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# Bioavailability of Polycyclic Aromatic Hydrocarbons Studied Through Single-Species Ecotoxicity Tests and Laboratory Microcosm Assays

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## 1. Introduction

The bioavailability of a pollutant is defined as its capacity to transfer from the surrounding environment (water and sediments) to organisms and is one of the key factors governing its bioaccumulation and toxicity. Knowledge of the bioavailable fraction of a compound is therefore vital in order to evaluate environmental risks and in particular to study its effects on living organisms. We began works on this topic at the laboratory Transfert et Effets des Polluants sur l'Environnement (TEPE) of Université de Savoie by studying the toxicity of a mixture of three polycyclic aromatic hydrocarbons (PAHs) (phenanthrene, fluoranthene and benzo(k)fluoranthene) in the framework of a PNETOX (programme national d'écotoxicologie) 1998-2001 program (Verrhiest, 2001; Verrhiest *et al.* 2000, 2001, 2002a, 2002b). The aim of this program, which brought together three research laboratories (TEPE of the Université de Savoie, Centre d'Etudes du Machinisme Agricole, du Génie Rural, des Eaux et Forêts (CEMAGREF) Lyon, INERIS, Institut Français pour la Recherche et les Etudes en Mer (IFREMER)), was to contribute to the development of a method of evaluating the risks of freshwater sediments contaminated by PAHs *via* in-depth changes to methods and better knowledge of the fate and effects of PAHs in aquatic ecosystems. The choice of PAHs was motivated by the fact that they are:

- ubiquitous organic contaminants (**table 1**), stemming from incomplete combustion (pyrolytic origin) or from the slow maturation of organic matter (petroleum hydrocarbons), and mostly emitted by anthropic activities (industry, transport),
- hydrophobic compounds which, after being introduced in a water column, rapidly become linked to colloidal and suspended matter, thus they enter the sediment where their effects depend on their bioavailability and fate, in relation with the physicochemical and microbiological properties of the sediment and interstitial water,
- toxic (**tables 2 and 3**) or/and mutagenic and carcinogenic compounds.

2-L microcosm assays (28 days) were performed on different types of sediment (artificial and natural) spiked with a mixture of PAHs (fluoranthene, phenanthrene, benzo(k)fluoranthene, **table 4**). The effects were evaluated on higher organisms and on the bacterial compartment of the sediment. Details on the protocols can be found in Verrhiest *et*

*al.* (2000, 2001, 2002a, 2002b). The microcosms consisted in glass beakers filled with artificial sediment (300 g sand + kaolinite + alpha-cellulose + TetraMin®) or natural sediment, synthetic water (2000 mL), and inoculated with daphnids (*Daphnia magna*), micro-algae (*Pseudokirchneriella subcapitata*), duckweeds (*Lemna minor*), chironomids (*Chironomus riparius*) and amphipods (*Hyalella azteca*). The organisms were exposed 28 days to PAH-spiked sediment, measurements were performed on PAH contents, organism development (survival, growth, reproduction), bacterial exo-enzymatic activities involved in the nitrogen (leucine-aminopeptidase) and carbon ( $\beta$ -glucosidase) cycles, and bacterial density (direct counting of bacteria by epi-fluorescence or Colony Forming Units after spreading on agar). The fate and the effects of PAHs were analysed as a function of the sediments' characteristics, especially organic carbon content and sediment type (artificial sediment conditioned or not, natural sediment). Studying the bioavailability of PAHs has been extended through a program on the impact of pyrene (**table 4**) on the organisms of an aquatic ecosystem (Clément *et al.*, 2005b). This study, performed in the framework of the Centre National de la Recherche Scientifique (CNRS) Life and Societies Environment Program (Ecosystems and Environment), was performed in 2001-2002 (Jouanneau *et al.*, 2003). It brought together four laboratories: the Laboratory of Biochemistry and Biophysics of Integrated Systems, the Department of Molecular and Structural Biology, Unité Mixte de Recherche (UMR) 5092, Commissariat à l'Energie Atomique (CEA) Grenoble (Yves Jouanneau), the TEPE Laboratory, Université de Savoie (Pr Gérard Blake), the Laboratoire Chimie Moléculaire et Environnement (LCME) Laboratory, Université de Savoie (Emmanuel Naffrechoux), and the Laboratoire des Sciences de l'Environnement (L.S.E.) (Bernard Clément). During the first phase (2001), the toxicity of pyrene was evaluated at the L.S.E. by single-species assays (daphnia, algae, duckweed, chironomidae, amphipods), and a bioaccumulation measurement protocol for *D. magna* and *H. azteca* was formulated. During the second year, 2-litre microcosm tests were performed in the presence of two lacustrine sediments. As in the case of Verrhiest's (2001) study of PAH mixtures, we completed monitoring of the higher organisms of the microcosm by measuring enzymatic activity and bacterial density in the bacterial compartment of the sediments

Place		Area studied	PHE (mg/kg)	FLU (mg/kg)	BKFLU (mg/kg)	Pyrene (mg/kg)	Sum of 16 PAHs (mg/kg)	References
Marine sediments	West of the sea Méditerranée	N+C	0.06 to 1.86	0.001 to 3.18	–	dna	1.18 to 20.44	Baumard <i>et al.</i> , 1998
	Bassin d'Arcachon	C	–	–	–	dna	5 to 10	Geffard <i>et al.</i> , 1999
	West of the Beaufort Sea (Alaska)	N	–	–	–	dna	0.16 to 1.1	Raymond <i>et al.</i> , 1999
	Baltic Sea	N	0.03 to 0.08	0.05 to 0.27	0.05 to 0.16	dna	0.72 to 1.9	Valette-Silver <i>et al.</i> , 1999

	Ain	N *	0.05 to 0.12	0.25 (mean)	0.07 to 0.3	dna	–	Verrhiest, 2001
	Neyrieux	N *	<0.05	<0.05	0.03	dna	–	Verrhiest, 2001
	Confluence of Seine-Marne	N	0.5 (mean)	0.82 (mean)	–	dna	1.5 to 7.4	Garban and Ollivon (1995)
	Rhone, Saone and Ain	N+C	<0.05 to 0.55	< 0.04 to 0.88	< 0.01 to 0.67	dna	0.52 to 14.64	Bonnet, 2000
	Moselle and Meurthe	N+C	<0.53 to 3.44	0.75 to 6.3	0.38 to 1.5	dna	6.68 to 32	Bonnet, 2000
	Seine	N C	– –	– –	– –	dna	2 to 4 60	Ollivon <i>et al.</i> , 1995
	Lake Annecy	C	–	–	–	dna	1.43	Naffrechoux <i>et al.</i> , 1999
	Niagara river (EU)	N C	0.01 0.4	0.04 0.9	–	dna	0.4 (mean) 3.3 to 5.4	Eisler, 2000
Fresh water sediments	Black river (EU)	C	52 (mean)	33 (mean)	1.5 (mean)	dna	–	Eisler, 2000
	CEBS code B2	C*	0.51	0.87	0.26	0.95	5.86	Bray <i>et al.</i> , 2001
	CEBS code B13	C*	1.3	1.6	0.48	1.5	9.58	Bray <i>et al.</i> , 2001
	CEBS code B22	C*	0.84	1.45	0.46	1.33	9.59	Bray <i>et al.</i> , 2001
	North 13990	C*	0.53	0.68	0.89	1.84	10.09	Bray <i>et al.</i> , 2003
	North 17000	C*	3.96	2.53	0.15	7.00	19.11	Bray <i>et al.</i> , 2003
	North 12570	C*	1.23	1.64	2.04	3.31	17.04	Bray <i>et al.</i> , 2003
	North 12730	C*	2.29	7.36	4.43	6.34	37.64	Bray <i>et al.</i> , 2003
	North 12800	C*	2.52	4.67	3.71	5.59	62.54	Bray <i>et al.</i> , 2003

Table 1. PAH concentrations of several sediments (according to Verrhiest 2001 and completed by the author) (PHE: phenanthrene; FLU: fluoranthene; BKFLU: benzo(k)fluoranthene; N: normal; C: contaminated; CEBS: Canal de l’Est Branche Sud; dna: data non available; \* sediments used in our studies).

