
Organic and inorganic human-induced contamination of *Posidonia oceanica* meadows

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Abstract :

In coastal environments, plants are used for phytoremediation of contamination. Organic and inorganic contaminants may be due to natural and/or anthropogenic sources. The aim of this study is to compare inorganic (trace metal) and organic (PAH) contamination in *Posidonia oceanica* and to analyse the relationship between these types of pollutants indeed very few studies have been interested in their correlations and common sources. *P. oceanica* leaves were collected in two sites exhibiting different levels of human-induced pressure. Higher values were recorded in the more polluted site (Toulon) for trace metals (Ag, Hg, Pb) as well as for PAHs (Medium Molecular Weight and High Molecular Weight) due to the presence of the city and/or harbour in proximity. For the first time in a coastal environment, correlations were observed between metals and PAHs.

Keywords : Trace metals ; Polycyclic aromatic hydrocarbons ; Contamination ; Seagrass ; *Posidonia oceanica* ; Mediterranean

1. Introduction

The Mediterranean sea, characterised by a rich biodiversity (7% of world marine species in 0.8% of the ocean surface), is threatened (Benoit & Comeau, 2005; EEA, 2006). Indeed, its coastal area is characterised high anthropogenic activities such as tourism, transport and industrial activities; responsible of chemical contaminations such as trace metals and hydrocarbons (Benoit & Comeau, 2005). Some of these substances present accumulative properties, as well as in some cases mutagenic and/or carcinogenic properties (Belpomme et al., 2007).

Trace metals are introduced in costal environments through run-off and atmospheric deposition (Benoit & Comeau, 2005). They may be present as natural compounds but they have also been linked to domestic, agricultural and industrial activities (Murray et al., 2004). Mercury (Hg) and lead (Pb) are listed as priority substances of the European Union Water Framework Directive (WFD) and as priority pollutant by several international organizations (e.g. US Environmental Protection Agency, Barcelona Convention). Silver (Ag), very toxic compound, is produced by metallurgy, industries and introduced in sea by run-off and sewer (Kantin & Pergent, 2007).

Among organic contaminants, hydrocarbons produced by terrestrial sources are more represented than those produced accidentally or operationally (Benoit & Comeau, 2005; NRC, 2003). For instance, Polycyclic Aromatic Hydrocarbons (PAHs) are naturally produced (e.g. natural seep) but contamination is mainly of anthropogenic origin (e.g. extraction, transportation and consumption of petroleum; NRC, 2003).

There is currently a great interest in the use of living organisms as pollution biomonitors in aquatic ecosystems (Goldberg, 1986; Pergent-Martini & Pergent, 2000; Usero et al., 2005). Most of these studies are based on the use of caged mussels, *Mytilus galloprovincialis* (Andral et al., 2004; Baumard et al., 1998a; Stella et al., 2002). However, in coastal environments seagrass are naturally widespread (Short et al., 2001) and have already been adopted for a number of monitoring purposes (trace metals: Lafabrie et al., 2007; radionuclide: Baysal & Tuncer, 1994; Calmet et al., 1991; Warnau et al., 1996; PCB: Doust et al., 1994; Haynes et al., 2000). Plants are also used for phytoremediation (Huesemann et al., 2009; Lin & Mendelssohn, 2009; White et al., 2002). The Mediterranean endemic seagrass, *Posidonia oceanica* (L.) Delile, is used in a number of monitoring programs (Calmet et al., 1991; Lopez y Royo et al., 2007; Pergent et al., 2007) and is acknowledged to be a powerful metal bioindicator (Lafabrie et al., 2007; Pergent-Martini & Pergent, 2000).

Even if trace metals and PAHs are currently used in monitoring programs (Andral et al., 2004), very few studies have been interesting in their correlations and common sources (Alfani et al., 2001; Maisto et al., 2004). The aim of this study is to compare inorganic (trace metal) and organic (PAH) contamination in *P. oceanica* leaves, and to identify the relationship between these types of pollutants, in order to constitute a first step in the identification of contamination sources and fundamental data for ecological restoration and reconstruction design.

