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# Optimization of pressurized liquid extraction (PLE) of dioxin-furans and dioxin-like PCBs from environmental samples

Pedro Antunes[a], Paula Viana[a], Tereza Vinhas[a], J.L. Capelo[b],
J. Rivera[c], Elvira M.S.M. Gaspar[d,*]

[a] *Reference Laboratory of Environmental Institute, Rua da Murgueira Zambujal, Apartado 7585, Alfragide, 2721-865 Amadora, Portugal*
[b] *Requimte, Department of Chemistry, Faculty of Science and Technology, New University of Lisbon,*
*Quinta da Torre, 2825-114 Caparica, Portugal*
[c] *Instituto de Investigaciones Quimicas y Ambientales,CSIC, Departamento de Ecotecnologías, Laboratorio de Dioxinas,*
*c/Jordi Girona, 18, 08034 Barcelona, Spain*
[d] *Department of Chemistry, CQFB-Requimte, Faculty of Science and Technology, New University of Lisbon,*
*Quinta da Torre, 2825-114 Caparica, Portugal*

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## Abstract

Pressurized liquid extraction (PLE) applying three extraction cycles, temperature and pressure, improved the efficiency of solvent extraction when compared with the classical Soxhlet extraction. Polychlorinated-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like PCBs (coplanar polychlorinated biphenyls (Co-PCBs)) in two Certified Reference Materials [DX-1 (sediment) and BCR 529 (soil)] and in two contaminated environmental samples (sediment and soil) were extracted by ASE and Soxhlet methods. Unlike data previously reported by other authors, results demonstrated that ASE using *n*-hexane as solvent and three extraction cycles, 12.4 MPa (1800 psi) and 150 °C achieves similar recovery results than the classical Soxhlet extraction for PCDFs and Co-PCBs, and better recovery results for PCDDs. ASE extraction, performed in less time and with less solvent proved to be, under optimized conditions, an excellent extraction technique for the simultaneous analysis of PCDD/PCDFs and Co-PCBs from environmental samples. Such fast analytical methodology, having the best cost-efficiency ratio, will improve the control and will provide more information about the occurrence of dioxins and the levels of toxicity and thereby will contribute to increase human health.
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*Keywords:* Simultaneous PLE extraction; Pressurized liquid extraction; Dioxins; PCDDs; PCDFs; PCBs; Persistent organic pollutants

## 1. Introduction

The extreme toxicity of dioxins and furans has made their analysis in environmental samples increasingly important. They are well known as persistent and highly toxic organic pollutants (POPs). Pressurized liquid extraction (PLE, sometimes designated by, accelerated solvent extraction ASE) has been used in the last years with the aim of reducing the solvent consumption and the sample preparation time.

PLE technique [1–3] uses conventional liquid solvents at elevated pressures (10.3–13.8 MPa) and temperatures (40–200 °C) to extract solid samples quickly, and uses less solvent than the classical Soxhlet procedure. For instance, the Soxhlet technique for the extraction of polychlorinated-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) can take over 18 h and uses from 50 to 400 mL. With ASE, a solid sample is enclosed in a stainless steel vessel which is filled with an extraction solvent and heated to temperature. The sample is allowed to statically extract for 5–10 min. Extraction solvents of PLE are commonly used at temperatures that are higher than their respective boiling points and at high pressures to increase the analyte solubilities in solvents. The complete procedure is finished in 15–25 min, using about 15 mL of solvent for a 10 g sample. PLE has been approved by the United States Environmental Protection Agency (USEPA) as proposed Method 3545 [4] which includes polychlorinated biphenyls (PCBs), organochlorine and

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* Corresponding author.
*E-mail address:* emg@dq.fct.unl.pt (E.M.S.M. Gaspar).

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organophosphorous pesticides (OCP and OPP), semi-volatiles or BNAs, chlorinated phenoxy herbicides and polycyclic aromatic hydrocarbons (PAHs).

Optimization studies of PLE procedure, such as choice of solvents and temperatures, have been reported [5] because recoveries of POPs from environmental samples depend strongly on these conditions. However, few studies have been done for quantitative and simultaneous extractions of PCDDs, PCDFs and PCBs from environmental samples [5,6]. The main reason is that PLE conditions for each compound are indicated separately by the USEPA method.

