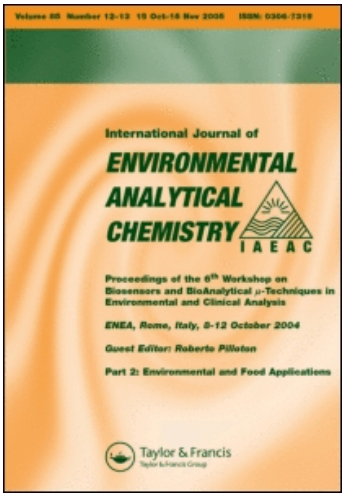

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In Situ Correlations Between Polycyclic Aromatic Hydrocarbons (PAH) and Pah Metabolizing System Activities in Mussels and Fish in the Mediterranean Sea: Preliminary Results

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IN SITU CORRELATIONS BETWEEN POLYCYCLIC AROMATIC HYDROCARBONS (PAH) AND PAH METABOLIZING SYSTEM ACTIVITIES IN MUSSELS AND FISH IN THE MEDITERRANEAN SEA: PRELIMINARY RESULTS*

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Biochemical indices based on enzymatic activities have been determined in fish and mussels sampled in various different coastal locations in the Mediterranean Sea. Preliminary results show a good agreement between biochemical measurements in marine organisms and chemical analyses of polycyclic aromatic hydrocarbons present in sediments. The results obtained suggest the use of biochemical indices for application in chemical contaminant biomonitoring.

KEY WORDS: Polycyclic aromatic hydrocarbons (PAH), carcinogenic compounds, Mediterranean Sea, *Serranus scriba*, *Mytilus galloprovincialis*, biological marker, enzyme activity, marine waters and sediments, pollution biomonitoring.

INTRODUCTION

Many analytical studies have been devoted in the past to the chemical measurements of organic/inorganic contaminants in various marine environments for the

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assessment of contamination levels.[1,2] However, biological systems such as molluscs, fishes, plants give biochemical/physiological responses as a result of a stress caused by chemical contaminant especially carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAH). Such responses, well known in fish,[3–8] involve enzymatic systems of biotransformation of toxic compounds: in the first stage, there occurs a phase of functionalization of the xenobiotics (phase I) mostly cytochrome P-450 dependant, then a phase of conjugation and/or elimination of the xenobiotic metabolites (phase II). Biochemical changes, especially related to Mixed Function Oxidases (MFO) occur even at low concentration before physiological damage. Fish metabolise PAH[3] very rapidly and this leads to an increase of MFO activities. Ultimate carcinogens could be formed then and linked to DNA. Such MFO activities in fish have been suggested by several authors as pollution bioindicators.[5,6] For molluscs, many previous studies[10–14] have also shown that components of the MFO system increase with an increased hydrocarbon gradient; however, our knowledge of mollusc enzymes needs further fundamental investigation.[11]

In these studies, enzymatic activities related to PAH biotransformation have been determined in whole mussels and livers of fish caught in various sites of the coastal mediterranean ecosystem. PAH content has been determined in marine waters and the uppermost layer of the marine sediment to investigate the capability of biochemical tests for chemical contamination biomonitoring.

EXPERIMENTAL SECTION

Sampling Sites

The oceanographic sampling cruises took place in June and September 1987 along the Côte d'Azur and on the West Coast of Corse aboard the research vessel "Winnaretta Singer" (Oceanographic Institute of Monaco). The sampling stations have been reported in Table 1 and in Figure 1. Some are characterized by high PAH concentration (Marseille, Toulon, Monaco) whereas other locations are moderately contaminated (Cannes) and also quite unpolluted (Galeria, Stareso in Corse).

Sediments and Water Samples

Sediment samples were cored using a Petersen corer.[15] Only the superficial sediment (about 10 cm) was collected and frozen aboard. The procedure for the extraction of the organic matter involving Soxhlet extraction is detailed elsewhere.[15] Water samples (81 to 12 l) were collected with bottles and stored on board in glass bottles after addition of *n*-hexane. Solvent extraction was performed in the laboratory with *n*-hexane and the extract was concentrated to about 0.5 ml. Aromatic compounds were determined on the total organic extract by High Performance Liquid Chromatography (HPLC) with fluorescence detection as reported previously.[16] The so-called PAH content represents the total concentration of 12 priority pollutant unsubstituted PAHs.[16]

Table 1 PAH content (sum of 12 PAH[16]) in waters and sediments in the sampling sites.

