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Ecotoxicological characterization of sediments from stormwater retention basins

Caractérisation écotoxicologique des sédiments issus de bassins de rétention d'eaux pluviales

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RÉSUMÉ

Les bassins de retenue-décantation correspondent à des ouvrages importants de la gestion des eaux pluviales. Leur fonctionnement à long terme pose cependant le problème de la gestion des sédiments accumulés en leur sein. Des pistes de valorisation ont été envisagées mais toute filière nécessite toutefois de caractériser au préalable l'écotoxicité des sédiments pour vérifier leur innocuité. Pour ce faire, une batterie de tests d'écotoxicité (ou bio-essais), spécialement adaptée à ces sédiments a été proposée, et testée sur des échantillons issus de quatre bassins de rétention de la communauté urbaine de Lyon (France). Cette batterie porte à la fois sur les effets toxiques liés à la phase solide (essais ostracodes et Microtox®-phase solide), et à la phase liquide (eau interstitielle) des sédiments (essais rotifères et Microtox®-phase liquide). Les résultats obtenus permettent de faire le tri entre les sédiments peu toxiques issus de certains bassins, et donc potentiellement valorisables, et les sédiments moyennement écotoxiques issus d'un bassin de rétention d'une zone industrielle, dont la valorisation est plus problématique. Ces résultats doivent maintenant être validés sur un nombre plus important de bassins de rétention. Ils pourraient ensuite être intégrés dans une méthodologie opérationnelle pour la réalisation de l'étude d'impact d'un projet de remblaiement urbain comprenant la valorisation de sédiments urbains.

ABSTRACT

Retention-detention basins are important structures for managing stormwater. However, their long-term operation raises the problem managing the sediments they accumulate. Different ways to valorize them have been envisaged but each one requires prior characterisation of their ecotoxicity to verify that they are harmless. To do this a battery of bioassays specifically adapted to these sediments has been proposed and tested on samples taken from four retention basins of Greater Lyon (France). This battery focuses on both the toxic effects linked to 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 sorting sediments presenting little toxicity and which could therefore be potentially exploitable, and sediments presenting average toxicity coming from a retention basin in an industrial area whose exploitation is more problematic. These results must now be validated by for a larger number of retention basins. They could then be incorporated in an operational methodology to carry out the impact study of an urban fill project comprising the use of urban sediments.

MOTS CLÉS

Ecotoxicity, Exploitation, Methodology, Retention basin, Sediment

1 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 risk of flooding and contribute to protecting receiving environments (Marsalek & Chocat 2002).

The work 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 (Randall 1982; Marsalek *et al.* 1992; Matthews *et al.* 1997; Chebbo *et al.* 2003). Thus detention basins are used for the efficient settling of metallic pollution and hydrocarbons mainly bound to suspended materials (Schueler 1987; Marsalek *et al.* 1997; Aires *et al.* 2003; Persson & Wittgren 2003; Strecker *et al.* 2004; Weiss *et al.* 2007).

However, the operation of these basins raises the problem of the fate and sustainable management of the sediments accumulated over the time. Different ways to valorise them 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.

For that purpose, 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). Thus bioassays (or ecotoxicity tests) can contribute towards solving these problems (ADEME 2005; Perrodin *et al.* 2010).

In this context, a battery of bioassays specifically adapted to urban sediments has been proposed and tested on sediments taken from four retention basins of Greater Lyon (France). 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.).



Figure 1. Localisation of retention basins in east Lyon (France).

Name of basin	Chemin de Feyzin	Grange Blanche	Zac de Pivolles	Django Reinhardt
Municipality	Mions	St Symphorien d'Ozon	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

Table 1. Retention basin characteristics

2 DESCRIPTION OF SITES

Four retention basins were selected. They are all integrated in structures that comprise stormwater infiltration basins downstream 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 (Figure 1). Table 1 shows the main characteristics of these retention basins (ARTELIA 2012).

3 MATERIALS AND METHODS

3.1 Preparation of samples

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.

