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Miniaturised analytical procedure of determining polycyclic aromatic hydrocarbons and polychlorinated biphenyls in bottom sediments

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Abstract

This work concerns the determination of very low polychlorinated biphenyl (PCB) concentrations in bottom sediment samples in the presence of large amounts of polycyclic aromatic hydrocarbons (PAHs). A procedure for preparing 1 g bottom sediment samples for GC–MS analysis for the content of PAHs and PCBs is proposed. It consumes a few times smaller amounts of solvents than conventionally used procedures. Naphthalene-d8, benzo[*a*]anthracene-d12 and PCB 209 were used as internal standards for quantitation purposes; average recoveries of these standards were 65, 55 and 60%, respectively. A home-made glass column filled with ca. 500 mg of activated silica gel was used to isolate the PCB fraction. This has no significant effect on the recovery level of PCB (92–119%). Studies of the effect of homogenisation of a bottom sediment sample on the results of PAH and PCB were conducted. Grinding of bottom sediment samples to a particle size of 0.2 mm had no statistically significant effect on the analytical results and can therefore be omitted, which makes the preparation of the samples definitely less labour- and time-consuming. The interlaboratory study proved that the developed procedure for the simultaneous determination of PAHs and PCBs at largely different concentrations in sediment gives accurate and precise results.

Keywords: Polynuclear aromatic hydrocarbons; Polychlorinated biphenyls

1. Introduction

Bottom sediments are an important element of aquatic ecosystems. They constitute ecological niches supporting benthic organisms, i.e. animals and plants living on the bottom of bodies of water, and are a source of nutrients for aquatic organisms such as small invertebrates and protozoans.

An assessment of the effect of pollution on life in water bodies requires the sources and concentrations of the pollutants to be determined. In this respect, bottom sediments are very useful material for investigation, because they act as sorption column and provide a clear picture of events taking place in the overlying water.

The structure of bottom sediments and their extensive surface allow them to be treated as natural sorbent filtering out a variety of components, such as heavy metals, volatile organohalogen compounds, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phenols, and pesticides [1–3]. Having accumulated in the sediments,

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the bottom sediment may become a secondary source of pollution and keep contaminating water for many more years, despite the cutting off of primary sources [4].

Bottom sediments are, due to their complex nature, difficult to analyse directly for trace contaminants using e.g. chromatographic techniques. Owing to their highly complicated physico-chemical structure, the processing of such samples has to be carried out in several successive stages to widely remove any interfering substances. The compounds to be removed include:

- elemental sulphur (S_8);
- macromolecular compounds (e.g. fats, waxes) with molecular masses ranging between 600 and 1500 g mol^{-1}. They usually contain polar groups that can form hydrogen bonds, and are characterised by high molecular masses and low volatility; and
- compounds whose molecules are similar in size to those of the analytes.

The choice of the technique of analyte extraction from the environmental matrix largely depends on its physical state. The extraction techniques employed in the case of such a complex matrix as a bottom sediment can be divided into those dealing with wet or suitably dried samples [5].

The raw extract is generally subjected to additional treatment, i.e. to clean-up and/or fractionation. A proper choice of fractionating the raw extract allows an analysis of many groups of compounds simultaneously. Fig. 1 presents various procedures described in the literature which are employed in the fractionation of the extracts [6–9].

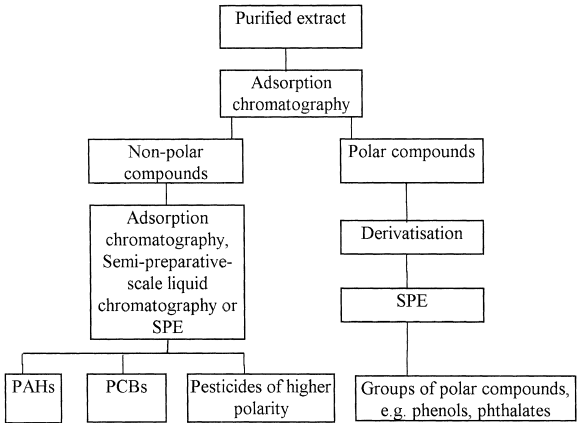

Fig. 1. Diagram of the procedure during the fractionation of extracts [6–9].

