PAPER

Polycyclic aromatic hydrocarbons in sediments and mussels of Corral Bay, south central Chile

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PAHs were measured in sediments and mussels (*Mytilus chilensis*) from Carboneros and Puerto Claro, located in Corral Bay, Valdivia. According to the ratio of phenanthrene/anthracene and fluoranthene/pyrene concentrations, these sites are medium polluted with PAHs originating mainly from pyrolytic sources. Fluoranthene was the major component measured in mussels (3.1–390 ng g^{-1} dry weight) and sediments (6.9–74.1 ng g^{-1} dry weight). In general, mussels were mainly exposed to the dissolved fraction of the lower molecular weight PAHs (tri- and tetra-aromatics) while the higher molecular ring systems (penta- and hexa-aromatics) were more bioavailable to sediments. Mussel PAHs content was relatively constant, with the exception of the 1999 summer season (March), when higher concentration values were found in both sites; however, PAHs residues in sediments showed a temporal variation.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are regarded as a widespread class of environmental persistent organic pollutants (POPs) which are suspected of toxicity in aquatic organisms.[1–3] Due to their mutagenic and procarcinogenic activity,[4–6] these compounds have been extensively measured in numerous sites and compartments.[7–12] Because of their lipophilic character, these compounds are readily absorbed by organic matter of sediments and accumulated by marine organisms from waters polluted by anthropogenic activity.[8] Sediment-associated organisms, like marine bivalves have been used to monitor pollution since they exhibit not only the behaviour trends of lipophilic contaminants but also the bioavailabilty portion of environmental contamination.[13] Bivalves tend to accumulate high PAH levels due to their inability to metabolize and excrete them, probably due to inefficient or missing mixed-function oxidase (MFO) enzymes that use the cytochrome P-450 electron transfer system (CYP1A), a protein that is induced by and plays a critical role in the oxidation of numerous xenobiotics, including PAHs. The metabolism of PAHs by CYP1A yields to oxidized products with different levels of toxicity, resulting in deleterious effects on the organisms.[14] Steady-state levels of these organic hydrophobic compounds are attained when bivalves are exposed to polluted waters, which result from a balance between uptake and depuration processes.[15] Two main routes are recognized for the PAH uptake by bivalves. Lower molecular weight PAHs (the water-soluble portion) are taken up through the gills, and the higher molecular weight PAHs are absorbed through the digestive tract supplied by the smallest grain-size fraction uptake of sediments (<60 μm).[8] Uptake of PAHs depends on their bioavailability which in turns depends on their solubility in water. While the lowest is the octanol–water partition coefficient (K_{ow}) the largest is the PAH bioavailability. Since PAH solubility decreases with increasing number of aromatic rings (increased molecular weight), the bioaccumulation of these compounds from sediments by mussels is usually greater for lower molecular weight than for higher molecular weight PAHs.[8] The most important decomposition mechanisms of PAHs, like photo-oxidation and biodegradation by micro-organisms are of little significance and its global balance is almost not affected.[16–18]

PAH speciation in sediments and mussels as well as the comparison of its fingerprints and concentrations can provide useful information on the bioavailability of the various PAHs, the pollution level at specific sites, the sources (pyrolytic, petrogenic, diagenic) and the temporal fluctuations.

The aim of this study was to assess the level and sources of PAHs pollution and the bioavailable fraction of the pollution at two different sites of Corral Bay at Valdivia, in the south of Chile. A group of 16 priority PAHs recommended by the USEPA (US Environmental Protection Agency) was chosen as target analytes in sediments and mussels (*Mytilus chilensis*)

Experimental

Dichloromethane and hexane used for extraction and clean-up were pesticide residue grade (Mallinckrodt, USA). Glass distillation was used when solvent quality did not meet the requirement of purity specified by standard operation procedures (SOP). Water of high purity grade, suitable for PAHs analysis, was obtained by elution through an ion exchange cartridge and then by boiling for 2 h with nitrogen bubbling.

