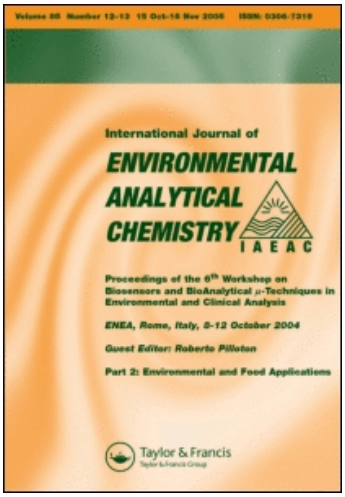

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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Online publication date: 11 June 2010

To cite this Article Bustamante, Julen , Albisu, Amaia , Bartolomé, Luis , Prieto, Ailette , Atutxa, Alaitz , Arrasate, Sonia , Anakabe, Eneritz , De Diego, Alberto , Usobiaga, Aresatz and Zuloaga, Olatz(2010) 'Levels of polycyclic aromatic hydrocarbons, polychlorinated byphenyls, methylmercury and butyltins in the natural UNESCO reserve of the biosphere of Urdaibai (Bay of Biscay, Spain)', International Journal of Environmental Analytical Chemistry, 90: 9, 722 — 736
To link to this Article: DOI: 10.1080/03067310902995155
URL: http://dx.doi.org/10.1080/03067310902995155

Intern. J. Environ. Anal. Chem.
Vol. 90, No. 9, 10 August 2010, 722–736

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Levels of polycyclic aromatic hydrocarbons, polychlorinated byphenyls, methylmercury and butyltins in the natural UNESCO reserve of the biosphere of Urdaibai (Bay of Biscay, Spain)

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(*Received 16 January 2009; final version received 24 April 2009*)

Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), methylmercury ($MeHg^+$) and butyltins (mono-, di- and tri-butyltin, MBT, DBT and TBT) were monitored in oysters (*Crassostrea* sp.) and sediments collected in different sampling points of the UNESCO reserve of the biosphere of Urdaibai (Bay of Biscay) from March 2006 to June 2007. In the case of oyster samples, concentrations in the 290–1814 $\mu g\,kg^{-1}$ (PAHs), 70–475 $\mu g\,kg^{-1}$ (PCBs), 75–644 $\mu g\,kg^{-1}$ ($MeHg^+$) and 200–1300 $\mu g\,kg^{-1}$ (as a sum of the three butyltins) ranges were obtained. In most samples TBT was the most abundant butyltin, followed by DBT and MBT. It should be highlighted that most samples exceeded the highest range (367 $\mu g\,kg^{-1}$) found in the last mussel watch programme carried out by the National Oceanic and Atmospheric Administration (NOAA) for butyltins in oyster samples. This could be due to the presence of a shipyard in the estuary. Sediment concentrations ranged as follows: total PAHs (856–3495 $\mu g\,kg^{-1}$) and total PCBs (58–220 $\mu g\,kg^{-1}$). Organometallic species were always below the limits of detection (LODs) (0.24 $\mu g\,kg^{-1}$ for $MeHg^+$, 0.6 $\mu g\,kg^{-1}$ for MBT, 0.48 $\mu g\,kg^{-1}$ for DBT and 1.1 $\mu g\,kg^{-1}$ for TBT). In both sediment and oyster PAH sources were mostly combustion. In the case of PCBs, 4-6 chlorine-atom congeners were the most abundant ones. Slight differences in the profile of PAHs as well as PCBs can be detected when the matrices were compared with each other. Finally, in the case of PAHs, sediment and water column played the main role in the accumulation pathway into the organisms in all the sampling stations.

Keywords: PAH; PCB; methyl mercury; sediments; oysters; aquatic organisms; Urdaibai

1. Introduction

The European Water Framework Directive (WFD, 2000/60) considers water management from a wide perspective, looking for the prevention of any future deterioration of water bodies, as well as the protection and improvement of the state of marine ecosystems, in order to obtain 'a good state' of water bodies [1]. According to the WFD, the good state of the aquatic bodies is obtained when the concentration of the priority substances in water,

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ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310902995155
http://www.informaworld.com

sediment and biota are below the established Environmental Quality Standards (EQSs). All the European Union member states should implement management plans in their river basins including monitoring programmes.

Concentrations of different trace contaminants are used in order to assess the quality and status of the environment and to be aware of their risks [2]. The contaminants of major concern that deserve monitoring are usually those of high chemical stability and, therefore, high persistence in the environment, of hydrophobic behaviour (given as the octanol-water partition coefficient, K_{ow}), thus able to bioaccumulate, and, finally, those exhibiting acute toxicity [3].

In marine monitoring, sediments and biota, such as mussels and oysters, are commonly used, since sediments act as the ultimate sink of contaminants and due to the ability of mussels and oysters are able to accumulate pollutants at concentrations much higher than in the water column [4].

The estuary of Urdaibai (Bay of Biscay, northern Spain), and almost the whole basin of the Oka river, is a natural UNESCO reserve of the biosphere since 1984. This estuary is an unique habitat for many species (birds, fishes, amphibious, etc.) but in spite of the ecological richness of the area there are urban inputs, especially from the village of Gernika (20,000 inhabitants), and industrial and leisure activities as well as fisheries that have to be considered [5]. As a consequence of those features, considerable efforts are taken in order to monitor and minimise any anthropogenic harm on the environment [6].

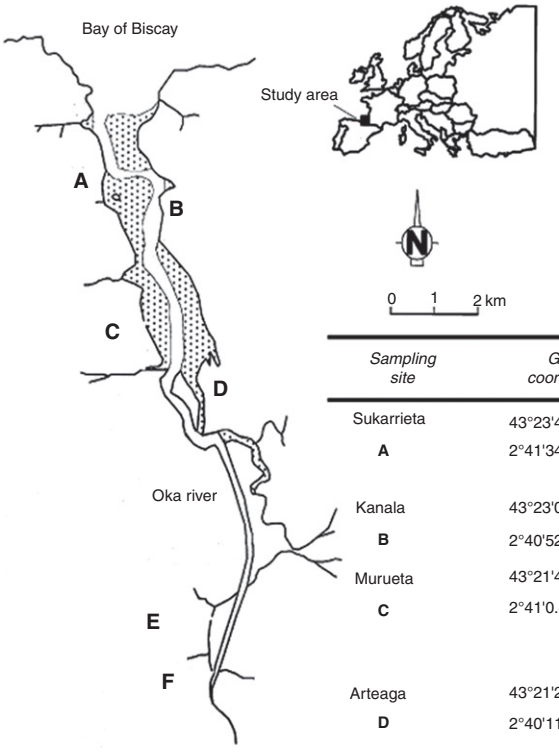
The contamination detected in the estuary is mainly of industrial origin. The main sources are metallurgic and motoring industry (Gernika) and shipping industry in Murueta [7]. In addition, urban pressure together with agricultural, farming and leisure activities are also partially responsible for soil and water contamination.

