UPTAKE OF FOUR PAHS OCCURRING INTO AERIAL PLANT TISSUE ARTIFICIALLY EXPOSED TO HEAVY FUEL OIL IN *SALICORNIA*

Anna Meudec[1], Jacques Dussauze[2], Eric Deslandes[1], Nathalie Poupart[1].

ABSTRACT

Coastal salt-marsh vegetations are directly exposed to accidental marine pollution by oil spill, as it was the case in winter of the year 2000 following Erika tanker oil spill in France. As petroleum is incorporated in sediment, it tends to coat aerial parts of plants. Among fuel hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) are the most toxic compounds known in marine organisms. Although their low water solubility, they can be taken up and bioaccumulated by plants. This work was conducted to determine whether PAHs, like naphthalene, phenanthrene, pyrene and benzo(a)pyrene, present in artificially fuel contaminated soils are transferred or not to aerial part of the coastal and edible plant, Salicornia fragilis. Sediments were mixed up with N°6. heavy fuel oil. Young plants of Salicornia were sampled in spring at the "Aber du Conquet" (Finistère, France), and cultured artificially in three different mixture conditions of oil and sediment: 0.2%, 2% or 20%. Two durations of culture were tested: one or five weeks. At the end of the culture, shoot are then cut off and PAHs concentrations were determinate by (GC-MS). Results showed that whatever the time of exposure and the concentrations of fuel oil in soil, significant PAHs concentrations were measured in Salicornia tissues. Phenanthrene and pyrene are the most abundant compounds. The particular morphology of Salicornia plants and the absence of PAHs in control also suggest that root uptake was the main pathway for accumulation of PAHs in this halophytic plant. By this capacity to uptake PAHs from fuel oil contaminated soil, Salicornia fragilis appears as a potential bioindicator of marine pollution by petroleum and may have a role in remediating contaminated soil.

Keywords: *polycyclic aromatic hydrocarbons, PAHs; Fuel oil; Halophytic plant; Bioaccumulation; Soil*

INTRODUCTION

Oil spills posed a serious risk to the health of wetland systems throughout the world. Coastal salt-marsh vegetations can be directly exposed to accidental marine pollution by oil spill. On December the 12th, 1999, the tanker Erika sank off the coast of Brittany, leading to the spill of 20,000 tons of bunker C fuel (N°6 heavy fuel). Pollution reached about 400km of coastline. All types of habitats were concerned, especially lichens and terrestrial vegetation growing in the upper intertidal and low supratidal zones. In this case, substratum and vegetation was covered at the time with the withdrawal of the tide (Poncet, et al., 2000). Ecological studies reported that Erika's oil spill had a limited impact on vegetation but locally, chlorosis, drying and death of leaves, reduction of germination and growth or a complete mortality of plants had been observed (Meudec and Poupart, 2004; Poupart, 2004).

Effects of oil on plants result from physical effects and chemical toxicity. Physical impacts of oil on plants act primarily through the coating of aerial parts of plant and soil surface. This creates a black screen which absorb the light radiations necessary for photosynthesis and an impermeable film which by blocking stomata, modifies or makes impossible the gaseous exchange (DeLaune, et al., 1979; Pezeshki, et al., 2000). The movement of oxygen into the soil are also restricted and gaseous exchanges between roots and soil may be reduced. The chemical effects of petroleum are due to the toxic compounds which compose it. Fuel oil is a complex mixture of hydrocarbons (paraffins, naphtenes, olefins and aromatics), resins and metals (Baker, 1970). Among this compounds, polycyclic aromatic hydrocarbons (PAHs) are an important class of marine contaminants and are the most toxic compounds known in marine organisms (Van Overbeek and Blondeau, 1954). They can affect physiological mechanisms such as photosynthesis, respiration, transpiration and nutrients absorption (Baker, 1970; Chaîneau, et al., 1997).

Studies reported that PAHs can be taken up by plants leaves through air deposition in polluted atmosphere or by partitioning from PAH contaminated soil to the roots (Simonich and Hites, 1994, 1995; Kipopoulou, et al., 1999; Fismes, et al., 2002; Tao, et al., 2004). Indeed, caused to their high lipophility, they are adsorbed on leaves cuticle and passed through it by solubilization in waxes or adsorbed on root suberine cortical zones and absorbed by roots cell (Simonich and Hites, 1994; Kipopoulou, et al., 1999; Fismes, et al., 2002). The aim of this study is to investigate the uptake of fuel oil PAHs by an halophytic plant and the bioaccumulation of this compounds into shoot tissues in the upper aerial part of the plant.

