

Application of Comprehensive Two-Dimensional Chromatography for Assessment of Pahas in Estuary Sediments in the Patos Lagoon (Pelotas-Rs, Brazil) Undergoing Oil Contamination

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Abstract

The purpose of this work is to use a comprehensive two-dimensional gas chromatography to assess retention capacity of polycyclic aromatic hydrocarbons (PAHs) in the estuarine sediments contaminated by simulating oil spill in laboratory scale. The experiment allowed us to show that accidents involving spills in the water column take longer to reduce contamination with higher impact in the estuary. The GCxGC/TOFMS has separated and identified a number of compounds present together with HPAs, which would co elute the one-dimensional chromatography, and ensure a more secure identification. The extracts were complex mixtures containing many other compounds beside the PAHs. The application of GCxGC/TOFMS as an analytical tool, allowed detecting PAHs in all samples. Sediment samples without added petroleum had a lower molecular diversity, generally unbranched rings.

Keywords: Sediment; Hydrocarbons; Oil contamination; Chromatography; GCxGC

Introduction

The southern area of the Patos Lagoon, Brazil, is composed of a large estuary. This region receives tidal influence since it is closely connected with the Atlantic Ocean, and also because of the rainfalls that comes into the water, pouring down in the region and flowing directly into the lagoon from the marshes and the coast, mostly from the São Gonçalo Channel, which is a natural connection between the Mirim Lagoon and the Channel. Moreover, the characteristic of channels, the São Gonçalo in particular, is to show current reversals with variations in salinity, making dynamic changes in nutrients and also contaminants when this is possible [1].

It cannot be denied that the extreme dependence of the world on oil and its derivatives may come into our lives in several

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ways. The increasing demand generated by the population growth and their consumption habits, combined with the decrease of natural resources, and has led the world to search for oil in even further more complex places to be explored. The extraction technology applied and the high demand on operational obstacles are raising, for instance, the case of the Brazilian pre-salt extraction [2], which might increase the risk of accidents with oil spills, and therefore, having environmental consequences.

Oil spills that occur in water bodies, especially in the sea, are the cause of major environmental damage, such as the one occurred in 2001 at the Morretes-PR pipeline, the Roncador field (Campos Basin-RJ), and the Paranaguá Port [3].

Among the compounds that constitutes oil, they are mainly saturated hydrocarbons, and polycyclic aromatic hydrocarbons (PAHs), and they have partition coefficient octanol/water greater than 1000, showing high affinity lipophilic, which increases directly in relation to the number of aromatic rings of the molecule. These compounds have the following main features: high hydrophobicity, low reactivity in the environment and a high tendency to either accumulate or bioconcentrate in the tissues of living organisms [4].

The composition of hydrocarbons found in the sediments reflects the relative contribution from different biogenic, diagenetic, petrogenic and pyrogenic sources [5]. The sediments are recognized as final destinations for pollutants such as hydrocarbons, since these compounds are easily adsorbed in material particles, and the bottom sediment acts as a reservoir for hydrophobic contaminants [6]. Environmental damage is particularly severe when oil spill accidents happen because its range undergoes salinity variations, for instance, the estuaries, which work as nurseries and spawning for aquatic life [7]. In this context, the Mirim Lagoon is the final section of the Patos Lagoon. In connection with the sea, it is situated in the Rio Grande Port, which has an intense cargo movement, including oil that supplies refineries in the city. Given the frequent cases of worldwide accidents, companies that work with extraction and oil transportation have become interested in discovering how the sediments behave in case of oil spills in the region.

The GC/MS equipment is widely used in environmental analysis [8]. Yet, the one-dimensional chromatographic analysis has been used to obtain satisfactory analytical data, despite the complexity of samples, which sometimes exceeds the resolution capacity of a single chromatographic process. The comprehensive two-dimensional gas chromatography (GC×GC) technique presents itself as an alternative, and that includes two orthogonal mechanisms for separating several components of a sample carried out only once. The GC×GC has emerged as a powerful separation technique that is especially well-suited for a complex sample characterization [9-10]. The application of GC×GC in determination of PAHs in several environmental samples has been recently reviewed by Tran et al. [11]. The GC×GC combined with a time-of-flight mass spectrometric detector (GC×GC/TOFMS) and high acquisition rate provides compound-specific information. GC×GC/TOFMS allows an extensive description of a polluted sediment sample and a drawing “map” of different pollutant groups [12]. The chromatographic screening carried out by GC×GC allows rapidly providing information for characterization of the toxic potential from a sample.

The purpose of this work is to use a comprehensive two-dimensional gas chromatography to assess retention capacity of polycyclic aromatic hydrocarbons (PAHs) in the estuarine sediments contaminated by simulating oil spill in laboratory scale. The computer aided detection is used in the field of medicine recently to reduce the medical expenses. This technique is used to determine the doubtful lesion and characterize the malignancy of the specific lesion. The detection side of this technique, the CADe helps to recognize and locate the cancerous area from the input image whereas the CADx system helps to diagnose the disease and classify the cancerous location to be benign or malignant tissue [10-13]. The cancer detection tool CADe follows a region based or pixel based algorithm to extract the Region of interest (ROIs) from the mammographic images.