As a consequence of this complex scenario, different monitoring schemes and surveillance studies are in course in the whole estuary of Urdaibai [8]. In this framework, our research group has monitored different pollutants (e.g. metals, organometallic compounds, persistent organics, etc.) in sediment and oyster for the last few years. The last results included in this work concern polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), methylmercury ($MeHg^+$), and butyltins (tributyltin and its derivates, dibutyltin and monobutyltin. TBT, DBT and MBT, respectively), which are considered priority pollutants by different organisations and legislations such as the Oslo and Paris Commission for the Protection of the Marine Environment of the North East Atlantic (OSPAR) or the WFD [9].

2. Experimental

2.1 *Study area*

The estuary of Urdaibai is a shallow, meso-tidal estuary located in the Basque Country (Bay of Biscay, 43°22′N, 2°40′W) northern Spain (Figure 1). Physically it is a rather shallow estuary (average 2.6 m) and it extends roughly 12 km and exhibits a maximum width of 1.2 km. Additionally, the high variations in its volume and flushing rates are two features of this estuary. Geomorphologically extensive sand-mud flats in the lower zone, which become exposed during the low tide, together with salt marshes and reclaimed land in the intermediate zone, can be distinguished [5,7]. The samples were collected from six stations, from the low estuary to the upper zone in Gernika (two sampling points before and after a wastewater treatment plant). From the stations located in Gernika, only



Sampling site	*GPS coordinate*	*Observations*
Sukarrieta **A**	43°23'43.41"N 2°41'34.99"E	Fishing and leisures activities. Urban emissary
Kanala **B**	43°23'0.58"N 2°40'52.64"E	Leisure activities
Murueta **C**	43°21'45.25"N 2°41'0.48"E	Shipping industry. Murueta site is enclosed in a shipyard
Arteaga **D**	43°21'20.06"N 2°40'11.37"E	Leisure activities Urban area (20000 inhabitants)
Gernika **E**	43°19'1.83"N 2°40'32.10"E	Metallurgic and motoring industry.
Gernika **F**		Sampling before and after a wastewater treatment plant

Figure 1. Map of the study area with sampling points: A: Sukarrieta; B: Kanala; C: Murueta; D: Arteaga; E: Gernika wastewater treatment plant; and F: Gernika city. Small map shows estuary of Urdaibai position in the north-east Iberian Peninsula.

sediments were taken since no oysters are found in these locations. All the sampling was performed at low tide.

Approximately 100 g of sediment samples were collected manually from the upper sediment during two years in eight sampling periods (March, June, September, December 2006 and March and June 2007), stored in pre-cleaned flasks (10% (v/v) nitric acid bath for 24 hours and dried at 110°C overnight) and 1 mL of 4% (v/v) formaldehyde was added to avoid bacteria growing. Oyster samples (20 individuals per sample point) were collected during one year in four sampling periods (March, June, September and December 2006) close to the water line, rinsed with natural water and placed into plastic bags. All the samples were transported in cooled boxes to the laboratory.

2.2 *Sample pre-treatment*

Sediment samples were frozen (−20°C) and freeze-dried at low temperatures (−46/−52°C) and pressures (0.17/0.22 mbar) in a Cryodos-50 apparatus (Telstar, Spain). Freeze-dried sediment samples were sieved at different particle sizes (PS) (PS < 63 μm, 63 μm < PS < 250 μm and PS > 250 μm). Only the smallest fraction was analysed. Samples were kept in the refrigerator at 4°C until analysis.

The individual oysters were dissected with a clean scalpel blade to separate the soft tissues from the shells. Around 20 individuals were dissected, frozen, homogenised and freeze-dried as explained above. Freeze-dried samples were ground in a ball mill and kept in the fridge at 4°C until analysis.

Once the samples were freeze-dried, the residual water content was measured, especially in oyster samples. The sample was placed in a previously pre-weighed Pt crucible and heated at 105°C. The water content was measured by weight difference. Concentrations were always given in a dry weight basis.

2.3 *Total Organic Carbon (TOC) determination in sediment*

Different methods can be found in the literature to determine the TOC [10,11,12]. The Wakley-Black titration was used in this work [10]. The method consists of a redox titration, using $K_2Cr_2O_7$ and assumes that only the organic carbon consumes the $K_2Cr_2O_7$ and that the oxidation of the organic carbon is zero.

Approximately 0.25–0.5 g of sample, an excess of $K_2Cr_2O_7$ ($K_2Cr_2O_7$, Q.P. %99, Probus, Barcelona, Spain) and 10 mL of sulphuric acid (H_2SO_4, Q.P. %96, Panreac Barcelona, Spain) were added and shaken for one minute. The solution was left to stand for 30 minutes and, before the dichromate excess was titrated, 10 mL of phosphoric acid (H_3PO_4, PRS %96, Panreac) and Milli-Q water (Millipore, Bedford, MA, USA) up to 100 mL were added. The titration was carried out using Fe (II) (Mohr salt SV 0.1 mol L^{-1}, Panreac) as titration agent and ferroine as indicator in an automatic burette (Metromh 665 Dosimat, Herisau, Switzerland).

2.4 *Determination of the lipid content in oysters*

Bligh's method [13] was used for this determination. Approximately 1 g of freeze-dried oyster was taken and extracted under magnetic stirring with 10 mL of dichloromethane (HPLC, Lab-Scan, Dublin, Ireland) for 4 hours. After the extraction, the samples were centrifuged at 500 rpm and the solutions were heated at 40°C in order to evaporate the organic solvent. The dried residual was weighed.