This paper reports on, for the first time, PLE optimized conditions for quantitative and simultaneous extraction of PCDDs, PCDFs and dioxin-like PCBs (Co-PCBs) from two Certified Reference Materials DX-1 (sediment) and BCR 529 (soil) and also from two real environmental samples. Unlike data previously reported by other authors, the results indicate that an exhaustive and simultaneous extraction was achieved using *n*-hexane as single solvent at optimized conditions (three cycles, 12.4 MPa and 150 °C) and recoveries were surprisingly high for PCDDs when compared to those obtained using Soxhlet extraction. PLE method is performed in less time and with less solvent than Soxhlet methodology, being an excellent sustainable extraction technique for the simultaneous analysis of chlorinated dioxin-like compounds. The method is a good alternative to European Community needs for information about production and release of organohalogen compounds to the environment.

## 2. Experimental

### 2.1. Samples

Two Certified Reference Materials DX-1 (sediment), National Water Research Institute, Canada and BCR-529 (sandy soil), European Commission—Joint Research Center, Institute for Reference Materials and Measurements, Belgium were used in this study. The water content, according to certificate values, were residual (<5%); the organic matter for DX-1 was 3.5% and for BCR-529 was 0.8%. The authors would like to test this method with a previously mentioned Certified Reference Material CRM 0422 [5,6]. However, internationally, this reference corresponds to a fish muscle, instead of a forest soil sample [7].

The real samples are one sediment sample from Castelo de Bode dum, Portugal, and a Portuguese forest soil. The real samples were sieved to particles ≤106 μm and lyophilized during 72 h (water content <5%).

### 2.2. Standards and reagents

PCDD/PCDFs internal standards for extraction (EPA 1613 LCS), PCDD/PCDFs injection standards (EPA 1613 ISS) and PCDD/PCDFs calibration standards (EPA 1613 CSL, CS1–CS5), all in nonane solution, were purchased from Wellington Laboratories (Ont., Canada). Co-PCBs extraction standards (WP–LCS), Co-PCBs injection standards (WP–ISS) and Co-PCBs calibration standards (WP–CS1–CS7), all in nonane solution, were also purchased from Wellington Laboratories (Ont., Canada). All internal standards are $^{13}C_{12}$-labelled compounds. All used solvents are from Merck (pesticide grade; Darmstad, Germany). Diatomaceous earth (Hidromatix), ASE PreP DE, is from Dionex (Sunnyvale, CA, USA). Soxhlet cellulose extraction thimbles (30 mm × 80 mm) are from Schleicher & Schuell (Dassel, Germany). Power-prep columns are from Power Prep (Fluid Management System, Waltham, MA, USA); silica (19 cm), basic alumina (11 g, 19 cm) and carbon/celite (0.34 g, 4 cm) column were used.

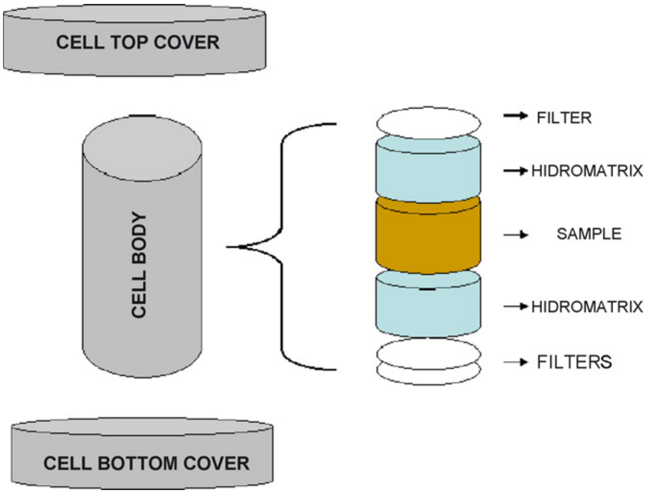

Fig. 1. Packing diagram of the extraction cell of ASE system.

### 2.3. Soxhlet extraction

Soxhlet apparatus were used with a cellulose thimble containing 1 or 2 g samples. The extractions were performed with and without copper, following USEPA 1613 Method for PCDD/PCDFs and USEPA Method 1688 for Co-PCBs. Both USEPA methods have similar procedures; the difference is the internal standards. The copper treatment included the addition of copper powder to the sample (1/2) contained in the cellulose thimble. Toluene was used as solvent and the extraction was performed during 48 h. Triplicate extractions were always performed, using ca. 350 mL of toluene. The certified values were based on Soxhlet extraction using toluene as solvent.