These methods consider the size, color, and morphology of the tumor. When the input feed of the size and morphology of the tumor is fed to the algorithm helps in detecting the region to be cancerous or normal. By sharpness and the margin distinction in any mammography image the computer aided design searches for a specific mass that denotes greater possibility to malignancy. From the image after the distinction of the mass there needs to be an extraction of the region with a higher resolution. The next step is the classification of the extracted image to be benign or malignant [14-17]. Microcalcifications are small calcium deposits that might be a symbol of starting stage of cancer in the future. Techniques like wavelet transforms, local area threshold are used to locate high spatial frequencies in the image to locate the calcification [18,19]. Right after mammography, MRI and PET biopsy is a compulsory option for the confirmation of grades of cancer. By using a CADx system the image extracted and expected to be the tumor mass can be predicted as benign or malignant [20]. The main aim of this work is to use image processing technique, neural networking to predict tumor and by using back propagation and support vector machine algorithm to detect whether the extracted tumor is benign or malignant. Here as a next level of computer aided prediction we propose a method to determine the extracted tumor image as stage I, II, III or IV. This method gives better accuracy and reduces the time and cost consumption compared to the traditional methods of cancer stage detection.

Experimental

Materials

Analytical grade of organic solvents (dichloromethane (DCM), methanol, acetone and *n*-hexane), anhydrous sodium sulfate (Na₂SO₄) and silica gel 60 (70-230 mesh ASTM) were purchased from Merck (Darmstadt, Germany). Neutral alumina (type 507, 70-290 mesh ASTM) was obtained from Fluka AG (Switzerland). Cellulose extraction cartridges (20 mm i.d. and 80 mm long) were purchased from Whatman (UK). Silica, alumina and anhydrous sodium sulfate were activated at 400 °C for 4 hours before use. Aluminum foil was rinsed with acetone and left to dry at ambient temperature prior to use. Copper foils (Sigma –Aldrich, USA) were activated by being treated with concentrated hydrochloric acid (Merck, p.a. grade) and washed off with purified water, methanol, DCM and *n*-hexane, in this order, before being stored in *n*-hexane [13]. Nitrogen (purity 99.9999%) was supplied by White Martins (Porto Alegre, Brazil).

The PAHs mixture (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indene[1,2,3-cd]pyrene, benzo[ghi]perylene, dibenzo[ah]anthracene, containing 2.0 mg.mL⁻¹ of each compound, was purchased from Supelco (Bellefonte, PA, USA). Working solutions were obtained by appropriate dilution of this standard solution in *n*-hexane. All solutions were stored in amber vials at – 20 °C. The internal standard (biphenyl) was purchased from Supelco (Bellefonte, PA, USA).

Collection

The collection of the sediment was held in May 2010 in the São Gonçalo Channel Inlet at a point identified by GPS - Global Positioning System - accurate to 15m (Garmin brand & Trax), at the coordinates: 31° 47' 20.22"S and 52° 13' 20.01"W, and it occurred on the surface of the sediment with a depth between 5 and 10 cm, specifically the interface sediment / water, being

carried out with the aid of a dredger Van Veen. We collected five aliquots, and the sample material was discarded from the top, as well as what came into contact with the edges of the sampler. The samples were stored at $-4\text{ }^{\circ}\text{C}$, and all analyzes were performed in triplicate.

The site was monitored for pH (homis, MOD. 801), portable conductivity meter for conductivity (Instruterm MOD. CD-830), and water temperature was measured with mercury thermometer, in order to determine the characteristics of the water body in the sampled point. The choice of this point was for convenience, since it has been held collection for analysis of other contaminants in the same location.

Physical and chemical characterization of sediments

Collected sediments were sub-sampled for determination of water content, organic matter and grain size distribution. The size of sediment grain is a determinant factor in the cumulative capacity of contaminants, being determined by the Standard Method Sieve [14]. Sediment size fractions were classified as fine (silt/clay -diameter $<0.063\text{ mm}$) and sand ($0.063\text{ to }2\text{ mm}$). The moisture content was obtained by gravimetric method [15]. The total of organic matter (TOM) was determined by the loss of weight after combustion at 450°C for 4 h [16].

Contamination of samples

The sediment contamination (20g) was made by adding 1.64 g of oil (about 2 ml) in two ways: In the first beaker, the oil was added directly into the sediment (sample SD). The second addition took place over a column of water, being collected at the same point (SC). The samples were gently mixed with a glass rod, and allowed to stand for 7 days at room temperature.

The exposure time was chosen based on the rate of the weathering process [17], and also the attempt to estimate a response time for a possible accident in the region. The characterization of the oil used was made accordingly to the method ARDS [18-19], and showed the following characteristics: 46.41% saturated hydrocarbons and PAHs (F1 and F2); 23.70% nitrogen (F3); 14.09% oxygenates (F4); 5.10% asphaltenes in the form of a precipitate, and 10.70% volatiles, disengaged in the first step of preparation. These features indicate that the oil used in the experiment can be considered as light oil.

Extraction of hydrocarbons and fractioning samples

Extraction of hydrocarbon sand fraction samples was extracted in triplicate: one from the natural sediment (SN) and two from contaminated samples with oil directly (SD) and water column (SC). During the extraction of analytes [20], it was used ultrasound device (bath type) Fischer Scientific Model-FS14H with a power of 90 W, frequency of 40 kHz and radiation intensity of $0.27\text{ W}\cdot\text{cm}^{-2}$. The extract was purified by preparative liquid chromatography column according to the protocol of UNEP [21] with layers constituted by 1g of anhydrous Na_2SO_4 , 3.2 g of silica (5% deactivated), 1.8 g of alumina (2% deactivated) and 1 g of anhydrous Na_2SO_4 , on top. The fractions were eluted with solvents in order of increasing polarity (hexane and dichloromethane). The extracts obtained in Fraction1 (aliphatic) were separated and in Fraction 2 (PAHs), they were quantified by Gas chromatography combined with mass spectrometry (monodimensional) and qualitative study by GC \times GC/TOFMS.

Chromatographic analysis

The conditions of chromatographic analysis are shown in TABLE 1 and the specifications of the equipment used are described in three, corresponding to the item below.