2.5 *PAH and PCB determination in sediment and oyster*

The methods for PAH and PCB determination have been described elsewhere [14,15]. Briefly, approximately 1.0 g of dry sediment or 2.0 g of dry oyster sample were accurately weighed and submitted to microwave-assisted extraction in 15 mL of acetone. The extracts were concentrated and submitted to Florisil® cartridge (Supelco, Walton-on-Thames, UK) clean-up. The eluates were concentrated to dryness, re-dissolved in 500 μL (sediments) or 200 μL (oysters) of isooctane and kept in the dark at −18°C until analysis. Dilutions of the NPD Control Standard S-4089 (16 EPA PAHs + 9

alkyl-derivatives + dibenzothiophene and alkylderivative) from Chiron (Trondheim, Norway) and CEN PCB Congener Mix 1 CB-18, CB-28, CB-31, CB-52, CB-44, CB-101, CB-153, CB, 118, CB-138, CB-149, CB-180 and CB-194 [16] from Supelco were used to prepare calibration standards. The extracts were analysed in a 6890N Agilent gas chromatograph (GC) coupled to a 5973N Agilent mass spectrometer (MS) (Agilent Technologies, Avondale, PA, USA) with a 7683 Agilent autosampler. The method summarised above was validated in our laboratories using the certified reference marine sediment NIST 1944 (National Institute of Standards and Technology, USA) and certified reference mussel tissue NIST 2977 (National Institute of Standards and Technology, USA) [14,15]. The results obtained were within the uncertainties of the reference values.

Blank samples were processed together with samples and limits of detection (LODs) were estimated as the average signal of the blanks ($n = 3$) plus three times the standard deviation of the signals of the blanks [17]. LODs in the 0.56–69.12 $\mu g\,kg^{-1}$ and 0.09–12.39 $\mu g\,kg^{-1}$ ranges were obtained for sediment and oyster samples, respectively.

Deuterated analogues (naphthalene-d_8, biphenyl-d_{10}, phenanthrene-d_{10}, pyrene-d_{10}, benzo[*a*]anthracene-d_{12}, benzo[*a*]pyrene-d_{12} and benzo[*ghi*]-perylene-d_{10}) were used for both recovery and quantification corrections.

2.6 *Organometallic species*

The methods for the determination of $MeHg^+$ and butyltins have been described elsewhere [19]. Briefly, approximately 1 g of sediment or 0.3 g of oyster tissue were weighed in pre-cleaned 25 mL glass vials and ultrasound extracted with 10 mL of 2 mol dm^{-3} hydrochloric acid on methanolic potassium hydroxide for 1 hour. The extracts were diluted to 25 mL with Milli Q water (Millipore, Bedford, USA) and submitted to derivatisation with sodium tetraethylborate (Alfa Aestar, Ward Hill, USA) at pH 4.5. The derivatised analytes were pre-concentrated in 500 μL of *n*-hexane and the organic layer was submitted to GC-MS analysis. Quantification was performed using the standard addition method to minimise matrix effect. The method summarised above was validated in our laboratories using the following certified reference materials (CRMs): IAEA-406 (MeHg in marine sediment, International Atomic Energy Agency, Vienna, Austria), BCR-477 (butyltins in mussel tissue, Community Bureau of Reference, European Union), DOLT-2 (MeHg in dogfish liver, National Research Council of Canada, Canada), BCR-463 (MeHg in tuna fish, Community Bureau of Reference, European Union), NIST 2976 (MeHg in mussel tissue, National Institute of Standards and Technology, USA) [18,19]. The results obtained were within the uncertainties of the reference values.

Blank samples were processed together with samples and limits of detection (LODs) were estimated as the average signal of the blanks ($n = 3$) plus three times the standard deviation of the signals of the blanks. LODs in the 0.24–1.1 $\mu g\,kg^{-1}$ and 3–21 $\mu g\,kg^{-1}$ ranges were obtained for sediment and oyster samples, respectively.

3. Results and discussion

3.1 *Sediments*

In the case of sediment samples, the concentrations of all the organometallic species were below the LOD of the method used (0.24 $\mu g\,kg^{-1}$ for $MeHg^+$, 0.6 $\mu g\,kg^{-1}$ for MBT,

0.48 μg kg^{-1} for DBT and 1.1 μg kg^{-1} for TBT) and, thus, no quantification of $MeHg^+$ and butyltins in the sediment was possible.

In order to establish the toxicity of the sediments studied the ERL (effects range low) and ERM (effects range medium) parameters defined by the NOAA (National and Oceanic and Atmospheric Administration) were used [20]. When total concentrations of PAHs were considered, the concentrations were below the ERL value (see Table 1). However, when PAHs were divided in low molecular mass (LPAHs) and high molecular mass (HPAHs), in some cases the ERL values were exceeded (see Table 1). Since different PAHs show a different toxicity, it is clear that single PAHs should be studied in order to allow discussion on the sediment quality.

To simplify the study of the distribution of PAHs in sediments, five groups of PAHs were selected taking into account their properties and relevance in the environmental studies: (i) fraction 0: sum of methylnaphthalenes (C1-C3), methylphenanthrenes (C1-C3) and methyldibenzothiophenes (C1-C3), (ii) fraction 1: sum of naphthalene, acenaphthylene, acenaphthene and fluorene, (iii) fraction 2: phenanthrene and anthracene, (iv) fraction 3: fluoranthene and pyrene and (v) fraction 4: benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*ah*]anthracene and benzo[*ghi*]perylene (see Figure 2). A similar distribution for PAH compounds were found in all the locations sampled with a predominance of fraction 4 over the rest of fractions along the estuary of Urdaibai (see Figure 2). The highest values for this fraction were found in Gernika (E, F) and Murueta (C) where more urban and industrial discharges occur.

Table 1. Total mean concentration (in μg kg^{-1}) for PAHs and PCBs in sediment for the six sampling points of the estuary of Urdaibai. The highest and lowest concentrations for each group of contaminant are shown in brackects. Total concentration of low molecular mass PAHs (LPAHs: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene) and total concentration of high molecular mass PAHs (HPAHs: fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*ah*]anthracene and benzo[*ghi*]perylene). Comparison with the ERL (effect range low) and ERM (effect range medium) values established by the NOAA.

	μg kg^{-1} (dry weight)			
Sampling point	ΣPAHs[a]	ΣPCBs[b]	LPAHs[c]	HPAHs[d]
Gernika (F)	1225.5 (953.4–1544.3)	144 (116.5–169.0)	595.6 (385.9–803.3)	491.9 (429.0–548.6)
Gernika (E)	1388.1 (855.9–2180.6)	130.8 (90.3–190.9)	564.1 (362.8–859.0)	687.1 (309.4–1193.1)
Arteaga (D)	1549.3 (995.9–2460.4)	93.1 (58.3–146.6)	855.8 (412.8–1643.1)	602.4 (485.2–727.4)
Murueta (C)	1234.4 (870.5–1692.4)	157.7 (137.5–220.2)	615.4 (357.2–1059.6)	497.1 (425.6–550.4)
Kanala (B)	1967.3 (1113.7–3494.7)	102.9 (70.1–149.0)	1026.7 (471.7–2365.2)	827.7 (551.5–1195.4)
Sukarrieta (A)	2466.1 (1992.4–2747.4)	104.9 (73.7–153.7)	1022.7 (685.6–1246.2)	1314 (1154–1406.6)

[a]ERL for ΣPAHs = 4000 μg kg^{-1}.
[b]ERL for ΣPCBs = 30 μg kg^{-1}; ERM for ΣPCBs = 180 μg kg^{-1}.
[c]ERL for ΣLPAHs = 600 μg kg^{-1}; ERM for ΣLPAHs = 3100 μg kg^{-1}.
[d]ERL for ΣHPAHs = 1700 μg kg^{-1}.