The ultimate separation of an extract into fractions is achieved via elution of a particular fraction with the help of various solvents used successively in suitable amounts, e.g. hexane and dichloromethane/hexane in various proportions, in order to increase the polarity gradually [7,8].

Although determinations of PAH and PCB in sediments are carried out on a routine basis in many laboratories worldwide, the procedures usually require 5–100 g of sediment and include many time- and solvent-consuming steps.

This paper presents the procedure for determination of analytes from both groups of compounds (PAH and PCB) simultaneously in a single solvent extract obtained from 1 g sediment sample.

2. Experimental

2.1. Chemicals

All solvents were GC-pure quality and were purchased from Merck (Germany). Silica gel 40 (J.T. Baker, Holland) was used as an adsorbent. A working stock solution was prepared from individual non-coplanar PCB standards (PCBs IUPAC Nos. 28, 52, 101, 118, 138, 153, 180) containing 200 ng of each in 1 ml of isooctane. PCB 209 was used as an internal standard. PCB solutions were purchased from Ehrenstorfer (Germany). A mixture of 16 PAHs at a concentration of 2000 μg ml^{-1} for each was from Resteck Corporation (USA). Naphthalene-d8 and benzo[*a*]anthracene-d12 were used as internal standards in dichloromethane at a concentration of 2000 μg ml^{-1}.

Silica gel (particle size 40 μm; J.T. Baker, Deventer, Holland) was used as an extract cleaning material. Copper (1 g) was treated with ca. 5 ml concentrated hydrochloric acid+water (1:1), rinsed with bidistilled water and dried with ca. 2 ml acetone.

2.2. Instrumentation

A GC 8000 (Fisons—Italy) gas chromatograph equipped with a 30 m×0.25 mm I.D., 0.25 μm Rtx-5MS capillary column coupled to a mass spectrometer detector MD 800 (Fisons) and an on-

column injection system was used to analyse PAHs and PCBs. In both analyses, the mass spectrometer was operated in the selected ion monitoring (SIM) mode, and the pollutants determination was based on the three selected ions monitored (Table 1) in small windows (width of 0.1 a.m.u.). The analyte concentration was the average calculated only from these data, which did not differ by more than 20%. The carrier gas was helium (inlet pressure 70 kPa). GC–MS was calibrated with six calibration standard solutions in the concentration range 0.06–0.7 μg ml^{-1} for PAH and 16–200 ng ml^{-1} for PCB.

2.2.1. GC conditions for PAH analysis

The temperature programme applied in GC was as follows: initial temperature 40 °C, 40–120 °C at 40 °C min^{-1}, 120–280 °C at 5 °C min^{-1}, 280 °C for 17 min.

2.2.2. GC conditions for PCB analysis

The temperature programme applied in GC was as follows: initial temperature 40 °C, 40–120 °C at 40 °C min^{-1}, 120–280 °C at 5 °C min^{-1}, 280 °C for 5 min. Under those conditions, PCB 28 co-eluted with PCB 31.

Table 1
Ion selected for MS analysis of PAH and PCB

Analyte	Ion selected for MS analysis
Naphthalene	129, 128, 127
Acenaphthylene	153, 152, 151
Acenaphthene	154, 153, 152
Fluorene	167, 166, 165
Phenanthrene	179, 178, 176
Anthracene	179, 178, 176
Fluoranthene	203, 202, 201
Pyrene	203, 202, 201
Benzo[*a*]anthracene	229, 228, 226
Chrysene	229, 228, 226
Benzo[*b*]fluoranthene	253, 252, 250
Benzo[*k*]fluoranthene	253, 252, 250
Benzo[*a*]pyrene	253, 252, 250
Indeno[1,2,3,-*cd*]pyrene	277, 276, 138
Dibenzo[*a,h*]anthracene	279, 278, 139
Benzo[*g,h,i*]perylene	277, 276, 138
PCB 28	258, 256, 150
PCB 52	292, 290, 257
PCB 101	326, 324, 254
PCB 118	326, 324, 254
PCB 138	360, 358, 292
PCB 153	360, 358, 292
PCB 180	394, 392, 326

2.3. Study of the PCB elution profile in a glass column filled with silica gel

A part of the PCB and PAH determination procedure proposed in this paper is the stage of the separation of PCB fractions from all other dichloromethane-eluted contaminants during sediment extraction. Sediment containing no PCBs was selected for studies. A 20-g sample of this sediment was extracted with 200 ml dichloromethane. The extract obtained was decanted and then evaporated to a volume of 100 ml under a stream of nitrogen. For determination of PCB elution profile, a 5 ml extract aliquot was used, which corresponds to 1 g sediment.