TABLE 1. Conditions of chromatographic analysis for PAHs

Analysys	Identification PAHs	Quantification PAHs
Equipment	GCxGC/TOFMS	GC/MS
Column	1 ^a : column DB-5 (30mx0.25mmx0.25µm) 2 ^a : column DB-17 (1m x 0.18mm x 0.18µm)	OV-5 (30mx0.25mmx0.25µm)
Injection mode	Splitless 1 minute	Splitless 1 minute
Temperature	280°C	280°C
Ionization	impact 70 eV	impact 70 eV
Carrier gas	Helium	Helium
Heating ramp	80°C(2min), 5°C/min to 260°C and 3°C/min to 280°C (10 minutes) The second-dimension column was maintained at 15°C above the temperature of the column of the first dimension	80°C(0min), 5°C/min to 260°C and 3°C/min to 280°C. (10 minutes)

Methodology for PAHs

The identification of polycyclic aromatic hydrocarbons contained in Fraction 2 was performed by Comprehensive Two-Dimensional Gas Chromatography. The GCxGC/TOFMS system consisted of an Agilent 7890A gas chromatograph (Santa Clara, USA) equipped with a split/splitless injector and interfaced with a LECO Pegasus® 4D TOFMS system (LECO, St. Joseph, MI, USA). The Agilent GC was equipped with a secondary oven and a dual stage thermal modulator. The column set (TABLE 1) was chosen based on literature data and the physicochemical properties of the analytes [22]. The two columns were connected by a Silket® Treated Universal Press-Tight® connector (20480) (Restek, Bellefonte, PA, USA). The analytical conditions were: 1 µl sample was introduced by pulsed splitless injection (injector temperature 250 °C); helium (Linde Gases, Canoas, Brazil, 99.999% purity) was used as carrier gas in constant flow mode (0.80 mL.min⁻¹). The transfer line between the second-dimension column and the detector was maintained at 280 °C and the ionization source at 250°C. Electron impact ionization (70 eV) was used, and mass range of 45–500 Daltons was recorded with an acquisition rate of 100 Hz and a detector voltage of -1700 V also was applied. The main parameters of the cryogenic modulator were programmed as it follows: the modulator temperature offset, relative to the main GC oven, was 30 °C and the optimum modulation time was 6 s with a 2.4 s hot pulse time, and a 0.60 s cooling time between stages. Cooling was done with pressurized nitrogen gas cooled by liquid nitrogen (Linde Gases Ltda, São Paulo, Brazil).

Quantitative evaluation of the 16 PAHs was considered priority pollutants by the US-EPA (U.S. Environmental Protection Agency), and it was used Selective Detector Gas Chromatography with Mass Spectrometry (GC/MS) in SIM mode, Shimadzu QP2010 Plus equipped with quadrupole analyzer and split/splitless injector. MS detection was applied since it has great sensitivity and more widespread use of this equipment [23].

The mixture containing the 16 PAHs standards, used in quantitative and qualitative analysis, was purchased from Supelco (99.99%, Bellefonte, USA) and patterns of deuterated PAHs (phenanthrene-d 10, chrysene-d 12, and perylene-d 12) Analytical Solution of Ultra Scientific (North Kingstown, RI, USA). The standard mixture obtained was diluted with dichloromethane until reaching concentration of 200 mg L^{-1} . From this stock solution, working solutions (10.0 to 200.0 mg L^{-1}) were stored at 4°C in amber glass ampoules. A stock solution of biphenyl (internal standard) was prepared with concentration of 1000 mg.L^{-1} , and from this, it was added $100 \text{ } \mu\text{g L}^{-1}$ into the samples and standards.

The calibration curves were constructed by injecting standard solutions prepared in dichloromethane in the range of 10 - $200 \text{ } \mu\text{g.L}^{-1}$ containing the internal standard (Biphenyl) at fixed concentration of $100 \text{ } \mu\text{g L}^{-1}$. The limits of detection (LOD) and quantification (LOQ) were based on the parameters of the calibration curve, as recommended by IUPAC - International Union of Pure and Applied Chemistry [24,25] and obtained from the equations: $\text{LOD}=\text{YB} + 3 \text{ SB}$, and $\text{LOQ}=\text{YB} + 10 \text{ SB}$ (YB: average signal white; and SB: standard deviation of blank signal).

The recovery study was conducted with samples marked with deuterated aromatic hydrocarbon $200 \text{ } \mu\text{g.kg}^{-1}$ (3, 4 and 5 rings) according to Numata et al. [26].

Data handling

Identification of PAHs in the fraction F2 of sediment was achieved based on seven categories of analytical information available: Retention time in the first (1tR) and the second (2tR) dimensions, co-injection of analytical standards, the structured pattern of the separation space in the TID (defined as the Total Ion Diagram), the structured pattern of the separation space observed when specific m/z values were selected (defined as the Selected Ion Diagram; SID), the library mass spectral match factor and/or the characteristic of deconvoluted mass spectra alone, comparing to the acquired mass spectra with NIST 2.0 library (they were considered positive identifications for similarity over 800 %).

For data processing, it was used LECO® Chroma TOFTM optimized for the Pegasus® 4D software (version 3.32). For the purposes of this study, the maximum number of processed peaks was limited to 1000 (peaks with $\text{S/N}>10$).

Results and Discussion

Physical and chemical characterization of sediments

Water parameters measured the time of collection, and showed the following figures: temperature de 18°C , pH 8.05 and electrical conductivity (EC) of 168.8 mS cm^{-1} , corrected to 25°C .

TABLE 2. Grain size distribution of sediment samples.

mm	2.0	1.5	1.00	0.500	0.250	0.125	<0.063
Particle	Grain	Very coarse	Coarse	Avered	Fine	Very fine	Botton
		sand	sand	sand	sand	sand	
Content (%)	zero	5.43 ± 1.71	6.923 ± 0.18	19.41 ± 2.23	16.91 ± 0.85	17.68 ± 2.08	33.64 ± 1.97

As shown in TABLE 2, there is great predominance of fines, which indicates a good adsorption ability of this sediment.