Different tools are available in order to discuss PAH sources. In general, while petrogenic origin PAHs are usually rich in 2-3 ring PAHs (LPAHs), pyrolytic origin PAHs are rich in 4-6 ring PAHs (HPAHs) [21,22]. Thus, if $r = \text{LPAH/HPAH}$ is defined, $r > 1$ indicates a petrogenic origin and a pyrolytic origin if $r < 1$. Taking into account this

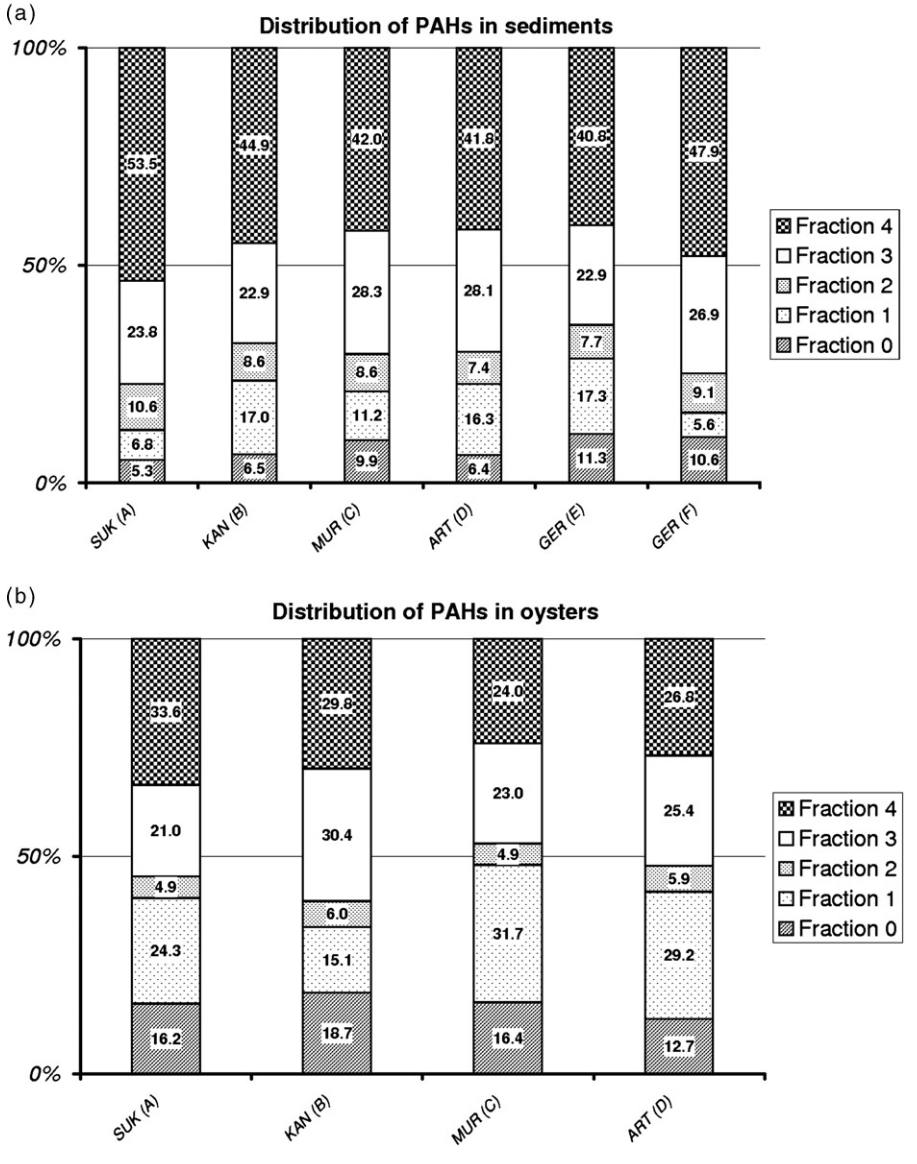


Figure 2. Distribution of PAHs in sediments (a) and oysters (b). The percentages were calculated as the mean of all campaigns sampled in each location: (i) fraction 0: sum of methylnaphthalenes (C1-C3), methylphenanthrenes (C1-C3) and methyldibenzothiophenes (C1-C3); (ii) fraction 1: sum of naphthalene, acenaphthylene, acenaphthene and fuorene; (iii) fraction 2: sum of phenanthrene and anthracene; (iv) fraction 3: sum of fluoranthene and pyrene; and (v) fraction 4: benzo[*a*] anthracene, chrysene, benzo[*b*]fluoranthene, benzo[k]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*ah*]anthracene and benzo[*ghi*]perylene.

parameter, most of the sediment samples analysed in the estuary of Urdaibai exhibit a pyrolytic origin.

Another way to determinate PAH origin is based on the relative distribution of some PAH isomers [23]. As can be observed in Figure 3, indeno[1,2,3-*cd*]pyrene/(indeno[1,2,3-*cd*]pyrene + benzo[*ghi*]perylene) and fluoranthene/(fluoranthene + pyrene), once again a combustion origin (both coal and fuel) seems to describe best the origin of PAHs in the sediments of the estuary. In this sense, it could be underlined that the concentrations were lower than those found in a previous monitoring study after the *Prestige* oil spill [24].

In the case of PCBs, total concentrations ranged between 58 and 220 $\mu g\,kg^{-1}$ and, since no significant correlation was found between those concentrations and TOC ($R^2 = 0.05$), no normalisation of the concentrations was performed. Besides, 4-6 chlorine-atom PCBs were the most abundant, with a predominance of CB-194 isomer.

The profile of the PCBs selected was studied along the sampling period in each sampling location. As can be seen in Figure 4, while a singular pattern was found for each sampling location during 2006, the same profile was found in all the sediments along the estuary of Urdaibai in the two last sampling campaigns, which suggests a common source of PCB during this period of time (2007). Besides, this common profile found in the last two sampling campaigns, have been already observed along the 2006 period in the G-ARAZ site (just after a sewage treatment plant). This fact suggests that this wastewater plant could act as a source of PCBs in the estuary during the 2007 sampling campaign. In previous studies carried out in other estuaries, a relation between this kind of treatment plant and PCBs contamination have been already determined by means of statistical analysis [25].