The halophytic species *Salicornia fragilis*, named glasswort, has been chosen as plant model. This annual plant of the littoral coast is an edible plant, harvesting by local population. Caused to their low localization on intertidal area, they can be potentially exposed to oil spill. Although numerous data exist about ecologi-

[1] Laboratoire d'Ecophysiologie et de Biotechnologie des Halophytes et des Algues Marines (LEBHAM), Institut Universitaire Européen de la Mer – Université de Bretagne Occidentale (U.B.O), France.
anna.meudec@univ-brest.fr, eric.deslandes@univ-brest.fr, nathalie.poupart@univ-brest.fr
[2] Laboratoire d'Analyse de Brest Océan- Pôle Analytique des Eaux, France.
polluants.organiques@pae-brest.fr

cal impact of an oil spill on terrestrial vegetation, particularly on salt marshes, there is a lake of data on the possible transfer of PAHs from petroleum fuel oil contained in sediment to aerial parts of halophytic plants through the root system.

MATERIAL AND METHODS

Plant material

Young plants of glasswort were sampled in spring 2003 (May, the 7th) in a natural salt-marsh, "Aber du Conquet" (Finistère, France). This site is dominated by a population of *Salicornia fragilis*. The plants present four segments and a shoot length of 2,5 cm in mean.

For the experiments, *Salicornia* plants are grown in greenhouse. After a washing, they are placed individually in bucket containing natural sediment sampled *in situ*. Cultures are placed in vats and a nutritive solution was added. Salinity of mineral solution is fixed artificially with NaCl.

Artificial exposure to fuel oil

The artificial exposures to fuel oil are realized using heavy N°6 fuel oil. Natural substratum of *Salicornia* collected *in situ* is manually mixed up with fuel oil. Each contaminated sediment condition is then separated and placed into buckets. For each experiment, control plants represented by twenty plants grown in uncontaminated sediments were realized.

In first experiment, one kilogram of sediment is mixed up with 2 g of petroleum (i.e. 0.2% fuel oil in sediment). To monitor the uptake of PAHs in *Salicornia* tissues according to the time of exposure, plants have been grown five weeks with this treatment.

Second experiments studied the accumulation of PAHs according to the degree of soil contamination. For that, one kilogram of sediment is mixed up with 2, 20 and 200g of petroleum and twenty plants were grown only one week under these conditions.

Analysis of Polycyclic Aromatic Hydrocarbons in Glasswort tissues

Weekly, unoiled aerial green (i.e. without any trace of petroleum on surface tissues) parts are cut off the plant for the assays of PAHs by gaseous chromatography coupled to mass spectrometry (GC-MS) according to the method developed by the authors (unpublished data). All plant samples were stored in aluminium foil at –20°C.

Extraction of PAHs is performed by saponification. Five grams of frozen tissues are crushed in ethanol containing internal standard of deuterated Benzo(a)Pyrene (BaP D12). After addition of KOH, the sampled are shaken one hour at 140 tr/min and placed three hours at 80°C. The extracted hydrocarbons are then recovered by addition of hexane. By addition of distilled water, the hexanic phase is recovered and purified on a florisil column with hexane/dichloromethane (80/20) (unpublished method). Extracts are concentrated by evaporations and analyzed GC-MS according to the method SIM (Single Ion Monitoring). In this study, naphthalene, phenanthrene, pyrene and benzo(a)pyrene were identified based on the retention time and the ion m/z ratio and quantified as referred to the internal standard BaP D12 (unpublished method).

RESULTS

Among the sixteen EPA PAH, the accumulation of only four of thems was described in this study : naphthalene, phenanthrene, pyrene and benzo(a)pyrene. Naphtalene is composed by two benzenic rings and is the most volatiles PAHs quantified. Phenanthrene and pyrene are composed respectively by three and four benzenic rings and are generally the most abundant PAHs in environment. Benzo(a)pyrene, the most carcinogenic PAHs, is composed by five benzenic rings. These four PAHs belong to sixteen priority PAHs described by USEPA. Their lipophility, water solubility, volatility and bioavailability depend directly on the number and the configuration of benzene rings. Their concentrations were quantified by GC-MS in tissues of *Salicornia* plants exposed to heavy fuel oil contaminated soil. Only shoots showing no visual fuel oil traces were cut off.