This protocol was set up in order to highlight: i) the potential ecotoxic effects of the sediments; 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).

3.2 Chemical analyses

The chemical characterisation of the sediments accumulated in the four retention basins was performed in the framework of research carried out by (Sébastien *et al.* 2011), and the engineering office ARTELIA (ARTELIA 2012). In particular the chemical characterisation of the sediments provided information on the levels of potentially toxic concentrations of each of the pollutants present in the samples.

Chemical characterisation was performed on the pH (ISO 10390 2005 and ISO 10523 2008), the total organic carbon (TOC) content of the soil (ISO 10694 1995). The determination of the hydrocarbon index C10-C40 was carried out as per standard (ISO 9377-2 2000). Sixteen PAHs classified by the US Environmental Protection Agency (U.S. EPA) as priority pollutants were determinate per standard (ISO 18287 2006). The determination of volatile halogenated organic compounds (VHOC) and all Benzene, Toluene, Ethylbenzene and Xylenes (BTEX) were evaluated by gas phase chromatography-mass spectrometry with a mass selective detector (GC-MS). Seven congeners Polychlorobiphenyl (PCB: 101, 118, 138, 153, 180, 28, 52) content were determinate per standard (ISO 10382 2002). The analysis of arsenic and metals (*i.e.* Cd, Cr,Cu, Pb, Ni, Zn) was performed by ICP-MS (ISO 17294-1/2 2004). The total organic chloride pesticides, Phosphorous and Nitrogen containing pesticides were analysed. The evaluation of pesticides was performed by liquid or gas phase chromatography as a function of the characteristics of each pesticide studied.

3.3 Ecotoxicity tests

Four additional tests were performed on the four sediments studied.

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

This acute toxicity test was performed as per standard (ISO 11348 2009). The protocol of the latter permits evaluating 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 is recorded first before they are brought into contact with the sample, then after incubation periods of 15 and 30 minutes following the contact.

The bioassay with the bacteria *V. fischeri*, marketed at the beginning of the 1980s under the name Microtox® (developed by Azur Environment), then Lumistox® (developed by Dr Lange), met with rapid success for detecting the toxic effects of effluents of domestic and industrial waste water 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 at 0.45 µm of the sample to be tested. The dilution medium used was distilled water with salt at 20 g/L. The range of dilutions generally

comprised 8 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 most often presented in the form of EC50 (efficient concentration inhibiting 50% of the luminescence of the suspension of bacteria at the end of the test period).

3.3.2 Solid phase Microtox® test

This test permits detecting acute toxicity linked to the particle fraction of the sediment. The material and the biological reagent (strain *Vibrio fischeri*) are the same as those used for the test on the liquid matrix. The protocol applied for performing the test is that of standard ISO 11348, 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 *V. fischeri* is 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 again following an incubation period of 30 minutes.

The results are most often presented in the form of EC50 (Efficient Concentration inhibiting 50% of luminescence of the bacteria suspension following the test period).

3.3.3 Rotifer test (*Brachionus calyciflorus*)

This test of chronic toxicity was implemented as per the indications of standard (ISO 20666 2007). Its marketing in the form of Toxkit, as well as the sensitivity of the organism and its fast reproduction feature among the criteria leading to the choice of this test for studying the toxic effects of a sample.

This bioassay is used to determine the inhibition of the growth of a population of rotifers *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 most often presented in the form of EC50 (efficient concentration inhibiting 50% of the growth of the population of rotifers *Brachionus calyciflorus* following the test period).

When toxicity is low, it is also possible to present the percentage of inhibition of the growth of a rotifer population with non-diluted effluent (100%).

3.3.4 Ostracod test (*Heterocypris incongruens*).

This subchronic toxicity test performed in 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 a reference sediment is used for comparison to assess the effect of the sample on the organisms. The test is carried out on six well microplates using one microplate per sample and a microplate for the control. The criteria of the effects are the mortality of the organisms and their growth in comparison to their initial size.

