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Chemical and ecotoxicological characterization of the "*Erika*" petroleum: Bio-tests applied to petroleum water-accommodated fractions and natural contaminated samples

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Abstract – Oil spills are an important source of PAHs in marine and coastal areas and comprise a short- and long-term threat for aquatic organisms. Some PAHs are known to be toxic, in particular mutagenic and/or carcinogenic, and their toxicological effects must be evaluated. Here, the impact of the "*Erika*" oil spill, which occurred at the end of 1999, was studied by combining chemical (PAH analyses) and toxicological approaches (biological effect assessment). "*Erika*" elutriates have been found to be more toxic than the elutriate obtained with a crude oil, Bal 250, inducing deleterious effects in *Mytilus galloprovincialis* and *Crassostrea gigas* embryos and in *Isochrysis galbana* algae. The embryotoxicity test in mussel is more sensitive than growth test in *I. galbana*. Naphthalenic compounds make up more than 95% of total PAHs quantified in elutriates. "*Erika*" elutriates are enriched with naphthalene, methyl-naphthalene, anthracene and higher-molecular-weight compounds. On the contrary, Bal 250 elutriate is characterized by the highest dibenzothiophene, methyl-dibenzothiophene and dimethyl-dibenzothiophene levels. Weathering does not highly affect the toxicity of the "*Erika*" oil. This study also confirms the potential impact of the "*Erika*" fuel on the biological quality of sea water and sediments from Traict du Croisic on the Atlantic coast of France.

Key words: Toxicity / "*Erika*" oil spill / Elutriate / Sea water / Sediment / Bio-assays / *Mytilus galloprovincialis* / *Crassostrea gigas* and *Isochryisis galbana*

Résumé – Caractérisation chimique et écotoxicologique du fioul de l'« *Erika* » : Bio-tests appliqués à la fraction soluble de pétrole et à des échantillons naturels contaminés. Les marées noires sont une source importante d'hydrocarbures aromatiques polycycliques (HAP) dans les milieux marins et côtiers, constituant un risque à court et long terme pour les organismes aquatiques. De nombreux HAP sont connus pour être toxiques, en particulier mutagène et cancérigène, dont les effets toxicologiques doivent être évalués. Dans cette étude, l'impact du naufrage de l'« *Erika* », qui a eu lieu à la fin de l'année 1999, a été étudié en combinant une approche chimique (analyse des HAP) et une approche toxicologique (étude d'effet biologique). Les élutriats du fioul de l'« *Erika* » se sont révélés plus toxiques que celui obtenu à l'aide du pétrole brut Bal 250, entraînant des effets délétères sur les embryons de *Mytilus galloprovincialis* et de *Crassostrea gigas*, ainsi que sur l'algue *Isochrysis galbana*. Le test d'embryotoxicité chez la moule est apparu plus sensible que le test de croissance chez l'algue *I. galbana*. Les composés naphtaléniques représentent plus de 95 % des HAP totaux quantifiés dans les élutriats. Les élutriats du fioul de l'« *Erika* » sont enrichis en naphthalène, méthyl-naphthalène, anthracène et en composés de haut poids moléculaire. En revanche, l'élutriat du pétrole brut Bal 250 est caractérisé par les plus fortes teneurs en dibenzothiophène, methyl-dibenzothiophène et dimethyl-dibenzothiophène. Le vieillissement en mer du fioul de l'« *Erika* » n'a pas d'impact sur sa toxicité. Cette étude confirme l'impact potentiel du fioul de l'« *Erika* » sur la qualité biologique des eaux et des sédiments du Traict du Croisic situé sur la côte atlantique française.

1 Introduction

Marine and coastal habitats are constantly stressed by the introduction of anthropogenic pollutants, from indirect (river, atmosphere, land) and direct (oil spill, industrial and urban effluents) sources, constituting a threat for biota (Chapman and Long 1983). Among all these contaminants, polycyclic aromatic hydrocarbons (PAHs) are widely studied because they are ubiquitous in the environment, hydrophobic and are known

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to be toxic (carcinogenic and/or mutagenic) (White 1986; Belkin et al. 1994). An oil spill is an important local source of PAHs. After an oil release, the toxicological risk is particularly high for pelagic species, which are exposed to the highest concentrations of water-soluble PAHs and PAH transformation products, particularly after photo-oxidation. Several studies have shown that the toxicity of some PAHs may increase from 2 to 1000 times in the presence of UV radiation (Allred and Giesy 1985; Pelletier et al. 1997; Barron and Ka'aihue 2001). Once PAHs are transferred into sea water, they adsorb on suspended particles and then subsequently accumulate in the sediments. These PAHs constitute a threat for benthic organisms that are directly exposed to PAHs adsorbed on the particles and to PAHs dissolved in the pore water (Ho et al. 1999; Fukuyama et al. 2000).