Sediments rich in fine fraction are more likely to contain contaminants.

The values for moisture, organic matter (OM) and its standard deviation (in%) for the sediments collected were respectively $73.0\% \pm 2.8\%$ and $10.8\% \pm 1.9\%$. Sediment OM content that is larger than 10% is classified as organic compounds [27]. As the organic matter has the characteristic of adsorbing contaminants, the content is found in large sorption potential, as well as collaborating with the aggregation of sedimentary particles.

The high content of organic matter added to obtain the particle size, being predominantly the fine sediment studied, has showed a good capability to retain contaminants.

The FIG.1a shows the GC×GC/TOFMS chromatogram 3D of the Fraction F2 obtained from the extract of contaminated sample in the water column (SC), but the other fractions displayed similar behavior, allowing a general characterization of fraction F2 for all the samples. It was found that even after the clean-up procedure, done by preparative column chromatography, the extracts are complex mixtures, which brings together several functions, especially oxygen, sulfur, and nitrogen compounds, beside other mixed functions. It can be observed that the chromatogram is composed mainly of oxygenated compounds (such as esters, acids, aldehydes) and hydrocarbons, which are found in three lines in the central part of the chromatogram. Line 1: mainly saturated hydrocarbon; line 2, especially olefins, and line 3 mainly oxygenates such as acids, esters, aldehydes and others. (FIG. 1a). It was also noted that the optimized conditions in the GC×GC ensured the distribution of the compounds in the chromatographic space with the PAHs preferentially located in different zones of the other functions, except for the sulfur compounds which co-eluted with dimethyl, trimethyl and tetramethylphenanthrene. The circles indicate the regions where the PAHs have been found, illustrate the separation of the compounds of the matrix, which enables and enhances the ability to identify chromatography.

FIG. 1b presents the characteristics of regions (marked by colored circles) from different classes of compounds as well as the location of the main groups of PAHs. In this Figure, it is also observed that when specific m/z values were selected, the PAHs showed a structured pattern of separation space. This behavior was employed in identification of families of compounds present in different extracts.

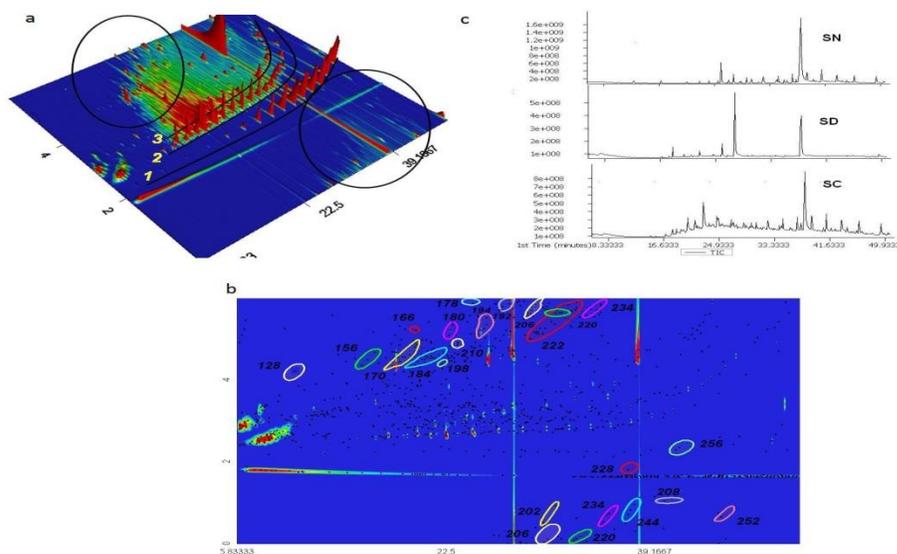


FIG. 1a. GC×GC/TOFMS chromatogram 3D of the Fraction F2 obtained from the extract of contaminated sample in the water column (SC), 1b: Characteristics of regions (marked by colored circles) from different classes of compounds

and the main groups of PAHs. 1c: Chromatogram of the sediment samples (SN); contaminated direct in the sediment, samples (SD) and contaminated in the sediment by water column (SC).

The presence of saturated hydrocarbons was mainly determined by branched ones, alkenes and monoaromatic, which were supposed to remain in Fraction F1. Many of the PAHs were detected with lower intensities, which hinder the visualization of the peak when analyzed in TDI (FIG. 1c).

Qualitative determination

TABLE 3 shows the PAH identified and the retention times are expressed in two dimensions (1TR and 2TR) and a single mass of compounds (U). For the sample in the contaminated water column (SC), 161 PAHs have been found containing from 2 to 5 ring alkylated. In the sample with direct contamination (SD), it was detected 33 compounds, mainly fluorenes, anthracenes, phenanthrenes and pyrenes, also presenting many PAHs with radical methyl, a characteristic of petrogenic contamination source.

In the sediment sampled (SN) 24 HPA's were identified being mostly heavier and without ramifications. The presence of compounds that have 4 to 6 ring natural sediment is due to its greater persistence in the environment and they are typical contaminants from pyrolytic sources. These results are in agreement with the results obtained by Luz et al. [28], and Sanches Filho et al. [29] in studies conducted in the Patos Lagoon.