Besides, it is worth noting that concentrations of PCBs in all the sediment samples analysed in the estuary exceeded the ERL values and in a few cases [20] even the ERM values were exceeded (see Table 1). In this sense, it could be concluded that the quality of the sediment from Urdaibai is no good with respect to PCBs.

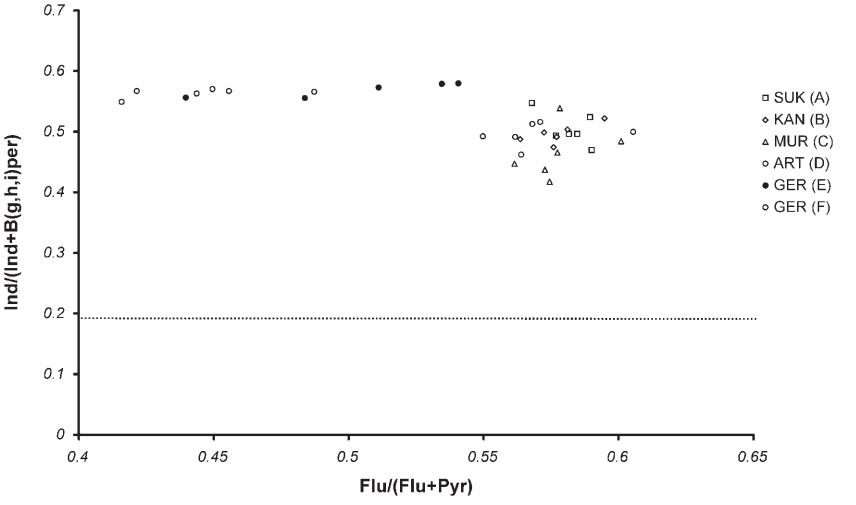


Figure 3. Indeno[*cd*]pyrene/indeno[*cd*]pyrene + benzo[*ghi*]perylene) versus fluoranthene/(fluoranthene + pyrene) in order to determinate PAH source in sediment samples of the estuary of Urdaibai.

3.2 *Oysters*

In the case of organometallic species in oysters from the estuary of Urdaibai, no significant correlation was found between the concentrations observed and the lipid content ($R^2_{MeHg} = -0.08$; $R^2_{MBT} = -0.11$; $R^2_{DBT} = 0.04$ and $R^2_{TBT} = 0.02$) and thus the concentrations were not normalised. Consistent with our results, Kannan [26] and Liu [27] also found that the presence of organotin compounds in tissues did not seem to be related to their lipid

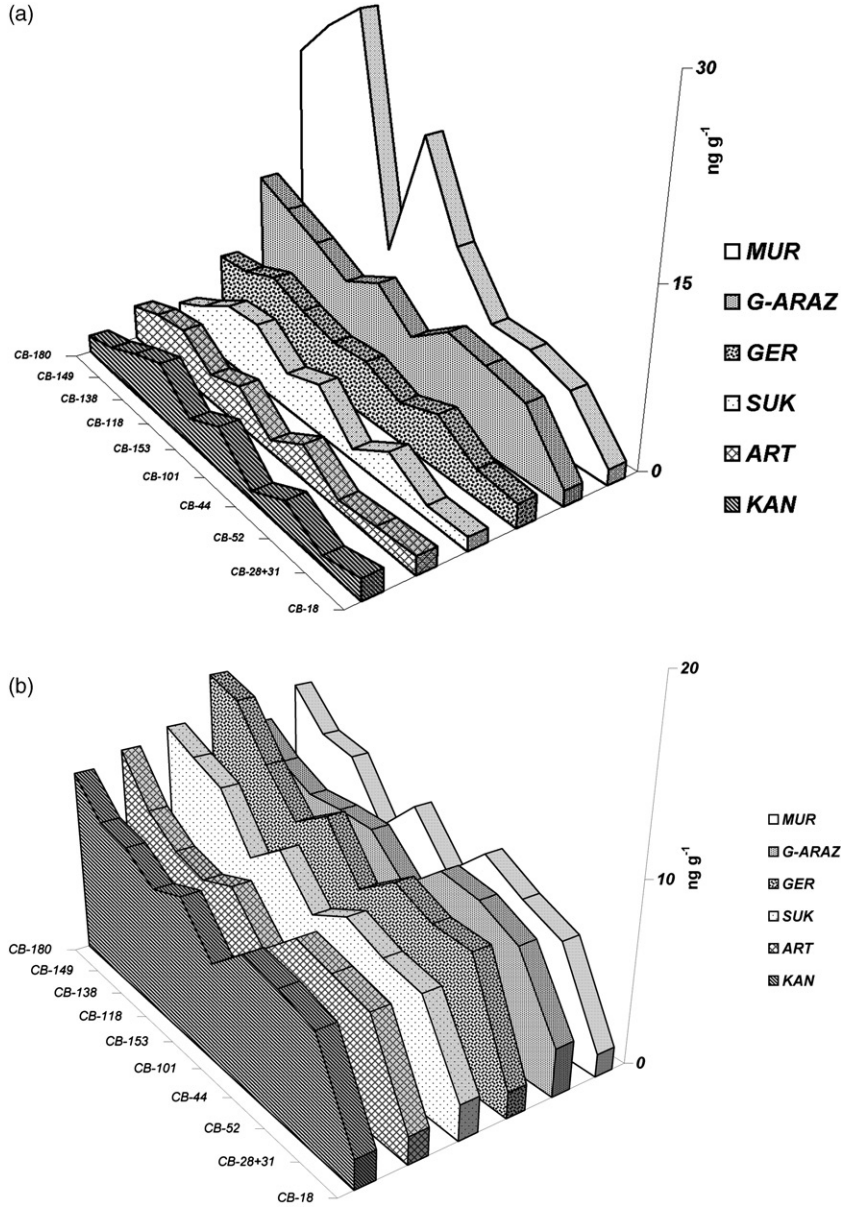


Figure 4. PCBs profile in sediments from all the sites sampled in September 2006 (a) and June 2007 (b). The CB 194 was omitted in the figure for clarity.

content. Concentrations of $MeHg^+$ and butyltins ranged from 70 to 475 and 200 to 1300 $\mu g\,kg^{-1}$, respectively. In general, TBT was the most abundant butyltin, followed by DBT and MBT. The BT concentrations were higher than those found in the last NOAA mussel watch programme [28], which suggests high butyltin contamination in oysters of the estuary. This could be due to the presence of a shipyard in Murueta and leisure ports in Kanala and Sukarrieta.