Sampling

Two seasonal campaigns per year were selected, March and September 1999 and 2000 at Carboneros and Puerto Claro sites of Corral Bay (39°52′ S, 73°25′ W) (Fig. 1). Triplicate samples were collected at each sampling station.

Surface sediments were collected by snorkel divers using stainless steel cores. Subsamples were taken from the center of the core to a depth of 1 cm, sieved at 1 mm and frozen at −20 °C. Sediments were freeze-dried and finally stored in glass containers until further analysis.

Mussels were collected by snorkel divers near the air–water interface (1–2 m from the surface) in natural banks. Specimens of approximately the same size were wrapped in aluminium foil and immediately transported to the laboratory under wet conditions. Ten to fifteen specimens were pooled for each triplicate sample, freeze-dried and stored frozen in glass-amber flasks until further analysis.

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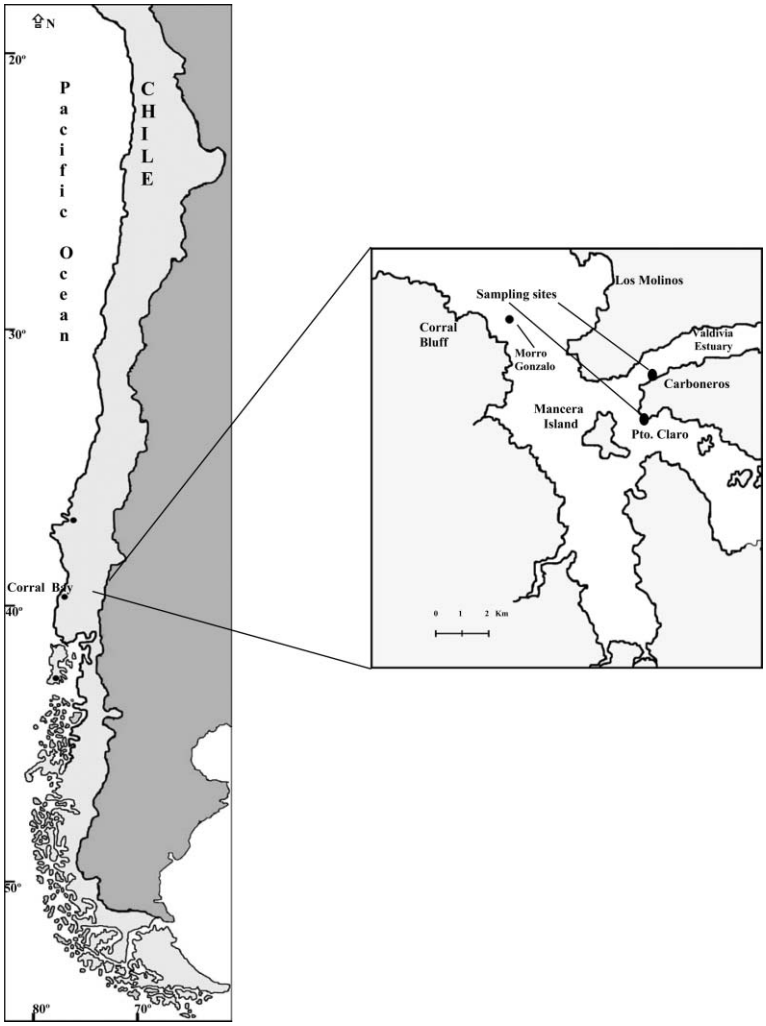

Fig. 1 Sampling sites: Carboneros and Puerto Claro.

