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Static extraction with modified pressurized liquid and on-line fluorescence monitoring
Independent matrix approach for the removal of polycyclic aromatic hydrocarbons from environmental solid samples

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Abstract

The coupling of a static pressurized liquid extractor to a flow injection manifold has allowed real-time on-line fluorescence monitoring of the polycyclic aromatic hydrocarbons (PAHs) extracted from environmental solid samples, which can be used for either screening or semiquantitative purposes. Sodium dodecyl sulfate (SDS) was added to the water for favoring the extraction of the low-polar analytes. Different solid samples such as sandy soil, river sediments, trout and sardine spiked with the target PAHs were subjected to several 15-min static extraction cycles with SDS–water, at 200 °C. The results obtained demonstrated that fluorometric monitoring of static pressurized liquid extraction constitutes an approach as efficient as conventional Soxhlet for the extraction of PAHs from solid samples but with the following positive features: (a) drastic reduction of the extraction time as the extraction kinetics can be monitored and thus the end of the leaching step determined independently of the sample matrix; (b) use of water as extractant thus given place to an environmentally friendly method; and (c) coupling of static extraction to subsequent dynamic steps. The method has been applied to a certified reference material (CRM 524, BCR, industrial soil/organics) for quality assurance/validation. The total content of each analyte was determined by HPLC–fluorimetric detection.

Keywords: Pressurized liquid extraction; Extraction methods; Polynuclear aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent a class of nonionic, poorly water-soluble and toxic organic compounds which are ubiquitous environmental contaminants [1]. Because of their high mutagenicity and carcinogenicity [2], the presence/absence and existing levels of PAHs in a wide range of natural samples has produced high interest among analytical chemists. Soil and sediments are universal sinks for these pollutants, being the last a drain and a source for these toxicants to impose adverse effects on aquatic organisms [3].

The use of liquid water at high pressure and temperature provides a promising alternative to other sample pretreatments, as it is the ideal solvent for the establishment of environmental friendly methods.

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Water at high pressure and temperature has been traditionally named as subcritical water. The outstanding feature of subcritical water as a leaching agent is its capacity for altering its dielectric constant (ε). In fact, this parameter can be changed within a wide range by changing the temperature under moderate pressure, because it depends mainly on temperature and only slightly on pressure (the lower the pressure, the lower the ε). At ambient pressure and temperature, water has a dielectric constant of ca. 80, which is drastically lowered by raising the temperature under moderate pressure. For example, subcritical water at 250 °C (and pressure >40 atm to maintain the liquid state) has ε=27, which is between to those of ethanol (ε=24) and methanol (ε=33). This significant drop in dielectric constant allows hydrophobic compounds, which have limited water solubility at room temperature, to be more readily dissolved in water. Nevertheless, the corrosiveness of water increases with increased temperature; so, low-temperature extractions are desirable in order to decrease both deterioration of the experimental set-up and costs. In order to reduce the temperature needed for the extraction of very low-polar compounds such as PAHs using water, micelle-based methodologies have been successfully used [4–6].

Traditionally, leaching of PAHs from environmental solid samples has been carried out by conventional Soxhlet extraction, the advantages and disadvantages of which have been exhaustively referred in the literature [7,8]. However, a number of papers have appeared in the last two decades which show that PAHs can be extracted from environmental matrices with relative ease using new technologies such as supercritical fluid extraction (SFE) [9,10], static pressurized liquid extraction (static PLE; the Dionex trade name for PLE is ASE, for accelerated solvent extraction) [11,12], and microwave-assisted extraction (MAE) [13,14], working either in the mono-mode [15] or multimode manner [16]. The usual way of verifying the effectiveness of these techniques in extracting PAHs from solid samples is to compare the results they provide with those obtained by conventional Soxhlet. The use of certified reference materials (CRMs) in both method development and quality assurance applications is effective in order to optimize a new method for the analytes of interest and the type of sample matrix and also to ensure that the optimized method is under control for routine application.