Total concentrations of PAHs in oysters of the estuary of Urdaibai ranged from 290 to 1814 $\mu g\,kg^{-1}$ but, since no significant correlation was found with respect to the lipid content ($R^2 = -0.11$), no normalisation was performed [29]. In general (see Table 2) oyster concentration of PAHs was below the highest range (more than 2512 $\mu g\,kg^{-1}$) established by the NOAA [26]. Actually, the highest value (1814 $\mu g\,kg^{-1}$) was found in Sukarrieta in September 2006. It should be mentioned that during the summertime many leisure boats can be found in the surroundings of Sukarrieta. The four sampling points followed the same PAH concentration profile if concentration for Sukarrieta in September 2006 was considered an outlier, showing lower concentrations in summer and higher in winter.

As can be seen in Figure 2, when PAHs were divided into the five groups mentioned above for sediments (see Section 3.1), the distribution of PAHs was clearly different from the sediment distribution. While heavy PAHs were the most abundant in sediments in all the locations, fraction 1 and fraction 3 showed the highest contribution in most of the oyster populations sampled. A significant increase in fraction 0 was also noticed in the oyster tissues.

Once again, the main source of PAH appears to be combustion, both when LPAH concentrations were compared with HPAH concentrations or when isomer relations were studied.

When the concentrations found from March 2006 to March 2007 were compared with the data of previous monitoring in the estuary [24], it could be concluded that the increase in concentration observed during 2003 and 2004 after the *Prestige* oil spill had stopped and the concentrations have decreased to their former levels (see Figure 5). This fact is very obvious in the stations located in the outer part of the estuary as Kanala (B) in which a downward trend can be observed at $\alpha = 0.1$ level of significance by means of Mann Kendall non-parametric test. In the case of Murueta (C), the impact can be smoothed because the site is located close to an industrial area.

In the case of PCBs, the concentrations observed (50–250 $\mu g\,kg^{-1}$) were, in general, in the medium or high NOAA established thresholds [28]. As happened with PCBs found in sediments, 4 to 6 chlorine PCBs were the most abundant.

In the case of the seasonal distribution of PCBs in oyster tissue, similar profiles were observed along the sampling period of 2006 in all the locations with CB-194 as the main congener followed by CB-138, CB-149 and CB-153. If those results were compared with PCB profile in sediments, a different behaviour was clearly highlighted with regards to some PCB congeners. While the same congener was the most abundant in both matrices (CB-194), the relevance of the other congeners (CB-138) differed in both samples. This fact suggests the convenience of a bioavailability study.

3.3 *Bioavailability*

The mere presence of the substances of concern is not sufficient to evaluate the risk of pollutants to ecosystems and, therefore, the bioaccesibility and bioavailability of

Table 2. Total mean concentration (in $\mu g\,kg^{-1}$) for PAHs, PCBs, $MeHg^{+}$, and butyltins (MBT, DBT, TBT) in oysters from four of the six sampling points in the estuary of Urdaibai. The highest and the lowest concentration for each group of contaminants are shown in brackets.

	$\mu g\,kg^{-1}$ (dry weight)						
Site	ΣPAHs[a]	ΣPCBs[b]	MeHg+	MBT	DBT	TBT	ΣOT[c]
Arteaga (D)	570.9 (389.2–793.5)	239.4 (122.5–473.4)	264.2 (78–644)	139 (17.7–448.4)	70.1 (<l.d–223.4)	136.5 (71.8–204.0)	345.6 (226.9–577.2)
Murueta (C)	588.7 (333.6–854.4)	170.2 (83.5–218.9)	149.0 (131–175)	125.1 (18.1–434.5)	195.8 (66.9–352.1)	137.5 <l.d–280.6)	458.3 195.5–580.2)
Kanala (B)	415.6 (290.9–534.5)	115.6 (94.5–138.4)	184.7 (113–328)	107.9 (<l.d–431.6)	454.1 (<l.d–1478.6)	152.7 (<l.d–419.9)	714.6 (389.2–1478.6)
Sukarrieta (A)	816.9 (314.1–1813.6)	113.6 (70.0–162.3)	118.8 (78–211)	97.5 (<l.d–314.1)	41.9 (<l.d–88.0	371.3 (<l.d–556.7)	510.7 (<l.d–788.9)

<l.d: value below the limit of detection.
(a) NOAA ranges values for PAHs: low: 47–828 $\mu g\,kg^{-1}$; medium: 829–2511 $\mu g\,kg^{-1}$, high: $>2512\,\mu g\,kg^{-1}$.
(b) NOAA ranges values for PCBs: low: 4–38 $\mu g\,kg^{-1}$; medium: 39–87 $\mu g\,kg^{-1}$, high: $>88\,\mu g\,kg^{-1}$.
(c) NOAA ranges values for butyltins: low: 2–87 $\mu g\,kg^{-1}$; medium: 88–366 $\mu g\,kg^{-1}$; high: $>367\,\mu g\,kg^{-1}$.

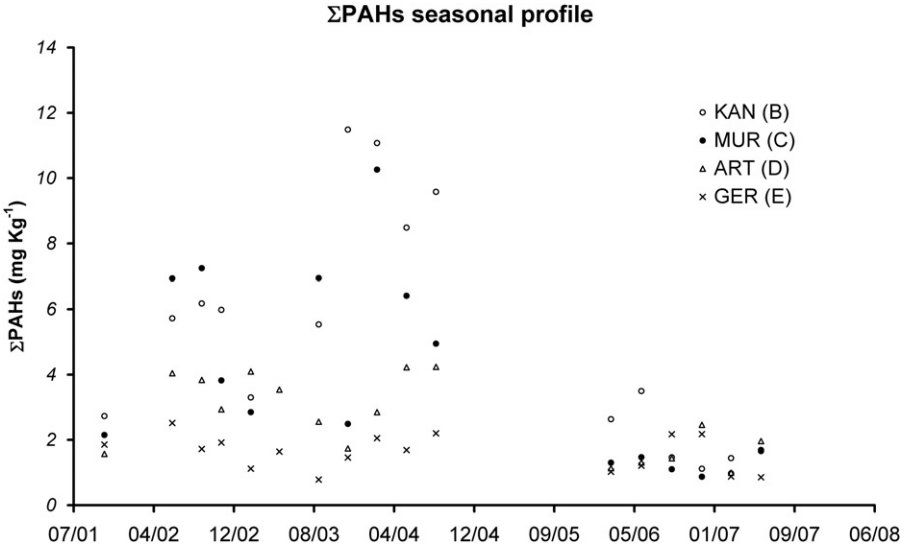


Figure 5. Evolution of total PAH concentration from September 2002 to March 2007 in Arteaga sampling point.