Sample analysis

Sediment samples were analyzed according to the method described by UNEP (1992).[19] Briefly, 10–20 g of homogenized samples were exhaustively extracted with dichloromethane using Soxhlet extraction (about 4–5 cycles h^{-1} during 8 h) after addition of perdeuterated PAHs used as internal standards (naphtahalene-d8, acenaphthene-d10, chrysene-d12 and perylene-d12). The sample was reduced to a small volume using a rotary evaporator and then purified into aliphatic and aromatic fractions by alumina–silica gel column chromatography. Mussel samples were analyzed according to the method described by Granby *et al.* (1995).[20] In brief, approximately 10 g of mussel homogenate was saponified by refluxing for 2 h with 50 mL ethanol–KOH (1 M) and the warm sample was transferred to a separating funnel, 20 mL of 0.25 M sulfuric acid were added and the sample was twice extracted with 25 mL dichloromethane. The dichloromethane phase was washed with 50 mL n-hexane reagent grade water, dried by filtering through anhydrous sodium sulfate and vacuum evaporated to 1 mL after adding 5 mL n-hexane. The sample was further purified into aliphatic and aromatic fractions by alumina-silica gel column chromatography.

The aromatic fraction was analyzed by gas chromatography coupled to a mass selective detector (GC-MSD). An HP 6890 PLUS gas chromatograph equipped with a programmable temperature vaporizing inlet (PTV) and coupled to an HP 5973 mass selective detector (Hewlett-Packard, Palo Alto, CA, USA) was used. The injector temperature was maintained at 280 °C in a pulsed splitless mode. A GC program temperature ramp from 60 °C for 4 min and then at a rate of 10 °C min^{-1} up to 300 °C was used to afford the best separation of PAHs by using a capillary HP-5 MS column, 30 m × 0.32 mm id × 0.25 μm film thickness (Hewlett-Packard, Palo Alto, CA, USA). The MSD was operated under Single Ion Monitoring mode (SIM). Naphthalene (N), acenaphthylene (Ac), acenaphthene (Acn), fluorene (Flu), phenanthrene (P), anthracene (A), fluoranthene (Fln), pyrene (Py), benzo[*a*]-anthracene (BaA), chrysene (Chry), benzo[*b*]fluoranthene (Bbf), benzo[*k*]fluoranthene (Bkf), benzo[*a*]pyrene (BaPy), indene[*1,2,3-cd*]pyrene (IPy), dibenzo[*a,h*]anthracene (DahA) and benzo[*ghi*]perylene (BgPer) were quantified on a dry weight basis relative to perdeuterated PAHs added to the methylene chloride extracts. Unresolved peaks of benzo [*k*]- and [*b*]fluoranthenes are indicated as ΣBF. Total PAHs correspond to the sum of the 16 parent compounds. Analytical protocols were validated by using certified marine sediment SRM 1941a (NIST) and certified mussel tissue SRM 2974 (NIST). The recoveries of PAHs on both matrices were between 50% and 118%. The Relative Percent Difference (RPD) for analytical duplicates were less than 25%. Detection limits for PAHs were about 0.020 ng g^{-1} dry weight for sediments and 0.200 ng g^{-1} dry weight for mussels.

Results and discussion

During 1999, the total PAHs concentration levels in sediments of Carboneros fluctuated from about 33 to 72 ng g^{-1} whereas sediments of Puerto Claro fluctuated from 198 to 292 ng g^{-1}. During 2000, PAHs levels in sediments were between 99 and 203 ng g^{-1}, and 55 and 205 ng g^{-1} for Carboneros and Puerto

Table 1 PAHs in sediments (ng g^{-1} dry weight) from Carboneros and Puerto Claro during 1999 and 2000