Chemical analyses can characterize the contamination levels of sea waters and sediments, determine compound sources, but they are limited for biological quality assessment. Only living systems are able to integrate the effects of contaminants that are truly bio-available. Numerous bio-assays, standardized to varying degrees and designed to study the toxicity of contaminants, industrial and urban effluents, natural seawater and sediments, have been developed during the last two decades using algae, amphipods, annelids, crustaceans, molluscs, echinoderms and bacteria (see reviews by Burton 1992 and His et al. 1999). Among these bio-assays, the embryotoxicity test with bivalve embryos is one of the most sensitive tests (Taylor 1978; His et al. 1999; McPherson and Chapman 2000). The embryotoxicity test is usually conducted with aqueous media. Potential toxicity of sediments is currently assessed with sediment pore water and/or elutriate. Recently, the embryotoxicity test in bivalve and sea urchin has been modified to be directly conducted with decanted sediments (Geffard et al. 2001, 2002, 2004a).

On December 12th 1999, the Maltese oil tanker *Erika* wrecked, causing a spillage of approximately 10 000 tons of oil, contaminating 400–500 km of the French coast. The purpose of this study was to evaluate the potential toxicity of *Erika* oil, combining chemical (PAH analyses) and toxicological approaches (biological effect assessment). First, the contamination level and the toxicity of petroleum water-accommodated fractions (before and after natural light exposure) were investigated. Three fuels were compared, the *Erika*'s original fuel (*Erika*-O, obtained from the refinery), the *Erika* fuel sampled in the sea (*Erika*-B, several days after the oil spill) and a crude oil (BAL 250, obtained from ELF). The toxicity of petroleum water-accommodated fractions was assessed using embryotoxicity tests in *Mytilus galloprovincialis* and *Crassostrea gigas* and the growth test in the unicellular algae *Isochrysis galbana*. Then, the embryotoxicity (in *C. gigas*) of sea waters and sediments from an *Erika* oil-spill-impacted area (Traict du Croisic) was assessed. The Traict du Croisic area was selected because it is the source of sea water for numerous salterns that are known to produce salt of very good quality. The aim of the work was to follow the remediation of the site after cleaning operations. These operations terminated in summer/autumn 2000. Considering this date, sediments sampled in summer 2001, i.e., 1 year after the end of cleaning operations, and then in 2003 were investigated.

2 Material and methods

2.1 Samples

2.1.1 *Petroleum water-accommodated fractions (elutriate)*

Each fuel (10 g) was stirred with a magnetic stirrer with 1 litre of natural filtered (0.45 μm) sea water (from Arcachon Bay with a salinity of 33‰) for 12 hours and allowed to settle for 1 hour. The stirring speed was adjusted so that the oil would break down into droplets. Elutriate was then recovered by filtration on dichloromethane-washed cotton. The potential impact of natural light on the toxicity of elutriate was studied with *Erika*-B fuel at the concentration of 40 g L^{-1}. The PAH contamination level and toxicity of the elutriate were assessed before (T0) and after (T1) an exposure of 7 days to natural light (5−13 July 2003) at the ambient temperature (between 24 and 35 °C).

In all cases, elutriate was mixed with natural filtered (0.45 μm) sea water at the following concentrations: 0 (control), 12.5, 25, 50 and 100% (v/v) and was then immediately tested. An aliquot of each elutriate was frozen (−30 °C) for less than 1 month prior to being used for PAH analyses.

2.1.2 *Natural samples*

Sea waters were collected at seven sites (Pouliguen ponton, Pouliguen jetée, Grand Bal, Plinet, Siberon, Penbron and Rostu) from Traict du Croisic on the French Atlantic coast on August 30–31, 2000. Salinity values were ranged between 35 and 37‰ The sites were chosen because they are situated at the entrance of the system that brings water to the saltern system. Unfiltered sea waters were directly tested (without dilution). However, PAH analyses were performed on filtered (0.45 μm) sea waters, as soon as they arrived in the laboratory.

