DETERMINATION OF SIXTEEN POLYCYCLIC AROMATIC HYDROCARBONS IN AQUEOUS AND SOLID SAMPLES FROM AN ITALIAN WASTEWATER TREATMENT PLANT

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ABSTRACT

A robust procedure for the determination of 16 US EPA PAHs in both aqueous (e.g. wastewaters, industrial discharges, treated effluents) and solid samples (e.g. suspended solids and sludge) from a wastewater treatment plant (WWTP) is presented. Recovery experiments using different percentages of organic modifier, sorbents and eluting solvent mixtures were carried out in Milli-Q water (1000 mL) spiked with a mixture of the PAH analytes (100 ng/L of each analyte). The solid phase extraction (SPE) procedures applied to spiked waste water samples (1000 mL; 100 ng/L spiking level) permitted simultaneous recovery of all the 16 PAHs with yields >70% (6-13% RSD). SPE clean up procedures applied to sewage and stabilized sludge extracts, showed percent recoveries in the range 73-92% (7-13% RSD) and 71-89% (7-12% RSD) respectively. The methods were used for the determination of PAHs in aqueous and solid samples from the WWTP of Fusina (Venice, Italy). Mean concentrations, as the sum of the 16 PAHs in aqueous and suspended solid samples, were found to be approx. in the 1.12-4.62 μg/L range. Sewage and stabilized sludge samples contained mean PAH concentrations, as sum of 16 compounds, in the concentration range of 1.44-1.26 mg/kg, respectively. Extraction and clean up procedures for sludge samples were validated using EPA certified reference material IRM-104 (CRM No.912). Instrumental analyses were performed by coupling HPLC with UV- diode array detection (UV-DAD) and fluorescence detection (FLD).

INTRODUCTION

Wastewater treatment plants (WWTPs), especially those serving both urban and industrial areas, consistently receive complex mixtures, containing a wide variety of organic pollutants. Groups of compounds present in these mixtures include polycyclic aromatic hydrocarbons (PAHs), which are listed as US-EPA and EU priority pollutants, and their concentrations therefore need to be controlled in treated wastewater effluents [1-3]. PAHs are ubiquitous environmental pollutants with carcinogenic and mutagenic properties, which were also included in the Italian guidelines (Decree of April 23, 1998 for Water quality requirements and characteristics of purification plants to safeguard the Venice Lagoon) for treated waste monitoring programs [4]. As a consequence of their strongly hydrophobic properties and their resistance to biodegradation, PAHs are almost quantitatively removed from wastewaters by activated sludge treatments, which very efficiently relocate them into sludges [3]. Hence, residual suspended solids in treated effluents and in sludges from WWTPs may contain very high concentrations of PAHs [5-7]. Moreover, the practice of recycling sewage sludges directly, or after composting, onto agricultural lands, poses an additional risk of soil contamination *via* leaching of PAHs. As a consequence, a new draft directive of the Council of the European Community has been released regulating the maximum allowable concentrations of PAHs in the sewage sludges used in agriculture [8].

At present, many excellent analytical methods for extraction and determination of PAHs in wastewater and sludge, are available from the literature [1, 9-14]; this class of analytes are commonly analyzed by gas chromatography coupled with mass spectrometric detection (GC-MS) or by liquid chromatography (LC) coupled with fluorescence (FL) and UV-DAD detectors. Separation and detection steps are

extensively described by US-EPA Method 8100 and 8310, respectively. Both techniques possess high sensitivity and selectivity but MS provides greater specificity than FL or UV detectors, which is important since confirmation of the identities of some PAHs in complex environmental samples can be very difficult. Recently, however, commercial availability of LC detectors providing both UV-DAD and FL spectra has permitted improved confirmation of analyte identity by matching emission fluorescence or absorbance UV-DAD spectra with reference standard spectra [12, 15-16]. Furthermore, analysis of PAHs can now be performed by coupling LC with mass selective detectors *via* an atmospheric pressure photoionisation interface (APPI) which offers a different mechanism of ionization and provides greater sensitivity than electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) [17]. In general, LC analytical procedures require less intensive sample clean up procedures than the GC-based techniques mainly because LC analytical columns are normally equipped with guard pre-columns [11, 16]; furthermore the LC chromatographic system permits injection of much larger sample volumes (up to 200 μL) than GC-based techniques. GC-MS systems allow injection volumes of only a few microlitres, and hence samples are usually pre-concentrated by volatilization of the solvent. However, substantial volume reduction *via* solvent volatilization may results in losses of the more volatile compounds [11, 18]. Reliable Large Volume Injection (LVI) systems for GC have only recently become commercially available. These systems allow for the injection of larger extract volumes in the GC-MS system and are good rapid screening tools for environmental investigations [19].

Common analytical methods for the extraction of PAHs from wastewater samples involve liquid-liquid extraction using non-polar solvents or solid phase extraction (SPE)

using reverse phase or polymeric sorbing materials [1, 10, 15]. Because of their demonstrated versatility, selectivity and reproducibility [20], SPE techniques have been successfully applied, as extraction-enrichment-clean up procedures, to a wide range of environmental aqueous matrices such as surface water [21], precipitation [22], seawater [23] and wastewater [1, 9] samples. Adverse effects related to losses of PAHs during SPE procedures, have been extensively discussed by many authors. SPE parameters such as sorbing materials [24-25], flow rate through cartridges [22], organic modifier quality and/or content [22, 24], breakthrough volumes [22], eluting solvents [21, 22, 25-27] and interfering effects of humic acids [21], have been demonstrated to affect recovery rates and method reproducibility. The low solubility and hydrophobicity of the PAHs leads to problems of adsorption during sampling, storage and SPE procedures, and, in order to avoid these negative interferences, selected organic solvents, such as acetonitrile, 2-propanol or methanol are usually added as modifiers to the water samples [22, 24, 28]. In this work, the effects of different percentages of 2-propanol as an organic modifier were tested, processing large volume of aqueous samples through C_{18} cartridges. The studies were based on the research of El ArracK et al. (1996) [24], Kiss et al. (1996) [22] and Urbe et al. (1997) [28], who investigated the influence of different SPE conditions applied to the recovery of the PAHs from fortified water samples.

Many methods to extract PAHs have been successfully applied to a wide range of solid samples. Some of these have used mechanical shaking [29], Soxhlet extraction and ultrasonic extraction [13, 29] as well as alternative extraction techniques such as pressurized hot water extraction (PHWE) [30], supercritical fluid extraction, pressurized liquid extraction, focused microwave extraction in open vessels [13]. Irrespective of the extraction procedure used, the high content of organic matter has posed a major problem

in trace analysis of organic compounds in sewage sludges. Fats, proteins, carbohydrates, amino acids, lignin, sugar celluloses, humic materials and fatty acids constitute about 40-80% of sewage sludge dry weight, and a great proportion of these interfering compounds are co-extracted with the analytes [31-32]. Raw extracts from solid samples, are typically subjected to clean up treatments based on normal phase extractions using glass columns filled with Alumina, Silica gel and magnesium silicate (Florisil) [3, 11, 29]. These procedures are laborious, not easily automated and time consuming [14, 33-36]. In routine environmental analysis, in order to simplify and shorten extraction and clean up procedures, a faster and more reliable method is required. The aim of this study was to improve the SPE procedures for the recovery of the 16 US EPA PAHs by means of recovery experiments involving the processing of large sample volumes (900 mL), spiked analyte levels to match the expected environmental concentrations of PAHs (1-100 ng/L) and using different percentages of 2-propanol as organic modifier and eluting solvent mixtures not commonly employed. The analytical method presented in this work was developed in Milli-Q water, tested in wastewater samples and then applied to the determination of the PAHs from large volumes of polluted WWTP aqueous samples (industrial wastewaters, sewage influents, treated effluents). The proposed method was also extended to include the determination of the 16 PAHs in WWTP solid samples (i.e. suspend solids and sludge). In particular, a clean up procedure based on SPE on C_{18} sorbing material was successfully applied to raw extracts from sludge samples. Extraction and clean up procedures in WWTP sludges were validated using an EPA certified reference material. Instrumental analyses were performed using HPLC coupled with both UV-DAD and fluorescence (FL) detection.