By preliminary observations, the culture of plants in 0.2% fuel oil/sediments mixture appeared as the best condition for the study of PAHs accumulation in time. Indeed, growth and development of *Saliconia* plants seems to be affected by petroleum. As most of plants grown on 2 and 20% of fuel oil died after two weeks of treatment, the time of exposure of the plants in these conditions was limited to one week. Consequently, the study of accumulation of PAHs according to the concentration of petroleum in sediments was performed only during one week.

Time monitoring of PAH content in *Salicornia* tissues was carried out during five weeks of culture on 0.2% fuel oil. After one week of exposure, the most abundant compounds were phenanthrene and pyrene. Their concentrations were significant and reached respectively 656.9 and 1352.7 µg/kg dry weight (dw). After four weeks, a 2 and 5 fold increase concentrations were found, respectively (Table 1). By contrast, low proportions were observed for naphthalene and benzo(a)pyrene : 166.8 and 17.4 µg/kg dw after one week of exposure and 431.9 and 93.9 µg/kg dw after four weeks. As observed on the figure 1, the distributions of these four PAHs were rather similar after one and four weeks of culture. However, a light decrease of naphthalene and phenanthrene was observed after four weeks of treatment.

Table 1 : PAH concentrations in Salicornia tissues grown five weeks with 0.2% of fuel oil in sediments (mg/kg dw) (n=20)

	Control	one week	two weeks	three weeks	four weeks	five weeks
naphtalene	<1,0	166.8 ± 18.1	191.5 ± 9.7	322.9 ± 20.5	358.1 ± 25.5	431.9 ± 6.1
phenanthrene	0.55 ± 0.12	656.9 ± 7.6	541.5 ± 64.4	1166.7 ± 27.7	1257.8 ± 46.3	1082.4 ± 28.2
pyrene	<0.5	1352.7 ± 69.2	3303.4 ± 328.2	3738.5 ± 354.9	4606.0 ± 365.7	4395.0 ± 243.9
benzo(a)pyrene	<2.0	17.4 ± 1.7	11.7 ± 1.6	15.6 ± 2.2	93.9 ± 6.6	114.7 ± 6.9

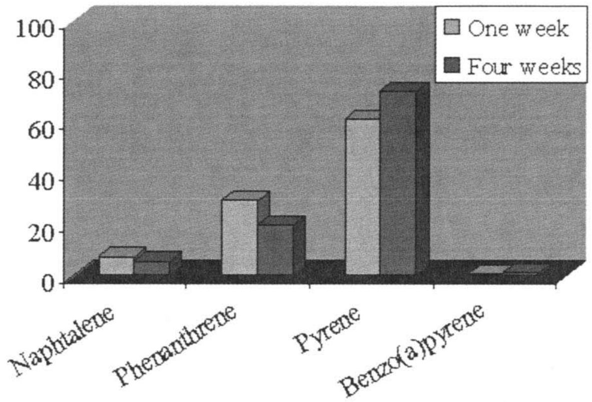

FIGURE 1: DISTRIBUTIONS (% OF THE CONCENTRATION TOTAL THE FOUR PAHS) OF NAPHTHALENE, PHENANTHRENE, PYRENE AND BENZO(A)PYRENE IN *SALICORNIA* TISSUES AFTER ONE WEEK AND FOUR WEEKS OF EXPOSURE TO CONTAMINATED SOIL

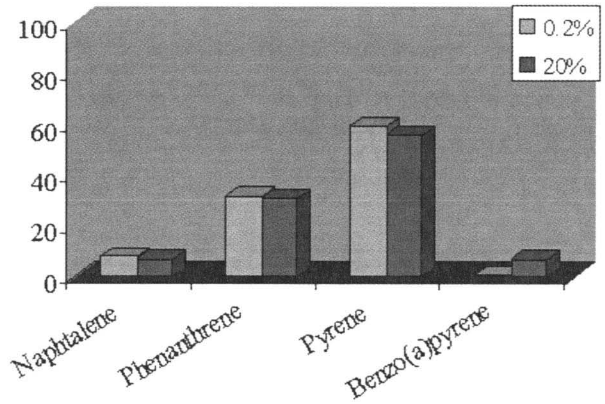

FIGURE 2 : DISTRIBUTIONS (% OF THE CONCENTRATION TOTAL THE FOUR PAHS) OF NAPHTHALENE, PHENANTHRENE, PYRENE AND BENZO(A)PYRENE IN SALICORNIA TISSUES ACCORDING TO THE RATE OF FUEL OIL CONTAMINATION OF SOIL