PAH	Organism	Type	EC50/LC50 (µg/l overlaying water or mg/kg dry sediment)	Duration of exposure/ Endpoint
	Hydra <i>Hydra sp.</i>	Water	96 µg/l	_/mortality
	Daphnia <i>Daphnia magna</i>	Water	700 µg/l	_/mortality
	<i>Daphnia pulex</i>		734 µg/l	_/mortality
Phenanthrene	Amphipod <i>Gammarus pseudolimnaeus</i>	Water & sediment (epibenthic)	126 µg/l	_/mortality
	Insect chironomid <i>Chironomus tentans</i>	Sediment (benthic)	490 µg/l	_/mortality
	Annelid <i>Lumbriculus variegatus</i>	Sediment (benthic)	> 419 µg/l	_/mortality
Fluoranthene	Hydra <i>Hydra americana</i>	Water	70.06 µg/l	_/mortality
	Daphnia <i>Ceriodaphnia dubia</i>	Water	45 µg/l	_/mortality
	<i>Daphnia magna</i>		102.8 µg/l	_/mortality
			43 to 92 µg/l	10 days/mortality
			4.2 to 15 mg/kg	10 days /mortality
	Amphipod <i>Hyaella azteca</i>	Water & sediment (epibenthic)	97 to 114 µg/l	10 days/mortality
			32 to 54 µg/l	10 days/mortality
			2.3 to 7.4 mg/kg	10 days/mortality
	Insect <i>Chironomus tentans</i>	Sediment (benthic)	30 to 61 µg/l	10 days/mortality
	<i>Chironomus riparius</i>		3 to 8.7 mg/kg	10 days/mortality
			29 to 41 µg/l	11 days/mortality
			170 mg/kg	28 days /emergence
	Annelid <i>Lumbriculus variegatus</i>	Sediment (benthic)	> 178.5 µg/l	mortality

Table 2. Acute toxicity data for phenanthrene and fluoranthene towards water invertebrates (Verrhiest, 2001) (EC/LC50: concentration which produces 50% effect on a given end point or kills half of the organisms initially present).

2. Results and main findings

2.1 Difficulties specific to experiments on PAHs

PAHs are difficult to handle due to their properties. Indeed, their strong affinity for particular phases and low hydrosolubility complicate assays when spiking sediments and studying the fate of these substances in experimental systems. The sediment has to be spiked so that the PAHs introduced become homogenously distributed within the matrix. However, their poor solubility does not allow spiking with an aqueous solution. This makes it necessary to use an apolar solvent which makes it possible to concentrate PAHs, although this may prove toxic for organisms. We used the "wall-coating" method (Ditsworth *et al.*, 1990) for all the assays. This method consists in distributing the PAHs on the wall of a glass flask after adding an acetone solution, evaporating the solvent by rotating the flask horizontally, and then adding the wet sediment that comes into contact with the PAHs inside the flask as it is being rotated. High spiking yields were always obtained during the assays performed on fluoranthene, phenanthrene, benzo(k)fluoranthene and their mixtures (>80%) (Verrhiest *et al.*, 2000, 2001, 2002a). On the contrary, the assays on pyrene provided generally variable and much lower yields (**table 5**). This may have been due to the different characteristics of the sediments used in these assays, as the efficiency of the spiking depends on the way in which sediments come into contact with the flask wall. Chevron (2004) proposed another more classical method that consists in dissolving pyrene in DMSO (dimethylsulfoxide) at a given concentration, then mixing it with an organic matrix. The low toxicity of this solvent to microorganisms means that it can be used, as she did, in biodegradation assays. It remains to be determined, however, whether DMSO has no effect on higher organisms in contact with the sediment.

µg/L	Daphnia		Algae		Ceriodaphnia	
	LC50-48 h survival	IC95%	EC10 growth	IC95%	EC10 reprod 7 d	IC95%
Benzo(k)fluoranthene	> 1.1		> 1.5		> 1.5	
Benzo(a)anthracene	> 9.1		4.1	3.8-4.6	> 13	
Benzo(b)fluoranthene	> 1.1		> 1.5		> 1.5	
Benzo(ghi)perylene	> 0.2		> 0.26		0.124	0-0.17
Benzo(a)pyrene	> 2.7		1.54	1.52-1.57	0.77	0.03-2.2
Dibenzo(a,h)anthracene	> 0.35		0.73	0.52-1.31	> 0.046	
Acenaphtene	958	916-994	308	266-371	64	50-100
Acenaphtylene	1800	1731-1956	595	505-703	95	47-231
Anthracene	> 25		23.3	18.6-27.8	> 4.9	
Fluorene	408	368-449	485	411-540	33	28-45
Chrysene	> 1.3		> 3.9		> 0.13	
Fluoranthene	> 112		33.1	27.8-37.5	1.2	0.2-4.9
Indeno(1,2,3,cd)pyrene	> 357		5.7	3.6-9.8	0.38	0-9.49
Naphtalene	1664	1441-1902	> 8024		999	761-1331
Phenanthrene	> 400		123.5	80.1-170.4	15	4.7-19
Pyrene	24.6	21.6-28.4	12.4	6.7-17.9	2.1	1.3-3.1

Table 3. Toxicity data of PAHs in aqueous phase for three pelagic organisms (Vindimian *et al.*, 2000) (EC10: concentration which produces 10% effect on a given end point; LC50 concentration which kills half of the organisms initially present; IC95: confidence interval at 95%).



The affinity of PAHs for solid surfaces can also bias results when studying their effects in the aqueous phase. PAHs dissolved in water tend to adsorb onto the walls of beakers, even glass ones, as already observed (Clément *et al.*, 2005b), thus confirming the results of other experiments (McCarthy, 1983; Gauthier *et al.*, 1986; Pelletier *et al.*, 1997; Miller, 1999). Therefore a solution of 10 µg/L pyrene contains only 4 µg/L pyrene after 30 days in the absence of light to avoid any photodegradation. Finally, the same problem exists when conserving aqueous samples before analysis. Since we were unable to carry out the first assays on pyrene ourselves, we had to subcontract this task to another laboratory which, due to the lead times given, provided results that greatly underestimated the real values that we were able to check against solutions spiked at known concentration. We strongly recommend strict control over the analytical part (extraction and analyses) when working on PAHs, otherwise there is a risk of coming up against the same problems.