Sediments were sampled monthly from April to August 2001 and in June 2003 at three sites (Penbron, Siberon and Plinet) at Traict du Croisic. Immediately after sampling, the sediment was sieved at 2 mm to eliminate debris, homogenized, frozen (−30 °C) and finally freeze-dried prior to being used for the chemical analyses and the toxicity tests. Freeze-drying increases the toxicity of sediment (Beiras and His 1995; Geffard et al. 2002; Geffard et al. 2004b), but in a previous study, we showed that the toxicity of fresh sediments is correlated with that of freeze-dried sediments (Geffard et al. 2002). Moreover, we demonstrated that the toxicity of freeze-dried sediments does not change during storage (Geffard et al. 2004b). Thus freeze-drying makes it possible to study the temporal variability in the potential toxicity of sediments that have not been sampled at the same time using the same biological material.

The bioassays were conducted with decanted sediment which was prepared by resuspending remoistened freeze-dried sediment in natural filtered (0.45 μm) seawater at a salinity of 33‰. Sediment suspensions of 0 (control), 1 and 5 g L^{-1} were prepared in 500-ml flasks, mixed for 10 s, and 30 ml of suspension was then transferred into the test vessels (see below). To avoid mechanical effects from suspended particles, the sediments were allowed to settle for 2 h before the biological material was introduced (Geffard et al. 2002).

2.2 Bioassays

2.2.1 *Embryo-larval test*

Mature *Mytilus galloprovincialis* and *Crassostrea gigas* were collected in the field (Arcachon Bay) the day of the experiments. They were induced to spawn by thermal stimulation according to standard procedures (His et al. 1997). Oocytes and sperm were mixed in sterile glass beakers with filtered seawater for 15 min to achieve fertilization. Using an automatic micropipette with an enlarged tip, volumes corresponding to 1200 fertilized eggs were transferred into 30-ml glass vials for elutriates and natural sea waters, so as to avoid the loss of PAHs by evaporation and/or adsorption processes, and into 30-ml polystyrene vials for decanted sediments (five replicates per treatment). The embryos were incubated without light for 48 h at 18 ± 1 °C in *M. galloprovincialis* and for 24 h at 24 ± 1 °C in *C. gigas*. After the incubation period, buffered formalin (100 μl) was added to each vial. We also added 2 ml of Rose Bengal (Sigma Aldrich, Saint Quentin Fallavier, France) to vials with decanted sediment. This produces coloured larvae and facilitates assessment of the percentage of abnormal D larvae. The percentage of abnormal larvae was determined according to the criteria of His et al. (1997), by direct observation through an inverted microscope of 100 randomly selected individuals per vial.

M. galloprovincialis embryos were used for *Erika*-O, *Erika*-B and Bal-250 elutriate toxicity assessment. The impact of natural light on the toxicity of *Erika*-B elutriate was studied using *C. gigas* embryos. In summer, only mature oysters are available. Finally, toxicity assessment of natural samples was studied with oyster embryos.

2.2.2 *Algal growth inhibition test*

Tests were carried out in 100-ml glass flasks with 50 ml of test solution (three replicates per treatment) and under artificial light (Sylvania Gro-lux 18 W, 60 cm, special artificial light for algal culture). The test species was *Isochrysis galbana*, which was cultivated on Conway medium with and without elutriate (Walne 1970). Algal culture medium was prepared by adding Conway medium to natural filtered sea water or to the different concentrations of elutriates at a 1% concentration. Consequently, the highest elutriate concentration used with the algal growth test was 99% instead of 100%. Studied elutriates have pH and salinity values similar to those observed with the control sea water. Inocula were taken from exponentially growing pre-cultures. The initial cell density in the test flasks was 10^6 cells ml^{-1}. The algae were incubated at 19 ± 1 °C under continuous white light and for 4 days. After the incubation, the algal concentration of each flask was determined.