TABLE 3. Compounds identified by GCxGC / TOFMS, using patterns or by similarity with the equipment's internal library, in the SA, SD and SC samples

Peak	N° rings	Compound	1 _{tr}	2 _{tr}	U	MF	SA	SD	SC	Similar. %
1	2	Naphthalene	10.63	4.21	128	C8H10	x		x	
2	2	C2 Naphthalene	17.03	4.73	156	C12H12	x	x	x	911
3	2	C2 Naphthalene	15.83	4.36	156	C12H12	x	x	x	929
4	2	C2 Naphthalene	16.23	4.50	156	C12H12	x	x	x	859
5	2	C3 Naphthalene	18.03	4.49	170	C13H14		x	x	873
6	2	C3 Naphthalene	17.03	4.27	170	C13H14			x	923
7	2	C3 Naphthalene	18.43	4.51	170	C13H14		x	x	868
8	2	C3 Naphthalene	19.33	4.50	170	C13H14			x	945
9	2	C3 Naphthalene	20.53	4.94	170	C13H14			x	865
10	2	C3 Naphthalene	19.63	4.63	170	C13H14			x	907
11	2	C3 Naphthalene	21.63	4.18	170	C13H14			x	717
12	2	C3 Naphthalene	22.83	4.33	170	C13H14			x	702
13	2	C3 Naphthalene	20.13	4.86	170	C13H14		x	x	947
14	2	C3 Naphthalene	22.83	4.34	170	C13H14			x	702
15	2	C3 Naphthalene	20.23	4.81	170	C13H14		x	x	950
16	2	C3 Naphthalene	18.83	4.46	170	C13H14		x	x	947

17	2	C3 Naphthalene	18.33	4.43	170	C13H14			x	903
18	2	C3 Naphthalene	21.83	4.24	170	C13H14			x	557
19	2	C3 Naphthalene	20.73	5.14	168	C13H12			x	857
20	2	C3 Naphthalene	20.43	5.32	168	C13H12			x	874
21	2	C3 Naphthalene	17.83	4.35	170	C13H14		x	x	813
22	2	C4 Naphthalene	19.13	4.28	184	C14H16			x	734
23	2	C4 Naphthalene	20.43	4.31	184	C14H16		x	x	807
24	2	C4 Naphthalene	20.73	4.61	184	C14H16			x	780
25	2	C4 Naphthalene	20.53	4.45	184	C14H16			x	747
26	2	C4 Naphthalene	22.63	4.62	184	C14H16		x	x	799
27	2	C4 Naphthalene	22.03	4.55	184	C14H16		x	x	897
28	2	C4 Naphthalene	19.13	4.23	184	C14H16		x	x	734
29	2	C4 Naphthalene	21.03	4.41	184	C14H16		x	x	813
30	2	C4 Naphthalene	20.03	4.38	184	C14H16		x	x	764
31	2	C4 Naphthalene	22.83	4.76	184	C14H16			x	886
32	2	C4 Naphthalene	21.63	4.45	184	C14H16			x	833
33	2	C4 Naphthalene	23.03	4.81	184	C14H16		x	x	868
34	2	C4 Naphthalene	19.63	4.24	184	C14H16		x	x	824
35	2	C4 Naphthalene	20.23	4.43	184	C14H16			x	804
36	2	C4 Naphthalene	25.43	4.34	184	C14H16			x	653
37	2	C4 Naphthalene	20.83	4.48	184	C14H16			x	805
38	2	C4 Naphthalene	23.43	4.94	184	C14H16			x	881
39	2	C4 Naphthalene	19.73	4.37	184	C14H16			x	835
40	2	C5 Naphthalene	25.03	4.37	198	C15H18			x	594
41	2	C5 Naphthalene	22.13	4.39	184	C15H18			x	853
42	2	C5 Naphthalene	26.43	5.03	184	C15H18			x	834
43	2	C5 Naphthalene	23.43	4.49	184	C15H18			x	760
44	2	C5 Naphthalene	23.33	4.44	184	C15H18			x	789
45	2	C5 Naphthalene	23.63	4.47	198	C15H18			x	774
46	2	C6 Naphthalene	24.23	4.29	212	C16H20	x		x	740
47	2	C6 Naphthalene	21.43	4.32	210	C16H18			x	804
48	2	C6 Naphthalene	23.73	4.72	210	C16H18		x	x	847
49	2	C6 Naphthalene	26.23	4.76	210	C16H18			x	893
50	2	C6 Naphthalene	23.13	4.55	210	C16H18			x	767
51	2	C6 Naphthalene	24.63	4.74	210	C16H18			x	816
52	2	C6 Naphthalene	25.73	4.86	210	C16H18			x	735
53	2	C6 Naphthalene	24.43	4.88	210	C16H18			x	779