### 2.4. PLE extraction

Pressurized liquid extraction was carried out using a PLE system (ASE 200; Dionex, Sunnyvale, CA, USA) equipped with 33 mL stainless-steel extraction cells. All samples were packed according to Fig. 1 . All samples were placed into the stainless-steel extraction cells prepared with a cellulose filter (Dionex), then a portion (ca. 1/3 of cell's volume) of diatomaceous earth was placed and the sample (ca. 1/3 of cell's volume) was added, enriched with $^{13}C_{12}$-labelled internal extraction standards. The cell stands by 2 h to incorporate the standards and then were filled with a second portion of diatomaceous earth (ca. 1/3 of cell volume) and closed with a cellulose filter (Dionex, USA). During the process, the material in cell was being compressed

Table 1
Copper and sulphuric acid cleanup effect on Soxhlet extraction and pressurized liquid extraction (PLE) of PCDD/Fs and dioxin-like PCBs (Co-PCBs) from Certified Reference Material DX-1 (sediment).

| PCDD/Fs and Co-PCBs isomers (IUPAC number) | DX-1 certificate value ($ng\,kg^{-1}$) | Soxhlet with $Cu_{(s)}$ and $H_2SO_4$ ($n = 3$) | | | | Soxhlet without $Cu_{(s)}$ and $H_2SO_4$ ($n = 3$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Concentration[a] ($ng\,kg^{-1}$) | Observed/certified value (%) | RSD (%) | Recovery[b] (%) | Concentration[a] ($ng\,kg^{-1}$) | Observed/certified value (%) | RSD (%) | Recovery[b] (%) |
| 2,3,7,8-TCDF (F1) | 89 ± 44 | 51 | 58 | 24 | 85 | 32 | 36 | 29 | 103 |
| 1,2,3,7,8-PeCDF (F2) | 39 ± 14 | 45 | 114 | 18 | 79 | 40 | 101 | 4.9 | 88 |
| 2,3,4,7,8-PeCDF (F3) | 62 ± 32 | 67 | 109 | 9.3 | 82 | 81 | 130 | 9.0 | 85 |
| 1,2,3,4,7,8-HxCDF (F4) | 714 ± 276 | 669 | 94 | 6.2 | 82 | 639 | 90 | 1.9 | 96 |
| 1,2,3,6,7,8-HxCDF (F5) | 116 ± 37 | 128 | 110 | 2.1 | 72 | 111 | 96 | 7.9 | 88 |
| 2,3,4,6,7,8-HxCDF (F6) | 57 ± 36 | 63 | 110 | 4.6 | 76 | 56 | 98 | 6.6 | 86 |
| 1,2,3,7,8,9-HxCDF (F7) | 28 ± 42 | 48 | 172 | 2.1 | 86 | 44 | 156 | 1.1 | 91 |
| 1,2,3,4,6,7,8-HpCDF (F8) | 2397 ± 796 | 2468 | 103 | 2.6 | 71 | 2467 | 103 | 1.3 | 67 |
| 1,2,3,4,7,8,9-HpCDF (F9) | 137 ± 62 | 157 | 115 | 2.1 | 84 | 152 | 111 | 1.6 | 69 |
| OCDF (F10) | 7122 ± 2406 | 7669 | 108 | 20 | – | 6362 | 89 | 2.4 | – |
| 2,3,7,8-TCDD (D1) | 263 ± 53 | 260 | 99 | 0.6 | 81 | 216 | 82 | 0.6 | 104 |
| 1,2,3,7,8-PeCDD (D2) | 22 ± 8 | 28 | 129 | 13 | 79 | 28 | 129 | 2.5 | 87 |
| 1,2,3,4,7,8-HxCDD (D3) | 23 ± 7 | 22 | 96 | 5.2 | 87 | 25 | 107 | 20 | 85 |
| 1,2,3,6,7,8-HxCDD (D4) | 77 ± 27 | 88 | 114 | 12 | 70 | 75 | 98 | 2.4 | 92 |
| 1,2,3,7,8,9-HxCDD (D5) | 53 ± 24 | 41 | 77 | 27 | – | 42 | 79 | 4.4 | – |
| 1,2,3,4,6,7,8-HpCDD (D6) | 634 ± 182 | 640 | 101 | 11 | 74 | 654 | 103 | 1.0 | 70 |
| OCDD (D7) | 3932 ± 933 | 4187 | 106 | 8.9 | 69 | 3904 | 99 | 2.4 | 52 |
| 3,4,4′,5-TCB (#81) | – | 475 | – | 7.2 | 51 | 421 | – | 16 | 103 |
| 3,3′,4,4′-TCB (#77) | – | 6244 | – | 2.1 | 49 | 6519 | – | 22 | 110 |
| 2′,3,4,4′,5-PeCB (#123) | – | 1377 | – | 7.1 | 64 | 991 | – | 22 | 90 |