The next stage consists of the following operations:

- evaporation of a specified extract volume to dryness;
- extraction with pentane (3×100 μl) of the dry residue in an ultrasonic bath;
- fractionation of the pentane extract in glass columns filled with freshly conditioned silica gel;
- collection of the fraction containing PCBs and evaporating it to dryness under a gentle stream of nitrogen; and
- dissolution of the dry residue in 30 μl of hexane.

Then, 2-μl aliquots of the hexane extract are injected into the chromatographic column.

The change in the solvent from dichloromethane to pentane allows a preliminary purification of the extract through the separation primarily of polar impurities. The use of a column with silica gel that offers a longer elution pathway than commercial columns makes it possible to separate PCBs from PAHs.

A 20-μl aliquot of PCB standard solution at a concentration of 1.25 μg l^{-1} was added to 5 ml of a methyl chloride extract (this corresponds to 1 g of bottom sediment). This mixture was evaporated to dryness under a gentle stream of nitrogen. The dry residue was extracted with 100 μl of pentane, assisted by ultrasonication; the extraction was repeated three times. The pentane extract was transferred to a glass column 120 mm in length and 5 mm in diameter, filled with ca. 500 mg of activated silica gel (8 h at 140 °C). Directly before use, the gel-filled

column was activated at 140 °C for about 1 h and ca. 100 mg of freshly activated copper was placed at the column front.

The analytes were eluted from the column with pentane (1 ml min^{-1}) and 1-ml fractions were collected; then an internal standard (PCB 209) was added to the successive fractions which where evaporated to dryness. The dry residues each were dissolved in 30 μl of hexane and analysed by means of GC–MS (injection volume 2 μl).

2.4. Study of the effect of grinding of the samples on the results of PAH and PCB determination

Four samples of bottom sediments were collected in various places in the Odra river (Poland). All were freeze-dried and divided into two parts, one was ground with a laboratory hammer mill with a 0.2-mm mesh sieve. Before extraction, each sample was wetted with acetone, supplemented with a dose of an internal standard (naphthalene-d8, benzo[*a*]-anthracene-d12, PCB 209), mixed, and left overnight for the acetone to evaporate. Then, 1-g aliquots of the samples (ground and not ground) were extracted with 5 ml of methyl chloride for 24 h in a shaker. The supernatant was cleaned up in glass solid-phase extraction (SPE) columns filled with 500 mg freshly activated silica gel and a layer of activated copper (100 mg). The eluate (ca. 10 ml methyl chloride) was evaporated to a volume of 1 ml, and 2 μl of it was analysed by GC–MS for PAHs under the conditions given in Section 2.2. The remaining extract was treated and analysed as described in Section 2.3; however, during PCBs fractionation on the home-made silica gel column, the activated copper layer was not added, as the sulphur had been removed from the extract in the preceding step, prior to the PAH analysis.

2.5. Validation of the procedure for determining PAHs and PCBs in bottom sediments

The procedure of bottom sediment sample preparation was verified during an international inter-laboratory study organised by the International Atomic Energy Agency Marine Environment Laboratory in 1998. All participants obtained a sediment sample prepared as follows. A large sediment sample was collected from the intertidal mudflats of the Tagus estuary for use as intercomparison material. The sediment was deep-frozen, freeze-dried, ground, and sieved through a 0.15-mm stainless-steel sieve. It was further homogenised by mixing in a stainless-steel rotating drum for 2 weeks. Thus prepared, the sediment was sent to laboratories.

A 1-g sample was analysed employing the procedure described in Section 2.4, and the analysis was repeated three times. Fig. 2 shows a diagram of the preparation of bottom sediment samples for the analysis of PAHs and PCBs resulting from the present studies. The final quantitative results were averages of those calculated from each selected peak separately (Table 1). Average recoveries of standards in the following validation studies were 65, 55 and 60% for naphthalene-d8 and benzo[*a*]anthracene-d12, and PCB 209, respectively. The values were close to those obtained by other authors [10–14].