54	2	C6 Naphthalene	24.13	4.67	210	C16H18			x	760
55	2	C3tetrahydroNaphthalene	21.33	3.62	174	C13H18			x	581
56	2	C4 tetrahydroNaphthalene	17.73	3.91	188	C14H20			x	782
57	2	C8 tetrahydroNaphthalene	23.93	3.66	202	C16H10			x	749
58	2	C8 tetrahydroNaphthalene	24.93	3.74	202	C16H10			x	614
59	3	C1Phenylethyl Naphthalene	36.83	0.59	244	C19H16			x	771
60	3	C1Phenylethyl Naphthalene	37.03	0.73	244	C16H16			x	746
61	3	C1Phenylethyl Naphthalene	37.23	0.83	244	C19H16			x	766
62	3	Acenaphthene	20.53	5.09	154	C12H10			x	702
63	3	Fluorene	20.13	5.20	166	C13H10	x	x	x	915
64	3	C1 fluorene	23.13	5.35	180	C14H12	x		x	916
65	3	C1 fluorene	22.73	5.11	180	C14H12		x	x	925
66	3	C1 fluorene	20.63	4.98	180	C14H12		x	x	895
67	3	C1 fluorene	22.83	5.25	180	C14H12		x	x	918
68	3	C2 fluorene	24.83	5.15	194	C15H14		x	x	809
69	3	C2 fluorene	22.23	4.96	194	C15H14			x	769
70	3	C2 fluorene	23.33	5.11	194	C15H14	x	x	x	325
71	3	C2 fluorene	23.03	4.93	194	C15H14		x	x	850
72	3	C2 fluorene	26.03	5.39	194	C15H14			x	873
73	3	C2 fluorene	20.03	4.70	194	C15H14		x	x	870
74	3	C2 fluorene	25.73	5.36	194	C15H14		x	x	872
75	3	Tetrahydro Anthracene	23.93	5.31	182	C14H14			x	863
76	3	C1 dihydro Anthracene	25.13	5.11	194	C15H14		x	x	873
77	3	C1 dihydro Anthracene	25.33	5.20	194	C15H14	x	x	x	879
78	3	C1 dihydro Anthracene	25.53	5.27	194	C15H14			x	831
79	3	C2 dihydro Anthracene	27.93	5.28	208	C16H16			x	819
80	3	C2 dihydro Anthracene	27.43	5.02	208	C16H16			x	799
81	3	C2 dihydro Anthracene	28.03	5.34	208	C16H16		x	x	833
82	3	C2 dihydro Anthracene	22.83	4.90	208	C16H16			x	774
83	3	C2 dihydro Anthracene	27.53	5.11	208	C16H16		x	x	799
84	3	C2 dihydro Anthracene	25.33	4.88	208	C16H16			x	716
85	3	C2 dihydro Anthracene	28.73	5.49	208	C16H16		x	x	820
86	3	C2 dihydro Anthracene	27.63	5.18	208	C16H16		x	x	799
87	3	C2 dihydro Anthracene	25.63	5.04	208	C16H16		x	x	716
88	3	C2 dihydro Anthracene	28.33	5.29	208	C16H16			x	843
89	3	C2 dihydro Anthracene	28.43	5.35	208	C16H16		x	x	841
90	3	C2 dihydro Anthracene	31.23	4.94	222	C17H18			x	622

91	3	C2 dihydro Anthracene	31.13	5.37	222	C17H18		x	x	732
92	3	C2 dihydro Anthracene	30.43	5.28	222	C17H18		x	x	796
93	3	C2 dihydro Anthracene	29.73	5.13	222	C17H18			x	693
94	3	C2 dihydro Anthracene	31.03	5.39	222	C17H18			x	681
95	3	C2 dihydro Anthracene	24.93	4.83	222	C17H18			x	660
96	3	C2 dihydro Anthracene	30.23	5.15	222	C17H18		x	x	687
97	3	C2 dihydro Anthracene	24.63	4.55	222	C17H18			x	770
98	3	C2 dihydro Anthracene	24.83	4.75	222	C17H18	x		x	783
99	3	C2 dihydro Anthracene	24.53	4.62	222	C17H18	x		x	758
100	3	C3 dihydro Anthracene	29.73	5.18	222	C17H18		x	x	693
101	3	C3 dihydro Anthracene	29.03	5.00	222	C17H18		x	x	577
102	3	C3 dihydro Anthracene	29.23	5.02	222	C17H18		x	x	756
103	3	C3 dihydro Anthracene	29.53	5.18	222	C17H18		x	x	765
104	3	C2 Anthracene	30.03	0.01	206	C16H14		x	x	874
105	3	C2 Anthracene	29.03	5.67	206	C16H14		x	x	887
106	3	C3 Anthracene	29.63	5.39	220	C17H16			x	716
107	3	C3 Anthracene	30.43	5.73	218	C17H14			x	823
108	3	C3 Anthracene	33.53	0.80	218	C17H14			x	873
109	3	C3 Anthracene	32.13	0.12	218	C17H14			x	800
110	3	C3 Anthracene	39.93	1.07	218	C17H14			x	693
111	4	Benzo[a]Anthracene	37.03	1.82	228	C18H12	x	x	x	912
112	4	C1 benzo[a]Anthracene	39.63	2.62	242	C19H41			x	910
113	4	C1 benzo[a]Anthracene	39.33	2.45	242	C19H14			x	886
114	3	Phenanthrene	24.53	5.95	178	C14H10	x	x	x	949
115	3	C1 Phenanthrene	26.93	5.72	192	C15H12	x	x	x	936
116	3	C1 Phenanthrene	27.53	0.00	192	C15H12			x	936
117	3	C1 Phenanthrene	27.43	0.02	192	C15H12		x	x	927
118	3	C1 Phenanthrene	27.53	5.98	192	C15H12		x	x	934
119	3	C2 Phenanthrene	29.33	5.71	206	C16H14		x	x	791
120	3	C2 Phenanthrene	29.63	5.86	206	C16H14	x	x	x	891
121	3	C2 Phenanthrene	29.73	5.95	206	C16H14	x	x	x	892
122	3	C2 Phenanthrene	30.33	0.06	206	C16H14	x		x	885
123	3	C2 Phenanthrene	29.03	5.58	206	C16H14	x	x	x	887
124	3	C2 Phenanthrene	28.73	5.57	206	C16H14	x		x	809
125	3	C2 Phenanthrene	29.23	5.64	206	C16H14	x		x	883
126	3	C2 Phenanthrene	30.63	0.14	206	C16H14		x	x	885
127	3	C3 Phenanthrene	31.23	5.64	220	C17H16		x	x	762