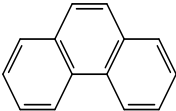
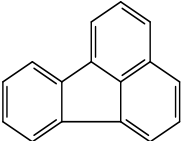
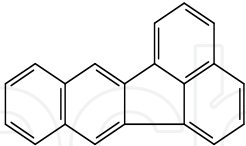
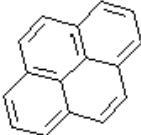
PAH	Structure	Hydrosolubility (µg/L) to 25°C	log Kow	Photosensitivity
Phenanthrene C <sub>14</sub> H <sub>10</sub>		1000 (May, 1980)	4.29 (May, 1980)	Non absorbent (Newsted and Giesy, 1987) UV <sub>C</sub> (Huovinen <i>et al.</i> , 2001)
Fluoranthene C <sub>16</sub> H <sub>10</sub>		206 (May, 1980)	5 (May, 1980)	UV <sub>A</sub> , UV <sub>B</sub> (Newsted and Giesy, 1987)
Benzo(k)fluoranthene C <sub>20</sub> H <sub>12</sub>		1.5 (Swartz <i>et al.</i> , 1995)	6.72 (Pelletier <i>et al.</i> , 1997)	UV <sub>A</sub> , UV <sub>B</sub> and visible (Pelletier <i>et al.</i> , 1997)
Pyrene C <sub>16</sub> H <sub>10</sub>		160	5.18	UV <sub>A</sub> and above all UV <sub>B</sub> (Huovinen <i>et al.</i> , 2001)

Table 4. Physicochemical properties of phenanthrene, fluoranthene, benzo(k)fluoranthene, and pyrene (according to Verrhiest *et al.* (2001) completed by data on pyrene)  
(Kow: water/octanol partition coefficient; UV: ultra-violet).

µg/g nominal f.w.	Test	Carbonate sediment		Peat sediment	
		µg/g measured f.w.	Spiking rate, %	µg/g measured f.w.	Spiking rate, %
2	ss	4.9 <sup>a</sup>	243	1.5 <sup>a</sup>	73.5
20	ss	15.1 <sup>a</sup>	75.7	11.5 <sup>a</sup>	57.7
50	sp	31.6 <sup>b</sup> ± 7.9	63.2 ± 15.8	23.6 <sup>b</sup> ± 7.6	47.3 ± 15.2
50	m1	24.1 <sup>a</sup>	48.2	1.4 <sup>a</sup>	2.7
50	m2	33.6 <sup>a</sup>	67.2	3.9 <sup>a</sup>	7.8
50	m3	29.1 <sup>b</sup>	58.2	7.2 <sup>b</sup>	14.4
200	ss	175.3 <sup>a</sup>	87.7	194.6 <sup>a</sup>	97.3

Table 5. Nominal and measured concentrations of sediments from lake Aiguebelette spiked with pyrene, with spiking rates (p.f.: fresh weight; ss: amphipod test; sp: spiking assay; m1: 1<sup>st</sup> microcosm assay; m2: 2<sup>nd</sup> microcosm assay; m3: microcosm assay without organisms; carbonate sediment; peat sediment; <sup>a</sup> concentrations measured on D0, after 7 days underwater ; <sup>b</sup> concentrations measured on 3 samples immediately after spiking the sediment) (source: Clément *et al.*, 2005b).

2.2 Toxicity in the aqueous phase towards microcosm organisms exposed during single-specific bioassays and modulating factors

The results of single-species bioassays in the aqueous phase on *Daphnia magna* and *Hyaella azteca* (**table 6**) confirm the acute toxicity of the PAH studies on these crustaceans, except for benzo(k)fluoranthene, which has very weak hydrosolubility (1.5 µg/L) (Verrhiest *et al.*, 2001; Clément *et al.*, 2000; Clément *et al.*, 2005b).

The absence of acute toxicity of benzo(k)fluoranthene was confirmed by the results of Vindimian *et al.* (2000), who also showed the absence of chronic toxicity (**table 3**). When organisms are exposed in obscurity, toxicity at 24 and 48 h is observed at contents close to solubility. Conversely, in the presence of the fluorescent light usually used in the laboratory, particularly in all microcosm assays, toxicity is higher, though the induction of toxicity is more significant for fluoranthene than for phenanthrene, generally recognised as non phototoxic (Newsted and Giesy, 1987; Swartz *et al.*, 1997; Boese *et al.*, 1998), and pyrene. This type of light (*cool-white fluorescent*) contains a fraction of UV (Clément *et al.*, 2000) which probably explains the increase in toxicity. Phototoxicity mechanisms are recalled in **figure 1**. For the most part, phototoxicity can be attributed to photosensitisation (Arfsten *et al.*, 1996). Furthermore, the phototoxicity of phenanthrene and fluoranthene has been confirmed by the post-exposure of organisms for 2 hours to pure UV radiation (A or C) (according to the method of Wernersson and Dave, 1997). This post-exposure could not be performed with pyrene. Our results strengthen the hypothesis that, although many PAHs are not acutely toxic to aquatic organisms at concentrations corresponding to their range of solubility (National Research Council of Canada (NRCC), 1983), the presence of natural light containing UV rays represents a factor that considerably increases the risk of toxicity (Lyons *et al.*, 2002). This is especially the case for pelagic invertebrates such as *Daphnia magna*, which are positively heliotropic and are thus more exposed in the presence of light and PAHs in the water column (Wernersson *et al.*, 1999).



	24 h lux	48 h lux	48 h lux + UV	24 h dark	48 h dark	48 h dark + UV
<i>Daphnia magna</i>						
PHE	2500 lux: 678	2500 lux: 604	2500 lux: 273 <sup>a</sup>	854	731	725 <sup>a</sup>
FLU	1500 lux: 56 2500 lux: 63	1500 lux: 36 2500 lux: 34	1500 lux: 29 <sup>b</sup> 2500 lux: <18 <sup>a</sup>	>200 >180	201 >180	80 <sup>b</sup> 20 <sup>a</sup>
BKFLU	non toxic					
PYR	1500 lux: 139 2500 lux: 105 6000 lux: 161	1500 lux: 74 2500 lux: 48 6000 lux: 45	/	167	68	/
<i>Hyalella azteca</i>						
PYR	1500 lux: 80 2500 lux: 134 6000 lux: 77	1500 lux: 41 2500 lux: 41 6000 lux: 26	/	82	63	/

Table 6. Toxicity in the aqueous phase of PAHs to *Daphnia magna* and *Hyalella azteca* (according to Verrhiest *et al.* (2001), Clément *et al.* (2000), and Clément *et al.* (2005b); lux: exposure to 1500 and 2500 lux 16h/ day; dark: obscurity; lux+UV <sup>a</sup>: post-exposure 2 h to UV-A (365 nm, 247 μW/cm<sup>2</sup>); lux+UV <sup>b</sup>: post-exposure 2 h to UV-C (365 nm, 247 μW/cm<sup>2</sup>).