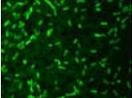
At the start of the test, the size of a set of 10 organisms representative of the batch used for the test is measured. Contact is ensured with 10 organisms per well for each microplate. The microplates were then incubated for 6 days according to the instructions of the standard. At this stage, a population of 60 organisms were 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.

The data was processed following a visual assessment (using a binocular magnifying glass) of the two effect criteria of the test (organism mortality and growth). Mortality was expressed as the average percentage of dead organisms at the end of the incubation period. For growth, we proceeded to calculate the average size of the organisms by test well, then evaluated for both the control and the sample 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 sample tested. The results are therefore expressed in percentage of effects observed for the non-diluted sediment.

A synthesis of the characteristics of the bioassays used is presented in Table 2.

Table 2: Bioassays used for the ecotoxicological characterisation of the sediments

Organism		Type of exposure	Criterion of effect/duration of exposure	Standard	Type of sample
<i>Heterocypris incongruens</i> (Ostracods)		Chronic	Inhibition of growth (6j)	(ISO 14371 2012)	Raw sediment
<i>Brachionus calyciflorus</i> (Rotifers)		Chronic	inhibition of reproduction (48h)	(ISO 20666 2007)	Interstitial water
<i>Vibrio fischeri</i> (Bacteria)		Acute	inhibition luminescence (30 minutes)	(ISO 11348 2009)	Raw sediment & interstitial water

3.3.5 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 comparing 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 does not use the values taken by the observations, but their ranks, nor does it make any hypothesis on the shapes of the distributions.

Table 3 summarises the significance thresholds chosen for the different bioassays.

Table 3. Significance thresholds of bioassays

Organism	Effect criteria	Thresholds	Significance of biological effects according to
<i>Heterocypris incongruens</i> (adapted to the standard)	Mortality and growth of the organisms tested	(> 0% and $\alpha < 0.05$) significant inhibition of growth	Wilcoxon test
		$\alpha > 0.05$ no significant effect	
		(< 0% and $\alpha < 0.05$) stimulation of growth	
<i>Brachionus calyciflorus</i>	Reproduction	> 30% significant inhibition of reproduction	(ISO 17616 2008)
		< 30% no significant effect	
		< -30% stimulation of reproduction	
<i>Vibrio fischeri</i>	Luminescence	> 20% significant inhibition of luminescence	(ISO 17616 2008)
		< 20% no significant effect	
		< -20% stimulation of luminescence	

4 RESULTS

4.1 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 were fairly homogeneous.

The results of the other chemical analyses are summarised in table 4.

Table 4: 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	Industrial	Industrial
Dry content (% mass)	43	47	50	25-60
TOC (mg/kg)	110 000	130 000	110 000	-
TOC in liquid (mg/kg)	770	120	170	-
s: TCH C10-C40 (mg/kg-MS)	560	600	3600	685-3472
s: PAH (mg/kg MS)	2.4	3.3	3.4	0.52-3.55
s: PCB (mg/kg MS)	<.014	0.047	0.1	0.2-2
s: VHOC (mg/kg MS)	<0.02	<0.02	<0.02	<0.02
s: BTEX (mg/kg MS)	<0.2	<0.2	<0.2	<0.2
As (mg/kg MS)	13	11	6.6	-
Cd (mg/kg MS)	0.7	<0.4	0.5	2.5-8.3
Cr (mg/kg MS)	40	41	77	-
Cu (mg/kg MS)	130	150	160	174-349
Hg (mg/kg MS)	0.46	0.81	0.14	-
Pb (mg/kg MS)	88	85	93	97-323
Ni (mg/kg MS)	32	32	37	45-54
Zn (mg/kg MS)	640	650	870	927-1809

s: total concentration of each pollutant type

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 area (stormwater and water from clean industrial processes like cooling).

Cu concentrations were higher than local background geochemical values for all sediments.

The highest TCH content values were measured in the two basins (“Django Reinhardt” and “Zac de Pivolles”) which are of industrial and commercial types.

The PAH contents of the studied retention basins were similar to each other. All the samples analysed presented BTEX and VHOC values lower than the quantification thresholds.