One of the main drawbacks of the methods based on new technologies is the lack of a way for determining when the target compounds have been completely removed from the matrix without prolonging unnecessarily the extraction time. Several papers have shown that when static pressurised liquid extraction is used for the removal of PAHs from several matrices, or even from the same matrix but differently aged, the results obtained differ greatly from one case to the other [17–19].

In 1996, the US Environmental Protection Agency (EPA) adopted the PLE technique in producing Method 3545 [20] in view of the good results it provided at an early research stage. This official method is a procedure for extracting water-insoluble or slightly water-soluble semi-volatile organic compounds such as chlorinated herbicides, organophosphorus and organochlorine pesticides, PAHs, polychlorinated biphenyls (PCBs), and polydibenzodioxins and polybenzofurans from soils, clays, sediments, sludges and waste solids. The method uses a temperature of 100 °C and high pressures (1500–2000 p.s.i.; 1 p.s.i.=6894.76 Pa) to achieve analyte recoveries equivalent to those from Soxhlet extraction but using less solvent and taking significantly shorter time. It has been validated for a number of analytes in different materials such as pesticides and PAHs in soils [21,22], and PCBs in both sediments [23,24] and sewage sludge [23]; all providing results similar to, or even better than, those provided by conventional methods such as Soxhlet or shaking extraction. However, the recommended conditions for implementation of this method (viz. 5 min of static extraction, 5 min of pre-heat equilibration and one static cycle) are not always sufficient to ensure quantitative extraction, particularly with natural or aged samples which retain analytes more strongly than freshly spiked samples; they often require altering the methods by using a static extraction time of 5–50 min [25,26], raising the extraction temperature to 150–200 °C [27,28] or performing several extraction cycles. In some cases, the extraction must be extended to as long as 90 min to ensure acceptable results [29].

The extraction monitoring approach proposed in

this paper is suitable to solve the matrix effect problem. This assertion is based on the following facts: (1) the system works as a commercial accelerated solvent extractor (ASE-200)—thus, it can provide, at least, the same results; (2) the system allows monitoring of the extraction process; hence the extraction is halted just when removal of the analytes is complete. The construction of the approach has been supported on the coupling of the following devices: (a) a laboratory-made static pressurized liquid extractor that uses water modified with sodium dodecyl sulfate (SDS) as leaching agent with fundamentals and performance similar to that of the commercial ASE-200; (b) a simple flow injection manifold which acts as an interface between the extractor and a fluorimetric detector for transporting the extract to the flow cell at the required intervals.

The choice of a molecular fluorescence detector for the on-line monitoring of PAHs is based on the higher selectivity and sensitivity of fluorimetry as compared with other more available techniques such as molecular absorption. This approach only allows monitoring of the overall content of fluorescent species as it is not free from fluorescent interferences nor able to give individual determination of the target PAHs, the latter requiring the use of chromatography.

2. Experimental

2.1. Instruments and apparatus

Static PLE was performed using the following assembly: (1) a Shimadzu (Tokyo, Japan) LC10AD pump with digital flow-rate and pressure readouts used to propel the extractant through the system. (2) An extraction chamber consisting of a stainless steel cylinder (100×10 mm I.D., 8 ml internal volume) (Análisis Vínicos, C. Real, Spain) closed with screws at either end, which permits the circulation of the leaching fluid through them. Both screw caps contain stainless steel filter plates (2 μm in thickness and 1/4-in. I.D.; 1 in=2.54 cm) to ensure that the sample remains in the extraction chamber. (3) A gas chromatograph oven (HP 5720A, Hewlett-Packard, Wilmington, DE, USA) used as heating source and where the chamber was located. (4) A cooler system (consisting of a loop made from 1-m length stainless steel tubing and cooled with water) was used to cool the fluid from the oven to a temperature close to 25 °C. (5) A pressure needle valve coupled to the outlet of the cooler. (6) A selecting valve located between the high-pressure pump and the oven allows flush the extract with N_2 after extraction.