2.3 PAH analyses

In all the samples, the following PAHs were analysed as representative of PAHs present in the *Erika* fuel: naphthalene (Naph), methyl-naphthalene (C1-Naph), dimethyl-naphthalene (C2-Naph), trimethyl-naphthalene (C3-Naph), dibenzothiophene (DBT), methyl-dibenzothiophene (C1-DBT), dimethyl-dibenzothiophene (C2-DBT), anthracene (An), phenanthrene (Phe), methyl-phenanthrene (C1-Phe), dimethyl-phenanthrene (C2-Phe), fluoranthene (Fluo), pyrene (Pyr), benzo(a)anthracene (BaA), triphenylene (Triph), chrysene (Chrys), methyl-chrysene (C1-Chrys), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(j)fluoranthene (BjF), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), perylene (Per), indeno(123-cd)pyrene (IP), benzo(ghi)perylene (BP) and dibenz(ah+ac)anthracene (DaA). After adding perdeuterated aromatic compounds (naphthalene, phenanthrene, fluoranthene, chrysene, benzo(e)pyrene, benzo(a)pyrene and benzo(ghi)perylene) to 100 ml of each elutriate, to 2 L of each natural filtered (0.45 μm) sea water or to 1–3 g of dry sediment, the PAHs were extracted using a liquid-liquid extraction method with dichloromethane for liquid samples and using a microwave-assisted extraction method with dichloromethane for sediments (Budzinski et al. 1995). The organic phase was then recovered and dried with anhydrous sodium sulfate (Na_2SO_4). Next, the organic extracts were purified on a micro-column containing alumina and fractionated on a micro-column containing silica to collect only the aromatic compounds (Budzinski et al. 1999). Aromatic fractions were analysed by gas chromatography/mass spectrometry (GC/MS) using an HP 6890, Agilent Technologies (Bios Analytique, l'Union, France) (splitless injection; purge delay: 1 min; purge flow: 60 ml min^{-1}; injector temperature: 270 °C; interface temperature: 290 °C; oven temperature: from 50 °C (2 min) to 290 °C (20 min) at 5 °C min^{-1}) coupled to an HP MSD 5972 mass spectrometer (electron impact: 70 eV; voltage: 2000 V; source temperature: 150 °C) operated in SIM (selected ion monitoring) mode using the molecular ions of the studied compounds at 1.2 scan s^{-1}. A 30 m × 0.25 mm i.d. × 0.25 μm film thickness capillary column (HP5-MS, Agilent Technologies) was used with helium as the carrier gas at a constant flow of 1 ml min^{-1}.

2.4 Statistical analyses

To test the null hypothesis that the different treatments had no effect on larval development and algal growth, the percentages of normal larvae and the algal densities of each concentration were compared to the control concentration using a one-way ANOVA (Sokal and Rolhf 1981), after conducting Cochran's test for homogeneity of variance. For data expressed as percentages, Arcsin $P^{1/2}$ transformation was applied to achieve homogeneity. Significant differences (at the 95% level) were then determined using the Tukey test.

3 Results and discussion

3.1 PAH contamination level and toxicity of elutriates

The PAH content of *Erika*-O elutriate (ΣPAHs = 520 ng ml^{-1}) was higher than the PAH content of the *Erika*-B elutriate (ΣPAHs = 360 ng ml^{-1}), which was in turn

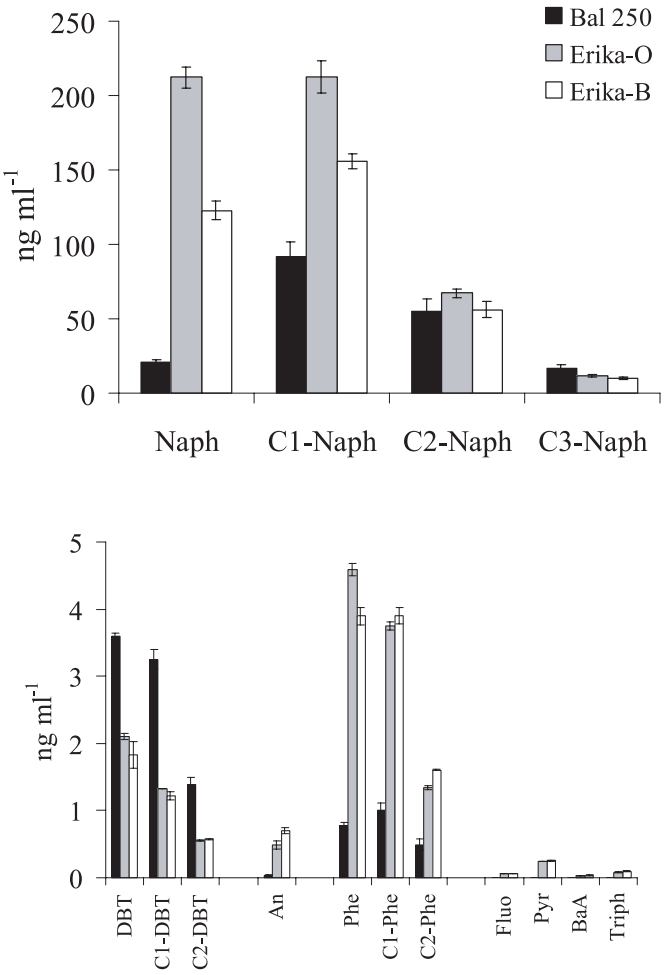

Fig. 1. Total PAH contents (ng ml^{-1}) of elutriates obtained from Bal 250, *Erika*-O and *Erika*-B fuels (10 g of fuel L^{-1} of filtered sea water).