3. Results and discussion

3.1. PCB elution profile in a glass column filled with silica gel

The determination of very low PCB concentrations in bottom sediment samples in the presence of large amounts of PAHs is subject to much error when a typical, 30-m chromatographic column is used, owing to the co-elution of some PCB and PAH congeners as well as other compounds, such as phthalates or humic substances. The use of commercial SPE columns with silica gel to fractionate the extract does not solve the problem because of difficulties with the separation of PCB and PAH fractions.

Fig. 3 presents a typical elution profile of the analytes from the PCB group obtained when using the purification and fractionation procedure proposed.

Polychlorinated biphenyls were eluted from the proposed column completely in the first 7 ml of the solvent (pentane). The analytes from the PCB group were separated from aliphatic and polycyclic aromatic hydrocarbons as well as from coloured compounds dissolved in pentane.

The estimated recovery values of particular PCBs

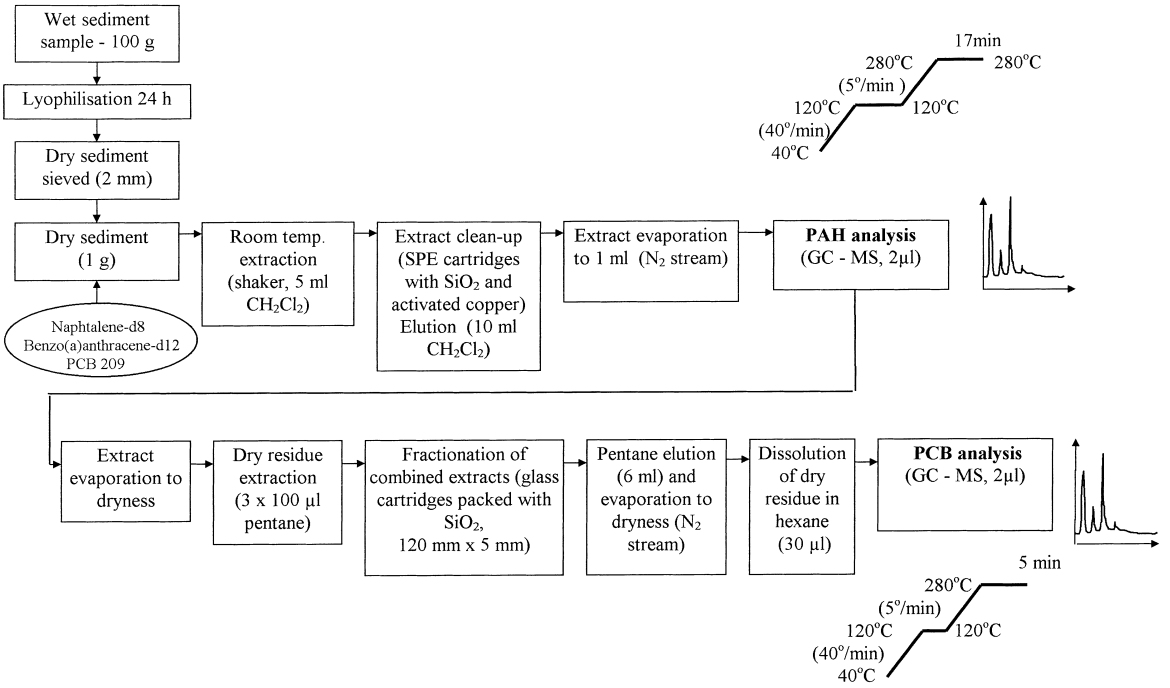

Fig. 2. Diagram of the procedure for determining analytes from the PAH and PCB groups in bottom sediment samples.

within this fractionation step are listed in Table 2. These figures provide additional confirmation that the proposed procedure of extract purification and fractionation does not bring about a significant loss of the compounds under study. When evaporated to dryness and dissolved in 30 µl of hexane, the combined fractions containing analytes from the PCB group were transparent, even when the extracts were highly polluted.