| 2,3′,4,4′,5-PeCB (#118) | – | 44670 | – | 3.4 | 67 | 41977 | – | 13 | 90 |
| 2,3,4,4′,5-PeCB (#114) | – | 1939 | – | 10 | 60 | 1902 | – | 15 | 93 |
| 2,3,3′,4,4′-PeCB (#105) | – | 19230 | – | 6.0 | 47 | 23557 | – | 5.5 | 96 |
| 3,3′,4,4′,5-PeCB (#126) | – | 328 | – | 29 | 49 | 206 | – | 16 | 116 |
| 2,3′,4,4′,5,5′-HxCB (#167) | – | 1549 | – | 4.3 | 68 | 1174 | – | 24 | 97 |
| 2,3,3′,4,4′,5-HxCB (#156) | – | 3934 | – | 5.6 | 56 | 3822 | – | 12 | 86 |
| 2,3,3′,4,4′,5′-HxCB (#157) | – | 761 | – | 12 | 51 | 727 | – | 3.6 | 91 |
| 3,3′,4,4′,5,5′-HxCB (#169) | – | 37 | – | 26 | 46 | 16 | – | 47 | 95 |
| 2,3,3′,4,4′,5,5′-HpCB (#189) | – | 410 | – | 9.0 | 66 | 337 | – | 8.7 | 85 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 2,3,7,8-TCDF (F1) | 89 ± 44 | 69 | 77 | 16 | 66 | 44 | 49 | 2.3 | 94 |
| 1,2,3,7,8-PeCDF (F2) | 39 ± 14 | 54 | 138 | 12 | 73 | 43 | 111 | 4.8 | 73 |
| 2,3,4,7,8-PeCDF (F3) | 62 ± 32 | 95 | 152 | 42 | 70 | 83 | 134 | 5.7 | 73 |
| 1,2,3,4,7,8-HxCDF (F4) | 714 ± 276 | 798 | 112 | 1.7 | 66 | 730 | 102 | 2.1 | 79 |
| 1,2,3,6,7,8-HxCDF (F5) | 116 ± 37 | 149 | 128 | 0.8 | 64 | 133 | 114 | 4.4 | 77 |
| 2,3,4,6,7,8-HxCDF (F6) | 57 ± 36 | 84 | 148 | 8.5 | 61 | 65 | 114 | 3.5 | 77 |
| 1,2,3,7,8,9-HxCDF (F7) | 28 ± 42 | 65 | 230 | 8.5 | 60 | 52 | 186 | 2.2 | 83 |
| 1,2,3,4,6,7,8-HpCDF (F8) | 2397 ± 796 | 3328 | 139 | 4.1 | 44 | 2764 | 115 | 2.3 | 49 |
| 1,2,3,4,7,8,9-HpCDF (F9) | 137 ± 62 | 232 | 169 | 8.2 | 41 | 163 | 119 | 3.5 | 41 |
| OCDF (F10) | 7122 ± 2406 | 7724 | 108 | 0.5 | - | 3401 | 48 | 33 | – |
| 2,3,7,8-TCDD (D1) | 263 ± 53 | 248 | 94 | 1.9 | 74 | 272 | 103 | 4.2 | 91 |
| 1,2,3,7,8-PeCDD (D2) | 22 ± 8 | 47 | 215 | 10 | 71 | 64 | 291 | 21 | 74 |
| 1,2,3,4,7,8-HxCDD (D3) | 23 ± 7 | 30 | 131 | 13 | 68 | 23 | 101 | 24 | 82 |
| 1,2,3,6,7,8-HxCDD (D4) | 77 ± 27 | 229 | 297 | 2.0 | 63 | 332 | 431 | 7.5 | 76 |
| 1,2,3,7,8,9-HxCDD (D5) | 53 ± 24 | 90 | 170 | 14 | - | 143 | 269 | 15 | - |
| 1,2,3,4,6,7,8-HpCDD (D6) | 634 ± 182 | 1956 | 308 | 0.2 | 57 | 2041 | 322 | 3.0 | 63 |
| OCDD (D7) | 3932 ± 933 | 9984 | 254 | 2.7 | 41 | 8349 | 212 | 1.2 | 45 |
| 3,4,4′,5-TCB (#81) | – | 600 | – | 13 | 45 | – | – | – | – |
| 3,3′,4,4′-TCB (#77) | – | 6819 | – | 5.8 | 54 | – | – | – | – |
| 2′,3,4,4′,5-PeCB (#123) | – | 1316 | – | 2.8 | 72 | – | – | – | – |
| 2,3′,4,4′,5-PeCB (#118) | – | 46868 | – | 2.1 | 71 | – | – | – | – |
| 2,3,4,4′,5-PeCB (#114) | – | 2364 | – | 16 | 65 | – | – | – | – |
| 2,3,3′,4,4′-PeCB (#105) | – | 20408 | – | 5.7 | 66 | – | – | – | – |
| 3,3′,4,4′,5-PeCB (#126) | – | 283 | – | 10 | 58 | – | – | – | – |
| 2,3′,4,4′,5,5′-HxCB (#167) | – | 1800 | – | 2.4 | 72 | 1361 | – | 27 | 87 |
| 2,3,3′,4,4′,5-HxCB (#156) | – | 4466 | – | 4.6 | 67 | 4208 | – | 12 | 88 |
| 2,3,3′,4,4′,5′-HxCB (#157) | – | 977 | – | 10 | 65 | 906 | – | 28 | 86 |
| 3,3′,4,4′,5,5′-HxCB (#169) | – | 27 | – | 41 | 55 | 34 | – | 12 | 41 |
| 2,3,3′,4,4′,5,5′-HpCB (#189) | – | 487 | – | 5.1 | 62 | 471 | – | 5.3 | 85 |