2 EXPERIMENTAL

2.1. Materials and methods

The standard solution PAHs Mix-13 (US EPA 16) naphthalene (NAPH; 100 ng/μL) acenaphthylene (ACY; 100 ng/μL), acenaphthene (ACE; 10 ng/μL), fluorene (FLU; 10 ng/μL), phenanthrene (PHEN; 10 ng/μL), anthracene (ANTH; 10 ng/μL), fluoranthene (FLT; 10 ng/μL), pyrene (PYR; 10 ng/μL), benzo[a]anthracene (BaA; 10 ng/μL), chrysene (CHRY; 10 ng/μL), benzo[b]fluoranthene (BbF; 10 ng/μL), benzo[k]fluoranthene (BkF; 10 ng/μL), benzo[a]pyrene (BaP; 10 ng/μL), dibenzo[ah]anthracene (DiahA; 10 ng/μL), benzo[g,h,i]perylene (BghiP; 10 ng/μL) and indeno[1,2,3-c,d]pyrene (INPY; 10 ng/μL) purity > 99% was supplied by Dr. Ehrenstorfer (Augsburg, Germany). Soil/Sediment IRM-104 EPA Certified Reference Material (CRM No.912) was supplied by VESTA Spa laboratories and was purchased from ULTRAcheck (North Kingstown, RI, USA). The sorbents tested were Supelclean ENVI-18 (Supelco, Bellefonte, PA, USA) and StrataE and StrataM (Phenomenex, Torrance, CA, USA). Glassfiber filters (GF/F, 0.7μm) were purchased from Whatman (Clifton, NJ, USA). Acetonitrile, 2-propanol, acetone, n-hexane, were HPLC ultra-gradient solvents (Romil, Dublin, Ireland). The water for chromatographic purposes was purified using a Milli-Q system (Millipore, Bedford, MS, USA). All the working standard solutions were prepared daily by diluting the PAHs Mix-13 standard solution in 2-propanol with an Agilent G1313A autosampler (Avondale, PA, USA). In order to prevent photochemical degradations, standard solutions and sample extracts, were stored in brown glass vials at 4 °C.

2.2. Sampling points and sample pre-treatments

Aqueous samples (municipal and industrial wastewater influent and effluent) and solid samples (suspended solids, biological and stabilized sludges), were manually collected in October 2002 from the wastewater treatment plant of Fusina (Venice, Italy). The grab samples were representative of the quality of both the influent and the effluent streams at the WWTP. The plant in question receives approx. 90000 m^3/d of raw wastewater from the major neighbouring residential districts (Mirese area, Marghera and the South-West area of Mestre city) and approx. 10000 m^3/d of pre-treated industrial waste from the industrial area of Porto Marghera-Fusina. The treatment plant also treats part of the local urban runoff, which is mainly composed of atmospheric deposition and traffic emissions deposited on the road surface, as well as approximately 300 m^3/d of untreated industrial waste, sewage from passenger ships and sludge from Imhoff tanks, which are transported to the plant by truck-tankers. The grab samples were collected from different points along the WWTP (see figure 1). A brief description of the sampling points chosen along the treatment plant is summarized in the following: (Site1) municipal wastewater from the sewerage serving urban districts of Venice; (Site2) pre-treated industrial wastewater from the sewerage serving a power station (ENEL); (Site 3) pre-treated industrial wastewater from the sewerage serving a petrochemical site (ENICHEM); (Site 4) interstitial water from the filtration of the secondary WWTP sludge; (Site 5): secondary WWTP sludge (biological sludge); (Site 6): stabilized sludge; (Site 7) treated effluent.

In order to prevent bacterial and photochemical degradation, a solution of $HgCl_2$ in water (10 mL; final concentration: 100 mg/L) was added to the liquid samples which were stored at 4 °C in amber glass bottles prior to extraction. The suspended solids in

the liquid phase were separated by filtration using glass fibre filters (0.7 μm). Filters were frozen (-40 °C), lyophilized, weighed and stored in the dark in aluminum foils at 4 °C prior to extraction. Bacterial degradation in sludge samples was prevented by adding $HgCl_2$ before homogenization of samples, lyophilization and storage in dark jars at 4 °C prior to extraction. Laboratory glassware for analytical purposes was cleaned with n-hexane, 2-propanol and Milli-Q water before use. Aluminum foils and Whatman GF/F filters were pre-cleaned by sonication using n-hexane and 2-propanol and then gently dried overnight (12h, 80 °C). All chemical analyses were performed within 96 h after sampling.

2.3. Recovery experiments in spiked Milli-Q water

Aliquots of 2-propanol (i.e. 5, 10 and 15% (*v/v*)) were added to Milli-Q water, and the samples were shaken and then spiked with 10 μL of the US EPA PAHs Mix-13 standard solution to obtain final concentrations of 1 μg/L for NAPH, ACY and 0.1 μg/L for the other 14 PAHs. The spiked samples (1L) were processed by SPE using an automated Aspec XL extractor (Gilson Middleton, WI, USA). Three different sorbents (StrataE, StrataM, and Supelclean Envi-18) were tested. The SPE stationary phases were conditioned at a flow rate of 3 mL/min with sequential elution of acetonitrile (9 mL), 2-propanol (9 mL) and a solution of Milli-Q water: 2-propanol (12 mL) proportional to the content of 2-propanol previously added into the water sample (e.g. Milli-Q water: 2-propanol 95:5 *v/v;* 90:10, *v/v;* 85:15 *v/v*, respectively) acidified at pH 2.5 with HCl (37%, *v/v*). The aqueous samples were passed through the cartridges at a flow rate of 10 mL/min. Cartridges were cleaned up by eluting a solution of Milli-Q water and 2-propanol (30 mL; 95:5 *v/v*, Milli-Q water: 2-propanol 95:5 *v/v;* 90:10, *v/v;* 85:15 *v/v*,

respectively) acidified at pH 2.5. The cartridges were dried under vacuum in a manifold system (Supelco) for 10 minutes. The analytes were subsequently eluted at a flow rate of 1 mL/min using three different solvent mixtures as eluents (4x3 mL): Sol-1: (n-hexane), Sol-2: (n-hexane: acetone, 90:10 *v/v*) and Sol-3: (n-hexane: acetone: 2-propanol, 90:5:5 *v/v*). The combined extracts were added to 2-propanol (approx. 1 mL) and then concentrated to 500 μL under a gentle stream of nitrogen in an automated evaporator (Turbovap II, Zymark Darlington, MA, USA) set at 25 °C. The final extracts were diluted to 1 mL with 2-propanol and then stored in 2 mL Teflon-lined screw capped brown-glass vials at 4 °C, until chemical analyses.

2.4. Wastewater samples treatment

Recovery experiments: an aliquot (900 mL, n=3) of each filtered aqueous sample collected from Site 1-4 and 7, was spiked with the US EPA Mix-13 standard mixture (10 μL). The spiked samples were homogenized by shaking and then processed by SPE on StrataE cartridges (1g, 6 mL) using the automated Aspec XL extractor. An organic modifier, 2-propanol (10% *v/v*), was added to aqueous samples before performing SPE procedures. Unspiked samples (i.e. “blank” samples) were also processed, in an identical manner to the spiked samples. The SPE stationary phases were conditioned at a flow rate of 3 mL/min with sequential elutions of acetonitrile (9 mL), 2-propanol (9 mL) and a solution of Milli-Q water: 2-propanol (12 mL; 90:10, *v/v*) acidified at pH 2.5 with HCl (37%, *v/v*). The aqueous samples were passed through the cartridges at a flow rate of 10 mL/min. Interferences were removed from sorbing material by eluting through the cartridges a solution of Milli-Q water and 2-propanol (30 mL; 90:10 *v/v*) acidified at pH 2.5. The cartridges were dried under vacuum in a manifold system

(Supelco) for 10 minutes. The analytes were subsequently eluted at a flow rate of 1 mL/min using a solvent mixture consisting of n-hexane: 2-propanol: acetone (90:5:5 *v/v*; 4x3 mL aliquots). The combinated extracts were then treated as described in Section 2.3 for the solvent removal step.

Environmental samples: the samples used for the analytical determination of PAHs in wastewaters, were treated as described above, except that the spiking solution was not added to them. Concurrently with wastewater samples, Milli-Q water (1000 mL) was extracted as a procedural "blank".

2.5. Spiking, extraction and clean up procedures applied to sludge samples from the WWTP

- Recovery experiments: samples (2.5 g dry weight, n=3) of lyophilised sludge collected from Site 5 (biological sludge) and Site 6 (stabilized sludge), were spiked with the US EPA PAHs Mix-13 (250 μL), and then extracted using a Branson 5510 ultrasonication bath. The extraction solution was 2-propanol: acetone (50:50, *v/v*) (3x20 mL, 1h extraction time for each solvent aliquot). The combined extracts (approx. 50 mL) were filtered through glass fibre filters (0.7 μm), dissolved in Milli-Q water (450 mL), and then processed by SPE as described in Section 2.3 for liquid samples. The cartridges were cleaned up by sequential elution of Milli-Q water: 2-propanol (90:10, *v/v*; 50 mL) followed by Milli-Q water (30 mL). The sludge extracts were then treated as described in Section 2.3 for the solvent removal step.

- Environmental samples: the samples used for the analytical determination of PAHs in sludges were treated as described above, except that the spiking solution was not added

to them.

- Reference material: in order to validate extraction and clean up procedures in sludge samples, a certified material was also tested: a sample (approx. 1 g, n=3) of PAHs contaminated Soil/Sediment IRM-104 EPA Certified Reference Material was weighed into a flask and then extracted using the method described above.