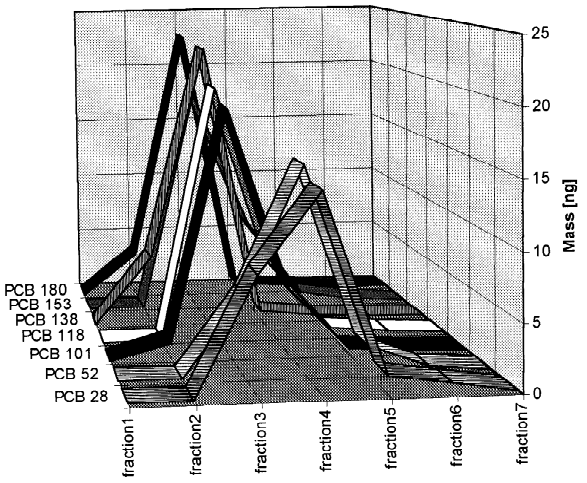

Fig. 3. Typical elution profile of analytes from the PCB group obtained with the use of the purification and fractionation procedure described (one fraction consists of 1 ml).

The proposed procedure of purification of dichloromethane extracts from bottom sediments has no significant effect on the recovery level of PCBs in the entire analytical procedure; it allows the determination limit to be achieved at 0.2 µg kg^{-1} with 1-g samples.

3.2. Study of the effect of grinding of the samples on the results of PAH and PCB determination

Grinding is an operation that results in an increase in the area of a solid in relation to its mass. Its aim is to enlarge the sample/solvent contact area during extraction in order to ensure a higher level of analyte recovery. The process also improves the homogeneity and representativeness of bottom sediment samples.

Table 2
Estimated recovery rates (mean of five measurements) of selected PCBs in the described procedure of purification and fractionation of extracts from bottom sediment samples

Analyte	Analyte concentration ($\mu g\ kg^{-1}$)	Fractions 1 to 7[a]		Fractions 2 to 4[a]	
		Recovery (%)	SD	Recovery (%)	SD
PCB 28—2,4,4′-trichlorobiphenyl	25	111	26	100	21
PCB 52—2,2′,5,5′-tetrachlorobiphenyl	25	92	14	81	10
PCB 101—2,2′,4,5,5′-pentachlorobiphenyl	25	99	20	96	22
PCB 118—2,3′,4,4′,5-pentachlorobiphenyl	25	97	20	93	20
PCB 138—2,2′,3,4,4′,5′-hexachlorobiphenyl	25	110	29	107	29
PCB 153—2,2′,4,4′,5,5′-hexachlorobiphenyl	25	119	38	111	34
PCB 180—2,2′,3,4,4′,5,5′-heptachlorobiphenyl	25	93	24	92	24

[a] One fraction consists of 1 ml.

The results for ground and non-ground samples were compared in a co-ordinate system, with concentrations obtained for non-ground samples plotted on the horizontal axis and the corresponding values for the same samples subjected to grinding plotted on the vertical axis. The evaluation was made on the basis of statistical criteria. The results were approximated using the least-squares method to obtain a linear function: $y = a + bx$, where a is the intercept; b, slope; y, analyte concentration in a bottom sediment sample subjected to grinding; and x, the analyte concentration in a bottom sediment sample not subjected to grinding.

Student's t-test was employed to examine the significance of the deviation of the bivariate correlation coefficients b from 1 and the intercept a from 0. The calculated experimental values t_{calc} (Table 3) are in each case smaller than the critical value t_{crit} read from tables for a specified degree of freedom ($f = n - 2$) and a 95% confidence level. This indicates

Table 3
Statistical assessment of the effect of grinding of bottom sediment samples on the results of determination of PAH and PCB analytes

Analyte	Slope b	Intercept a	Standard deviation of b, S_b	Standard deviation of a, S_a	No. of points (n)
Naphthalene	0.81	0.1	0.080	0.12	4
Acenaphthylene	2.1	0.0	0.76	0.033	4
Acenaphthene	1.2	0.01	0.18	0.095	4
Fluorene	1.3	0.01	0.26	0.096	4
Phenanthrene	1.13	0.0	0.034	0.046	4
Anthracene	1.3	0.0	0.43	0.15	4
Fluoranthene	1.0	0.1	0.13	0.28	4
Pyrene	0.93	0.03	0.056	0.086	4
Benzo[*a*]anthracene	1.2	0.2	0.79	0.84	4
Chrysene	0.93	0.02	0.034	0.027	4
Benzo[*b*]fluoranthene	0.99	0.01	0.27	0.033	4
Benzo[*k*]fluoranthene	1.0	0.1	0.26	0.28	4
Benzo[*a*]pyrene	1.1	0.2	0.48	0.41	4
Indeno[1,2,3,-*cd*]pyrene	1.3	0.0	0.53	0.17	4
Dibenzo[*a,h*]anthracene	1.4	0.0	0.66	0.17	4
Benzo[*g,h,i*]perylene	1.2	0.1	0.65	0.24	4
PCB 52	1.5	0.0	0.47	0.36	4
PCB 101	0.6	0.3	0.21	0.27	4
PCB 118	1.6	0.0	0.51	0.45	4
PCB 138	0.97	0.11	0.027	0.093	4
PCB 153	1.6	0.0	0.18	0.54	4
PCB 180	1.0	0.4	0.18	0.41	4

