitial water.

Vibrio fischeri tests performed on the liquid and solid phases showed that the solid fraction of the sediments is far more ecotoxic for this organism than the liquid fraction of the same samples (Figures 1(c) and 1(d)). They also showed that the commercial and industrial basins ('Zac de

Table 2 | Chemical characteristics of sediments stored in the retention basin

	Chemin de Feyzin	Grange Blanche	Zac de Pivolles	Django Reinhardt
Catchment type	Residential & agricultural	Residential & agricultural	Commercial	Industrial
Dry content (% mass)	43	47	50	43
TOC (mg/kg)	110 000	130 000	110 000	–
TOC in liquid (mg/kg)*	770	120	170	–
Σ: TCH C10-C40 (mg/kg-DM)	560	600	3600	2080
Σ: PAH (mg/kg DM)	2.4	3.3	3.4	3.6
Σ: PCB (mg/kg DM)	< 0.014	0.047	0.1	1.1
Σ: VHOC (mg/kg DM)	< 0.02	< 0.02	< 0.02	< 0.02
Σ: BTEX (mg/kg DM)	< 0.2	< 0.2	< 0.2	< 0.2
Cd (mg/kg DM)	0.7	< 0.4	0.5	5.4
Cu (mg/kg DM)	130	150	160	223
Pb (mg/kg DM)	88	85	93	210
Ni (mg/kg DM)	32	32	37	50
Zn (mg/kg DM)	640	650	870	1370

Σ: total concentration of each pollutant family; DM: dry matter; -: not analysed; *: leachable fraction of TOC in mg/kg.

Pivolles' and 'Django Reinhardt') lead to greater effects on this organism.

DISCUSSION

The application of the assessment tools chosen led to the conclusion of low to moderate toxicity for the sediments taken from the study of four retention basins collecting stormwater from separated sewer systems. These results differ from those obtained previously with water taken from a combined sewer system (Angerville 2010; Becouze-Lareure *et al.* 2012). Indeed, in the case of the latter, the same type of ecotoxicity tests, in particular Microtox® and ostracod, highlighted far higher ecotoxicity. This difference could be explained by higher concentrations of pollutants in the case of the combined sewer system (Angerville 2010; Becouze-Lareure *et al.* 2012).

The effects of inhibition were clearly greater for the solid phase for all the samples analysed. This is consistent with several studies in which the particle fraction was observed to be the main vector of pollution in stormwater (Matthews *et al.* 1997; Pitt 2003).

Furthermore, within the four samples tested in this study, the sediment taken from the 'Django Reinhardt' basin was generally observed to be the most toxic. This is consistent with the chemical analyses performed that showed that concentrations in metals and PCB were higher in this basin than in the others. These pollutants could be the cause of part of the ecotoxicity measured. They can also be indicators of more global industrial pollution involving other pollutants, not dosed here, and indicating illegal discharges.

CONCLUSION

The present study showed that the ecotoxicity of sediments accumulated in stormwater retention basins can be monitored using a relatively simple battery of bioassays adapted for this purpose.

The results obtained show firstly that the bioassays selected exhibit good sensitivity to sediment toxicity, especially on the solid phase. Thus, the toxicity effects of sediments were more remarkable for 'Ostracods' test and 'Microtox®' assessed in solid phase.

The comparison between the four detention basins highlighted a link between toxic effect and the catchment types located upstream of each basin. The results also show that the urban sediments from stormwater detention basins are moderately toxic.

However, the results obtained might be further supported by considering other types of catchment area. For example, analyses confined exclusively to urban stormwater retention basins could be performed in future studies.

Lastly, the elements of ecotoxicological characterisation obtained could be used to formulate ecotoxicological risk assessment methodologies dedicated to each of the sediment management procedures considered (Perrodin *et al.* 2011).

ACKNOWLEDGEMENTS

This work was funded by the ZABR- Rhone Mediterranean & Corsica Water Agency (BRTOX Action), the OTHU (Field Observatory in Urban Hydrology) and Greater Lyon. We are particularly grateful to Mathilde Inglot and Celine Bécouze-Lareure for their help in the experimental work.

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First received 16 July 2013; accepted in revised form 19 December 2013. Available online 3 January 2014