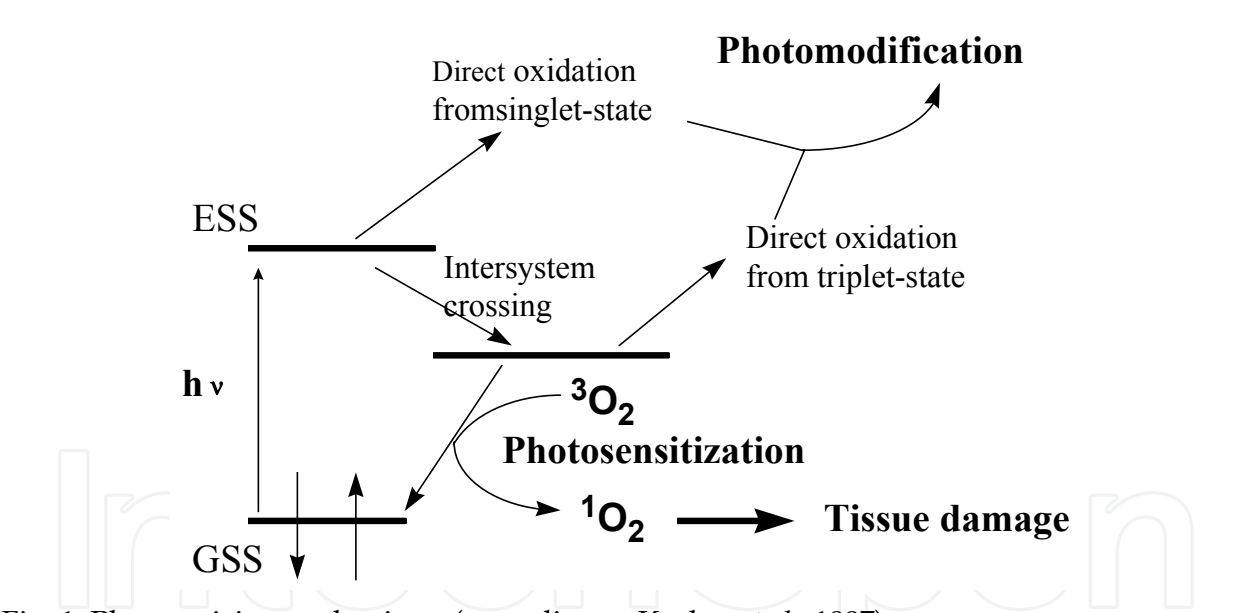


Fig. 1. Phototoxicity mechanisms (according to Krylov *et al.*, 1997).

The absence of phototoxicity of benzo(k)fluoranthène to *D. magna* (Verrhiest *et al.*, 2001) is probably due to the fact that the weak hydrosolubility of this PAH (1.5 μg/L) does not allow sufficient bioaccumulation to cause effects, as suggested by Boese *et al.* (1998) for benzo(b)fluoranthene (hydrosolubility: 6 μg/L). Although fluoranthene has been shown to be stable in the aqueous phase for 48 h in light or obscurity (Clément *et al.*, 2000), under the same conditions, pyrene is much more sensitive to light (30 to 44% degradation over 24 h). When algae were added in the beakers in which the daphnia were exposed, significant adsorption of fluoranthene occurred on the algae over 48 h, and there was a considerable

reduction of toxicity in the presence of light. This phenomenon of reduced toxicity in the presence of algae is not attributed to adsorption, which remains limited; rather we presume that a possible protective effect may be due to algal pigments of type  $\beta$ -carotene (Bennett *et al.*, 1986), or more simply, that the algae absorb part of the light, though this absorption could not be quantified.

Algae are highly sensitive to pyrene (Clément *et al.*, 2005b), with an EC-72 h  $< 10 \mu\text{g/L}$ . According to Vindimian *et al.* (2000), they are also more sensitive to fluoranthene and phenanthrene than *Daphnia magna*. Conditions of exposure to very intense light (6000 lux) to ensure sufficient algal growth can induce significant phototoxicity, thereby explaining this higher sensitivity. Nonetheless, Warshawsky *et al.* (1995) obtained only weak inhibition of the growth of *Selenastrum capricornutum*, another species used by us, exposed to  $400 \mu\text{g}$  pyrene/L for 4 days under fluorescent light similar to ours. According to Lei *et al.* (2001), *Selenastrum capricornutum* is resistant to pyrene. Furthermore, pyrene stimulates its glutathione-S-transferase (GST) activity during exposures of several days to concentrations ranging from 0.1 to 1 mg/L (Lei *et al.*, 2003). This enzymatic activity involves an enzyme, GST, that catalyses the binding reaction between an endogenous bio-molecule and the toxic substance, thereby detoxifying the contaminants. The authors associate the stimulation of GST activity with the degradation of pyrene observed, to suggest that the GST activity is responsible for the metabolism of pyrene. It is possible that the differences in culture and assay conditions between the experiments performed by Lei *et al.* (2001, 2003) and us resulted in very different expressions of GST activity. The same authors observed that pyrene inhibited the growth of another species of Chlorophyceae, *Scenedesmus quadricauda* (Lei *et al.*, 2003). The type of light, containing more or less UV, could have an impact on the cytotoxicity of PAHs to algae, due to its importance in biotransformation, highlighted with benzo(a)pyrene and *Selenastrum capricornutum* (Warshawsky *et al.*, 1995).

Contrary to algae, duckweed is not or only slightly sensitive to PAHs. We observed a value close to the solubility limit for pyrene, which proved to be non toxic in a single-species bioassay for an exposure of 6 days at  $180 \mu\text{g/L}$  (3500 lux 16 h/day). An absence of sensitivity was also observed in another species of duckweed, *Lemna gibba*, by different authors (Huang *et al.*, 1995; Ren *et al.*, 1994). This resistance may be linked to the strong metabolism related to GST activity.

### 2.3 Toxicity to pelagic organisms in microcosms

In microcosms containing sediments contaminated by PAHs, organisms of the water column were exposed to PAHs present in the latter in dissolved, colloidal and particulate form, and some were also exposed to PAHs of the sediment with which they can be in contact for long periods (sedimented algae) or occasionally (daphnia consuming particles on the surface of the sediment). The results obtained on the daphnia exposed in beakers containing the sediment spiked with fluoranthene or phenanthrene (Verrhiest *et al.*, 2001) showed increased toxicity in comparison to bioassays in the aqueous phase, by taking into account in both cases the contents measured in the supernatant water. This increase can be attributed to different exposure routes, as the daphnia above the sediment can be brought into contact with the sediment or ingest fine particles returned to suspension. Other results take the same direction. During microcosm bioassays on a mixture of fluoranthene, phenanthrene and benzo(k)fluoranthene (Verrhiest, 2001), we observed strong toxicity after a week's exposure to *Daphnia magna*, for a natural sediment (Neyrieux) spiked at  $300 \text{ mg/kg}$ ,

whereas the contents measured in the supernatant water after filtration at 0.8  $\mu\text{m}$  did not exceed 1  $\mu\text{g/L}$ . Conversely, in microcosm bioassays on pyrene (carbonate and peat sediments from lake Aiguebelette spiked at 50 mg/kg), the response of *Daphnia magna* was likely related to contents measured in the supernatant water: a significant effect on the survival above the carbonate sediment in water containing  $30 \pm 9 \mu\text{g pyrene/L}$ ; absence of effect above the peat in water containing  $5 \pm 1 \mu\text{g pyrene/L}$  (Clément *et al.*, 2005b). The same correlations were observed in single-species bioassays in small beakers (Jouanneau *et al.*, 2003), where the survival of daphnia above the contaminated sediment was reduced by about 50% to contents close to EC50.