that the slope does not differ from 1 and the intercept does not differ from 0 in a statistically significant way. Hence, it can be assumed that the process of grinding (homogenisation) of bottom sediment samples has no statistically significant effect on the analytical results obtained with the procedure described here and therefore can be omitted, which makes the process of preparing the samples less labour- and time-consuming.

As has been shown, slight differences in the concentration levels are statistically insignificant (95% confidence level) and result from random errors.

3.3. Validation of the procedure for determining PAHs and PCBs in bottom sediments

The procedure was verified during an international interlaboratory study organised by the International Atomic Energy Agency Marine Environment Laboratory in 1998. Forty-eight laboratories from 36 countries participated in the test. Table 4 contains the results of determination of the analytes from the PAH and PCB groups in a bottom sediment sample analysed during that test.

During the interlab test, the method was evaluated with respect to accuracy defined as the difference between the average value of the results of parallel determinations made when employing the new procedure and the actual analyte content in the sample examined.

For the assessment of laboratory performance, a *Z*-score was calculated according to the formula:

$$Z = (x_i - x_a)/s_b$$

where x_i is the robust mean of the reported values of the analyte concentration in the sample; x_a is the assigned value (the mean value of acceptable results in the worldwide intercomparison studies); s_b is the target standard deviation.

The *Z*-score effectively expresses the difference between the robust mean of the laboratory and the assigned value in s_b units. Performance is considered acceptable if $|z|<2$. The measurement is regarded as out of control when $|z|>3$. It represents a simple method of giving each participant a normalised performance score for bias. The procedure has been accepted as a standard by ISO/IUPAC [15,16].

Table 4
Statistical assessment of the results of determination of PAH and PCB analytes in the bottom sediment sample analysed in the interlaboratory test

Analyte	No. of repetitions	Mean ($\mu g\ kg^{-1}$)	Standard deviation
Naphthalene	3	27	4.5
Acenaphthylene	3	6	2.4
Acenaphthene	3	2	0.1
Fluorene	3	5	0.8
Phenanthrene	3	19	1.8
Anthracene	3	8	4.3
Fluoranthene	3	38	6.3
Pyrene	3	38	6.9
Benzo[*a*]anthracene	3	27	4.0
Chrysene	3	32	5.3
Benzo[*b*]fluoranthene	3	30	4.8
Benzo[*k*]fluoranthene	3	15	10.1
Benzo[*a*]pyrene	3	28	5.5
Indeno[1,2,3,-*cd*]pyrene	3	12	5.2
Dibenzo[*a*,*h*]anthracene	3	6	2.3
Benzo[*g*,*h*,*i*]perylene	3	16	3.9
PCB 28	3	0.8	0.3
PCB 52	3	0.6	0.2
PCB 101	3	1.0	0.4
PCB 118	3	1.1	0.4
PCB 138	3	2.2	0.4
PCB 153	3	2.3	0.5
PCB 180	3	1.1	0.2

All the analysed compounds from the PAH and PCB groups fell within $|z|<2$.

The presented results of the interlaboratory study prove that the developed procedure for preparing bottom sediment samples for analysis for the content of analytes from the PAH and PCB groups gives accurate and precise results.

Table 4 presents a statistical evaluation of the determination of PAHs and PCBs in the bottom sediment sample that was analysed in the interlaboratory test mentioned above.

4. Conclusions

The presented procedure for the preparation of bottom sediment samples makes it possible:

- to reduce the sample mass to 1 g, as opposed to

5–100 g samples in other methods described in the literature [11,17–19];
- to determine analytes from both (PAH and PCB) groups of compounds simultaneously in a single solvent extract;
- to minimise the use of solvents; and
- to reduce the labour- and time intensity of the entire procedure.

It should be emphasized that the procedure is accurate as confirmed by an interlaboratory test.

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