	Carboneros				Puerto Claro			
	1999		2000		1999		2000	
	March	September	March	September	March	September	March	September
N	1.40 ± 1.37	3.70 ± 3.02	0.25 ± 0.15	18.72 ± 3.13	11.67 ± 0.78	5.03 ± 0.18	1.08 ± 0.5	0.49 ± 0.31
Ac	0.40 ± 0.21	0.90 ± 0.38	0.72 ± 0.30	0.71 ± 0.23	0.40 ± 0.20	0.98 ± 0.41	1.25 ± 0.80	0.36 ± 0.08
Acn	2.70 ± 1.47	2.80 ± 1.99	5.04 ± 1.03	1.82 ± 0.30	3.94 ± 0.14	0.52 ± 0.06	17.97 ± 3.25	0.59 ± 0.26
Flu	5.20 ± 0.42	6.60 ± 4.19	10.19± 2.10	2.42 ± 2.19	3.59 ± 0.13	1.18 ± 0.66	36.46 ± 5.80	0.73 ± 0.14
P	1.80 ± 0.42	3.30 ± 0.20	3.89 ± 1.80	3.78 ± 0.26	61.15 ± 2.00	5.62 ± 0.54	7.57 ± 1.95	0.87 ± 0.21
A	3.70 ± 0.65	5.20 ± 4.14	10.90 ± 1.90	5.22 ± 0.42	15.02 ± 1.02	2.35 ± 1.70	10.61 ± 2.30	3.58 ± 1.40
Fln	8.90 ± 0.39	6.90 ± 2.61	64.52 ± 4.5	10.25 ± 2.96	74.06 ± 11.51	28.08 ± 0.97	68.22 ± 6.44	8.02 ± 1.79
Py	8.40 ± 3.53	3.60 ± 0.07	23.81 ± 2.1	9.61 ± 1.41	36.88 ± 1.75	10.49 ± 4.08	10.07 ± 2.40	5.68 ± 2.10
BaA	nd	nd	nd	nd	23.53 ± 0.55	3.71 ± 0.44	nd	3.56 ± 1.29
Chry	nd	nd	nd	nd	34.48 ± 0.64	29.44 ± 1.60	nd	10.85 ± 1.85
ΣBF	nd	nd	nd	nd	17.8 ± 4.63	44.00 ± 3.37	nd	6.05 ± 1.60
BaPy	0.40 ± 0.19	20.32 ± 3.06	nd	4.85 ± 2.33	0.90 ± 0.42	34.43 ± 5.32	3.53 ± 0.97	0.85 ± 0.69
IPy	0.80 ± 0.7	12.60 ± 3.21	33.79 ± 2.50	13.54 ± 7.10	4.09 ± 0.14	12.19 ± 1.81	15.03 ± 3.8	2.86 ± 1.70
DahA	0.60 ± 0.28	3.10 ± 1.00	17.37 ± 3.20	6.57 ± 3.44	2.01± 0.38	7.71 ± 2.85	8.37 ± 2.70	4.08 ± 0.42
Bgper	1.00 ± 0.31	2.50 ± 0.80	32.39 ± 5.00	21.71 ± 2.96	2.01 ± 0.18	12.50 ± 1.22	24.42 ± 3.54	6.14 ± 1.00
ΣPAHs	33.26 ± 6.51	71.52 ± 8.20	202.87 ± 22.78	99.22 ± 17.48	291.59 ± 24.46	198.22 ± 10.73	204.58 ± 33.48	54.70 ± 9.15

Claro, respectively (Table 1). Sediments from both sites were low to moderately polluted compared to other sites such as the western Mediterranean Sea, with values around 20 500 ng g^{-1} in a heavily industrialized area (very high pollution).[8] Additionally, the total PAH levels found in Sori and Kumo Islands, five years after the *Sea Prince* spill in Korea, were 51 to 130 ng g^{-1} and 56 to 923 ng g^{-1}, respectively, thus indicating a low to medium pollution.[21] Surface sediments from the Gulf of Trieste showed total PAHs between 30 and 600 ng g^{-1} (low to medium pollution), being the highest in the vicinity of the Port of Trieste.[22] Another study on PAHs from the Savannnah river, in the southeastern state of Georgia, reports high spatial variability with concentration values ranging from 29 to 5375 ng g^{-1} (low to very high pollution) and showing the highest concentrations adjacent to urban and industrial areas.[23] The most important input of PAHs to the environment is from combustion of organic matter, giving rise to complex mixtures of PAHs characterized by a high abundance of parent PAHs and a low abundance of alkylated PAHs.[8] In this study, a relatively high abundance of parent PAHs and only trace amounts of PAH alkyl derivatives below the detection limit strongly suggest a pyrogenic source from combustion of organic matter.[9] The ratio of phenanthrene to anthracene concentrations (P/A) is lower than 1 whereas the ratio of fluoranthene to pyrene concentrations (Fln/Py) is greater than 1 for both sites. Since sediments and mussels are almost free of methyl phenanthrene derivatives, the ratio ΣMeP/P will be less than 1, thus supporting the view that the PAHs found in these sites are probably mainly of pyrogenic origin. Significant depletion of the mussel and oyster populations around the sites under study have been observed over time (unpublished observations). At present, mussels for culture purposes transferred from non-contaminated sites will not usually survive in either site. Pyrogenic PAHs have been highly associated with toxicity to benthic organisms.[23] Toro *et al.* (2003)[24] studied the giant mussel *Choromytilus chorus* present in Corral Bay, as a biomarker and found a significant negative relationship between PAH tissue pollution and the clearance rate of this species. This result argues in favor of a possible impact of environmental pollution on the survival of benthic species.