Sampling stations	*Water PAH(ppb) Mean ± SE*	*Sediment PAH(ng/g) Mean ± SE*
Le Planier Light house (Marseille)	4.35 ± 0.20	1165.0 ± 90
Lazaret Bay (Toulon)	ND	8535.0 ± 410
La Fourmigue Light house (Cannes)	5.40 ± 0.5	620.0 ± 50
St Marguerite Island (Cannes)	ND	470.0 ± 35
Roquebrune Bay (Roquebrune)	3.45 ± 0.45	3735.0 ± 400
Fontvieille (Monaco)	9.60 ± 0.80	3230.0 ± 190
Revelatta (Corse)	2.35 ± 0.30	35.0 ± 2
Galeria (Corse)	2.60 ± 0.30	12.6 ± 0.3
Pointe Canelle (Corse)	ND	3.0 ± 0.4

Mussels

The mussels (*Mytilus galloprovincialis*) were collected by skin divers. Each sample was composed of a pool of 1 male and 1 female. Ten samples were collected at each station for biochemical measurements. On board for each mussel, adductor and mantle retractor muscles, foot, byssus and cristalline style were removed. The rest of the mollusc was rinsed in buffer, damp-dried and frozen in liquid nitrogen.

In the laboratory, the samples were homogenized and treated as previously reported.[13,14,17] Epoxy Hydrolase (EH) and Benzo(a)PyreneMonoOxygenase (BaPMO) were determined on microsomal fraction as detailed in the previously cited references.

Fishes

The sea perchs (*Serranus scriba*) were caught by fishing. Such species have been chosen since they are hermaphrodites, thus eliminating possible variations due to sex.

The microsomal fractions were prepared as follows: on board, the livers were sliced and rapidly homogenized (pool of 2 or 3 fish livers) in a cold vol. of Tris 10 mM, sucrose 250 mM buffer pH 7.4 containing protease inhibitors and 20% (v/v) glycerol. The homogenates were frozen and stored in liquid nitrogen. In the laboratory, the homogenates were centrifuged at 10,000 g for 15 min and the supernatant collected and centrifuged at 105,000 for 60 min. The microsomal pellets were suspended in a Tris 10 mM sucrose 250 mM buffer at pH 7.4.

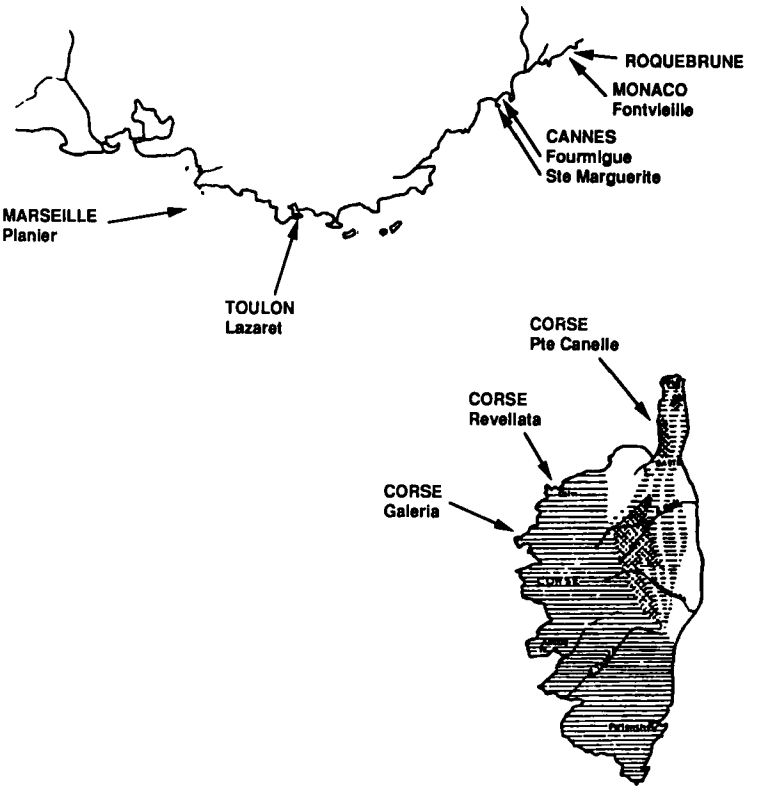

Figure 1 Locations of the coastal sampling in the Mediterranean Sea.

The stability of hepatic microsomal and cytosolic activities was investigated in order to develop a standardized freezing and storing procedure to maintain the enzyme optimum levels.[18]

EthoxyResofurin-O-Deethylase (EROD) activity was measured fluorimetrically as described by Burke and Mayer.[19] Protein content was quantified by the method of Lowry et al.[20] Cytochrome P-450 content was measured as reported previously.[21] Glutathione-S-Transferase (GST) were measured according to Habig et al.[22] All analyses were performed in duplicate and were run at 30 °C. All the enzyme reactions were performed under conditions of maximal velocity and were linear with time and protein concentration. All spectrophotometric measurements were performed with an UVIKON 930 spectrophotometer.