We have never been able to evaluate the effects of PAHs on algae in microcosms for various reasons : the difficulty of taking all the algae into account (in suspension or sedimented), the influence of browsing and/or insufficient growth, and in particular high variability.

As mentioned previously, duckweed is not sensitive to PAHs and, in general, we have not observed effects in microcosms, in the presence of artificial or natural sediments spiked with contents ranging from 4 to 300 mg/kg. This can be explained easily by the fact that PAH contents in the supernatant waters could not exceed the solubility limits of these contaminants. No effect on *Lemna minor* was observed at these values in our works (single-species bioassays) or in the literature. However, there was one noteworthy exception. In a microcosm bioassay on a natural sediment (Neyrieux) spiked with a mixture of PAHs (phenanthrene, fluoranthene, and benzo(k)fluoranthene) at 300 mg/kg, significant inhibition was demonstrated from 19 days onwards (Verrhiest, 2001). However, as the sum of the PAH contents of supernatant waters did not exceed 1  $\mu\text{g/L}$  during the test, it is possible that the inhibition of growth observed was due to a PAH degradation metabolite or the occurrence of an indirect effect. Verrhiest *et al.* (2002a) highlighted the effects on the bacterial compartment of a natural sediment spiked at 300 mg/kg. This disturbance perhaps concerned the sediment of Verrhiest (2001), possibly resulting in modifications of contents and flows of nutritive substances vital for *Lemna minor*. This hypothesis could not be supported by precise measurements.

#### 2.4 Toxicity to benthic organisms in single-species and microcosm bioassays

We were able to measure the sensitivity of the species *Hyaletta azteca* (crustacean-amphipod) and *Chironomus riparius* (dipterous insect) to several PAHs (fluoranthene, phenanthrene, benzo(k)fluoranthene, pyrene) alone and in mixture (for the first three), under different experimental conditions : single-species bioassays, microcosm bioassays, artificial sediments and natural sediments. Taking all the results into account, the first observation is that for exposures not exceeding one month, sediments contaminated by PAHs presented relatively weak acute toxicity as the first effects on benthic organisms (mortality and inhibited growth) were observed for contents of several mg/kg (or  $\mu\text{g/g}$ ), higher than 30 mg/kg for the mixture "phenanthrene + fluoranthene + benzo(k)fluoranthene", and from 20 mg/kg for pyrene. Such contents are those of strongly contaminated sediments (**table 1**) and these results correspond to the threshold values provided by McDonald *et al.* (2000) and Kalf *et al.* (1997), at least if we consider the PEC (probable effect concentration) and MPC (maximum permissible concentration), thresholds from which an effect is very much probable (**table 7**). What is more, it should be also be mentioned that these thresholds result from matching contamination data with data from bioassays on benthic organisms, the effects generally being imputable to all the contaminants present, especially total PAHs, whose effects are generally additive (Munoz and

Tarazona, 1993; Swartz *et al.*, 1995). However, it can be seen that effects are probable from 23 mg/kg in total PAHs, a value comparable to those for which we were able to show the effects in certain cases. The results obtained from microcosm bioassays were not different from those obtained from single-species tests, despite exposure being nearly twice as long. Other more sensitive sublethal effect criteria should be taken into account with these substances, some of which are known to be carcinogenic or mutagenic.

Substance	TEC (µg/g dw)	PEC (µg/g dw)	MPC (µg/g dw)
anthracene	0.0572	0.845	0.12
fluorene	0.0774	0.536	/
naphtalene	0.176	0.561	0.14
phenanthrene	0.204	1.170	0.51
benzo(a)anthracene	0.108	1.050	0.36
benzo(a)pyrene	0.150	1.450	2.70
chrysene	0.166	1.290	10.7
dibenzo(a,h)anthracene	0.033	/	/
fluoranthene	0.423	2.230	2.60
benzo(k)fluoranthene	/	/	2.40
benzo(ghi)perylene	/	/	7.50
indeno(1,2,3-cd)pyrene	/	/	5.90
pyrene	0.195	1.520	/
Total PAHs	1.610	22.800	/

Table 7. Toxicity thresholds in sediment for PAHs (TEC (threshold effect concentration) and PEC (probable effect concentration) taken from MacDonald *et al.*, 2000; MPC (maximum permissible concentration) taken from Kalf *et al.*, 1997).

The toxicity of mixtures of PAHs has rarely been studied, although natural sediments are contaminated by several substances. We approached this section with the study of the toxicity of the mixture of three PAHs (phenanthrene + fluoranthene + benzo(k)fluoranthene, Verrhiest *et al.*, 2001), and were able to highlight synergetic effects for this specific case, which contrasts with the hypothesis of additivity generally accepted. We also showed (Verrhiest, 2001; Verrhiest *et al.*, 2001) that the effects are more significant in artificial sediments than in natural sediments, a result that we impute to a partition between the particular and aqueous phases (interstitial water) favouring the aqueous phase, the main path of exposure for the organisms studied (Di Toro *et al.*, 1991), more in artificial sediment. These differences of partition are not only due to the quantity of organic matter in the sediments (content of total organic carbon for which PAHs have great affinity), but also seem to affect the quality of organic matter. (Grathwohl, 1990; DePaolis and Kukkonen, 1997; Haitzer *et al.*, 1999). More generally, the type of sediment (grain size, proportion of clay, sand, silt, etc.) influences the toxicity of PAHs (Landrum and Faust, 1994; Borglin *et al.*, 1996; Landrum *et al.*, 1997; Haitzer *et al.*, 1998). In the tests on the two natural sediments taken from Lake Aiguebelette ("carbonate sediment" and "peat sediment"), that differ in terms of grain size, composition, and above all the quantity of organic matter, we showed that the toxicity of the "peat" sediment was lower but that the partition coefficients between the dissolved fraction adsorbed on the organic carbon (Koc) were close, leading us to conclude that the quantity of organic matter was the main explanatory factor (Clément *et al.*, 2005b). The response of the



amphipod *Hyallela azteca* to pyrene in the two types of sediment can be explained for the most part by the contents measured in the interstitial water.

Toxicity to benthic organisms is also influenced by the fate of PAHs in the sediment. A balance in the partition of PAHs between solid and liquid phases occurs in the sediment after the latter has been spiked. A study performed on this point showed that the equilibration of artificial sediments for 8 hours led to lower PAH toxicity (Verrhiest, 2001). Beyond this period, other phenomena can contribute towards modifying PAH bioavailability. In natural sediments spiked with a mixture of PAHs (Verrhiest *et al.*, 2002a) over a period of 30 days, we highlighted that PAHs degraded with the exception of benzo(k)fluoranthene, a heavier and probably more recalcitrant PAH. We attributed this degradation to endogenous bacteria of the sediments, whose  $\beta$ -glucosidase activity, measured in parallel, was stimulated. In the studies on pyrene, we also observed a considerable reduction of content over 30 days. This reduction was presumed to be due to ageing, although biodegradation could have been partially responsible (Clément *et al.*, 2005b). This ageing, or reduced extractability (Guthrie *et al.*, 1999; Leppänen et Kukkonen, 2000; Alexander, 2000; Conrad *et al.*, 2002), could be due to the migration of PAHs inside particle pores, making their extraction more difficult and, in parallel, reducing their bioavailability to microorganisms (biodegradation) and higher organisms (toxicity). Therefore the tests on spiked sediments raise the question of sediment conditioning to take into account both the arrival at partition balancing between the different phases, by hypothesising that such a balance exists, and the influence of biodegradation and ageing phenomena on the bioavailability of PAHs in such a way as to reproduce the conditions prevailing for contaminated sediments.