The concentration levels of PAHs in mussels, *Mytilus chilensis*, sampled at Carboneros vary from 165 to 877 ng g^{-1} during 1999 and from 138 to 166 ng g^{-1} during 2000. Mussel samples collected during 1999 and 2000 from Puerto Claro vary from 145 to 446 ng g^{-1} and 150 to 389 ng g^{-1}, respectively (Table 2). According to the criteria proposed by Baumard *et al.* (1998),[8] Carboneros and Puerto Claro can be classified as sites of moderate pollution levels. Notar *et al.* (2000)[22] reported a total concentration of PAHs in *Mytilus galloprovinciallis* between 644 and 685 ng g^{-1} wet weight (on a 85% humidity base of the specimens, these values would be roundly 4293 and 4567 ng g^{-1}, respectively) in two bays on the Slovenian coastline of the Northern Adriatic Sea, which corresponds to high pollution sites. As for sediments, mussels show a PAH content with P/A < 10 and Fln/Py > 1 ratios that strongly suggest a mainly pyrolytic source of PAHs. Fluoranthene was the major component as it has been observed in mussels and sediments from other PAHs contaminated sites.[8,23,25] Moreover, lower levels of benzo[*a*]pirene, indene[*1,2,3-cd*]pyrene, dibenzo[*a,h*]anthracene and benzo[*ghi*]perylene were detected in sediments, while acenaphthene, fluorene, phenanthrene and anthracene were significantly detected in mussels.

Mussels showed abundance (patterns with greater concentrations) of low molecular weight (three and four ring aromatics) rather than high molecular weight PAHs (five and six ring aromatics), while in sediments the highest molecular weight components were the most prevalent. The aforementioned suggests a better bioavailability of the low molecular weight PAHs which agrees with their higher solubility in water, thus suggesting that the most probable route of PAHs ingestion would be through the gills[26] and not through the digestive tract. The turbidity, measured as suspended particulated matter, in both sampling sites was below 1 mg L^{-1}, therefore mussels were exposed to PAHs mainly associated with the water soluble fraction. Sediments in Carboneros and Puerto Claro are sandy sediments (*c.a.* 80% sand and 20% mud) with an organic matter content of about 6%.[27] Generally, mussel/sediment total PAH ratios were higher than one, revealing that PAHs were mostly partitioned in favor of the biota rather than sediments, thus agreeing with the fact that low PAHs concentrations in sediments are associated with sandy sediments, while high concentrations are associated with muddy sediments. Similar results have been reported by Baumard *et al.* (1998).[8] Significant temporal variation of PAH levels was observed in mussels and sediments. These changes of PAH concentration in mussels showed an unexpected decrease from 877 to 165 ng g^{-1} dry weight in Carboneros and from 423 to 145 ng g^{-1} dry weight in Puerto Claro during 1999, while a substantial increase was observed from March to September during 2000 in the last site. The sediments showed a similar trend. A temporal depuration process may be occurring in the estuarial system that could explain the elimination of PAHs from the mussels, one of these is the salinity, that substantially fluctuates