The study of the accumulation of PAHs according to the quantity of fuel oil in sediments was carried out by culture of *Salicornia* plants under 0.2, 2 or 20% of fuel oil during one week. Table 2 lists the concentrations of naphthalene, phenanthrene, pyrene and benzo(a)pyrene in the shoots of *Salicornia* plants grown. Phenanthrene and pyrene are the dominant compounds in the three exposure conditions tested.. After one week of culture on 2% oiled sediments, pyrene concentration (4195.4 µg/kg dw) is similar to those measured after five weeks on 0.2% (4606 µg/kg dw) (Table 2). When plants were grown with 20% of petroleum, tissues presented significant levels of almost all four PAHs. For example, concentration of benzo(a)pyrene which was lower (17.3 µg/kg dw) at 0.2%, reached 1869.5 µg/kg at 20% (Table 2). Despite the significant difference between the concentrations of these PAHs, distribution in *Salicornia* tissues are similar (Figure 2), excepted for benzo(a)pyrene which represented 0.8% of the total concentration under 0.2% fuel oil and 6.8% under 20%. These profiles are also rather similar to those observed in the monitoring of PAHs uptake during five weeks.

For each experiment, controls cultivated in sediments without addition of petroleum were realized. No significant PAHs concentrations were detected in all controls tested (n=20).

DISCUSSION

These results show that plants are able to uptake and to bioaccumulate polycyclic aromatic hydrocarbons originated from oil mixed to the sediments. This bioaccumulation depends on the time of exposure and on the quantity of fuel oil added to sediment. In this halophytic plant, the uptake by roots of PAHs contained in fuel oil contaminated sediments seems to be the main pathway. The particular morphology of *Salicornia* plants (no real leaf) and the absence of PAHs in control also suggest that PAHs detected are not originated from atmospheric contamination. Recovered PAHs in plants can be adsorbed both on root suberine cortical zones (lipophilic constituents) and by roots cells and subsequently transferred to the shoots (Fismes, et al., 2002). The different PAHs distribution suggests that low molecular weight PAHs, like phenanthrene and pyrene due to their higher solubility, are transported more easily in the phloem than high molecular weight PAHs like benzo(a)pyrene. Fismes et al. (2002) proposed that the transport of low molecular weight PAHs from soil through root system to aerial parts could be passive and driven by transpiration flux. By contrast, high molecular weight PAHs are more easily sequestered in cellular membranes but may slowly travel in the intercellular spaces (Baker, 1970; Chaîneau, et al., 1997). Caused to their high volatility, naphthalene may be lost from soil

Table 2 : PAH concentrations in *Salicornia* tissues grown one week with 0.2, 2 or 20% of fuel oil in sediments (µg/kg dw) (n=20)

	control	0.2%	2%	20%
naphtalene	<1.2	179.0 ± 8.1	578.4 ± 39.4	1863.7 ± 211.2
phenanthrene	0.6 ± 0.0	720.6 ± 88.0	1385.0 ± 43.1	8427.8 ± 546.2
pyrene	<0.5	1350.9 ± 97.7	4195.4 ± 63.5	15377.4± 1305.9
Benzo(a)pyrene	<2.0	170.3 ± 2.4	719.0 ± 123.0	1869.5 ± 95.4

and while its very low molecular weight PAHs, have low concentrations in plants tissues.

Reports on the ecological effect of an oil spill on salt-marsh plants are numerous but there is no data on the possible uptake by plants of petroleum compounds, like PAHs or heavy metals, contained in sediments after an oil spill. Effects of pollution by oil may vary according to the type and the amount of oil involved, the extend of coverage, the time of year, and the species and age of plants concerned (Baker, 1970). Death of *Spartina alterniflora* has been reported when high levels of crude oils accumulated in soil or remained in the marsh for extended period (Pezeshki, et al., 2000). Although initial short-term adverse effects of oil on plants are often dramatic, vegetation may recover quickly. However, oil spill that are stranded by high tides may allow oil to penetrate and accumulate in the soil. It appears that soil type and soil organic matter play important roles in the fate of petroleum hydrocarbons in soil, the extent of damage to vegetation and the rate of recovery of marsh plants. In saline marshes, slower degradation rates might then increase the time of exposure to toxins (Pezeshki, et al., 2000). In this case, although fuel oil is no more visible on the soil surface, plants, annual or perennial, are always exposed to the pollutant and may accumulated PAHs during a long time after oil spill. As described by Poupart (2004), after four years of monitoring, a long term persistence of the Erika fuel is locally observed in the polluted environment. For the halophytic plants growing in Erika sites previously exposed to fuel oil, PAHs were still detected in autumn 2003.