## 2.5 Bioaccumulation of PAHs during tests

In parallel with monitoring effects on *Daphnia magna*, we were able to measure this organism's bioaccumulation of fluoranthene (Clément *et al.*, 2000; **figure 2**) and pyrene (Jouanneau *et al.*, 2003), in single-species tests (fluoranthene and pyrene) and in microcosms (pyrene). In the studies on pyrene, measurements were also performed on other benthic organisms. The method employed consisted in an extraction procedure using acetone, followed by spectrofluorimetric analysis to identify the PAH spectrum and quantify the dose accumulated. The fact of working each time on a single PAH allowed us to use this simple method, without having to separate the compounds by chromatography.

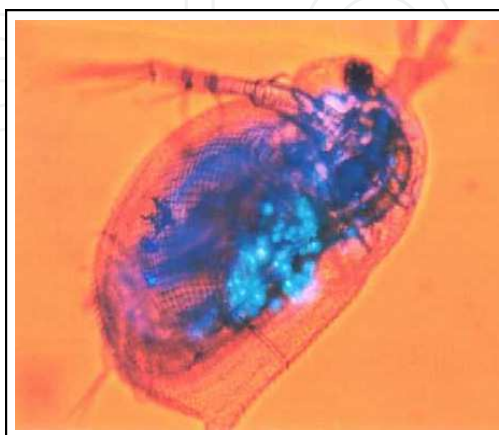


Fig. 2. Bioaccumulation of fluoranthene by *Daphnia magna*, visualised by epifluorescence microscopy (photo B. Clément).

In the single-species tests we showed significant bioaccumulation of fluoranthene and pyrene, directly correlated with the PAH content in the water. We found bioconcentration factors of 1000 L/kg (fresh weight) for fluoranthene and  $1986 \pm 445$  L/kg (fresh weight) for pyrene, the latter value being close to those found in the literature (1900 to 2000 L/kg for Nikkilä and Kukkonen (2001) in *D. magna*, 1700 to 3500 L/kg for Akkanen *et al.* (2001) in *D. magna* in river water whose dissolved organic carbon content varied from 0 to 18 mg C/L and spiked at 1 µg pyrene/L, 2702 L/kg for Southworth *et al.* (1978) in *Daphnia pulex*, values expressed in each case on the basis of fresh weight).

The comparison of bioaccumulation and effect data shows good correlation between them (figure 3). This is in line with the hypothesis of narcotic effects occurring after a given accumulation in tissues, with narcosis resulting from physical modifications and transformations of the phospholipidic membrane by adsorption of a hydrophobic compound. Disturbances of membrane functions occur when the quantity of compound adsorbed is sufficient (Driscoll *et al.*, 1997). Acute narcosis therefore occurs as a function of the quantity of PAHs bioaccumulated by the organism. According to Landrum *et al.* (1994) and Driscoll *et al.* (1997), EC50 is obtained for different organisms (e.g. the amphipod *Diporeia*) exposed to sediments spiked with PAH for internal doses close to 6 µmol/g (fresh weight of organism). In the tests on fluoranthene and pyrene, we obtained an EC50 of about 0.66 - 0.7 µmol/g (fresh weight), which is consistent with this theory, despite this value being only a tenth of that found by these authors. On the contrary, during the tests with "carbonate sediment" (Aiguebelette) spiked with pyrene on the amphipod *Hyaella azteca* (Jouanneau *et al.*, 2003), we observed that doses leading to effects were of about the same magnitude as the internal dose necessary to achieve 50% mortality in *Diporeia* spp., i.e. 6 µmol/g fresh weight of organism. In the "peat" sediment from Aiguebelette, we also observed an effect on the growth of *Hyaella azteca* for an internal dose of about 13 µmol/g.

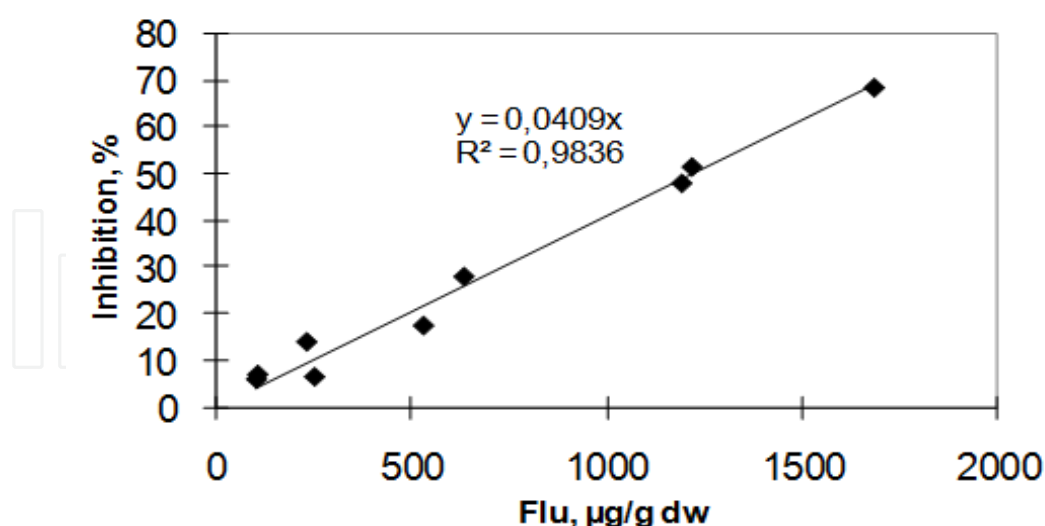


Fig. 3. Relation between the mean fluoranthene dose of *Daphnia magna* and the mean inhibition of mobility following 48 h exposure in darkness (Clément *et al.*, 2000).

During the microcosm bioassays on sediments spiked with pyrene, we were able to monitor the pyrene accumulated by daphnia introduced at the beginning of the assay and by their offspring. The mother daphnia recovered after 12 and 21 days exposure in microcosms



probably containing a compound derived from pyrene, as suggested by the differences observed between the fluorescence emission spectra (**figure 4**). It is noteworthy that the measurements performed on the young born in the microcosms and exposed for a maximum of 3 days in the systems, displayed the same type of spectra, whereas there was no modification in the accumulation of pyrene in the young bred by us and introduced in the microcosms for exposure for a maximum of 3 days. These observations suggest that the modified pyrene accumulated by the mothers was transmitted to the embryos and found in the young released during hatching. As with the daphnia in the microcosms, measurements of bioaccumulation in amphipods and chironomidae larvae showed that the modifications of pyrene spectra were similar to those observed for daphnia. Therefore it was not possible to quantify the doses of pyrene accumulated for any of these organisms. The modifications of the pyrene spectra suggest biotransformation processes that could occur in all the organisms of the microcosm, namely daphnia, chironomidae and amphipods. *C. riparius* is known to develop strong pyrene biotransformation activity (Guerrero *et al.*, 2002), and the results of Gourlay *et al.* (2002) showed that although *Daphnia magna* has little effect on fluoranthene, it is capable of biotransforming pyrene and benzo(a)pyrene. As in this study, these authors obtained a different spectrum of bioaccumulated pyrene (with a shift of peaks and an increase of ratio between peaks) that did not result from a matrix effect. As in the case of benzo(a)pyrene, they also highlighted strong fluorescence of the phase recovered in water and less fluorescence of the dichloromethane phase, tending to prove that the product derived from pyrene is more polar and thus clearly the result of biological transformation. This biotransformation of pyrene by *D. magna* was demonstrated by Akkanen and Kukkonen (2003), who showed the involvement of Cytochrome P450 monooxygenases in this elimination route.