Table 2 PAHs in *Mytilus chilensis* (ng g^{-1} dry weight) from Carboneros and Puerto Claro during 1999 and 2000

	Carboneros				Puerto Claro			
	1999		2000		1999		2000	
	March	September	March	September	March	September	March	September
N	13.00 ± 4.24	23.90 ± 2.97	10.65 ± 2.94	22.33 ± 2.99	23.59 ± 0.40	25.17 ± 5.92	6.6 ± 2.38	15.02 ± 3.34
Ac	4.55 ± 1.34	77.77 ± 7.00	1.64 ± 0.57	72.08 ± 18.84	2.48 ± 0.36	29.51 ± 1.06	2.1 ± 0.47	4.35 ± 1.78
Acn	122.51 ± 11.72	2.87 ± 0.23	11.15 ± 2.48	0.72 ± 0.01	29.04 ± 7.63	0.75 ± 0.08	14.55 ± 1.54	45.23 ± 4.62
Flu	310.81 ± 14.98	1.95 ± 0.64	31.27 ± 1.25	2.14 ± 0.55	122.06 ± 5.12	1.42 ± 0.50	37.6 ± 2.14	131.51 ± 17.24
P	6.82 ± 2.75	2.17 ± 0.01	5.85 ± 1.25	2.16 ± 0.02	71.55 ± 2.84	6.93 ± 0.10	5.54 ± 3.61	10.34 ± 4.43
A	10.44 ± 3.27	1.04 ± 0.35	9.17 ± 1.23	1.01 ± 0.21	159.40 ± 13.72	7.74 ± 2.75	7.96 ± 4.60	23.86 ± 1.90
Fln	389.64 ± 30.28	3.09 ± 0.71	27.40 ± 11.36	3.45 ± 0.16	23.15 ± 3.81	11.79 ± 3.28	28.22 ± 5.82	115.83 ± 8.44
Py	4.72 ± 2.57	1.20 ± 0.78	7.40 ± 2.92	0.80 ± 0.13	10.01 ± 2.57	3.44 ± 0.50	5.58 ± 1.36	25.71 ± 2.25
BaA	nd	9.53 ± 0.55	nd	4.41 ± 0.63	nd	41.30 ± 2.39	nd	nd
Chry	nd	nd	nd	nd	nd	0.31 ± 0.06	nd	nd
ΣBF	nd	0.50 ± 0.12	nd	nd	nd	0.21 ± 0.11	nd	nd
BaPy	0.52 ± 0.43	1.07 ± 0.88	6.84 ± 2.75	0.66 ± 0.09	0.33 ± 0.17	1.04 ± 0.57	4.24 ± 0.31	4.68 ± 4.45
IPy	6.75 ± 3.77	21.81 ± 4.31	32.34 ± 6.80	14.39 ± 2.58	2.78 ± 0.16	4.40 ± 2.02	21.65 ± 2.33	1.81 ± 0.30
DahA	1.62 ± 0.23	0.82 ± 0.63	7.51 ± 0.80	0.77 ± 0.45	0.82 ± 0.03	0.77 ± 0.55	9.65 ± 3.35	5.50 ± 2.43
Bgper	3.39 ± 0.28	16.76 ± 1.38	15.22 ± 6.48	12.79 ± 2.79	1.21 ± 0.13	10.70 ± 0.80	6.1 ± 0.28	5.03 ± 1.37
ΣPAHs	876.73 ± 6.91	164.47 ± 8.95	166.44 ± 20.44	137.73 ± 25.8	446.41 ± 16.16	145.49 ± 13.63	149.8 ± 7.24	388.86 ± 36.97

from approximately 5 to 33‰. Baumard *et al.* (1998)[28] found that mussels PAH contents of samples from different sites of the Mediterranean Sea, did not show variation in relation to sediment contamination but it remained fairly constant at low levels, thus concluding that the bioavailability variation of these compounds for the mussels was observed to be dependent on the origin of the compounds. The estuary under study is a very dynamic system as described by Pino *et al.* (1994)[29] and this could explain the temporal variations of the sediment and mussel PAH contents. The PAH pollution in the Corral Bay is widely spread over the coastline as it has been observed in parallel studies carried out on samples of the giant mussel, *Choromytilus chorus*, from Morro Gonzalo (39°52′ S, 73°25′ W), near a fishing port and a small town (1–2 km along the coast from the Corral Port). The medium level of PAHs pollution was found at this site with values ranging from 220 and 880 ng g^{-1} dry weight[30] (Fig. 1).