2. Materials and methods

21 orthotropic shoots of *Posidonia oceanica* were collected by scuba diving, at 10±2m depth, in October 2006, in two sites of the north-western Mediterranean Sea. Toulon (France, Fig. 1), in the vicinity of a large city with an industrial and military harbour, present high concentration in trace metals and PAHs (Andral et al., 2004). Calvi (France - Corsica, Fig. 1), a pristine site close to the Fango UNESCO Man And Biosphere Reserve. Corsica present low contamination levels of trace metals (Andral et al., 2004; Lafabrie et al., 2008) and PAHs (Andral et al., 2004).

For each site, collected shoots were divided into 3 replicates on a pool of adult leaves. Only the blades of adult leaves were retained, as it has been previously reported that most trace metals and PAHs accumulate preferentially in these tissues (Lafabrie et al., 2007). Epiphytes and sediments were removed from the blades using a glass slide. Samples were rinsed (ultrapure water), frozen (-20°C), freeze-dried (> 72h in Heto[®] FD4-85 freeze dryer, HetoHolten A/S) and then manually reduced to a coarse powder, for further metal and PAH analyses.

Mercury: 50 mg of each sample was weighed in a Teflon digestion vessel CEM[®] ACV of 100 ml (CEM Corporation, USA). 5 ml of 69% HNO₃ (Normapur 20 428.297 Prolabo[®]) and 1 ml of H₂O₂ 30% (Normapur 23 619.297 Prolabo[®]) were added. The vessels were sealed and placed into the CEM[®] MARS 5 chamber. The content of each vessel was poured into 25 ml volumetric flasks and diluted to volume with ultrapure water and then transferred to 60 ml polypropylene flasks. Mineralized samples were analysed with a cold vapour atomic absorption spectrometer (CV-AAS - Perkin Elmer[®]) equipped with a flow injection system (FIMS 100) and an autosampler (AS-90). A carrier solution of 5% (v/v) nitric acid and a reducing solution of 1.1% (p/v) tin chloride (23 742.260 Prolabo[®]) and 0.5% (p/v) hydroxylammonium chloride (24 708.235 Prolabo[®]) in 3% (v/v) hydrochloric acid (20 253.293 Prolabo[®]) were used. The standard addition method was applied for calibration. Calibration standards were prepared from a mercury standard solution of 1 000 mg.L⁻¹ (30 130.263 Prolabo[®]).

Lead and silver: The analyses of these elements were run with quality assurance procedures at the Laboratory of Rouen / ETSA (Rouen, France). They were performed using a graphite furnace atomic absorption spectrometer (GF-AAS).

The analytical procedures were verified using the certified reference material *Lagarosiphon major*, CRM 60 (Community Bureau of Reference - Commission of the European Communities).

PAH were extracted from 0.5 g (dry wt.) of *Posidonia oceanica* leaf powder using an accelerated solvent extractor (ASE 200, Dionex[®]). They were reduced and purified on a silica and a alumina spe column. The solution obtained was retaken in 1 ml of acetonitrile (hypergrade, Merk[®]). Extracts were analysed by High Performance Liquid Chromatography (HPLC; Dionex[®]) together with a fluorescence detector (RF 2000 Fluorescence detector, Dionex[®]) and an UV detector (UVD170U, Dionex[®]).

Sixteen PAHs were measured: naphthalene (Nap), acenaphtylene (Acy), acenaphthene (Ace), fluorene (Flr), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(a,h)anthracene (DahA), benzo(g,h,i)perylene (BghiP), indeno(1,2,3-cd)pyrene

(Ind, PAH-mix9 in acetonitrile Cluzeau®). Total PAHs was determined by the sum of these PAHs.

The analytical procedure was verified using certified reference material (*Fucus* sp, IAEA-140/OC) provided by the Analytical Quality Control Service (International Atomic Energy Agency).