128	3	C3 Phenanthrene	30.93	5.53	220	C17H16			x	738
129	3	C3 Phenanthrene	30.73	5.43	220	C17H16			x	724
130	3	C3 Phenanthrene	32.93	0.14	220	C17H16		x	x	830
131	3	C3 Phenanthrene	31.73	5.66	220	C17H16		x	x	859
132	3	C3 Phenanthrene	31.93	5.86	220	C17H16		x	x	855
133	3	C4 Phenanthrene	32.73	5.58	234	C18H18			x	740
134	3	C4 Phenanthrene	33.53	5.68	234	C18H18		x	x	769
135	3	C4 Phenanthrene	33.43	5.66	234	C18H18			x	672
136	3	C4 Phenanthrene	33.13	5.55	234	C18H18		x	x	775
137	3	C4 Phenanthrene	30.03	5.53	234	C18H18			x	
138	3	C4 Phenanthrene	35.33	0.17	234	C18H18			x	746
139	3	C4 Phenanthrene	34.23	5.76	234	C18H18			x	856
140	3	C4 Phenanthrene	33.73	5.70	234	C18H18			x	794
141	3	C4 Phenanthrene	34.03	5.74	234	C18H18		x	x	818
142	3	C4 Phenanthrene	35.03	0.04	234	C18H18			x	842
143	4	C2 benzo[c]Phenanthrene	41.63	2.82	256	C20H16			x	889
144	4	C2 benzo[c]Phenanthrene	41.73	3.08	256	C20H16			x	824
145	4	Pyrene	31.23	0.94	202	C16H10	x	x	x	915
146	4	C1 Pyrene	32.93	0.64	216	C17H12		x	x	872
147	4	C1 Pyrene	33.93	0.97	216	C17H12			x	904
148	4	C2 Pyrene	35.33	0.72	230	C18H14		x	x	844
149	4	C2 Pyrene	36.13	1.02	230	C18H14			x	816
150	4	C2 Pyrene	34.93	0.50	230	C18H14		x	x	845
151	4	C2 Pyrene	35.53	0.77	230	C18H14		x	x	838
152	4	C2 Pyrene	35.73	0.83	230	C18H14			x	864
153	4	C2 Pyrene	35.03	0.55	230	C18H14		x	x	849
154	4	Fluoranthene	30.23	0.52	202	C16H10	x	x	x	907
155	4	C1 Fluoranthene	33.23	0.76	216	C17H12			x	821
156	4	Chrysene	38.63	5.76	228	C18H12	x	x	x	
157	4	C1 chrysene	38.93	2.08	242	C19H14			x	875
158	5	Benzo[a]Pyrene	46.63	5.05	252	C20H12	x		x	
159	5	Benzo(aek)Fluoranthene	45.13	5.00	252	C20H12	x		x	
160	5	Perylene	44.43	0.67	252	C20H12		x	x	883
161	6t	Benzo[ghi]Perylene	54.23	3.94	276	C22H12			x	

Note: MF: molecular formula

The SC sample showed the highest number of compounds, including the lightest such as naphthalenes. Probably, during homogenization, the particulate matter was adsorbed, decanted and constituted the sediment and water column by acting as

a shield, and preventing the output of these compounds in the studied period [30].

Possibly, in the sample SD, there was loss of lighter compounds for being in direct contact with the atmosphere. PAHs with lower molecular weight have higher vapor pressure, and without the barrier of the water column, they could not be retained in the sediment. Yet, it is noticed that there was a significant increase in the number of compounds identified when the results are compared to the gross sediment (without added petroleum).

Quantitative determination

The recovery values found ranged from $75.47 \pm 2.57\%$ to $78.34 \pm 2.82\%$. The figures of merit and calibration curves obtained by the system GC/MS for the PAH's and deuterated standards for the mixture containing 16HPA's are shown in TABLE 4.

TABLE 4. Parameters of merit and analytical curves obtained by GC/MS for the PAHs and deuterated PAHs.

Compounds	Equation of the calibration curve			LOD ($\mu\text{g.L}^{-1}$)	LOQ ($\mu\text{g.L}^{-1}$)
	y=ax + b				
	a	b	r ²		
Phenanthrene D	1.82	-0.449	0.9967	0.576	1.92
Chrysene D	1.41	-0.354	0.9963	0.771	2.57
Perylene D	1.95	-0.469	0.9919	0.729	2.43
Naphthalene	0.0009	0.0012	0.9828	4.72	15.73
Acenaphthylene	0.0006	0.0018	0.9934	1.74	5.80
Acenaphthene	0.0006	0.0002	0.9919	2.32	7.73
Fluorene	0.0004	0.0013	0.9915	0.450	1.50
Phenanthrene	0.0006	0.0015	0.9865	2.23	7.43
Anthracene	0.0005	0.0006	0.9881	3.81	12.70
Fluoranthene	0.0004	0.0014	0.9868	3.02	10.07
Pyrene	0.0005	0.0011	0.9872	3.01	10.03
Benzo [a] anthracene	0.0004	0.0016	0.9802	3.05	10.17
Chrysene	0.0004	0.0012	0.9841	0.823	2.74
Benzo[b]Fluoranthene	0.0004	0.0013	0.9889	1.02	3.40
Benzo[k]Fluoranthene	0.0004	0.0007	0.9951	0.834	2.78
Benzo [a] Pyrene	0.0003	0.0005	0.9974	0.431	1.44
Indeno (1,2,3-cd) pyrene	0.0004	0.00003	0.9991	0.942	3.14
Dibenzo [ah] anthracene	0.0003	0.0002	0.9841	2.91	9.70
Benzo [ghi] Perylene	0.0002	0.0004	0.9923	1.44	4.80

y =relative area; a =slope; b =linear coefficient; r^2 =coefficient of linear correlation;
x =concentration in ($\mu\text{g L}^{-1}$); LOD= Limit of detection; LOQ=Limit of quantification

The results of quantitative analysis by GC/ MS of the 16 PAHS, considered priority pollutants present in the three pellet samples, as well as limits of detection and quantification are shown in TABLE 5.