A main source of pyrolitic PAHs could be the incomplete residential wood combustion in Valdivia city, located *c.a.* 20 km east of Corral Bay, for about 9–10 months per year. At present, there are no studies showing the total PAH mass balance of air emissions produced by wood combustion in Valdivia. It has been estimated that residential combustion accounts for more than 30% of anthropogenic PAHs emissions in eastern North America.[31] Studies carried out on biofuels by Oanh *et al.*(1999)[32] showed that assuming a combustion of 3 kg of wood fuel or 1.4 kg of coal briquettes or 1.5 kg of charcoal per daily cooking, the genotoxic PAHs emitted were 40 mg from wood fuel, 9 mg from coal briquettes, and 3.3 mg from charcoal. Valdivia is an area of high rainfall with an average of 2400 mm $year^{-1}$ for the last 29 years with 45% of the rainfall occuring in winter (May–July).[29] Therefore, it is likely that PAHs would be washed down to the estuary where sediments constitute a good natural trap for this type of hydrophobic compound.

The pyrolytic contamination of PAHs in the sites under study can also derive from airbone particle emissions of motor vehicle fuels and exhaust emissions. The high concentration of fluoranthene and pyrene found in both sediments and mussels correlates with the high concentration of these two compounds observed in the exhaust of outboard marine engines[33] and airbone particles from terrestrial traffic emissions.[34]

It is concluded that Carboneros and Puerto Claro sites, located in Corral Bay, are medium polluted sites with PAHs, according to the ratio of concentration of P/A and Fln/Pyr, originated mainly from pyrolytic sources. Generally, PAHs of lower molecular weight (tri- and tetra-aromatics) were more available for mussels rather than the higher number ring systems (penta- and hexa-aromatics), while the last compounds were more available for sediments. Mussel PAHs content was relatively constant, with the exception of the summer season during 1999 (March), where higher concentration values were found for both sites; however, PAHs content in sediments showed temporal variations.

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References

1 D. C. Malins and B. B. McCain, *Environ. Sci. Technol.*, 1984, **18**, 705.
2 R. Eisler, Polycyclic Aromatic Hydrocarbons, in *Handbook of Chemical Risk Assessment. Health Hazards to Humans, Plants and Animals. Vol. 2. Organics*, Lewis Publishers, Boca Raton, FL, 2000, 1500 pp.
3 K. V. Thomas, J. Balaam, N. Barnard, R. Dyer, C. Jones, J. McHugh and M. Lavender, *Chemosphere*, 2002, **49**, 247.
4 J. MacCann, E. Choi, E. Yamasaki and B. Ames, *Proc. Natl. Acad. Sci. U. S. A.*, 1975, **72**, 5135.
5 K. L. White, *Environ. Carcin. Rev.*, 1986, **2**, 163.
6 O. Geffard, H. Budzinski, E. His, M. N. L. Seaman and P. Garrigues, *Environ. Toxicol. Chem.*, 2002, **21**, 2310.
7 A. P. Murray, B. J. Richardson and C. F. Gibbs, *Mar. Pollut. Bull.*, 1991, **22**, 595.
8 P. Baumard, H. Budzinski and P. Garrigues, *Environ. Toxicol. Chem.*, 1998, **17**, 765.
9 P. Baumard, H. Budzinski, Q. Michon, P. Garrigues, J. F. Narbonne, T. Burgeot, X. Michel and J. Bellocq, *Mar. Environ. Res.*, 1999, **47**, 415.
10 M. M. Storelli and G. O. Marcotrigiano, *J. Food Prot.*, 2001, **64**, 405.
11 A. V. Botello, C. García-Ruelas and G. Ponce-Velez, *Bull. Environ. Contam. Toxicol.*, 2002, **69**, 486.
12 J. L. Zhou and K. Maskaoui, *Environ. Pollut.*, 2003, **12**, 269.
13 T. P. O'Connor, *Mar. Environ. Res.*, 1996, **41**, 183.
14 J. J. Stegeman, M. Brouwer, R. T. DiGiulio, L. Forlin, B. A. Fowler, B. M. Sanders and P. A. Van Veld, in *Biomarkers: Biochemical, Physiological, and Hystological Markers of Anthropogenic Stress*, ed. R. J. Huggett., R. A. Kimerle, P. M. Mehrle and H. L.Bergman, Proceedings of the Eighth Pellston Workshop, Keystone, CO, July 23–28, 1989, Boca Raton, FL, Lewis Publishers, pp. 235–236.