The presence of certain chlorous pesticides was detected in the samples from the “Grange Blanche” basin. Phosphorous and nitrogen containing pesticides were detected in the “Chemin de Feyzin” basin, and the herbicide Diuron was detected in the “Django Reinhardt” basin. The contents detected were nonetheless close to quantification thresholds in all these cases.

4.1.1 Characterisation of ecotoxicological effects

4.1.1.1 Ostracod chronic toxicity test (*Heterocypris incongruens*) – solid phase

Figure 2 corresponds to the mortality of organisms measured after 6 days testing. Figure 3 shows the growth in size of organisms in comparison to the control sample.

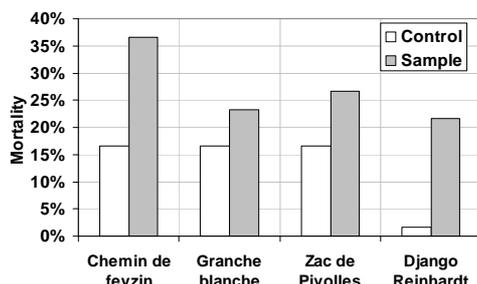


Figure 2. Mortality of organisms after 6 days contact with the sediments

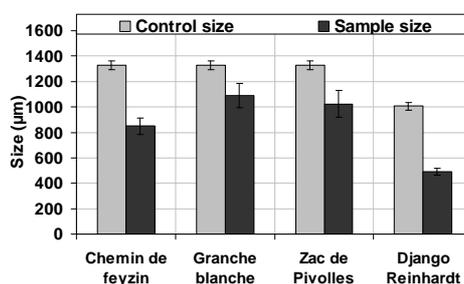


Figure 3. Average size of organisms

The highest mortality was observed for the sediments accumulated in the “Chemin de Feyzin” retention basin. This basin drains the stormwater of a residential and agricultural catchment area.

Figure 3 also shows that the four sediments tested significantly inhibit the growth of organisms. Thus 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 stormwater

of an industrial catchment area.

4.1.1.2 Rotifer chronic toxicity test (*Brachionus calyciflorus*) – liquid phase

Table shows the biological effects of the interstitial water obtained for the different concentrations tested.

Table 5 – Results of the rotifer tests

Retention tank	CE20	Growth %				
		Concentrations				
		10	25	50	75	100
Chemin de Feyzin	NT	Stimulation	Stimulation	Stimulation	Stimulation	No significant effect
		-79%	-52%	-69%	-38%	+14%
Grange Blanche	NT	No significant effect	Stimulation	No significant effect	Stimulation	No significant effect
		-14%	-41%	-27%	-38%	-8%
Zac de Pivolles	NT	Stimulation	Stimulation	Stimulation	Stimulation	No significant effect
		-33%	-90%	-43%	-77%	-27%
Django Reinhardt	NT	No significant effect				
		0%	-8%	-8%	8%	27%

Reminder: significant biological effects if inhibition or stimulation of population growth higher than 30%

None of the interstitial water led to significant inhibition of rotifer population growth.

The tests with the sediments of the “Chemin de Feyzin”, “Grange Blanche” and “Zac de Pivolles” retention basins, however, showed significant stimulation of organism population growth. This stimulation could be due to the presence of nutritive elements in the interstitial water.

4.1.1.3 *Vibrio fischeri* acute toxicity test – solid phase and liquid phase

Analysis of the biological effects of the *Vibrio fischeri* test focused on the inhibition of luminescence and on the calculation of EC50 (*i.e.* concentration for which biological effects are observed for 50% of the individuals tested). Figure 4 presents the EC50s obtained for the liquid and solid phases of the sediment sampled from each retention basin.