RESULTS AND DISCUSSIONS

Chemical Measurements

The sediment PAH content is presented in Table 1. The source of PAH in the studied coastal locations is essentially anthropogenic as evidenced by the PAH distribution criteria.[16] These compounds originate from combustion processes

Table 2 BaPMO and EH activities (per mg of microsomal proteins) measured in mussels (*Mytulis galloprovincialis*). Correlation coefficient (r) between logarithm of PAH concentration in waters (w) and sediments (s) and enzyme activity have been mentioned.

Sampling stations	*BaP MO pmole/min/mg M.P. Mean ± SE*	*EH nmole/min/mg M.P. Mean ± SE*
Le Planier Light house (Marseille)	53.4 ± 6.5	5.1 ± 1.80
Lazaret Bay (Toulon)	57.3 ± 5.3	5.9 ± 1.60
La Fourmigue Light house (Cannes)	46.1 ± 7.0	ND
Fontvieille (Monaco)	50.3 ± 6.0	ND
Revelatta (Corse)	43.4 ± 9.2	4.8 ± 1.7
Galeria (Corse)	29.7 ± 2.8	3.6 ± 0.6
Pointe Canelle (Corse)	36.4 ± 6.6	3.5 ± 1.0
log PAHs	r = 0.92	r = 0.90
log PAHw	r = 0.49	ND

(industrial activities, automobile exhaust, waste waters) and enter the marine environment via road water runoff and river transport. However, we observed a PAH gradient concentration between all the sampling sites. Certain locations highly industrialized and urbanized (Toulon, Monaco) are highly contaminated (near 10 μg/g of total PAH concentration) while others (Cannes) are moderately contaminated (near 0.5 μg/g of total PAH concentration) but nevertheless more polluted than other French coastal environments (south west Atlantic Coast). Different sampling stations in Corse appear to be good baseline stations since they are only slightly contaminated (about 0.01 μg/g of total PAH concentration). The lithology of the sediment (mud on the Côte d'Azur and sand in Corse) could also be responsible for a preferential trapping of pollutants.[16] The PAH content in waters does not reflect the PAH gradient concentration observed in the sediments and does not exhibit comparable differences among the sites.

Biochemical Indices

Mytilus galloprovincialis: Results are presented in Table 2 and showed that BaPMO and EH measurements activities were found to be significantly different ($p < 0.05$) between reference sites on Corse and other locations. A good correlation coefficient is obtained with concentration of PAH (log PAHs) in sediments. However, the dynamic range of these responses is small (maximum twice the reference values). Highly polluted locations exhibit low changes in BaPMO activities (maximum 14%) compared to reference sites whereas the change in PAH

Table 3 EROD and GST activities (per mg of total proteins) measured in fish (*Serranus scriba*). Numbers of pools of fish livers are mentioned in brackets.

Sampling stations	*EROD pmole/min/mg T.P. Mean ± SE*	*GST nmole/min Mean ± SE*
La Fourmigue Light house (Cannes)	15.1 (1)	46 (1)
Roquebrune Bay (Roquebrune)	20.9 ± 3.6 (2)	99.0 ± 13.0 (2)
Fontvieille (Monaco)	18.6 (1)	44 (1)
Revelatta (Corse)	8.3 ± 1.4 (3)	30.7 ± 3.8 (3)
Galeria (Corse)	6.7 ± 3.0 (2)	31.5 ± 2.5 (2)
Pointe Canelle (Corse)	5.7 ± 0.8 (2)	35.0 ± 7.0 (2)

concentration is about 10^3 times. The curve very rapidly reaches a plateau part as presented in Figure 3. Nevertheless, BaPMO measurements allow a differentiation between highly polluted and slightly polluted sites and could be recommended for survey of slightly polluted sites such as preserved areas. These BaPMO results are in agreement with previous field experiments.[13,14]

Serranus scriba: Fish have been caught only in Corse and in restricted stations in the Côte d'Azur (Cannes, Monaco). We noticed that a lack of caught fish is related to highly PAH contaminated area (see Table 1). EROD activities determined in the sea perchs are low (5 to 20 pmol/min/mg protein) compared to other fishes such as the trout[6,23,24] (*Salmo gairdneri*: 20 to 150 pmol/min/mg prot.), the killifish[25] (*Fundulus heteroclitus*: 135 to 185 pmol/min/mg prot.), the carp[26] (*Cyprinus carpio*: 0.1 to 2.5 nmol/min/mg prot.). However these data are close to those measured in the freshwater perch[6] (*Perca fluviatilis*: 10 to 50 pmol/min/mg prot.). GST activities show lower values than those determined in the trout (*Salmo gairdneri*) which is from 5 to about 800 nmol/min/mg prot. according to various authors.[26,28,29] Nevertheless, these data are in the same range as EROD and GST activities observed on *Serranus scriba* all year round.[30]