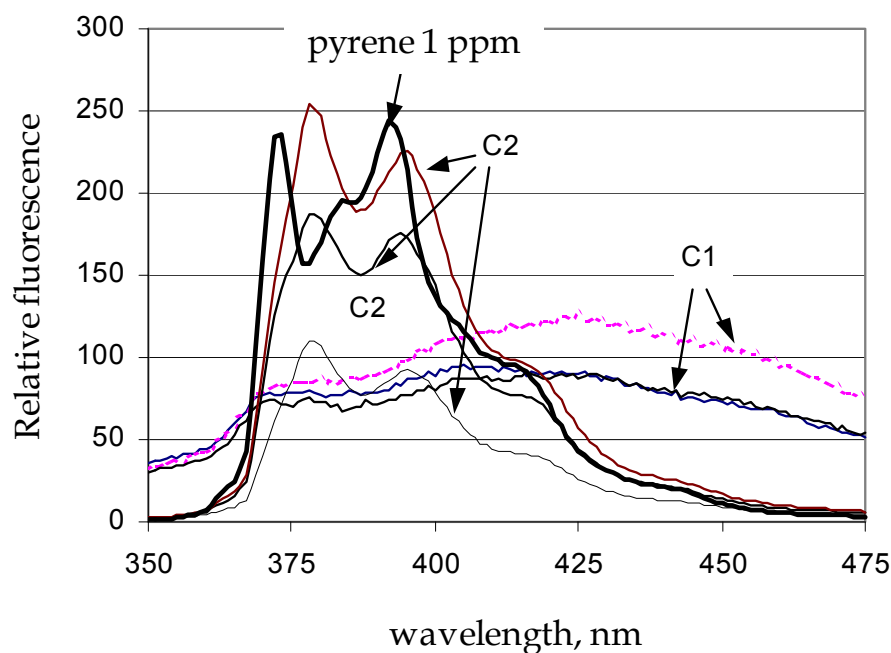


Fig. 4. Fluorescence emission spectra of methanol extracts of mother daphnia exposed for 12 days during microcosm bioassay no. 2 (carbonate sediment contaminated at 50 mg/kg) *versus* the spectrum of pure pyrene at 1 ppm in methanol.

## 2.6 Responses of the bacterial compartment to PAHs

Microorganisms play a vital role in environmental dynamics as they are involved in biogeochemical cycles acting as a medium through which matter and energy flow. As in any ecosystem, disturbing the microbial communities of a microcosm can impact the entire trophic chain and the balance of the environment. We also considered that it was important to take into account the microbial compartment of sediments in the microcosm bioassays. By using ecologically pertinent parameters (bacterial density and exoenzymatic activity), part of our work consisted in evaluating the responses of indigenous microorganisms in sediments to the contamination of the latter by PAHs.

Furthermore, the capacity of bacteria to biodegrade organic material as well as certain organic compounds, such as PAHs, influences the bioavailability of hydrophobic contaminants. This is why we chose, in addition to monitoring bacterial density, to focus on certain enzymatic activities involved in organic matter transformation processes, namely  $\beta$ -glucosidase (carbon cycle) and leucine-aminopeptidase (nitrogen cycle). In preliminary works on an artificial sediment spiked with fluoranthene (Verrhiest *et al.*, 2000), we also used the activity of INT-reductase by following the protocol of Merlin *et al.* (1995). Fluoranthene had no effect, even at 1000 mg/kg, on any of the parameters monitored (bacterial density of the sediment and supernatant water, INT-reductase and  $\beta$ -glucosidase activity of the sediment and the supernatant water). The study on the mixture of the three PAHs (phenanthrene + fluoranthene + benzo(k)fluoranthene) showed effects at high contents (300 mg/kg) on the bacterial populations of a natural sediment (Ain): reduction of the bacterial density of the sediments, partial inhibition of leucine-aminopeptidase activity, but stimulation of  $\beta$ -glucosidase activity, which it is tempting to parallel with the considerable degradation of fluoranthene and phenanthrene observed during the same bioassay (Verrhiest *et al.*, 2002a). Assays were also performed with pyrene (Jouanneau *et al.*, 2003), first under simple conditions (spiked sediment + water; bacterial density,  $\beta$ -glucosidase and leucine-aminopeptidase activities), then in microcosms (bacterial density and  $\beta$ -glucosidase). Over the range 1, 10, 50, 100 and 200 mg/kg, no significant effect was obtained in the single-species tests or in the microcosms.

In conclusion, PAHs do not appear to lead to effects on the bacterial compartment, at least as seen through the few parameters monitored in these works, except for very high concentrations rarely encountered in the environment. On the other hand, capacities to degrade phenanthrene, fluoranthene and pyrene (Jouanneau *et al.*, 2003) have been demonstrated.

## 3. General discussion and conclusion on the microcosm study of PAH toxicity

The purpose of the microcosm bioassays performed on sediments contaminated by PAHs was to evaluate the risks for lentic ecosystems related to the presence of these ubiquitous organic contaminants in this sediment compartment. We were able to obtain reasonably realistic results since it was possible to take into account different compartments of these ecosystems, and the relations between the different populations represented and between these populations and their environment.

We first confirmed the possibility of spiking initially non or only slightly contaminated artificial and natural sediments with PAHs, drawing attention to the need to ensure good spike rates and good distribution of PAHs in the sediment, as the physicochemical

properties of the latter can influence both rates and distribution. Once the sediment has been spiked, it is vital to observe the equilibration period, evaluated by us at being at one week (Verrhiest, 2001), which generally results in lower toxicity due to the higher adsorption of PAHs in the solid phase and which corresponds better to the situations most usually encountered in the environment (deposited, undisturbed sediment). We also looked into the possibility of extending this equilibration period to take into account ageing (migration of PAHs within particles, reducing their bioavailability) and biodegradation phenomena that we showed could be significant for certain PAHs over a few weeks (Verrhiest *et al.*, 2002a; Clément *et al.*, 2005b). In this framework we think that the use of natural sediments, with microflora more suitable for degrading PAHs and physicochemical and mineralogical characteristics more likely to simulate ageing, is probably preferable to using artificial sediments. The latter have other disadvantages related in particular to the impossibility of pertinently simulating the physicochemical properties impacting on the partition of PAHs between the dissolved and particular (mineral and organic) phases. On several occasions the comparative study of the fate and effects of PAHs in artificial and natural sediments showed large divergences between these two types of sediment, generally expressing an overestimation of risks of exposure and effects in artificial sediment (Verrhiest, 2001; Verrhiest *et al.*, 2001), not only for benthic microorganisms but also for pelagic organisms in contact directly with the sediment or/and *via* the water column. This overestimation is related to the difficulty of representing all the potential adsorption sites, in particular linked to a generally complex, natural organic material. Although we recommend giving up the use of artificial sediment in this type of research, the question of what natural sediment model should be used remains unanswered, given the great diversity of sediments. Here again, the *a priori* wide distribution of physicochemical properties of sediments can lead to behaviours that vary considerably as a function of the sediment chosen. The sediment should therefore be selected according to a site or a specific property under study.