contaminants should be studied. Bioaccumulation factors (BCFs) from sediment for PAHs and PCBs were firstly calculated in four of the six stations as the concentration of the compound in the oyster ($\mu g\,kg^{-1}$) divided by the concentration of the same compound in the sediment ($\mu g\,kg^{-1}$). Figure 6 shows mean log(BCF) values for PAHs and PCBs for each sampling location. In the case of PAHs, the BCF values decreased in this order: $BCF_C > BCF_D > BCF_B > BCF_A$. According to those results, the location where the concentration of the PAHs were the lowest, the BCF values were the highest since the concentrations in oysters along the estuary were very similar. An analysis of variance (ANOVA) was completed in order to know whether the BCFs were significantly different ($p < 0.05$) and was positive for some of the PAHs studied (phenanthrene, fluoranthene, pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and indeno[1,2,3-*cd*]pyrene). These differences were probably due to different physicochemical parameters in water that may affect to the PAH uptake [30]. In the case of PCBs, although heterogeneous results were observed when different sampling locations were compared, the mean log(BCF) value for three congeners (CB-153, CB-138, CB-149) was around 0.8 in all the oyster populations.

The BCF values obtained were lower than 1 for both PAHs and PCBs and, therefore, much lower than expected for a non-equilibrium state bioaccumulation model for hydrophobic compounds ($\log K_{ow} < 6$). Since molluscs are filter-feeding organisms that can retain particles larger than 4 μm and, since in our study the oysters were collected from the bed of the estuary, they could be influenced not only by the water column, but also, and more specifically, by particles coming from the sediment. This fact has been confirmed for PAHs following the study of Baumard and co-workers [23,31,32]. It is possible to define the pathway for hydrophobic contaminants such as PAHs from the correlation between BCF and $\log K_{ow}$, where K_{ow} is defined as the octanol/water partitioning coefficient for each congener. In order to compare the results from different stations, relative BCFs (BCF against the sum of 26 BCF × 100) were calculated. If the slopes

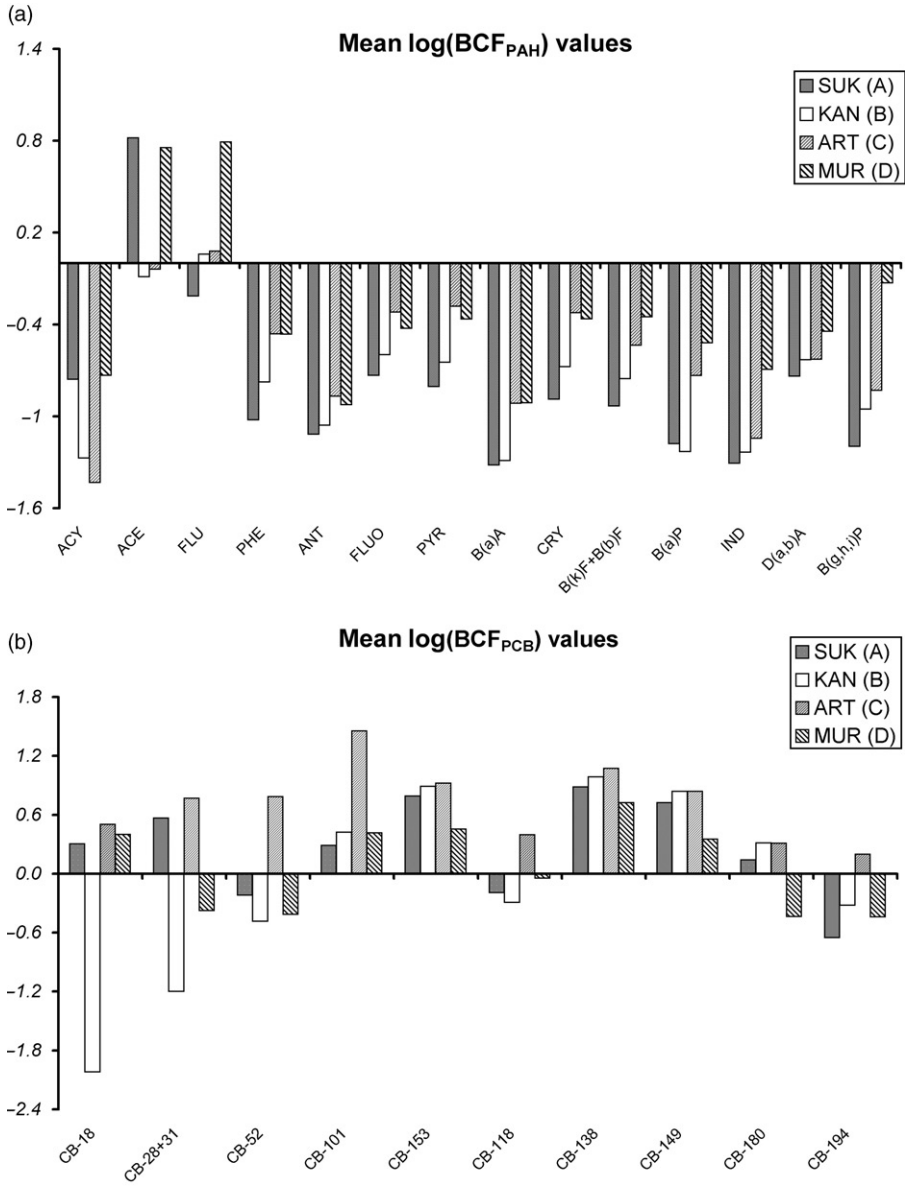


Figure 6. Mean log(BCF) values obtained for all the locations studied at the estuary of Urdaibai for (a) PAHs and (b) PCBs. Acenaphthylene ACY, acenaphthene ACE, fluorene FLU, phenanthrene PHE, anthracene ANT, fluoranthene FLUO, pyrene PYR, benzo[*a*]anthracene B[*a*]A, chrysene CRY, benzo[*b*]fluoranthene + benzo[*k*]fluoranthene B[*b*]F + B[*k*]F, benzo[a]pyrene B[*a*]P, indeno[1,2,3-*cd*]pyrene IND, dibenzo[*ah*]anthracene D[*ah*]A and benzo[*ghi*]perylene B[*ghi*]P.