CONCLUSION

The ability of *Salicornia fragilis*, an halophytic plants living in marine coast, to uptake rapidly fuel oil PAHs is very interesting. *Salicornia fragilis* appear as a potential bioindicator for PAH detection in soil. Indeed, as pollutants concentrations in vegetation have been used to indicate atmospheric contamination levels, to identify point sources of organic pollutants or to determine regional global contamination of organic pollutants (Simonich and Hites, 1995), halophytic plants could be used to evaluate and monitor, in time and space, the impact of an oil spill. Moreover, *Salicornia* plants take up higher amounts of PAHs and could be used in phytoremediation programs.

ACKNOWLEDGEMENTS

The authors would like to thank the "Ministère de l'Ecologie et du Développement" Durable, for funding this work. We are grateful to Mr Ronan Jezequel from the "CEDRE" for giving us the fuel oil.

BIOGRAPHY

Actually in third year of Ph.D in biological oceanology at the University of Occidental Brittany (Brest, France), I am working on the effects of petroleum compounds on an halophytic plants, *Salicornia*. Physiological, biochemical, cellular and molecular aspects are examined. In the same time, bioaccumulation of fuel oil compounds into *Salicornia* tissues is studied.

REFERENCES

Baker, J.M., 1970. The Effects of Oils on Plants. Environmental Pollution 1, 27-44.

Chaîneau, C.H., Morel, J.L., Oudot, J., 1997. Phytotoxicity and Plant Uptake of Fuel Oil Hydrocarbons. Journal Of Environmental Quality 26, 1478-1483.

DeLaune, R.D., Patrick, J., W. H., Buresh, R.J., 1979. Effect of crude oil on a Louisiana Spartina alterniflora salt marsh. Environmental Pollution (1970) 20, 21-31.

Fismes, J., Perrin-Ganier, C., Empereur-Bissonnet, P., Morel, J.L., 2002. Soil-to-Root Transfer and Translocation of Polycyclic Aromatic Hydrocarbons by Vegetables Grown on Industrial Contaminated Soils. J Environ Qual 31, 1649-1656.

Kipopoulou, A.M., Manoli, E., Samara, C., 1999. Bioconcentration of polycyclic aromatic hydrocarbons in vegetables grown in an industrial area. Environmental Pollution 106, 369-380.

Meudec, A., Poupart, N., 2004. Suivi écophysiologique et écotoxicologique des peuplements végétaux de la zone à halophiles soumise aux conséquences de la marée noire de l'Erika. Rapport d'activité Programme " suivi Erika ". IUEM-UBO, Brest, 84pp

Pezeshki, S.R., Hester, M.W., Lin, Q., Nyman, J.A., 2000. The Effects of Oil Spill and Clean-up on Dominant US Gulf Coast Marsh Macrophytes : a Review. Environmental Pollution 108, 129-139.

Poncet, F., Lachaud, A., Le Bail, J., Tintilier, F., Ragot, R., 2000. Impact de la marée noire de l'Erika. Conservatoire Botanique National de Brest, 57p

Poupart, N., 2004. Erika oil spill impact on terrestrial vegetation : ecologic, ecophysiology and ecotoxicology aspects. Aquatic Living Ressources. *In preparation.*

Simonich, S.L., Hites, R.A., 1994. Vegetation-Atmosphere Partitioning of Polycyclic Aromatic Hydrocarbons. Environmental Science & Technology 28, 939-943.

Simonich, S.L., Hites, R.A., 1995. Organic pollutant accumulation in vegetation. Environmental Science & Technology 29, 2905-2914.

Tao, S., Cui, Y.H., Xu, F.L., Li, B.G., Cao, J., Liu, W.X., Schmitt, G., Wang, X.J., Shen, W.R., Qing, B.P., Sun, R., 2004. Polycyclic aromatic hydrocarbons (PAHs) in agricultural soil and vegetables from Tianjin. Science of The Total Environment 320, 11-24.

Van Overbeek, J., Blondeau, R., 1954. Mode of Action of Phytotoxic Oils. Weeds 55-65.