2.6. Spiking and extraction procedures applied to suspended solid samples from the WWTP

- Recovery experiments: suspended solids (triplicate determination + "blank" sample) retained on filters from 1000 mL of untreated municipal wastewater sample (Site 1) and from an equal volume of treated effluent sample (Site 7) were lyophilized, weighed, spiked with the US EPA PAHs Mix-13 standard solution (50 μL), and then extracted by sonication using a solvent mixture of n-hexane: 2-propanol: acetone (90:5:5, *v/v*; 15x3 mL; 1h for each aliquot). No clean up procedure was applied to raw extracts which were combined, filtered through glass fibre filters (0.7 μm) and then treated as reported in Section 2.3 for the solvent removal step.

- Environmental samples: the sample procedure involving the determination of PAHs in suspended solids retained from wastewater samples was treated as described above, except that the spiking solution was not added to them.

2.7. Chromatographic separation and detection

The sample extracts were injected in an Agilent 1100 HPLC system (Palo Alto, CA, USA) using an Agilent G1313A autosampler. The chromatographic separation of the sixteen PAHs was performed using an Envirosep PP C_{18} column (125 X 3.2 mm I.D., 3

μm), protected by two C_{18} guard columns (4x3 mm) supplied by Phenomenex. The LC column temperature was set at 13 °C using an Agilent G1316A thermostated column compartment. The mobile phase was a mixture of acetonitrile (A)/ water (B) at 0.7 mL/min; the initial composition (40% (A)) was held for two minutes and then increased to 99% over a period of 28 minutes. The PAHs were analyzed using an Agilent 1100 series multi-wavelength Fluorescence Detector. The fluorescence detection conditions (excitation-Ex, emission-Em) were as follows: NAPH, ACE, FLU Ex=260 nm, Em=340 nm; PHEN, ANTH, PYR, BaA, CHRY Ex=260 nm, Em=392 nm; FLT, BbF, BkF, BaP, DiahA, BghiP Ex=260 nm, Em=432 nm; INPY Ex=260 nm, Em=520 nm. ACY was analyzed with a UV-DAD detector set at 210 nm. The quantification of PAHs in both spiked and unspiked wastewater samples was carried out using a standard addition method (see figure 2), while an external calibration method was used to quantify the analytes in recovery experiments involving spiked Milli-Q water. In both cases, five calibration levels in the range 1-250 ng for NAPH and ACY and in the range 0.1-25 ng for the other analytes (as the injected amount), were used to obtain the calibration lines which showed good linearities ($R^2 > 0.998$) for all the 16 PAHs investigated. The sensitivity and precision of the method were also evaluated. The precision, represented as % RSD, from 10 replicate analyses, was less than 6% for all the compounds except for ACE which was 8.2%. Limits of detection (LODs) and limits of quantitation (LOQs) were determined in real sample extracts (i.e. unspiked WWTP aqueous and solid samples), and were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10 times the baseline noise, respectively. LODs were in the range 0.52-1.2 ng/L and in the range 4.1-12.1 ng/g for extracts from liquid and solid samples, respectively, depending on the compound in question (Table 4). Identification of

analytes in the chromatograms was based on retention times, combined with structural confirmation, which was performed by matching the FLD and the UV-DAD analyte spectra with spectra from the reference library. The recovery percentages obtained from spiked WWTP samples (both in the liquid and solid phases), were corrected by subtracting the contributions attributed to PAHs in "blank" samples. Operation and setting of the HPLC system were controlled by Agilent Chemstation 08.03 software.

3 RESULTS AND DISCUSSION

3.1. Development of SPE method in spiked Milli-Q water

Various SPE conditions including sorbents, organic modifier percentages and eluting solvents, were tested in order to achieve acceptable recoveries upon extraction of large volumes (1000 mL) of Milli-Q water samples spiked with the PAH analytes. In order to avoid adsorption of spiked PAHs onto glass surfaces of equipment used during sample processing [28, 37-39], aliquots of 2-propanol (i.e. 5, 10 and 15% (*v/v*)) were added to Milli-Q water. The volume to be processed through the cartridges was 1000 mL, in order to test the ability of the cartridges to recover the spiked analytes from large volumes of aqueous samples. The SPE procedure employed is described in detail in Section 2.3 and 2.4. Three different sorbents (StrataE, StrataM, and Supelclean Envi-18) were tested. Moreover, three different solvent mixtures were used as eluents: Sol-1: (n-hexane), Sol-2: (n-hexane: acetone, 90:10 *v/v*) and Sol-3: (n-hexane: acetone: 2-propanol, 90:5:5 *v/v*). Dichloromethane, tetrahydrofuran and acetonitrile, which are frequently employed as eluting solvents, [22, 25, 40-41], were not considered for this work, since one aim of the study was to test the possibility of using different mixtures of

solvents that had not been commonly used in previous studies. Pure 2-propanol and methanol were not tested because of poor percentage recoveries achieved for the high molecular weight PAHs [22]. The results of these experiments are summarised in Tables 1-3, where the percentage recovery and the corresponding RSD for each US EPA PAHs are reported.

3.1.1. Performance of stationary phases

Marked differences were observed in the performance of the three stationary phases tested, both in their ability to recover analytes and in the reproducibility of the results. The lowest percentage recoveries and the highest standard deviations (Tables 1-3) were obtained using the StrataM cartridges, which gave the poorest results, irrespective of the eluent compostion and the proportion of organic modifier used. The superior performance of the Envi-18 and StrataE stationary phases was probably due to the fact that both of these consist of end-capped silica, while the StrataM is un-endcapped. The residual polar groups on un-endcapped silica may result in poor retention of the PAH analytes onto the stationary phase. In addition, surface area and carbon loading, which are parameters influencing the retention of compounds onto sorbing materials, are lower in StrataM than in StrataE and in Envi-18 (i.e. 307, 494; 481 (m^2/g) and 10.89, 17.52, 18.04 (%)) respectively. Both of the endcapped stationary phases (i.e. StrataE and Envi-18), gave satisfactory recoveries for the 4-5 rings PAHs, but StrataE exhibited better percentage recovery and reproducibility values for both 2-3 ring and 5-6 ring PAHs. Thus, only the data concerning Envi-18 and StrataE stationary phases are discussed in detail in the following paragraphs.

3.1.2. Effect of organic modifier

In agreement with the results obtained in previous studies [22, 24, 28], the concentration of organic modifier was found to be a key factor in the efficient recovery of the entire suite of PAHs. This was demonstrated by the decrease in the ratios of the percentage recoveries of the 16 PAHs that were obtained using 5% and 15% of 2-propanol in spiked Milli-Q water samples (Figures 3-5). The lowest concentration of 2-propanol (5%, *v/v*) promoted the recovery of 2-3 ring PAHs (ratio values >1), while 2-propanol at the highest concentration (15%, *v/v*) promoted the recovery of 5-6 aromatic ring compounds (ratio values <1). No significant differences in the percentage recoveries were observed for the 3-4 aromatic ring PAHs (approx. from ANTH to BkF), and these compounds appeared to be the least sensitive to the amount of organic modifier added to the water samples (ratio values remained $\approx$1). As shown in Figures 3-5, the ratios decreased consistently with increasing analyte molecular weight, and did not appear to be affected by the type of sorbing material or the solvent mixture used. These results were as expected, based on the increasing hydrophobic properties of these compounds with increasing molecular weight. Our observations are consistent with those reported by many authors who observed that the concentration of the organic modifier is a critical parameter influencing the quantitative recovery of PAHs from spiked aqueous samples [16, 22, 24, 28]. When the amount of 2-propanol added to water samples was set at 5% (*v/v*), the recoveries for the High Molecular Weight PAHs (HMW-PAHs) decreased because of their low water solubilities, leading to irreversible adsorption of these compounds onto glass surfaces during sample processing. In contrast, when the amount of 2-propanol added to water samples was set at 15% (*v/v*), the observed recovery yields increased for the HMW-PAHs, while a decrease in the percentage

recoveries was observed for the Low Molecular Weight PAHs (LMW-PAHs). This was due to the higher sample eluotrophic strength at high modifier concentrations, which resulted in lower breakthrough volumes for the 2-3 aromatic rings PAHs. Therefore, an organic modifier strength of 10% of 2-propanol was chosen as a good compromise (See on Table 2 and Figure 4), since this would adequately prevent losses due to surface adsorption of the HMW-PAHs, without excessive losses of LMW-PAHs due to breakthrough of the SPE phase. This point is especially important when large volumes (1000 mL) of spiked water samples are processed through the cartridges.