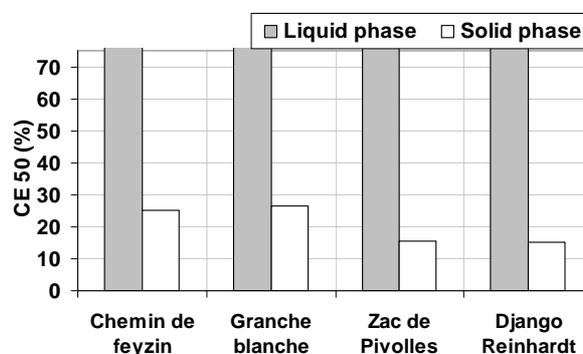


Figure 4. EC50 of sediments in solid phase (raw sediment diluted at 50%) and in aqueous phase (interstitial water).

These results show that the solid fraction of the sediments is far more ecotoxic for this organism than the liquid fraction of the same samples.

They also show that the commercial and industrial basins (“Zac de Pivolles” and “Django Reinhardt”) lead to greater effects on this organism.

5 DISCUSSION

5.1 Ecotoxicity of sediments

The application of the assessment tools chosen led to the conclusion of low to moderate toxicity for the sediments taken from the four retention basins studied 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).

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, HAP, 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.

5.1.1 Comparison of ecotoxic effects in the solid and liquid phases

Figure 5 permits comparing the percentages of inhibition and stimulation for the four ecotoxicity tests used.

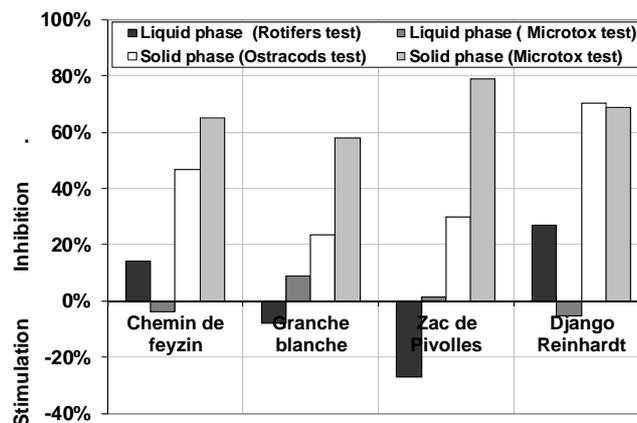


Figure 5. Effects of inhibition or stimulation on the 4 sediments studied

For all the samples analysed, the effects of inhibition were clearly greater for the solid phase. This is consistent with several studies that have observed that the particle fraction is the main vector of pollution in stormwater (Randall 1982; Matthews *et al.* 1997; Pitt 2003).

CONCLUSION

Retention-detention basins are valuable facilities for managing urban stormwater, from both the standpoint of flood control and that of retaining the particles and pollutants linked to them. Several previous studies have shown that the pollutants of urban stormwater (heavy metals, hydrocarbons, PCB, etc.) are most often found adsorbed on the particle fraction of stormwater (*e.g.* Schueler 1987), and are thus found in the sediments that accumulate in retention basins.

The operation of these basins raises the problem of the fate and possible exploitation of these sediments accumulated through time. This requires prior characterisation of the environmental safety of the sediments concerned.

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. Bioassays were implemented for the solid phase (particle phase) and for the liquid phase (interstitial water) of sediments. The “ostracod” test was used on the solid phase (raw sediment), the “rotifer” test was used on the liquid phase (interstitial water) of the sediments, and the “Microtox®” test was used on both phases, by adapting the experimental procedures to the phase concerned.

Given the results obtained during the study, it is now possible to suggest, in future, only performing bioassays on the solid phase as they appear more sensitive. It is nonetheless necessary, beforehand, to consolidate the first results obtained with a campaign of bioassays performed on a larger number of samples, and above all diversify still further the types of catchment areas collected and tested. Thus, for example, no strictly urban stormwater retention basin was tested in this initial study.

The methodology formulated could also be used to monitor the evolution of the ecotoxicity of

sediments during storage, so as to verify whether or not the bio-physico-chemical phenomena that occur (biodegradation, chelation, diffusion, adsorption, desorption, bioavailability of pollutant, etc.) reduce the ecotoxicity of sediments, and thus facilitate their exploitation outside the basin.

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).

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