TABLE 5. HPAS concentrations in the sediment on a dry basis determined by GC/MS-SIM mode.

Peak	Compounds	PAH concentrations*			LOD	LOQ
		SN	SD	SC		
1	Naphthalene	3.0 ± 7.2	2.2 ± 5.3	10.2 ± 4.2	0.5	1.6
2	Acenaphthylene	<LOQ	<LOQ	<LOQ	0.2	0.6
3	Acenaphthene	<LQ	<LQ	<LQ	0.2	0.8
4	Fluorene	<LQ	5.4 ± 4.2	38.8 ± 5.3	0.1	0.2
5	Phenanthrene	1.1 ± 5.2	32.9 ± 6.2	209.8 ± 6.1	0.2	0.7
6	Anthracene	<LOQ	<LOQ	<LOQ	0.4	1.3
7	Fluoranthene	4.9 ± 5.9	2.2 ± 1.2	178.3 ± 8.2	0.3	1.0
8	Pyrene	4.3 ± 5.3	1.6 ± 1.4	30.2 ± 2.3	0.3	1.0
9	Benzo [a] anthracene	<LOQ	4.6 ± 2.4	20.4 ± 2.4	0.3	1.0
10	Chrysene	10.3 ± 1.5	3.4 ± 3.5	21.4 ± 3.2	0.1	0.3
11	Benzo[b]Fluoranthene	0.4 ± 3.7	2.2 ± 5.2	19.3 ± 2.2	0.1	0.3
12	Benzo[k]Fluoranthene	<LOQ	0.5 ± 2.3	5.3 ± 3.1	0.1	0.3
13	Benzo [a] Pyrene	0.2 ± 2.4	0.9 ± 2.6	10.3 ± 4.2	0.1	0.2
14	Indeno (1,2,3-cd) pyrene	1.2 ± 2.9	0.7 ± 1.7	1.0 ± 3.7	0.1	0.3
15	Dibenzo [ah] anthracene	<LOQ	<LOQ	1.2 ± 2.5	0.3	1.0
16	Benzo [ghi] Perylene	1.8 ± 5.2	0.5 ± 1.8	2.0 ± 6.0	0.1	0.5
	Σ HPA's ($\mu\text{g kg}^{-1}$)	27.2	57.4	548.3		

Note: (*) $\mu\text{g kg}^{-1} \pm \text{RSD} (\%) (n=5)$

We observed that the major compounds in the samples without added oil appear from 4 rings, standing out from the Chrysene and Benzo (ghi) Perylene Pyrene and fluoranthene followed by the sum of PAHs under 4 rings, which represents 48.9% of the total, adding to sand oil chromatographic profile fluorene phenanthrene, benzo (a) anthracene, benzo (k) fluoranthene, dibenzo (ah) anthracene. The contaminated samples in the water column showed high amounts of fluorene, phenanthrene and fluoranthene figures that exceed the Threshold Effect Level (TEL), 21.2 mg.kg^{-1} for fluorene, 86.7 mg.kg^{-1} for phenanthrene and 113.0 mg.kg^{-1} for fluoranthene. This level established by the Canadian Environmental Agency [31], PAH concentrations below the TEL do not usually cause adverse effects on aquatic organisms.

The sum of PAHs with less than four rings rose from 77.2% to 85.2% of the total samples and direct contamination via water column, proving that the supply of this is the HPAS oil. These figures should actually be much higher, because they are being considered as compounds with nuclei without ramifications. What GCxGC showed is present in large quantity when

comparing samples under direct contamination and in the water column. We observed a dramatic reduction during the seven days of exposure of the sum PAHs since it was reduced to 89.5%. Suggested as a main route in the sediment concentration, this reduction is the displacement of these compounds into the atmosphere through evaporation.

Quantitative figures confirm the qualitative data analysis with a sum of the concentrations in the sample SC being much higher. So as to indicate the capacity of the water column associated with the characteristics of the sediment retaining the PAHs, which includes lower molecular weight. According to the classification suggested by Meire et al. [32], PAHs concentrations in sediments could be considered low for a sum situated in the range from 0 to 100 mg.kg⁻¹, moderate between 100 to 1000 mg.kg⁻¹, high between 1000 and 5000 mg.kg⁻¹ and very high when this figure is summed up. The sediment collected for studies shows low levels of contamination, and it presents mainly higher molecular weight PAHs.

The concentrations found in natural sediment are in agreement with bibliographic data in non-impacted areas (remote zone), similar to those obtained for the Black Sea [33] and the Patos Lagon, RS, Brazil [29-30].

Conclusion

The physico-chemical features of the sediment used for this experiment, especially with predominance of fine grain size and high organic matter content, provide a large sorption capacity and contaminant buildup, being confirmed by the results obtained in samples of quantitative and qualitative analysis that were contaminated with oil. It was also noted that the sediment exposed to air contamination was reduced around 90% in the first week. The experiment allowed us to show that accidents involving spills in the water column take longer to reduce contamination with higher impact in the estuary. The GCxGC/TOFMS has separated and identified a number of compounds present together with HPAs, which would co elute the one-dimensional chromatography, and ensure a more secure identification. The complex mixtures of hydrocarbons are appropriate samples to demonstrate the great separation power of GCxGC as well as its ability to generate chromatograms with highly organized profiles in the two-dimensional plan. The GCxGC also offers new opportunities to establish structured chromatograms (with contour lines based on the presence of structurally related compounds), which allows defining chromatographic spaces associated to certain molecular structures. This approach facilitates the identification of analytes in the samples.

The extracts were complex mixtures containing many other compounds beside the PAHs. The application of GCxGC/TOFMS as an analytical tool, allowed detecting PAHs in all samples. Sediment samples without added petroleum had a lower molecular diversity, generally unbranched rings.

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