3.1.3. Effects of SPE eluent composition

The optimum SPE conditions for the simultaneous recovery of the 16 PAHs from aqueous samples were determined by comparing the recoveries achieved by varying the two best sorbing materials (StrataE and Envi-18), the eluting mixtures and the concentrations of 2-propanol added to Milli-Q water. A careful analysis of these variables, plotted in Figures 6-8, shows that the best compromise, resulting in percentage recoveries higher than 82% for each US EPA PAHs was achieved using an organic modifier content of 10% of 2-propanol (*v/v*), with StrataE as the stationary phase and Sol-3 as the eluting mixture. The influence of the solvent mixture compositions on the recovery yields clearly emerges from comparison of the results obtained using StrataE and Sol-1, 2 or 3 as eluting mixtures. Sol-1 (n-hexane) gave unsatisfactory percentage recoveries for both LMW and HMW-PAHs. This may have been due to interference from residual water that was not completely removed from cartridges. In particular, Kiss et al. (1996) [22] reported the difference observed in the percentage recoveries of PAHs with or without drying the cartridges. Regardless of the

drying procedure applied in this work (e.g. vacuum system) we noticed that traces of water can be retained on the surface and within the pores of the SPE phase. Residual moisture can hinder the elution of analytes when a non-polar solvent (e.g. n-hexane), which has low miscibility with water, is used. Furthermore, losses by volatilization of LMW-PAHs, have been observed when SPE cartridges have been subjected to excessive drying procedures [42]. In order to avoid these negative effects, a small amount of a polar solvent such as 2-propanol or acetone was added to the n-hexane. The polar solvent added to the extraction solvent mixture thereby removed any residual water traces remaining within the cartridges, permitting a more efficient interaction between the analytes and the (non-polar) extraction solvent (non-polar fraction). As a result, Sol-2 (hexane: acetone, 90:10 *v/v*) and Sol-3 (hexane: acetone: 2-propanol, 90:5:5 *v/v*) which contained low amounts of polar solvents, gave improved recovery yields for the more volatile PAHs (i.e. 2-3 rings). However, Sol-3 gave the best recoveries for the less volatile and more hydrophobic PAHs (i.e. 5-6 rings) suggesting that the inclusion of 2-propanol (which is less polar than acetone) in the elution solvent mixture, may result in improved the recoveries of 5-6 aromatic ring PAHs. The percentage recoveries achieved by processing 1000 mL of a sample of Milli-Q water spiked with 10 μL of US EPA PAHs Mix-13, were very close to those obtained by El Harrak et al. (1996) [24] who processed samples of similar composition, but lower volume (200 mL). Our results are also in agreement with those obtained by Urbe et al. [28], who processed 1000 mL of Milli-Q (10% 2-propanol) through SPE glass fiber matrix (GFM) disks, obtaining similar recovery percentages. In conclusion, these studies demonstrate the advantages of using SPE tubes or SPE disks instead of classical liquid-liquid extraction methods for the analysis of PAHs. The ability to process large

sample volumes is advantageous especially where low LODs are required for very dilute samples (e.g. filtered effluent from WWTPs or similar environmental samples such as potable water or seawater samples).

3.2 Recovery of PAHs from spiked WWTP aqueous samples

With the optimal SPE conditions established for recovery of the analytes from spiked Milli-Q water, the percentage recoveries of the 16 US EPA PAHs were determined in spiked wastewater samples. The grab samples were collected from different points along the WWTP and from the sewerage which supplies it (see on Paragraph 2.2 and 2.4 for more details concerning sampling points and sample treatment, respectively). Quantification of PAHs in spiked samples was carried out using a standard addition method. The average percentage recoveries obtained from the spiked samples (Table 5) were corrected by subtracting the contributions attributed to PAHs in the corresponding "blank" samples. The resulting percentage recoveries of the 16 PAHs were in the range 70-95% (5-13% RSD). A comparison between these results with those obtained in spiked Milli-Q water showed that the matrix effects in the WWTP samples resulted in an average loss in the recoveries of analytes in the range of 5-15%. As previously reported, the observed losses in aqueous WWTP samples can be attributed to both the absorption of PAHs onto particulate matter in the samples [11], and to interferences in the SPE extraction step by organic material remaining in the filtered samples (< 0.7 μm). Non-analyte organic material present in wastes (e.g. humic substances, lipids, proteins, carbohydrates) can compete with the PAHs for adsorption sites on the solid phase, preventing efficient extraction of analytes from the aqueous phase. Surprisingly, regardless of the extent of wastewater treatment which efficiently removes much of the

organic content from wastes, no significant differences in recovery yields were observed between the treated and the untreated wastewater matrices. The spiked municipal wastes collected from the Site 1 showed average percentage recoveries in the range 70-93%, while the treated effluents collected at the end of the waste treatment plant (Site 7) showed average recovery percentages in the range 71-95% (Table 5). These results suggest that the organic modifier used to prevent unwanted adsorption of spiked HMW-PAHs onto surfaces during sample processing, can also help to prevent adsorption of the weakly retained interfering organic material onto the SPE phase.

3.3. Recovery of PAHs from spiked suspended solid samples from the WWTP

The percentage recoveries obtained in these experiments were corrected to account for the contribution of each PAH in the "blank" sample, and resulted in the range 71-95% (6-12% RSD) (See Table 5, Site 1* and Site 7*).

3.4. Recovery of PAHs from spiked sludge samples collected from the WWTP

The obtained recovery values and RSDs are listed in Table 5 (Sites 5 and Site 6, respectively). The contribution of each PAH in a "blank" sample was subtracted from the corresponding value obtained in each spiked samples to calculate the reported values. The sewage and the stabilized sludge extracts showed similar percentage recoveriss, ranging between 73-92% (7-13% RSD) and 71-89% (7-12% RSD), respectively. Considering the high matrix complexity of the WWTPs sludges due to their high content of organic matter, the clean up procedure based on C_{18} sorbents was shown to be suitable for routine analysis of PAHs in sludge samples, demonstrating the versatility of the SPE technique also when it was applied to critical environmental

samples such those considered in this work. These results are in agreement with those obtained by Sun et al. (1998) [26], who achieved the best results for the recovery rates of PAHs in soils using ultrasonication and acetone in the extracting step followed by a clean up procedure based on C_{18} cartridges using acetone:THF=1:1 (*v/v*) as the elution solution.

3.5 Validation of extraction/clean up procedures in sludge using a reference material

Listed in Table 6 are the reference values, the confidence intervals and the prediction intervals for the certified material, and the analytical mean values, the percent recoveries and the RSDs determined using the method described in this paper. The results for each PAH considered are in total agreement with those reported for the certified sample and very close to the results achieved with spiked sludge samples.

3.6. Concentrations of the selected PAHs in samples from the Fusina WWTP

The analytical methods developed and validated in aqueous and solid samples were applied to determine the 16 PAHs in real samples collected from the Fusina WWTP. Columns 2, 4, 6, 8 and 10 of Table 7 report the analytical concentrations recorded in filtered aqueous samples, while columns 3, 5, 7, 9 and 11 show the total concentrations as sum of the PAHs analytical concentrations found in the suspended solids and in the filtered aqueous phase. The data listed in the latter columns (i.e. columns 3, 5, 7, 9 and 11) represents the PAH concentrations determined in the filtered samples plus the PAH concentrations determined in the suspended solids retained from 900 mL of aqueous sample. The highest concentration of PAHs, expressed as the sum of the 16 compounds,

were found in wastes from Site 2 (4.62 μg/L), with similar values at the other sites: (Site 1) 3.77 μg/L; (Site 3) 3.56 μg/L; (Site 4) 3.77 μg/L respectively. The treated effluent sample (Site 7) showed the lower concentration level of PAHs with a recorded value of 1.12 μg/L.

Due to both the hydrophobic characteristics of the investigated compounds and the concentrations of suspended solids in the samples, the concentration levels of the 16 PAHs, were very low in the filtered phases of all of the WWTP liquid samples. Especially in the treated effluent samples, the concentrations of the individual compounds, were very low (2 - 8 ng/L) and in some cases close to the estimated LODs (see Table 4). This observation demonstrates that the very large sample volumes (~1000 mL) that were used in this study are necessary to detect PAHs in filtered aqueous samples from WWTPs. It is unlikely that processing volumes less than this through SPE cartridges, with percentage recoveries much lower than 70%, would detect the selected PAHs at the concentrations recorded here.

Table 8 shows the PAHs concentration levels determined in samples of activated and stabilized WWTP sludges. With few exceptions, the concentrations of the investigated PAHs in the activated sludges were higher than those recorded in the dried sludges. The concentrations of PAHs from Site 5 and Site 6 were 31.5 (NAPH) and 137.6 (PYR) ng/g and 28.4 (NAPH) and 98.6 (BaA) ng/g, respectively. In particular, a comparison among the PAHs concentrations detected in the activated sludge samples from the WWTP of Fusina with those recorded by Perez et al. (2001) [43], in two municipal WWTPs, and in three industrial/urban (about 60/30) Spanish WWTPs, showed comparable amounts of such pollutants in sludges.