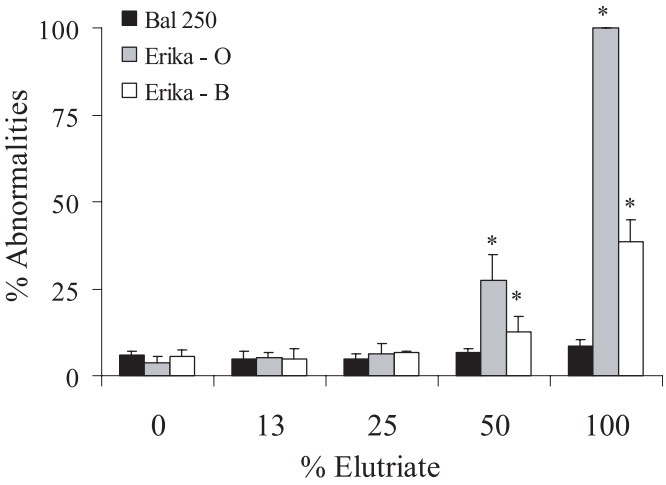

Fig. 2. Toxicity of elutriates obtained from *Erika*-O, *Erika*-B and Bal 250 fuels (10 g L^{-1} of filtered sea water) for *Mytilus galloprovincialis* larvae. Percentage of abnormal larvae (± standard deviation; n = 5). *: value significantly different from the control.

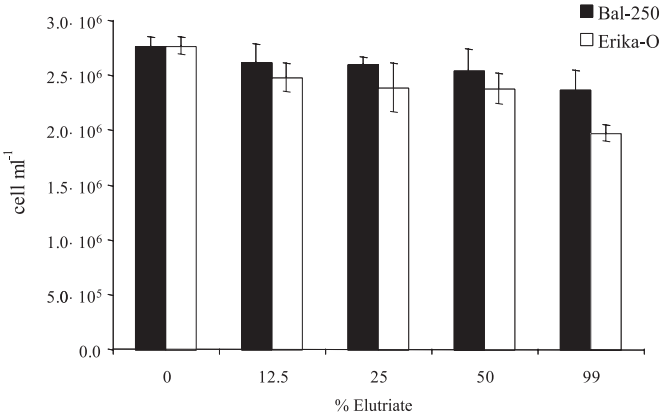

Fig. 3. Toxicity of elutriates obtained from *Erika*-O and Bal 250 fuels (10 g L^{-1} of filtered sea water) in *Isochrysis galbana* algae. Algal concentration (cell ml^{-1} ± standard deviation; n = 3).

higher than the PAH content of Bal 250 elutriate (ΣPAHs = 200 ng ml^{-1}) (Fig. 1). Naphthalenic compounds make up more than 95% of PAHs quantified in these elutriates. These results are in agreement with those of Saeed and Al-Mutairi (2000), who found that naphthalene and its homologs account for the major part of PAHs in petroleum water-accommodated fractions (ranging from 88 to 98% of the total PAHs). *Erika* elutriates are enriched with naphthalene, C1-naphthalene, anthracene and higher-molecular-weight compounds. On the contrary, Bal 250 elutriate is characterized by the highest dibenzothiophene, methyl-dibenzothiophene and dimethyl-dibenzothiophene levels. The two *Erika* elutriates show a similar PAH content level, except for naphthalene and C1-naphthalene.

For the two *Erika* elutriates, embryotoxicity effects in *M. galloprovincialis* were observed ($p < 0.0001$), with more abnormalities for *Erika*-O elutriate than for the *Erika*-B elutriate (Fig. 2). On the contrary, Bal-250 elutriate did not induce significant biological effects in mussel embryos ($p = 0.438$; Fig. 2). These results are in good agreement with the PAH level characterized for each elutriate. We obtained 100 and 50% of abnormal larvae with the highest concentration of *Erika*-O and *Erika*-B elutriates, respectively, whereas no effects were observed with Bal 250 elutriate. If the water content of *Erika*-B fuel (~50% in weight) is taken into account, results show that potential toxicity of *Erika*-O and *Erika*-B oils are similar.

Similarly, *Erika*-O elutriate induced growth inhibition on *I. galbana* alga (Fig. 3). At the two highest concentrations (50 and 99%), the concentrations of algae were significantly lower ($p = 0.00298$) than those observed in controls. On the contrary, with Bal-250 elutriate no significant effects ($p = 0.0815$) were observed. The algal growth test in *I. galbana* seems to be less sensitive than the embryotoxicity test in *M. galloprovincialis*. At the highest concentration of *Erika*-O elutriate, the inhibition of algal growth and the percentage of abnormal larvae were 30 and 100%, respectively.