The dynamic on-line monitoring was performed by a flow injection (FI) manifold constructed with a Gilson minipuls-3 low-pressure peristaltic pump (Gilson, Worthington, OH, USA), a Rheodyne 5041 low-pressure injection valve (Rheodyne, Cotati, CA, USA) and PTFE tubing of 0.5 mm I.D. (Scharlau, Barcelona, Spain), connected to a Kontron, model SFM 25 fluorimeter (Kontron, Zurich, Switzerland) equipped with an 18 μl flow cell from Hellma (Jamaica, NY, USA).

The coupling of the pressurized liquid extractor with the flow injection manifold and the fluorescence detector was as shown in Fig. 1.

A Vac elut sps 24 (Varian, USA) vacuum station incorporated to an Eye14 A-3S evaporator (Tokyo, Japan) and 500 mg C_{18} sorption cartridges from Análisis Vínicos were used in the clean-up step.

The individual separation of the analytes in the extract was performed by an HP1100 liquid chromatograph (Hewlett-Packard, Avondale, PA, USA) consisting of a G1311A high-pressure quaternary pump, a G1322A vacuum degasser, a Rheodyne 7725 high-pressure manual injector valve (20 μl injection loop) and a Hitachi, model F-1050 chromatographic fluorimeter detector (Hitachi, Tokyo, Japan), equipped with a 12 μl flow cell and a D-2500 integrator (Hitachi). An Ultrabase C_{18} (250×4.6 mm; 5 μm particle size from Scharlau) was used as the analytical column.

2.2. Reagents

Ultrapure water obtained from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout. Water modified with SDS (Merck, Darmstadt, Germany) as micelle former was used as extractant (2.5×10^{-2} *M* SDS aqueous solution). The value of the critical micellar concentration (CMC) is 5.4×10^{-3} *M*.

The PAHs {namely pyrene (Pyr), benzo[*a*]anth-

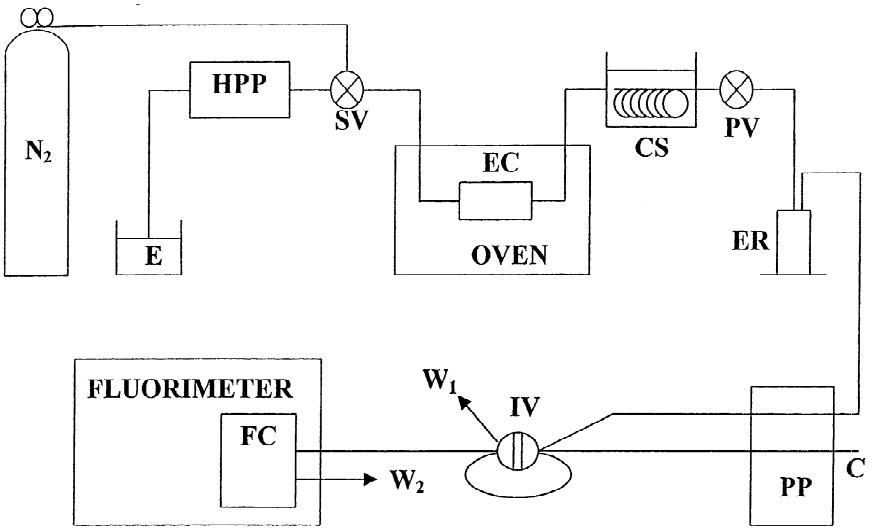

Fig. 1. Schematic diagram of the experimental set-up used. E, extractant; HPP, high-pressure pump; SV, selecting valve; EC, extraction cell; CS, cooler system; PV, pressure valve; ER, extract reservoir; C, carrier; PP, peristaltic pump; IV, injection valve; FC, flow cell; W, waste.

racene (b[*a*]ant), benzo[*ghi*]perylene (b[*ghi*]per), benzo[*a*]pyrene (b[*a*]pyr) and benzo[*k*]fluoranthene (b[*k*]flu)} were obtained from Aldrich (Milwaukee, WI, USA). These compounds were used for preparing the stock standard solutions (0.5 μg g^{-1} of each) in HPLC-grade acetonitrile (Merck). An N_2 stream (Carburos Metálicos, Barcelona, Spain) was used for removal the extract out from the extraction chamber. Diethyl ether (Panreac, Barcelona, Spain) was used for sample preparation.