4-CONCLUSIONS

The analytical methods decribed in this study were shown to be reliable for the determination of the 16 US EPA PAHs in both solid and liquid samples from a WWTP. In particular, it was demonstrated that large sample volumes, could be processed through C_{18} cartridges with minimal breakthrough of LMW-PAHs. Furthermore, the capacity of the method to analyse PAH concentrations in suspended solids from liquid samples, allows accurate evaluation of the total concentration and the partition coefficient of each investigated pollutant in real samples. The percentage recoveries of PAHs obtained from spiked sludges were consistent with recoveries reported using similar methods [26], and the validity of the method was also confirmed using a certified reference material. Moreover, the related clean up step applied to sludge extracts can be easily automated using an automated extractor, while alternative clean up procedures, such as those using Alumina, Florisil, Silica gel, cannot. It is intended to apply the analytical methods presented in this work to the monitoring of 16 US EPA priority pollutants in Fusina WWTP, by adopting sampling strategies based on composite samples obtained over 24 hour periods. In order to better characterize wastewater loads at the treatment plant and treated wastes discharged in Venice Lagoon, the methods will be further developed for additional investigations on oxygenated intermediates of chemical and biological degradation of PAHs (e.g. hydroxy and acid derivates).

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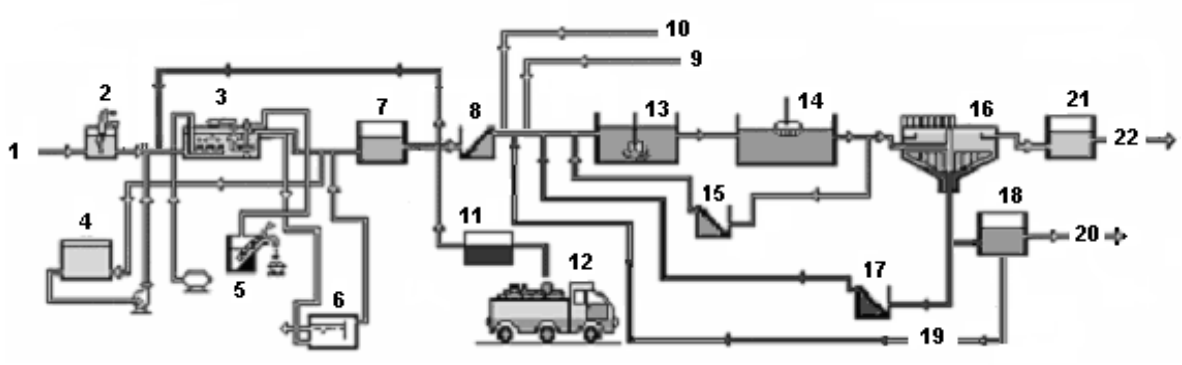
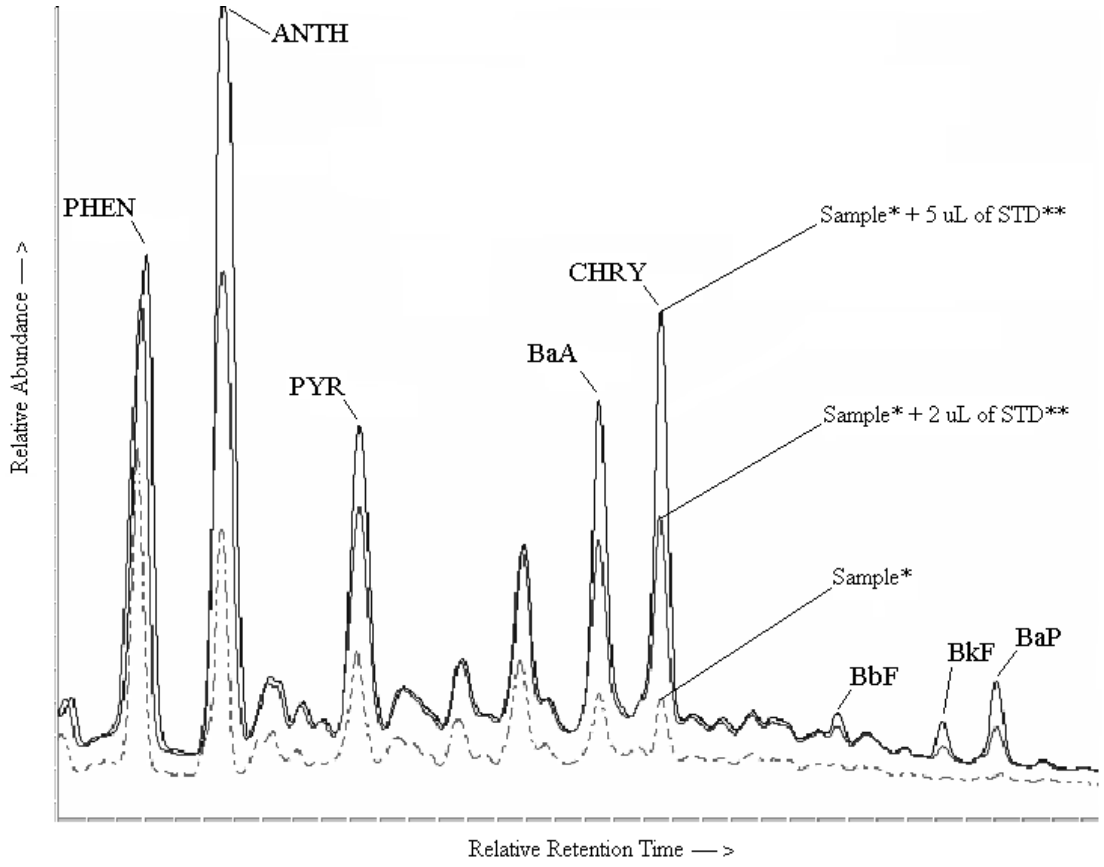
FIGURE 1. Flowchart of Fusina WWTP.



1) Municipal wastewater influent (Site 1); 2) Screening; 3) Oil and sand removal; 4) Accumulation tanks; 5) Sand collection sump; 6) Oil collection sump; 7) Equalization; 8) Screw Pumps; 9) Pre-treated industrial wastewater influent from ENEL (Site 2); 10) Pre-treated industrial wastewater influent from ENICHEM (Site 3); 11) Pre-treatment for municipal/industrial wastes truck transported; 12) Tank-truck; 13) Denitrification; 14) Oxidation and nitrification; 15) Recycle of primary sludge; 16) Sedimentation; 17) Recycle of secondary sludge (Site 5); 18) Waste sludge treatment; 19) Interstitial water from the filtration of the secondary WWTP sludge (Site 4); 20) Stabilized sludge (Site 6); 21) Disinfection; 22) Effluent (Site 7).

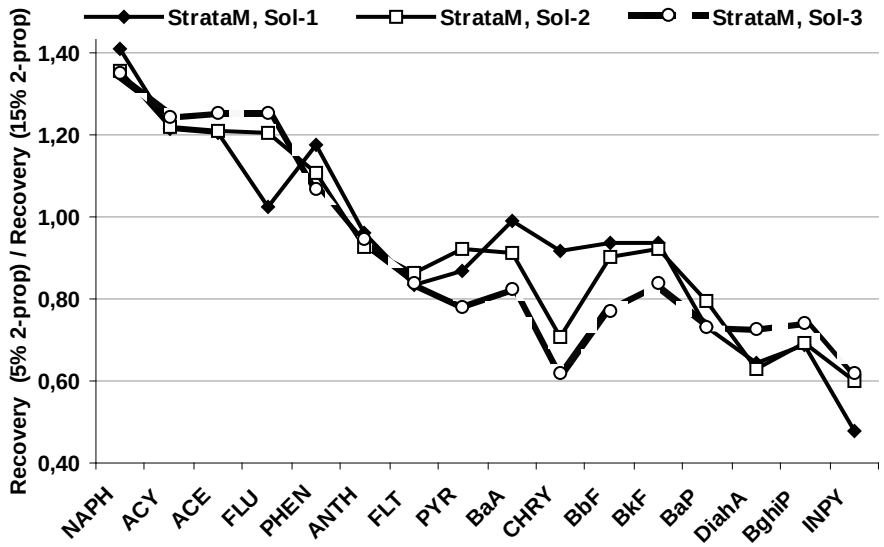
FIGURE 2. LC-FLD chromatogram showing the standard addition method applied to a real sample extract from the WWTP.