As compared to the crude oil Bal-250, *Erika* fuel shows a higher potential toxicity, with a higher amount of PAHs released into sea water. These results also show that weathering (several days in the ocean) does not highly affect the toxicity

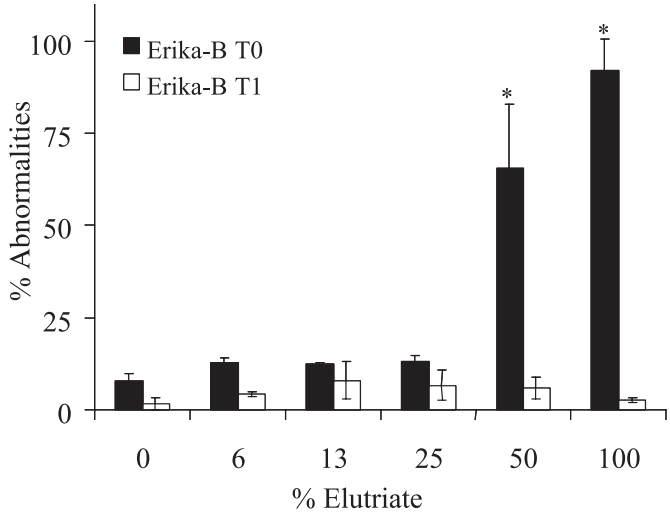

Fig. 4. Toxicity of *Erika*-B elutriate (40 g L^{-1}) in *Crassostrea gigas* before (T0) and after (T1) an exposure of 7 days to natural light. Percentage of abnormal larvae (± standard deviation; n = 5). *: value significantly different from the control.

of *Erika* oil. Elutriates obtained from original and weathered oils show similar toxicity and PAH contamination levels.

3.2 Impact of natural light on the toxicity of *Erika*-B elutriate

This study was conducted with *C. gigas* embryos because mature mussels are not available during the summer. However, some authors have shown that the sensitivity of *M. galloprovincialis* and *C. gigas* embryos is similar (see review by His et al. 1999).

The impact of natural light on the toxicity of *Erika*-B elutriate is highly pronounced (Fig. 4). At T0 (before light exposure), the two highest concentrations of elutriate resulted in 50 and 100% abnormalities, respectively. Observed biological effects were greater than those previously observed. These differences are explained by the oil concentration used to prepare the elutriate. Elutriates obtained with oil concentrations of 10 and 40 g L^{-1} show PAH contamination levels of 360 and 850 ng ml^{-1}, respectively. According to Page et al. (2000), these observations show that the PAH contamination level and the toxicity of elutriate are directly related to the oil concentration used to prepare the elutriate.

After the natural light exposure (7 days), the toxicity of the elutriate totally disappeared. The percentages of abnormal larvae were lower than 10% and were similar to those observed with reference filtered sea water. Analytical results show that studied PAHs were highly transformed by photo-oxidation. PAH contamination levels of the elutriates decreased from 850 to 0.8 μg L^{-1} after light exposure (data not shown). These toxicological observations are not in accordance with a number of previous studies, which showed that ultraviolet radiation may greatly increase the toxicity of petroleum products (Allred and Giesy 1985; Pelletier et al. 1997; Barron and Ka'aihue 2001). In these studies, petroleum products, PAHs and organisms were simultaneously exposed to light or UV, whereas in our study, *Erika*-B elutriate was exposed to natural light (UV) and then tested on oyster embryos. Photoenhanced toxicity to aquatic organisms can occur through two mechanisms: photosensitization and photomodification (Barron and Ka'aihue 2001). Allred and Giesy (1985) and Little et al. (2000) showed that oil, components of oil and some PAHs appear to act primarily through a photosensitization mechanism towards fish and aquatic invertebrates, rather than through structural modification of chemicals in the water column. Little et al. (2000) showed that fish exposed to water-accommodated fractions for 48 h in the dark exhibited no mortality, but 36% mortality occurred with 48 h of exposure to simulated sunlight. There was only 3% mortality of fish exposed to oil without UV. UV-exposed oil was not toxic to fish tested under fluorescent light, indicating that photomodification was not responsible for toxicity. Similar results were observed by Allred and Giesy (1985) with anthracene in *Daphnia pulex*. These authors showed that photoenhanced toxicity of anthracene results from activation by solar radiation of compounds present on or within the animals and not in the water. Our results are in accordance with these previous observations and show that photomodification does not induce toxicity and that photo-oxidated oil products seem to be less toxic than original PAHs. Moreover, these observations show that PAH are rapidly transformed by photo-oxydation.