2.3. Sample preparation

Four types of matrices (namely, sandy soil, sediment, trout and sardine) were selected for the study and the method was applied to a certified reference material (CRM 524, BCR, industrial soil/organics). The optimization of the extraction process was carried out using the sandy soil as it did not provide fluorescence signals due to other matrix components. A total of 400 g of air-dried sandy soil was sieved to a size smaller than 1 mm. Samples spiked with PAHs were prepared by adding 400 ml of ethyl ether, containing the necessary volume of stock standard solution of the PAHs, to the soil in order to obtain a total concentration of 2.5 μg g^{-1} (0.5 μg g^{-1} of each PAH, related to dry soil mass), within the usual concentration range of PAHs in natural samples. Then, the slurry was shaken for 72 h and, after evaporation of the solvent, the soil was completely dried under an N_2 stream and then stored at −20 °C for 3 months until use in order to allow sorption equilibrium to be established. Sediment samples were prepared and spiked in the same way as sandy soil.

Fish samples were purchased at a local market in Córdoba, Spain, and analyzed in the raw state. Sampling was done according to the protocol established by legislation [30]. The samples were washed with water and cut into pieces. Four grams of each sample was ground and spiked with PAHs by adding 20 μl of stock standard solution (100 μg g^{-1}) containing the necessary amount of PAHs to obtain a final total concentration in the fish tissue of 25 μg g^{-1} (0.5 μg g^{-1} of each PAH). Then, the samples were refrigerated for 2 h at 4 °C before extraction in order to simulate the normal contact between the fish and PAHs. A preliminary study showed an increase of the analytes retention in the spiked sample when the storage time (under refrigeration conditions) increased from 0 to 2 h, thus demonstrating the influence of this variable. Storage times between 2 and 24 h provided similar results, so the former was used in all the experiments. Neither of the samples had detectable levels of the target analytes before spiking.

2.4. Procedure

2.4.1. Static pressurized liquid extraction

Four grams of sample was weighed and placed into the extraction cell in all instances. After assembling the extraction cell in the oven, the unit was filled with solvent (2.5×10^{-2} *M* SDS aqueous solution) pumped at a flow-rate of 1 ml min^{-1} (the selecting valve SV and pressure valve PV remained in the adequate position). After the pre-set pressure needed to maintain the solvent in the liquid state was achieved, the oven was brought up to the working temperature (200 °C) and the extraction program, which consisted of a number of 15-min static cycles, was performed. The number of cycles depended on the extraction kinetics of the target compounds from the different matrices. After each cycle, the pressure valve was opened and fresh solvent (60% of the empty extraction cell volume) displaced the extract from the cell, which was collected in the extract reservoir after being cooled in the refrigerant at 25 °C. Then, the pressure valve was closed and a new extraction cycle was performed. After the last cycle, both the pressure and the selection valve were opened and a nitrogen stream purged the system for collecting the extract from the final cycle in the reservoir.

2.4.2. On-line fluorimetric monitoring of the extract

After collection of each extract, the peristaltic pump was activated and the extract aspirated to the FI manifold. The flow-rate of the FI pump was set at 4 ml min^{-1}. The fluorescence of the extract was monitored in triplicate by 500-μl injections of the extract into the carrier. The detector wavelengths were set at 300 and 400 nm for excitation and emission, respectively. The extraction was considered complete when the signal from the extract gave a value lower than 3.82 (fluorescence arbitrary units) corresponding to the detection limit of the method.

2.4.3. Clean up-preconcentration step

After each extraction cycle was completed and when the individual quantitation of the analytes was required, the extract (approximately 12 ml) was passed through a C_{18} bonded column where the PAHs included in the micelles were retained and the waste discarded. Acetonitrile (5 ml) was passed through the column for eluting the PAHs. A portion of the eluate (20 μl) was injected into the liquid chromatograph.