Treated effluent extract; injected amount: 25 µL.
*** PAHs Mix-13 (US EPA 16) diluted 1:10 in 2-propanol (v:v).*

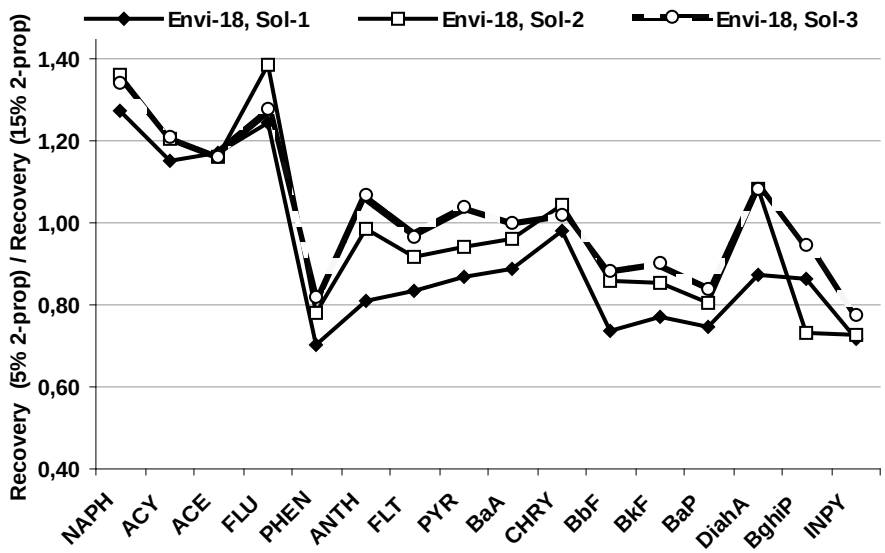
FIGURE 3. Ratios among the percentage recoveries of the 16 PAHs obtained using 5% and 15% of 2-propanol as organic modifier contents in spiked Milli-Q water samples

(1000 mL, 100 ng/L except for NAPH and ACY: 1000 ng/L), StrataM as stationary phase and, Sol-1, Sol-2 and Sol-3 as eluting solvents.



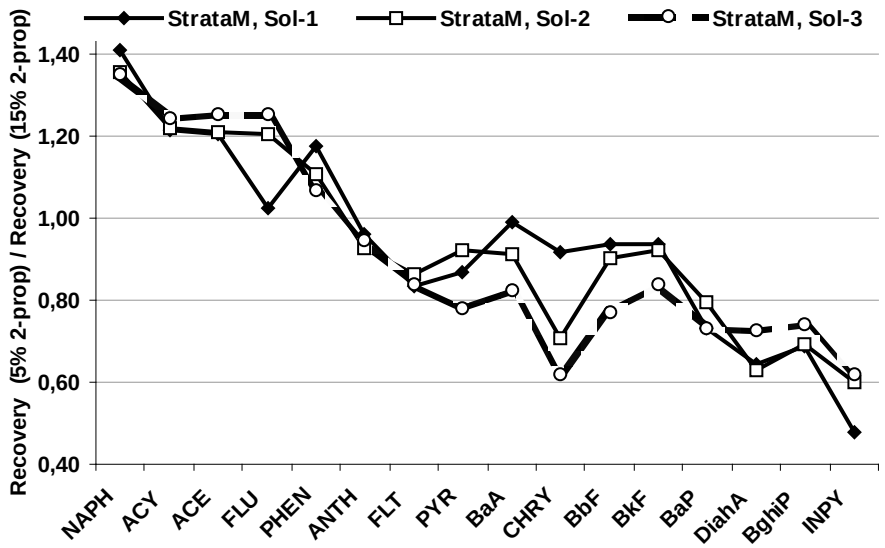
Sol-1: (n-hexane); Sol-2: (n-hexane: acetone, 90:10 v/v); Sol-3: (n-hexane: acetone: 2-propanol, 90:5:5 v/v).

FIGURE 4. Ratios among the percentage recoveries of the 16 PAHs obtained using 5% and 15% of 2-propanol as organic modifier contents in spiked Milli-Q water samples (1000 mL, 100 ng/L except for NAPH and ACY: 1000 ng/L), Envi-18 as stationary phase and, Sol-1, Sol-2 and Sol-3 as eluting solvents.



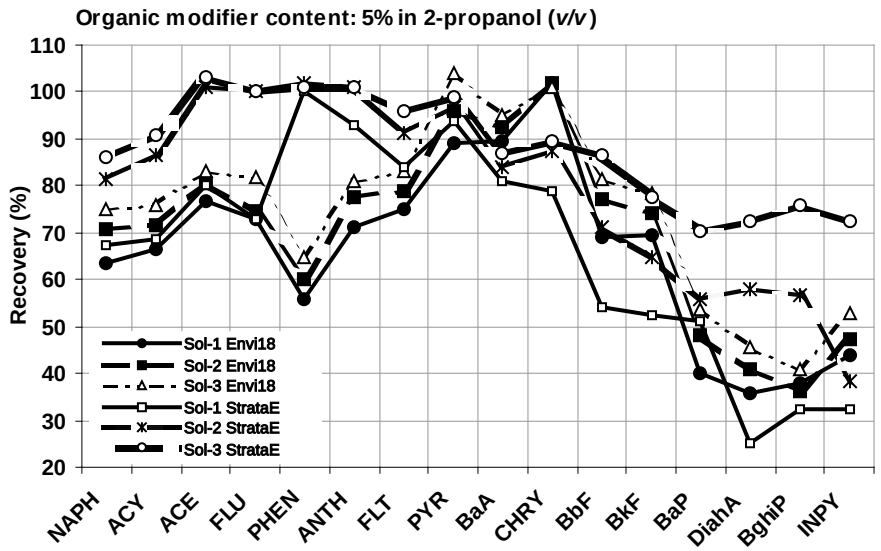
Sol-1: (n-hexane); Sol-2: (n-hexane: acetone, 90:10 v/v); Sol-3: (n-hexane: acetone: 2-propanol, 90:5:5 v/v).

FIGURE 5. Ratios among the percentage recoveries of the 16 PAHs obtained using 5% and 15% of 2-propanol as organic modifier contents in spiked Milli-Q water samples (1000 mL, 100 ng/L except for NAPH and ACY: 1000 ng/L), StrataE as stationary phase and, Sol-1, Sol-2 and Sol-3 as eluting solvents.



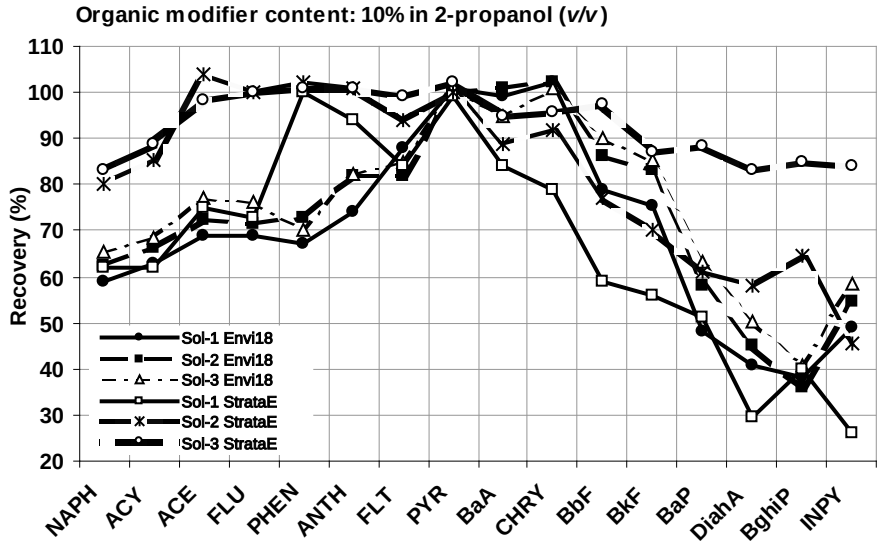
Sol-1: (n-hexane); Sol-2: (n-hexane: acetone, 90:10 v/v); Sol-3: (n-hexane: acetone: 2-propanol, 90:5:5 v/v).

FIGURE 6. Percentage recoveries (n=3) achieved using 5% of 2-propanol in spiked Milli-Q water samples (1000 mL, 100 ng/L spiking level except for NAPH and ACY: 1000 ng/L) and by varying the best stationary phases (StrataE and Envi-18) and the eluting mixtures (Sol-1, Sol-2 and Sol-3).