3.3 PAH contamination levels and toxicity of natural sea waters and sediments

PAH levels in sea waters range from 4 ng L^{-1} (Pouligen ponton) to 18 ng L^{-1} (Penbron) (Table 1). If we compare the PAH contamination levels of the studied sea waters with the data from Fernandes et al. (1997) for natural brackish water, the contamination level of Pouligen ponton is similar to that of a sea water sample from an uncontaminated area, whereas the sea water from Penbron shows a contamination level similar to that of Seine estuary waters known to be highly contaminated (Fernandes et al. 1997; Tronczynski et al. 1999). Despite different PAH contamination levels, sea waters from Grand Bal, Plinet, Pouligen and Penbron have molecular indices (Fluo/Pyr, PAH/Methyl-PAHs (Phe/C1-Phe, Chrys/C1-Chrys, etc.)) lower than 1, which is characteristic of petrogenic inputs (Soclo 1986; Garrigues et al. 1995). These results confirm the contamination of this coastal area by the *Erika* fuel. On the contrary, for Siberon and Rostu sea waters, these same molecular indices are higher than 1, showing a major pyrolytic input for PAHs.

All sea waters (except Penbron) show good biological quality, with percentages of abnormal larvae ranging from 5 to 11%, which are similar to those obtained with the control waters (Fig. 5). On the contrary, sea water from Penbron induces more than 50% abnormal larvae. These observations are in agreement with previous chemical analysis results. Penbron is the most heavily impacted site with the highest PAH contamination level and is characterized by a petrogenic-type contamination. These results confirm that the *Erika* oil release resulted in a risk for pelagic organisms.

Table 1. PAH contents (ng L^{-1}) and molecular indices for tested sea waters.

	Grand Bal	Plinet	Pouligen jetée	Pouligen ponton	Siberon	Rostu	Penbron
Σ PAHs ng L^{-1}	14	15	7	4	10	7	18
Chrys/C1-chrys	0.4	0.2	0.2	0.3	0.9	0.6	0.2
Fluo/Pyr	0.9	0.5	0.5	0.8	1.3	1.1	0.5

Table 2. Range of PAH concentrations (ng g^{-1} of dry weight) and molecular indices (Chrys/C1-Chrys) of sediments that were sampled monthly from April to August 2001 and on June 2003 in Traict du Croisic; nd: not determined.

	April 01	May 01	June 01	July 1	August 01	June 03
			Penbron			
Σ PAHs (16) ng g^{-1}		10	8	38	21	
Chrys/C1-chrys		0.2	0.4	0.3	0.5	
			Siberon			
Σ PAHs (16) ng g^{-1}	1550	3500	470	2300	2340	150
Chrys/C1-chrys	nd	1.9	1.6	1.5	1.5	1.4
			Plinet			
Σ PAHs (16) ng g^{-1}	570	710	1230	1980	1520	440
Chrys/C1-chrys	nd	0.4	0.6	0.9	0.6	0.9

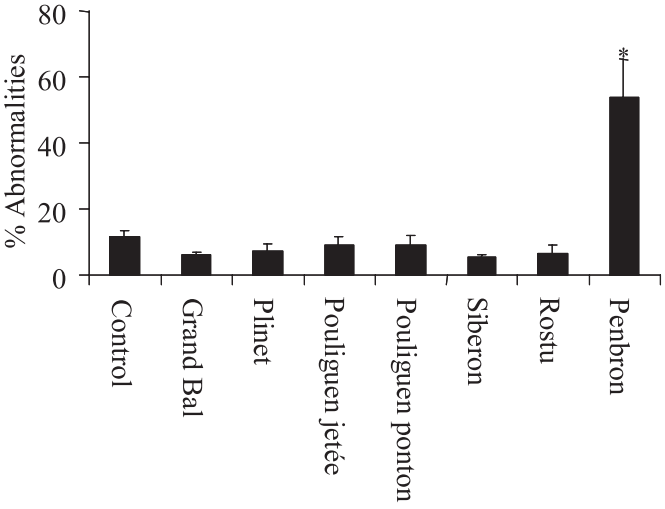

Fig. 5. Toxicity of sea waters sampled at different Traict du Croisic sites. Percentage of *Crassostrea gigas* abnormal larvae (± standard deviation; $n = 5$). *: value significantly different from the control.