2.4.4. Chromatographic determination

The HPLC separation of the PAHs was performed using a gradient elution program in which an acetonitrile–methanol–water (85:1.8:13.2) mixture was used as initial mobile phase at a flow-rate of 0.8 ml min^{-1}. The gradient program was as follows: (1) the initial mobile phase was hold for 5 min and then two linear gradients were established in order to reach first, a (90:1.8:8.2) composition in 15 min and then, a final (98.2:1.8:0) composition in 10 min more. Finally, 10 min was necessary for reestablishing the initial conditions. The injection volume was 20 μl. Fluorimetric detection was performed at 300 and 400 nm for the excitation and emission wavelengths, respectively. Quantitation of the analytes was carried out by running five calibration curves (one for each analyte) using standard solutions between 0.2 and 1.2 μg ml^{-1}.

3. Results and discussion

The method here proposed involves removal of the target analytes from the solid sample with continuous fluorescence monitoring of the extraction kinetics. When quantification of each analyte is required, the clean-up of the extract and the individual chromatographic separation/detection of the target compounds is also performed. The order used in the optimization of the steps was as follows: first, the chromatographic separation and fluorometric detection of the target analytes were optimized for checking the other previous steps; then, the clean-up step, followed by a preliminary study of the extraction step in order to make possible the optimization of the monitoring step and, finally, the extraction step was studied in depth. The ranges over which the variables were studied and the optimum values found are listed in Table 1.

Table 1
Optimization of the method

Step	Variable	Tested range	Optimum value
Static leaching	Temperature (°C)	100–250	200
	SDS concentration (M)	2.5×10^{-2}–7.5×10^{-2}	2.5×10^{-2}
	Extraction time (min)	5–15	15
Chromatographic separation	Q mobile phase (ml min^{-1}) elution (see text)	0.6–1.5	0.8
Fluorimetric detection	Excitation–emission (nm)	–	300–400

3.1. Optimization of the chromatographic separation

The experimental variables optimized in order to obtain appropriate separation of the analytes were the composition of the mobile phase, the flow-rate and injection volume (see Table 1). An injection volume of 20 μl was selected in order to avoid fluorimetric signal saturation, which occurred with higher volumes. The gradient program is given in Section 2. A chromatographic fluorescence detector was used after HPLC separation for proper monitoring.

3.2. Optimization of the clean up step

A solid-phase extraction step is required due to the necessity of removing the micelle former in excess from the extracts, thus obtaining an appropriate phase that can be introduced into the chromatograph. In addition to removal the surfactant, preconcentration of the target analytes is also achieved in this way. The study concerning this step consisted of selecting the best sorbent material in order to retain and separate the analytes from the extract as well as the solution required for their proper elution. Two types of sorbent were tested, C_{18} and C_{si}, and the best results were obtained with the former. After selection of the sorbent, different eluents were investigated in order to achieve elution of the PAHs from the solid-phase in a minimum volume. A 5-ml volume of acetonitrile was necessary for quantitative elution of the analytes.

3.3. Optimization of the extract monitoring

A preliminary study of the extraction step showed that the volume delivered from the extraction cell to the extract reservoir after each cycle was 12 ml and the time for each extraction cycle was not less than 15 min. With these premises, a flow-rate of 4 ml min^{-1} was established for aspiration of the extract reservoir by the peristaltic pump. At higher flow-rates the sampling frequency was not enough for performing three replicates; lower flow-rates were not necessary. This step started as soon as the extract was loaded in the extract reservoir. A 500-μl loop of the injection valve was selected as optimum as higher injection volumes—in the order of 1 ml—caused saturation of the fluorimetric detector when the extract was rich in the analytes; lower volumes—in the order of 250 μl—provided small signals for diluted extracts.

3.4. Features of the method for monitoring the extract

Taking into account that the subsequent study was the optimization of the extraction step, to be developed with a spiked soil that contained the same amount of all target analytes, the characterization of the monitoring step was as follows:

A calibration graph was run with equal concentration mixtures of the five PAHs. Solutions of the mixture in acetonitrile were prepared in the range 0.1–2 μg ml^{-1}; that is, ranging between 0.02 and 0.4 μg ml^{-1} for each individual PAH. Each solution was injected in triplicate. The calibration graph, which showed a linear shape within the range studied, was used for calculation of the extraction recovery. In this way, semiquantification of the analytes can be made. When quantification of the target analytes is required, the use of chromatography is mandatory.