Other procedures of the microcosm test protocol have a significant influence on the fate and effects of PAHs. We showed that the lighting chosen in our tests (classical laboratory fluorescent light) favoured the phototoxicity of certain PAHs (Clément *et al.*, 2000, 2005b). The adsorption of UV by PAHs governs photosensitisation and phototransformation phenomena, varying according to the type of radiation (UVA and UVB). Thus the choice of lighting more or less representative of the solar light spectrum influences the results observed (Wilcoxon *et al.*, 2003). Certain authors have also shown in the laboratory that the penetration of UV down to the sediment could generate phototoxicity for benthic organisms (Ankley *et al.*, 1994, 1995).

However, McDonald and Chapman (2002) questioned the ecological pertinence of phototoxicity, widely studied in the laboratory but, according to them, rarely expressed *in situ*. Indeed, many parameters have to be taken into account to estimate the probability of an organism bioaccumulating PAHs being subject to active solar radiation, as phototoxicity is essentially explained by photosensitisation. McDonald and Chapman (2002) showed that a large number of processes *in situ* allow organisms to avoid this exposure (reduction of bioavailability by organic and particular matter, protection mechanisms against UV in certain organisms, deep burrowing of benthic organisms, shade provided by aquatic plants, etc.). However, certain experimental parameters lead to overestimating exposure (glass flasks facilitate the passage of light in several directions, a shallow water column facilitates the passage of UVs down to the sediment, thin layers of sediment, radiation used at constant

intensity non representative of the variations observed during the day, water saturated with oxygen favouring photosensitisation, etc.). In tests performed in the presence of daphnia and algae, we were able to demonstrate their role in reducing the phototoxicity of PAHs to daphnia.

As mentioned earlier, the use of glass recipients does not prevent the adsorption of PAHs on their walls, a phenomenon that, for the generally low contents of PAHs found in the water column, can lead to underestimating the contents expected (Clément *et al.*, 2005b). Conversely, the use of synthetic environments generally free of dissolved organic materials leads to overestimating exposure. Regarding this, the incorporation of a sediment permits reducing this bias by enriching the water column with dissolved organic matter. Sediment also contributes particular and colloidal materials, the latter leading to an increase in the exposure of organisms in the water column to the PAHs adsorbed in it (Baumard, 1997). Similarly, the presence of micro-algae also modifies the exposure of daphnia and other consuming organisms (amphipods), though we did not have the opportunity to specify in which direction since although the algae capture some of the PAHs and thus reduce the dissolved fraction, they introduce an additional route of exposure for the organisms that consume them.

Although we studied monocontamination in most cases, we were also able to evaluate the effects of mixtures of PAHs, a situation closer to reality. Although the hypothesis of effect additivity is generally accepted, we showed that synergetic effects are possible (Verrhiest *et al.*, 2001). It is however necessary to go further by working on a mixture of a greater number of PAHs (for example the 16 priority PAHs of the USEPA), and by incorporating other types of pollutants, such as metals.

The microcosm bioassays made it possible to diversify the exposure routes of organisms, for example, daphnia present in the water column but which can also be in contact with sediment particles. The results of two different studies failed to converge: whereas the effects on daphnia were increased by sediment contaminated by PAHs in one (Verrhiest *et al.*, 2001), the other showed effects were essentially linked to pyrene contents in the water column. This absence of convergence can be explained by the different natures of the sediments used in these two studies and probably other parameters. Specific tests should be performed to study this point.

The toxicity criteria studied do not show that PAHs are very toxic to benthic organisms, even for exposures lasting a month. This is generally due to the high adsorption of PAHs on the particular and dissolved organic matter of the sediments which significantly reduces the bioavailability of these substances, a reduction that continues through time (ageing). It is reassuring in this initial approach to observe that chironomidae are capable of developing and emerging in nonetheless heavily contaminated sediments, a fact corroborated by results obtained on certain natural sediments also heavily contaminated by PAHs and heavy metals. We do not have data on the effects of PAHs on amphipod reproduction, due to the short time in which the tests were performed. It would be interesting to take this biological criterion into account by longer exposure or by the exposure of older individuals at the beginning of the test. Likewise, a study on several generations of chironomidae exposed to PAHs would make it possible to evaluate long term effects, by taking into account the number of hatched larvae and their capacity to pass through their life cycle. The few bioaccumulation measurements that we performed in the test on pyrene and the results in the literature encourage us to persevere along these lines, since the lower toxicity of PAHs



could be explained by the capacity of pelagic (daphnia, algae) and benthic (chironomidae and amphipods) organisms to biotransform PAHs. Given the measurements performed on the microbial compartment, it appears that the presence of high PAH contents does not significantly disturb this compartment. This result is important as the functioning of the ecosystem depends in part on the biological activities of sediment which contributes to recycling organic matter and renewing the mineral elements required for primary producers.

Although we were able to perform a global study of the fate of PAHs in microcosms, we were unable to identify the role played by organisms in this fate and in the effects stemming from them, thus this could form the basis for an additional path of research. The bioturbation activity of benthic organisms can contribute towards modifying the distribution of contaminants in sediment and stimulating their biodegradation through better oxygenation, for example, of superficial layers. When studying the fate of fluoranthene in the presence of the marine worm *Capitella*, Madsen *et al.* (1997) observed that bioturbation activity contributed towards burying fluoranthene, but the total loss of fluoranthene in the sediment was higher in the presence of the worms, whose activity increased the transfer of fluoranthene to the supernatant water or/and stimulated the biodegradation of this PAH. The role played on this level by the chironomidae and the amphipods used in our tests remains to be determined. Do they contribute to greater exposure of pelagic organisms or, on the contrary, do they reduce the risks to which they are exposed ? These questions are obviously far-reaching, as answering them requires a large number of microcosm bioassays in which certain populations are present simultaneously in order to highlight the interactions described above.

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## **Organic Pollutants Ten Years After the Stockholm Convention - Environmental and Analytical Update**

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Ten years after coming into force of the Stockholm Convention on Persistent Organic Pollutants (POPs), a wide range of organic chemicals (industrial formulations, plant protection products, pharmaceuticals and personal care products, etc.) still poses the highest priority environmental hazard. The broadening of knowledge of organic pollutants (OPs) environmental fate and effects, as well as the decontamination techniques, is accompanied by an increase in significance of certain pollution sources (e.g. sewage sludge and dredged sediments application, textile industry), associated with a potential generation of new dangers for humans and natural ecosystems. The present book addresses these aspects, especially in the light of Organic Pollutants risk assessment as well as the practical application of novel analytical methods and techniques for removing OPs from the environment. Providing analytical and environmental update, this contribution can be particularly valuable for engineers and environmental scientists.

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