In order to determine differences between sites, a Man-Whitney U test was performed on each measured compound. Correlations were determined by Spearman rank test.

3. Results

Ag, Hg and Pb concentrations are significantly higher in Toulon than in Calvi (Table I). Toulon presents 1.4 times higher Ag concentration than Calvi, 3.8 times higher Hg concentration, and 2.0 times higher Pb concentration (Table I).

Among the 14 PAHs detected, three of them exhibit significantly higher concentrations in Toulon (Phe $p < 0.10$, BghiP $p < 0.05$ and Ind $p < 0.05$) and 9 present maximum values in this site (Table II). Moreover, the sum of Medium Molecular Weight and the sum High Molecular Weight PAHs are significantly higher in Toulon (Man and Whithney U test, respectively $p < 0.10$ and $p < 0.05$).

4. Discussion and conclusion

Hg and Pb concentrations in *Posidonia oceanica* blades of Calvi are in same order than those measured along the Corsican coastline (Lafabrie et al., 2008). A study of Florida seagrasses recorded PAH contamination below detection levels in all tissues (Lewis et al., 2007). Whereas, a study on *Posidonia oceanica* leaves record higher concentration of PAHs (Bucalossi et al., 2006) may be due to sites, studied PAHs and seasonal variation. Toulon higher values are in accordance with a previous study carried out on mussels (*Mytilus galoprovincialis*), in which this site presented higher metal (Hg, Pb) and Flt (a MMW PAH) concentrations than Corsican sites (Andral et al., 2004).

Ag, Hg and Pb concentrations in Toulon confirm the anthropic pressure of the site (Andral et al., 2004) and the potential of *Posidonia oceanica* as a biointegrator of metal contamination (Campanella et al., 2001; Lafabrie et al., 2007; Marin-Guirao et al., 2005; Pergent-Martini & Pergent, 2000). In the same way, PAHs, and specifically Medium Molecular Weight (MMW) and High Molecular Weight (HMW) PAHs, exhibit higher concentrations in Toulon. Concentrations of MMW and HMW PAHs have been reported as related to human activities (Baumard et al., 1999).

In addition, Ag and Pb present a high positive correlation (Table III). These metals are used in pressure evaluations of urban and/or industrial centers (Murray et al., 2004; O'Connor, 1998). Indeed, Pb is related to atmospheric inputs (UNEP, 2006) and to gasoline (Zhang et al., 2009), and Ag to sewage treatment (Rozan & Hunter, 2001). The significant presence of these two pollutants is thus linked to the city of Toulon and

its industrial activities. This is in agreement with a study of Andral et al. (2004) which report more marked contamination in the vicinity of major urban and industrial centers (Gulf of Fos, Marseille, Toulon).

Concerning PAHs, BghiP is correlated to Phe and Ind (Table IV); moreover, HMW and MMW PAHs are correlated at 0.90 (spearman <0.05). Thus, human-induced pressures could be recorded by these types of PAHs.

Several ratios between specific PAHs can be used to determine the origin (petrogenic and/or pyrolytic) of these contaminants (Table V). In the present study, PAHs recorded in Toulon appear to be of petrogenic origine.

Moreover, correlations are observed between the inorganic and organic contaminants. Indeed, significant correlations are observed between (i) BghiP and the three metals, (ii) Ind and Ag/Pb, and (iii) Phe and Hg (Tab III).). Concerning the molecular weight, a significant positive correlation is observed between (i) the three metals and the High Molecular Weight (HMW), and (ii) Hg and Medium Molecular Weight (MMW) (Table VI).

Only few studies focused on the relationship between trace metals and PAHs contamination. In the *Quercus ilex* L. leaves, the correlation between sum of trace metals (Cd, Ni, Pb, V, Zn) and sum of PAHs is an evidence of vehicular traffic (Alfani et al., 2001). In terrestrial soil, the positive correlation between sum of 27 PAHs and Pb, Cu, Zn suggest a common source: vehicular traffic and its consequent deposition (Maisto et al., 2004). For the first time, a similar correlation is observed in a coastal environment, by using a seagrass bioindicator.