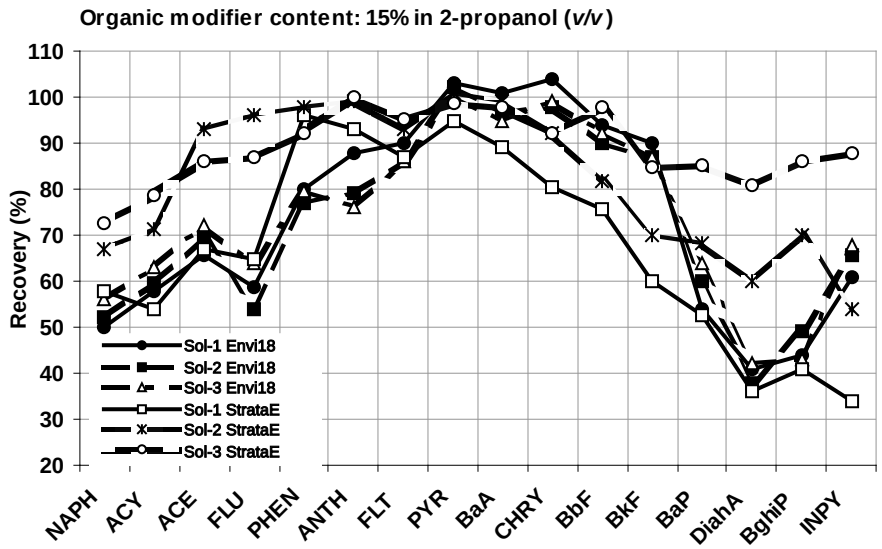
Sol-1: (n-hexane); Sol-2: (n-hexane: acetone, 90:10 v/v); Sol-3: (n-hexane: acetone: 2-propanol, 90:5:5 v/v).

FIGURE 7. Percentage recoveries (n=3) achieved using 10% of 2-propanol in spiked Milli-Q water samples (1000 mL, 100 ng/L spiking level except for NAPH and ACY: 1000 ng/L) and by varying the best stationary phases (StrataE and Envi-18) and the eluting mixtures (Sol-1, Sol-2 and Sol-3).



Sol-1: (n-hexane); Sol-2: (n-hexane: acetone, 90:10 v/v); Sol-3: (n-hexane: acetone: 2-propanol, 90:5:5 v/v).

FIGURE 8. Percentage recoveries (n=3) achieved using 15% of 2-propanol in spiked Milli-Q water samples (1000 mL, 100 ng/L spiking level except for NAPH and ACY: 1000 ng/L) and by varying the best stationary phases (StrataE and Envi-18) and the eluting mixtures (Sol-1, Sol-2 and Sol-3).



Sol-1: (n-hexane); Sol-2: (n-hexane: acetone, 90:10 v/v); Sol-3: (n-hexane: acetone: 2-propanol, 90:5:5 v/v).

TABLE 1. Recoveries and % RSDs (n=3) obtained processing 1000 mL of spiked Milli-Q water (100 ng/L spiking level except for NAPH and ACY: 1000 ng/L) containing 5% of 2-propanol and using StrataM, Envi-18 and StrataE as stationary phases and Sol-1, Sol-2 and Sol-3 as eluting solvents.

5% in 2-propanol	Sol-1						Sol-2						Sol-3					
	StrataM		Envi-18		StrataE		StrataM		Envi-18		StrataE		StrataM		Envi-18		StrataE	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
NAPH	52	13	64	10	67	9	54	8	71	7	82	6	58	9	75	6	86	6
ACY	42	14	67	13	69	12	46	10	72	9	87	8	49	10	76	8	91	8
ACE	45	13	77	12	80	11	49	10	81	9	101	8	56	9	83	8	103	8
FLU	50	11	73	11	73	10	52	9	75	8	100	7	58	7	82	7	100	7
PHEN	56	10	56	9	100	8	58	9	60	8	102	7	60	6	65	7	101	6
ANTH	73	9	71	10	93	9	64	8	78	7	101	6	69	5	81	6	101	5
FLT	43	11	75	11	84	10	47	9	79	8	91	7	56	7	83	7	96	6
PYR	55	9	89	10	94	9	59	8	96	7	97	6	61	5	104	6	99	5
BaA	49	11	90	12	81	11	52	9	93	8	84	7	59	7	95	7	87	6
CHRY	32	10	102	9	79	8	40	8	102	7	87	6	44	6	101	6	89	5
BbF	42	12	69	10	54	9	46	8	77	7	71	6	53	8	81	6	87	5
BkF	43	13	69	10	52	9	48	8	74	7	65	6	59	9	78	6	78	5
BaP	31	13	40	11	51	10	34	9	48	8	56	7	33	9	54	7	71	6
DiahA	18	14	36	10	25	9	18	8	41	7	58	6	24	7	45	6	73	5
BghiP	20	13	38	11	32	10	21	9	36	8	57	7	26	9	41	7	76	6
INPY	20	14	44	10	32	9	30	9	48	8	38	7	35	8	53	7	73	6

TABLE 2. Recoveries and % RSD (n=3) obtained processing 1000 mL of spiked Milli-Q water (100 ng/L spiking level except for NAPH and ACY: 1000 ng/L) containing 10% of 2-propanol and using StrataM, Envi-18 and StrataE as stationary phases and Sol-1, Sol-2 and Sol-3 as eluting solvents.

10% in 2-propanol	Sol-1						Sol-2						Sol-3					
	StrataM		Envi-18		StrataE		StrataM		Envi-18		StrataE		StrataM		Envi-18		StrataE	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
NAPH	50	10	59	9	62	8	49	8	63	9	80	8	55	8	66	9	83	7
ACY	38	13	63	12	62	10	43	9	66	10	85	11	45	11	68	10	89	10
ACE	42	12	69	11	75	10	43	10	72	8	104	6	49	7	77	9	98	6
FLU	50	11	69	10	73	9	52	10	71	9	100	7	56	8	76	7	100	7
PHEN	53	8	67	8	100	9	50	9	73	8	102	6	55	7	70	6	101	6
ANTH	73	8	74	9	94	8	64	9	82	5	101	5	69	4	82	5	101	3
FLT	49	9	88	10	84	9	50	11	82	5	94	5	67	4	85	7	99	3
PYR	59	8	101	9	99	8	59	9	100	7	100	5	77	6	101	5	102	5
BaA	47	10	99	11	84	9	52	9	101	6	89	6	70	5	95	7	95	4
CHRY	32	9	102	8	79	8	52	10	102	8	92	6	71	7	101	6	96	6
BbF	42	11	79	9	59	8	46	12	86	6	77	5	65	5	90	8	98	4
BkF	43	12	76	9	56	8	46	10	83	9	70	7	64	8	85	9	87	7
BaP	35	10	48	10	51	9	37	11	58	8	61	6	35	7	63	9	88	6
DiahA	20	13	41	9	29	8	22	11	45	7	58	5	24	6	50	10	83	5
BghiP	20	11	38	10	40	9	24	12	36	6	64	4	27	5	41	9	85	4
INPY	25	10	49	9	26	9	35	10	55	7	46	5	39	6	58	10	84	5

TABLE 3. Recoveries and % RSDs (n=3) obtained processing 1000 mL of spiked Milli-Q water (100 ng/L spiking level except for NAPH and ACY: 1000 ng/L) containing 15% of 2-propanol and using StrataM, Envi-18 and StrataE as stationary phases and Sol-1, Sol-2 and Sol-3 as eluting solvents.

15% in 2-propanol	Sol-1						Sol-2						Sol-3					
	StrataM		Envi-18		StrataE		StrataM		Envi-18		StrataE		StrataM		Envi-18		StrataE	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
NAPH	37	12	50	11	58	10	40	9	52	8	67	4	43	8	56	7	73	8
ACY	34	14	58	13	54	13	38	12	59	7	72	8	39	11	63	10	79	9
ACE	38	14	66	13	67	12	41	8	70	9	93	5	45	7	72	6	86	8
FLU	49	13	59	12	65	11	43	9	54	8	96	4	46	8	64	7	87	5
PHEN	48	9	80	8	96	11	52	8	77	7	98	6	56	7	80	6	92	5
ANTH	76	8	88	7	93	10	69	5	79	8	99	6	73	4	76	6	100	7
FLT	52	13	90	12	87	11	54	5	86	9	93	5	67	4	86	5	95	6
PYR	63	12	103	11	95	12	64	7	102	9	101	5	79	6	100	5	99	8
BaA	50	13	101	12	89	11	57	6	97	9	99	5	72	5	95	7	98	6
CHRY	35	12	104	11	80	10	57	8	98	8	92	7	71	7	99	6	92	9
BbF	45	8	94	7	76	10	51	6	90	7	82	7	69	5	92	5	98	8
BkF	46	8	90	7	60	10	52	9	87	6	70	6	70	8	87	7	85	7
BaP	42	9	54	8	53	11	43	8	60	7	68	8	45	7	64	6	85	6
DiahA	28	12	41	11	36	10	29	7	38	8	60	6	33	6	42	5	81	7
BghiP	29	10	44	10	41	10	30	6	49	9	70	5	35	5	43	6	86	6
INPY	42	9	61	9	34	11	50	7	66	8	54	8	56	6	68	5	88	5

TABLE 4. LODs and LOQs for the 16 PAHs determined in extracts from filtered liquid phases and from sludges.