The relative detection limit, was calculated by the equation $x_L = ks_{bl}/S$ where k is a constant, S the sensitivity of the analytical method corresponding to the slope of the calibration line, and s_{bl} the standard

deviation of the blank responses obtained from the analyses of 10 soil blanks. The value obtained was $x_{L(k=3)}=0.022$ μg ml^{-1}.

3.5. Optimization of the static pressurized liquid extraction

Preliminary experiments were carried out using pure water and water modified with a surfactant (SDS was used as recommended in the literature [4–6]) in order to obtain a micellar medium where the PAHs could be easily extracted. Poor recoveries were obtained with water which were improved when water modified with SDS was used. The variables affecting the static pressurized liquid extraction (namely, temperature, SDS concentration and extraction time) were studied in order to obtain the best recoveries in a single extraction cycle. A full two-levels factorial design involving an overall of $2^3=8$ experiments, in addition to three center points, was selected as screening method of the main variables affecting the extraction efficiency. The ranges within each variable was studied are shown in Table 1.

The study showed that the temperature and the extraction time are the key variables, while the SDS concentration appears to be not significant when working above the critical micellar concentration; thus, the lowest SDS concentration tested—2.5×10^{-2} *M*—was selected for subsequent experiments since higher concentrations provided a gel-like extract difficult to handle. Concerning both temperature and extraction time, higher values should be tested as their effects on the recovery were positive. However, when temperatures higher than 200 °C were tested, the extracts obtained had the appearance of a dense gel and caused blockage of the system; therefore, 200 °C was selected for further experiments. Extraction times from 15 to 30 min were tested in 5-min steps. The results showed that the extraction efficiency decreased with the increase of the extraction time, probably due to decomposition of the surfactant. Thus, 15 min was selected as optimum value.

Finally, the overall optimized procedure was applied to different samples (namely, sandy soil, sediment, trout and sardine) in order to check the influence of the sample matrix in the number of cycles needed for quantitative recovery. As can be seen in Fig. 2, the kinetics of the extraction for each analyte strongly depends on the type of matrix; nevertheless, four cycles were necessary for total removal of the analytes in all instances.

3.6. Application of the method to a certified reference material

The use of a CRM had a double objective. First, to demonstrate the efficiency of the method which uses micelle formation for the extraction of PAHs in natural samples; second, to show the independence of the system from the sample matrix effect due to its ability to provide a real-time monitoring of the extraction. The certified reference material used was CRM 524 (Community Bureau of Reference, BCR, Brussels, Belgium), an industrial soil in which eight PAHs are the certified compounds.

The optimized conditions were applied to 0.1 g of CRM. The on-line monitored fluorescence signal was not quantifiable in the effluent from the fourth cycle, thus showing total removal of the target compounds. Then, the individual PAHs were fluorimetrically quantified after HPLC separation, efficiencies close to 100%, ranging between 98.3 and 102.1%, were obtained in all instances. The within-laboratory reproducibility of the proposed method was studied by seven replicates of the CRM in different days, and relative standard deviations between 4.3 and 5.8%, depending on the analyte, were obtained.

4. Conclusions

After checking that in natural or aged samples the one-static cycle recommended by EPA Method 3545 was not enough for quantitative extraction of some analyte families, such as PAHs, several authors have proposed the application of more than one cycle, but how many are necessary as a function of both matrix and target analytes had not been reported so far. This is the first time that static pressurized liquid extraction has been on-line monitored by coupling the extractor to a flow injection manifold.

The proposed approach does not allow quantitation of the individual PAHs existing in a sample but it makes possible to reach the following objectives.