Results are presented in Table 3 and exhibit significant changes in EROD and GST activities between locations in Corse and the Côte d'Azur sites. However GST activity exhibits scattered values in highly polluted sites and does not correlate very well with PAH content in sediments, while EROD activity exhibits a high correlation coefficient value ($r = 0.98$). The magnitude of these responses is also greater than for the mussels. In particular a high PAH concentration in the Roquebrune station is associated with a high EROD activity (five fold higher than for the reference site). Such a trend allows a remarkable differentiation of the sites (slightly, moderately, highly contaminated) according to the PAH contamination gradient as presented in Figure 2 and Figure 3.

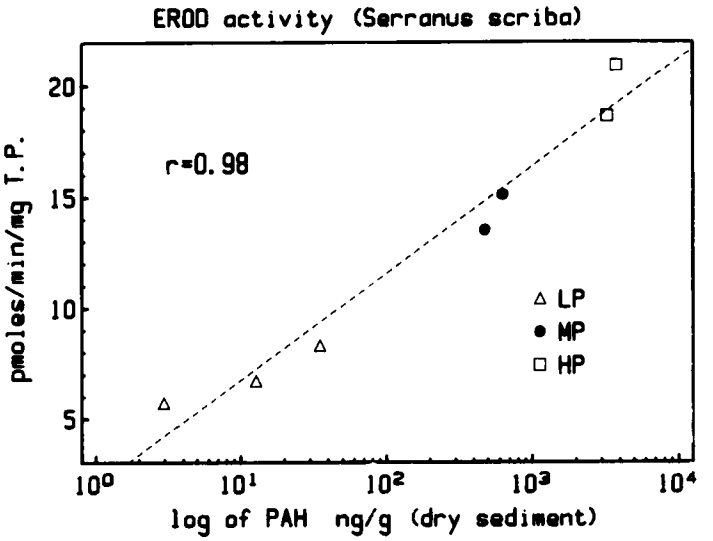

Figure 2 Correlation curve between EROD activity in fish and PAH content in sediments. LP, MP, and HP represent respectively low, medium and highly polluted sites (see also Table 1 and Table 3).

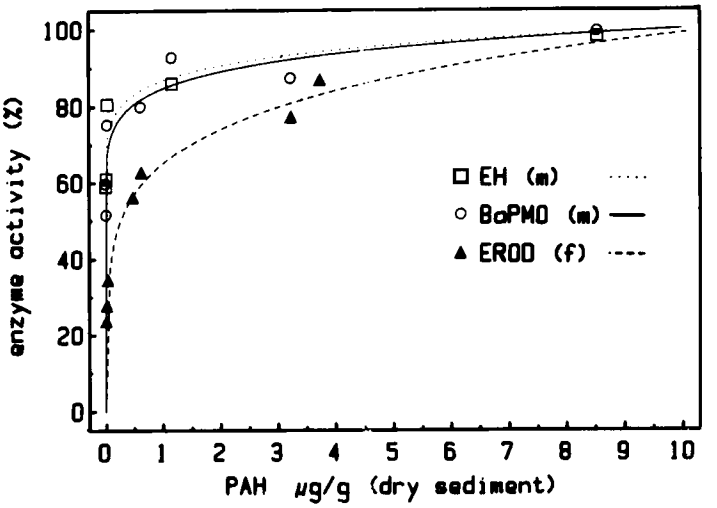

Figure 3 Correlation curves between EROD activities in fish (f), BaPMO and EH activities in mussels (m) and PAH content in sediments. Enzyme activity values have been normalized to the most contaminated site.

CONCLUSION

We observe a good correlation of EROD activities in fish and of BaPMO and EH activities in mussels with PAH content in sediment. Such results indicate: (a) the

sediment content is a good indicator of the average PAH contamination of the environment, (b) biochemical indices based on enzymatic activities in *Mytilus galloprovincialis* and *Serranus scriba* appear as valid bioindicators for pollution monitoring in the field.

Further studies will be developed on other marine organisms (*Posidonia oceanica*) and also on the chemical detection of other chemical contaminants (polychlorobiphenyls, pesticides, organometallic compounds). An extension of the sampling sites will be made also to include other North Mediterranean Sea coastal environments (Spain, Italy).

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