PAHs	LOD[a]	LOQ[a]	LOD[b]	LOQ[b]
	ng/L		ng/g	
NAPH	0.58	1.93	6.8	22.67
ACY	0.67	2.23	7.7	25.67
ACE	0.52	1.73	4.6	15.33
FLU	0.58	1.93	4.1	13.67
PHEN	0.52	1.73	4.6	15.33
ANTH	0.68	2.27	5.2	17.33
FLT	0.78	2.60	5.4	18.00
PYR	0.65	2.17	5.2	17.33
BaA	0.92	3.07	8.3	27.67
CHRY	0.73	2.43	8.4	28.00
BbF	0.78	2.60	9.2	30.67
BkF	0.76	2.53	6.2	20.67
BaP	0.79	2.63	7.1	23.67
DiahA	0.98	3.27	12.1	40.33
BghiP	0.92	3.07	8.2	27.33
INPY	1.2	4.00	7.2	24.00

[a] extracts from liquid samples
[b] extracts from sludge samples

TABLE 5. Recoveries and % RSDs (n=3) obtained from spiked WWTP aqueous samples[a] and from spiked suspended solids[b] and sludge samples[c]. ([a]1000 mL, 100 ng/L spiking level except for NAPH and ACY: 1000 ng/L; [b]spiking: 50 μL of US EPA PAHs Mix-13 standard solution; [c]2.5 g d.w., 1 μg/g spiking level except for NAPH and ACY: 10 μg/g).

PAHs	Site 1		Site 1*		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 7*	
	Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)
NAPH	78	9	75	10	75	11	73	10	75	10	74	12	71	9	79	8	78	10
ACY	82	12	79	11	79	11	86	13	74	11	77	13	72	12	82	11	80	12
ACE	93	8	91	9	89	10	85	11	91	9	87	11	81	12	92	10	90	9
FLU	91	9	90	8	92	11	87	10	86	8	86	9	77	9	91	8	89	7
PHEN	91	8	88	7	86	9	88	9	77	8	81	9	86	8	86	7	87	8
ANTH	91	6	89	8	87	8	89	7	85	6	78	11	81	10	82	6	80	8
FLT	89	6	90	8	79	7	76	6	71	7	86	7	81	9	84	6	84	7
PYR	91	7	91	7	86	9	86	8	78	7	78	11	78	11	87	6	88	7
BaA	89	6	89	8	90	8	91	7	90	8	89	10	87	12	91	7	93	8
CHRY	90	8	90	6	89	10	87	9	87	8	87	9	89	10	95	7	95	8
BbF	71	6	71	6	73	8	77	7	81	9	92	8	86	8	85	6	82	7
BkF	70	9	70	7	81	11	81	9	75	5	82	8	81	9	81	8	79	9
BaP	77	8	77	8	76	10	78	9	81	7	81	8	85	9	71	7	73	8
DiahA	78	7	76	7	81	9	79	8	74	6	73	7	75	7	75	7	78	8
BghiP	73	8	75	9	76	8	81	7	84	7	75	8	71	9	77	7	80	7
INPY	74	7	78	8	71	9	70	8	75	7	76	7	74	9	71	6	75	7

Site 1: Sewage inlet; Site 1: Suspended solids from filtered sewage inlet; Site 2: Enel power station; Site 3: Enichem petrochemical site; Site 4: Interstitial water; Site 5: Sewage sludges; Site 6: Stabilized sludges; Site 7: Final effluent; Site 7*: Suspended solids from filtered final effluent.*

TABLE 6. Soil/Sediment IRM-104 EPA Certified Reference Material.

Chemicals	Reference Value	Confidence Interval	Prediction Interval	Analytical Mean values	Recovery (%)	RSD (%)
NAPH	0.77	0.59-0.94	0.0-1.57	0.68	88	12
2-Me NAPH	trace	…	…	…	…	...
ACY	1.21	0.82-1.59	0.0-2.98	1.03	85	11
ACE	0.77	0.67-0.88	0.27-1.28	0.71	92	12
Dibenzofuran	0.66	0.55-0.77	0.17-1.14	…	…	...
FLU	0.65	0.56-0.74	0.25-1.05	0.56	86	8
PHEN	5.79	4.93-6.66	2.11-9.48	5.23	90	6
ANTH	1.44	1.15-1.73	0.08-2.80	1.38	96	5
Di-n-butyl phthalate	0.54*	…	…	…	…	...
FLT	24.60	19.7-29.4	4.53-44.6	21.40	87	5
PYR	15.00	11.6-18.5	0.0-30.7	13.70	91	6
Butyl Benzilphthalate	0.51*	…	…	…	…	...
BaA	7.98	6.70-9.26	2.09-13.9	7.42	93	5
Bis(2-ethylhexil)phthalate	1.64*	…	…	…	…	...
CHRY	8.60	7.05-10.14	3.39-13.8	7.65	89	6
BbF	9.69	…	…	8.53	88	6
BkF	5.1*	…	…	…	92	8
BaP	5.09	4.25-5.94	1.56-8.63	4.56	90	5
INPY	4.46	3.45-5.47	0.0-9.09	4.20	94	6
DiahA	1.55*	…	…	…	95	6
BghiP	3.58	2.65-4.51	0.0-8.08	3.12	87	5

*All values are expressed in mg/Kg on a dry wheight basis. Value listen with * are not certified and are reported for information only*

TABLE 7. PAH concentrations in the filtered aqueous samples and in the total aqueous samples (suspended solids + filtered liquid phase).

PAHs	Site 1[a] (ng/L)	Site 1[b] (ng/L)	Site 2[a] (ng/L)	Site 2[b] (ng/L)	Site 3[a] (ng/L)	Site 3[b] (ng/L)	Site 4[a] (ng/L)	Site 4[b] (ng/L)	Site 7[a] (ng/L)	Site 7[b] (ng/L)
NAPH	18.4±0.9	80±6	17.5±1.2	100±9	11.2±0.5	127±11	5.2±0.6	53±5	5.2±0.2	26±2
ACY	10.1±0.6	46±4	19.2±1.1	95±8	15.3±0.7	56±4	12.7±0.7	58±5	6.3±0.2	59±3
ACE	12.3±0.6	138±13	8.3±0.4	176±19	4.3±0.2	103±11	4.2±0.4	138±12	4.4±0.2	95±5
FLU	17.6±0.8	149±14	15.3±0.7	194±17	5.1±0.3	131±11	6.3±0.9	149±14	5.7±0.2	25±1
PHEN	19.2±1.1	399±35	30.7±1.8	464±37	3.2±0.2	154±13	14.6±0.2	399±35	8.2±0.3	87±4
ANTH	12.3±0.8	342±27	21.2±1.6	398±39	2.8±0.1	149±14	2.4±0.4	342±27	5.1±0.2	156±9
FLT	18.4±0.5	378±34	114.3±8.0	527±47	6.1±0.2	440±35	11.2±0.7	378±34	6.2±0.2	77±3
PYR	16.3±0.6	406±32	26.4±1.6	467±37	4.8±0.2	121±9	7.2±0.5	406±28	7.3±0.3	57±3
BaA	15.4±0.7	335±30	51.2±2.5	421±37	7.0±0.3	146±13	5.2±0.3	335±30	3.3±0.1	59±3
CHRY	8.3±0.4	218±13	13.2±0.7	266±18	6.3±0.3	125±10	13.4±0.3	218±15	4.2±0.2	58±3
BbF	15.3±0.6	275±22	18.3±1.2	328±29	9.1±0.4	603±54	18.1±1.1	275±19	3.2±0.2	56±2
BkF	12.1±0.4	204±18	18.1±1.1	244±19	4.4±0.1	298±23	18.2±0.8	204±16	3.3±0.1	86±4
BaP	17.4±0.6	297±26	19.3±1.1	338±27	3.8±0.1	172±15	15.3±0.7	297±23	2.8±0.1	66±3
DiahA	5.7±0.2	153±15	9.1±0.5	184±16	10.1±0.4	130±10	30.2±0.4	153±15	3.4±0.1	70±4
BghiP	4.2±0.2	192±19	10.2±0.5	225±18	11.2±0.4	315±22	21.3±0.9	193±17	3.4±0.2	60±3
INPY	8.3±0.3	158±12	11.7±0.5	191±17	10.4±0.5	488±43	10.2±0.7	168±13	4.2±0.2	67±2

[a]*aqueous samples*
[b]*suspended solids + filtered liquid phase*

TABLE 8. PAH concentrations in extracts from sewage sludge (Site 5) and stabilized sludge (Site 6) samples.

Sampling Site	NAPH	ACY	ACE	FLU	PHEN	ANTH	FLT	PYR
Site 5 (ng/g)	31.5	52.5	63.0	75.0	81.3	90.3	94.6	137.6
Site 6 (ng/g)	28.4	67.2	92.1	73.8	91.1	78.8	93.1	97.7
Sampling Site	**BaA**	**CHRY**	**BbF**	**BkF**	**BaP**	**DiahA**	**BghiP**	**INPY**
Site 5 (ng/g)	133.3	129.1	105.0	93.0	87.0	97.0	84.0	90.0
Site 6 (ng/g)	98.6	94.0	93.2	77.5	86.8	52.3	68.9	68.1