Siberon and Plinet sediments are more contaminated than Penbron sediment (Table 2). The difference can be partly explained by Penbron sediment being more coarse-grained than Siberon and Plinet sediments. In all cases, molecular indices (Chrys/C1-Chrys; Garrigues et al. 1995) for Siberon were higher than 1, which is characteristic of pyrolytic inputs. On the contrary, the molecular indices were lower than 1 for Penbron and Plinet, indicating the occurrence of petrogenic PAHs.

Embryotoxicity testing with oysters and decanted sediment can evaluate the toxicity of bio-available contaminants adsorbed to the sediment and/or dissolved in the water microlayer at the sediment–water interface (Geffard et al. 2004a). According to physical and chemical results, Penbron sediment did not induce significant ($p = 0.329$) biological effects on the embryonic development of *C. gigas* (Fig. 6). On the contrary, Siberon and Plinet sediments had a marked toxicity on oyster embryos. The percentages of abnormalities obtained for Siberon sediment (not impacted by the *Erika* oil) can be related to the PAH contamination levels, showing the high heterogeneity of the sampling site. For Plinet sediments, the toxicity remained constant between April 2001 and August 2001 and then decreased in June 2003 when abnormal larvae were only observed for the highest concentration. The results show that the impact of *Erika* oil (contamination and potential toxicity) at the Plinet site was lower in 2003 than in 2001. In 2001, Plinet sediment was less contaminated than Siberon sediment, but it was the most toxic, with abnormalities ranging from 20 to 40% at a concentration of 1 g L^{-1}. These results can be explained by the PAH contamination sources. Some authors have shown the influence of sediment-contact time on the bio-availability of sediment-associated organic compounds (Harkey et al. 1995; Lamoureux and Brownawell 1999; Leppänen and Kukkonen 2000). PAHs of petrogenic origin are recent and are more bio-available, whereas PAHs of pyrolytic origin are highly adsorbed and associated with the sediments, and consequently are less bio-available (Thorsen et al. 2004).

4 Conclusion

Erika oil is potentially more toxic and induces more PAH release in the sea water than the crude oil Bal-250. Several days' weathering in the ocean did not highly affect the toxicity

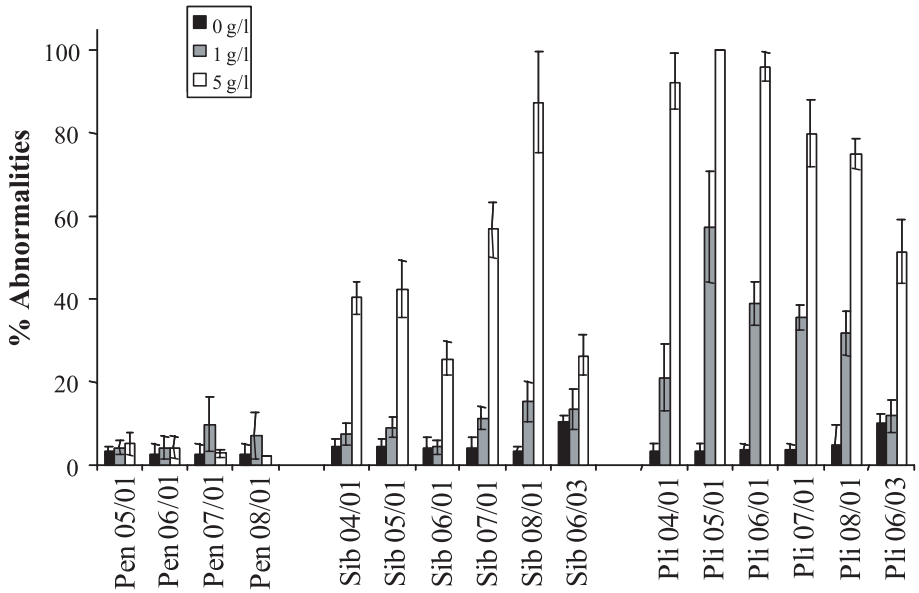

Fig. 6. Toxicity of freeze-dried sediments sampled at different Traict du Croisic sites. Percentage of *Crassostrea gigas* abnormal larvae (± standard deviation; $n = 5$. Pen: Penbron; Sib: Siberon; Pli: Plinet.)

of *Erika* oil. After a natural light exposure of 7 days, the PAH contamination level and the toxicity of *Erika* elutriate greatly decreased. Photo-oxidated PAHs seem to be less toxic than PAHs themselves. Sea waters and sediments from Traict du Croisic were impacted by the *Erika* oil spill.

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