These study underline the ability of the plant to accumulate heavy metals and PAHs from its surrounding environment in its leaves. Death leaves stay in situ or are exported to other ecosystem (Pergent et al., 1994). These results could constitute fundamental data for ecological restoration, reconstruction design or further phytoremediation test.

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Tables

Table 1. Trace metal concentrations (mean \pm S.E.; in $\mu\text{g}\cdot\text{g}^{-1}$ dry wt.; **: $p < 0.05$).

	Toulon	Calvi
Ag**	0.7 \pm 0.0	0.5 \pm 0.0
Hg**	0.15 \pm 0.03	0.04 \pm 0.01
Pb**	3.0 \pm 0.1	1.5 \pm 0.3

Table 2. PAHs concentrations (mean \pm S.E.; in $\mu\text{g.kg}^{-1}$ dry wt.; -: bellow detection level; **p<0.05, *p<0.10)

	Toulon		Calvi	
<i>Low Molecular Weight</i>				
Nap	-		-	
Acy	-		-	
Ace	4.4	\pm 0.6	5.2	\pm 1.1
Flr	19.3	\pm 1.9	20.8	\pm 5.7
<i>Medium Molecular Weight</i>				
Phe*	84.6	\pm 13.0	53.2	\pm 6.6
Ant	2.3	\pm 0.3	2.7	\pm 0.5
Flt	18.7	\pm 1.6	15.8	\pm 2.7
Pyr	18.6	\pm 1.0	15.0	\pm 3.0
BaA	22.8	\pm 1.2	20.1	\pm 2.0
Chry	12.0	\pm 1.1	11.7	\pm 2.5
<i>High Molecular weight</i>				
BbF	23.4	\pm 1.8	19.3	\pm 4.1
BkF	3.0	\pm 0.6	1.9	\pm 0.4
BaP	3.3	\pm 0.1	2.2	\pm 0.6
DahA	1.4	\pm 0.1	1.1	\pm 0.3
BghiP**	4.9	\pm 0.2	0.8	\pm 0.3
Ind**	8.9	\pm 0.4	0.7	\pm 0.2

Table 3. Spearman correlation coefficient between metals (**p<0.05, *p<0.10)

	Ag	Hg	Pb
Ag	1.00		
Hg	0.62	1.00	
Pb	0.90**	0.64	1.00

Table 4. Spearman correlation coefficient between significant PAHs (**p<0.05, *p<0.10)

	Phe	BghiP	Ind
Phe	1.00		
BghiP	0.87*	1.00	
Ind	0.70	0.75*	1.00

Table 5. Determination of contamination origin by the application of bibliographic ratios on *Posidonia oceanica* results (bold: more petrogenic site, **p<0.05, *p<0.10).

Ratios	<i>Posidonia</i> results		Origin		Authors
	Toulon	Calvi	Petrogeni c	Pyrolytic	
Phe/Ant*	37.05 ± 2.57	23.69 ± 1.15	>15	<10	Baumard et al., 1998b; Stella et al., 2002
Chry/BaA	0.53 ± 0.04	0.57 ± 0.06	>1	<1	Baumard et al., 1999; Stella et al., 2002
Flt/Pyr	1.00 ± 0.03	1.06 ± 0.07	<1	>1	Baumard et al., 1998b
BaP/BghiP**	0.67 ± 0.02	3.03 ± 0.66	Low	High	Stella et al., 2002

Table 6. correlation between metals and PAHs (**p<0.05, *p<0.10)

	Ag	Hg	Pb
Phe	0.67	0.90**	0.67
BghiP	0.76*	0.84**	0.88**
Ind	0.97**	0.66	0.81**
Sum	0.67	0.90**	0.67
LMW	0.09	0.31	0.46
MMW	0.67	0.90**	0.67
HMW	0.79*	0.77*	0.90**

Figures

Figure 1. Sampling sites (represented by stars) in the North-western Mediterranean sea.