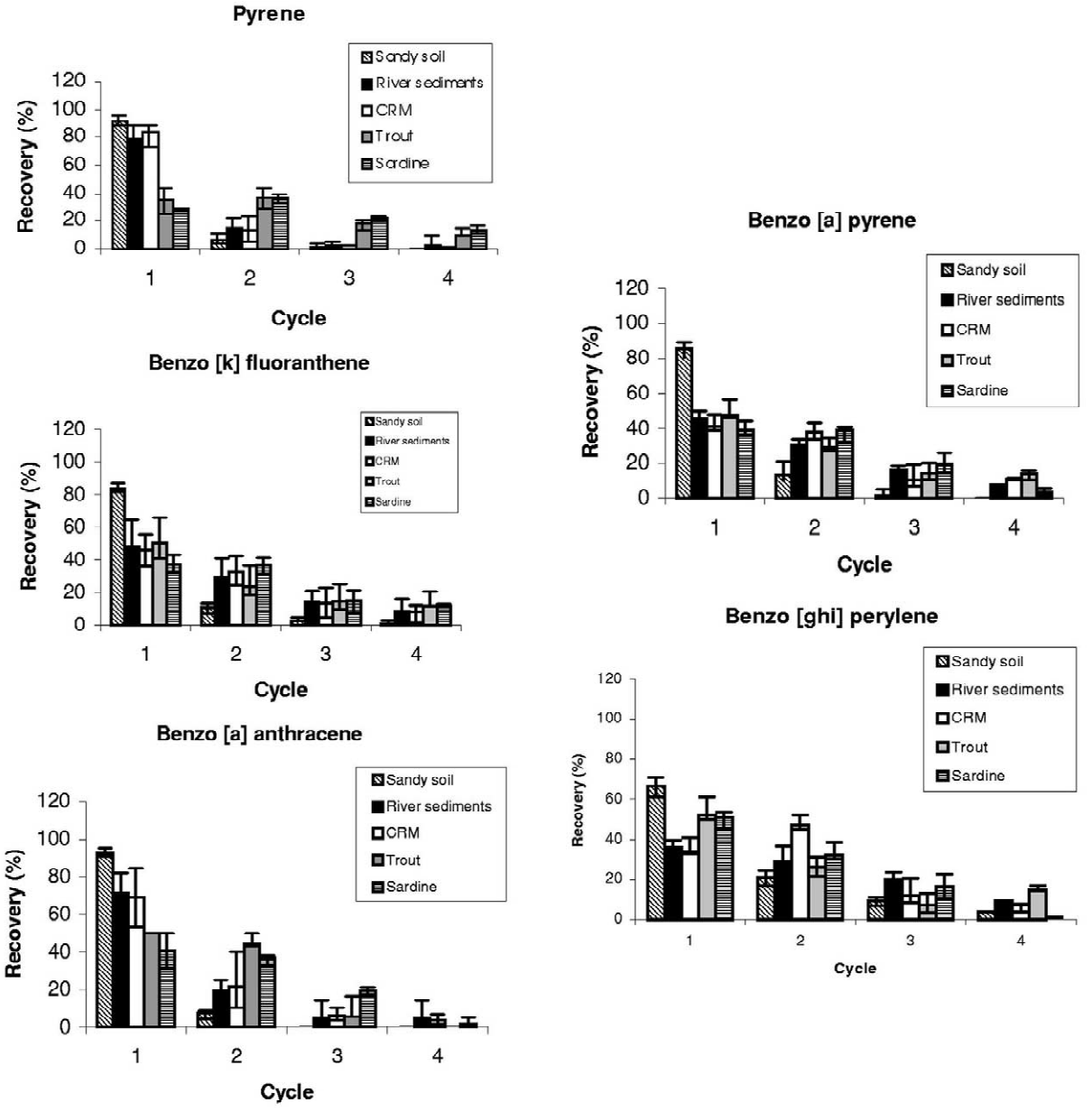

Fig. 2. Recoveries of the analytes from the different type of matrices obtained in each cycle.

(1) to work as a screening system (yes/no answer); (2) to monitor the extraction kinetics; (3) to complete a leaching stage in a time much shorter than that required by conventional Soxhlet; and (4) to semiquantify the analytes in routine analyses when the composition of the sample is approximately known.

Another advantage of the proposed approach is related to the growing concern for the health implications associated with the use of organic solvents

avoided using modified water as leaching agent, thus reducing the cost and providing an environmental friendly method.

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