15 R. V. Thomann and J. Komlos, *Environ. Toxicol. Chem.*, 1999, **18**, 1060.
16 P. Garrigues, H. Soclo and M. P. Marniesse, *J. Environ. Anal. Chem.*, 1987, **28**, 121.
17 Ch. A. Menzie, B. B. Potocki and J. Santodonato, *J. Environ. Sci. Technol.*, 1992, **26**, 1278.
18 B. G. McDonald and P. M. Chapman, *Mar. Pollut. Bull.*, 2002, **44**, 1321.
19 UNEP/IOC/IAEA, Determination of petroleum hydrocarbons in sediments, *Reference Methods for Marine Pollution Studies N° 20*, UNEP, 1992.
20 K. Granby and N. H. Spliid, *Mar. Pollut. Bull.*, 1995, **30**, 74.
21 U. H. Yim, J. R. Oh, S. H. Hong, S. H. Lee and W. J. Shim, *Environ. Forensics*, 2002, **3**, 357.
22 M. Notar, H. Faganeli and J. Leskovsek, *Mar. Pollut. Bull.*, 2001, **42**, 36.
23 M. Sanders, S. Scott and G. Sivertsen, *Arch. Environ. Contam. Toxicol.*, 2002, **43**, 438.
24 B. Toro, J. M. Navarro and H. Palma-Fleming, *Rev. Chil. Hist. Nat.*, 2003, **76**, 183.
25 R. Law and E. Andrulewicz, *Mar. Pollut. Bull.*, 1983, **14**, 289.
26 P. G. De Maagd, D. Th. E. M. Ten Hulscher, H. van den Heuvel, A. Opperhuizen and D. T. H. M. Sum, *Environ. Toxicol. Chem.*, 1998, **17**, 251.
27 H. Palma-Fleming, O. Espinoza, E. Gutierrez and M. Pino, *Bol. Soc. Chil. Quím.*, 1998, **43**, 435.
28 P. Baumard, H. Budzinski, Q. Michon, P. Garrigues, t. Burgeot and J. Bellocq, *Estuarine, Coastal Shelf Sci.*, 1998, **47**, 77.
29 M. Pino, M. E. Perillo and P. Santamarina, *Estuarine, Coastal Shelf Sci.*, 1994, **38**, 491.
30 B. Toro, J. M. Navarro and H. Palma-Fleming, *Aquatic Toxicol. 2003*, 2003, **63**, 207.
31 USEPA, *Deposition of Air Pollutants to the Great Lakes, First Report to Congress, EPA 453/R93-055*, 1994.
32 N. T. K. Oanh, L. B. Reutergardh and N. T. Dung, *Environ. Sci. Technol.*, 1999, **33**, 2703.
33 N. Y. Kado, R. A. Okamoto, J. Karim and P. A. Kuzmicky, *Environ. Sci. Technol.*, 2000, **34**, 2714.
34 L. H. Lim, R. M. Harrison and S. Harrad, *Environ. Sci. Technol.*, 1999, **33**, 3538.