(D:−0.98, C:0.32, B:−0.91, A:−0.36), ordinate (D:7.7, C:−0.5, B:7.3, A:2.8) and regression coefficients (D:0.27, C:0.21, B:0.18, A:0.24) of the correlations between BCF versus log K_{ow} were considered, it could be concluded that the main pathway for those populations along the estuary of Urdaibai was soluble and particulate fraction in all the

sampling stations. These results were fitted with other works carried out in sampling points where sentinels were sampled in sandy areas [24].

4. Conclusions

Concentrations of PAHs, PCBs, $MeHg^+$ and butyltins were measured in different sampling points in the estuary of Urdaibai. From a general point of view, PAH concentrations were lower than previous studies and PAH source was mainly combustion both in sediment and oyster samples. Thus, it could be concluded that both sediments and oysters from the estuary have recovered from the impact of the fuel oil arrivals after the *Prestige* wreck. In the case of PCBs in sediments and oysters and butyltins in oysters, it should be underlined that the concentrations found exceeded in some cases the NOAA established values, which indicates that the chemical state of the estuary is not as good as expected for a protected biosphere reserve. Regulatory authorities and marine scientists must work cooperatively to ensure the long-term health and sustainability of the coastal areas and recreational opportunities for future generations. Overall, results like the ones presented here may represent an important tool to influence the outcome of decision-making in coastal zone management. As well as highlight the need for more in-depth studies of the relationship between socioeconomic variables and local perspectives concerning environmental resources before endorsing one particular management approach.

Acknowledgements

This work has been financially supported by the University of the Basque Country through the UNESCO 05/12 project.

References

[1] M. Coquery, A. Morin, A. Bécue, and B. Lepot, Trends Anal. Chem. **24**, 117 (2005).
[2] P.M. Chapman and F. Wang, Environ. Toxicol. Chem. **20**, 3 (2001).
[3] C.G. Daughton, Environ. Impact. Assess. **24**, 711 (2004).
[4] R. Beiras, N. Fernández, J. Bellas, V. Besada, A. González-Quijano, and T. Nunez, Chemosphere **52**, 1209 (2003).
[5] I. Madariaga, Estuar. Coast. Shelf. S. **40**, 559 (1995).
[6] A. Borja, J. Solaun, J. Franco, and V. Pérez, in *Oceanography and Marine Environment of the Basque Country*, edited by A. Borja and M. Collins (Elsevier Oceanography Series, 70: 319–333, Elsevier, Amsterdam, 2004).
[7] M.J. Iraiben and F. Velasco, Environ. Geol. **37**, 54 (1999).
[8] A. Borja, J. Franco, V. Valencia, J. Bald, I. Muxika, M.J. Belzunce, and O. Solaun, Mar. Pollut. Bull. **48**, 209 (2004).
[9] P. Roose and U.A.Th. Brinkman, Trend. Anal. Chem.z **24**, 897 (2005).
[10] A. Wakley and I.A. Black, Soil Sci. **37**, 29 (1934).
[11] M.L. Jackson, *Soil Chemistry Analysis* (Prentice-Hall, New Jersey, 1958).
[12] A.L. Page, R.H. Miller, and D.R. Keeney, *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties* (ASA, Madison, 1982).
[13] E.G. Bligh and W.J. Dyer, Can. J. Bio. Phys. **37**, 911 (1959).

[14] L. Bartolomé, E. Cortazar, J.C. Raposo, A. Usobiaga, O. Zuloaga, N. Etxeabarria, and L.A. Fernández, J. Chromatogr. A. **1068**, 229 (2005).
[15] P. Navarro, E. Cortazar, L. Bartolomé, M. Deusto, J.C. Raposo, O. Zuloaga, G. Arana, and N. Etxebarria, J. Chromatogr. A **1128**, 10 (2006).
[16] S.A. Mills III, D.I. Thal, and J. Barney, Chemosphere **68**, 1603 (2007).
[17] J.R. Dean, *Methods for Environmental Trace Analysis* (Wiley, Chichester, 2003), pp. 14–25.
[18] A. Delgado, A. Prieto, O. Zuloaga, A. de Diego, and J.M. Madariaga, Anal. Chim. Acta. **582**, 109 (2007).
[19] M. Zabaljauregui, A. Delgado, A. Usobiaga, O. Zuloaga, A. de Diego, and J.M. Madariaga, J. Chromatogr. A. **1148**, 78 (2007).
[20] E.R. Long and L.G. Morgan, *The Potential for Biological Effects of Sediment-sorbed Contaminants Tested in the National Status and Trends Program*. NOAA Technical Memorandum NOS OMA 52 (NOAA Office of Oceanography and Marine Assessment, Ocean Assessment Division, Seattle, 1999).
[21] E.Ma. Peña-Méndez, Ma.S. Astorga-España, and F.J. García-Montelongo, Environ. Pollut. **111**, 177 (2001).
[22] J. Conde, E. Peña, and F. García-Montelongo, Int. J. Environ. An. Ch. **62**, 77 (1996).
[23] P. Baumard, H. Budzinski, and P. Garrigues, Environ. Tox. Chem. **17**, 765 (1998).
[24] E. Cortazar, L. Bartolomé, A. Usobiaga, O. Zuloaga, and N. Etxebarria, Chemosphere **72**, 1467 (2008).
[25] A. Prieto, O. Zuloaga, A. Usobiaga, L. Bartolomé, L.A. Fernández, N. Etxebarria, E. Ciprain, and A. Alonso, Mar. Pollut. Bull. **56**, 2082 (2008).
[26] K. Kannan, S. Tanabe, and R. Tatsukawa, Int. J. Environ. An. Ch. **61**, 263 (1995).
[27] S. Liu, M. Hsia, and C. Huang, Sci. Tot. Environ. **355**, 167 (2006).
[28] K.L. Kimbrough, W.E. Johnson, G.G. Lauenstein, J.D Christensen, and D.A. Apeti, *An Assessment of Two Decades of Contaminant Monitoring in the Nationś Coastal Zone*. NOAA Technical Memorandum NOS NCCOS 74 (Sylver Spring, Maryland, 2008), p. 105.
[29] C.E. Hebert and K.A. Keenleyside, Environ. Tox. Chem. **14**, 801 (1995).
[30] W.J. Langston and S.K. Spence, in *Metal Speciation and Bioavailability in Aquatic System*, edited by A. Tessier and D.R. Turner (Wiley, Chichester, UK, 1995).
[31] P. Baumard, H. Budzinski, P. Garrigues, H. Dizer, and P.D. Hansen, Mar. Environ. Res. **47**, 17 (1999).
[32] P. Baumard, H. Budzinski, Q. Michon, P. Garrigues, T. Burgeot, and J. Bellocq, Estuar. Coast. Shelf. S. **47**, 77 (1998).