[a] Extraction solvent: toluene.

[b] $^{13}$C: labelled compounds.

[c] Extraction solvent: *n*-hexane.

by hand. Then, the cell was sealed with the top cell cap. The dead volume was lower than 2 mm.

The optimal PLE conditions achieved for extractions were $P = 12.4$ MPa and $T = 150\,^{\circ}C$, with three (3) extraction cycles. Triplicate extractions were performed without and with copper (added to the sample), using *n*-hexane and toluene as solvents. The choice of these solvents was based on the USEPA Method 3545A for PCDD/PCDFs and PCBs, which recommends the use of single solvent.

### 2.5. Cleanup

The cleanup procedures included both possibilities—the treatment without and with sulphuric acid. The treatment with sulphuric acid was done submitting the extracts to three successive extraction steps with 50 mL of concentrated sulphuric acid. During the first extraction the acid stands by 24 h, while the second and third extractions were performed during 3 h. The organic phase was collected and submitted to multi-layered silica/alumina/active carbon columns (Power Prep/FMS apparatus). A gradient elution, using an automatic program, was done. PCDD/PCDFs were retained in the active carbon column, which was first eluted with mixed different solvents and, at the end, was eluted using toluene from the opposite flow direction. Co-PCBs were collected from all columns, using different solvents (dichloromethane/hexane and toluene). The PCDD/PCDFs fraction was concentrated to dryness using a rotary evaporator and a nitrogen stream, and after was diluted with nonane and internal standards (USEPA 1613-ISS solution; 5 μL of each).

The Co-PCBs sub-fraction (mono-*ortho*-PCBs) resulted from the silica and alumina columns' elution. This sub-fraction was concentrated to dryness using a rotary evaporator and nitrogen stream. A portion of this sub-fraction was combined with the PCDD/PCDFs fraction which contains the non-*ortho*-PCBs, to constitute the total Co-PCBs fraction [8]. Finally, the diluted $^{13}C_{12}$-labelled injection internal standards WP–ISS were added to each sample to calculate the recoveries of the extraction and cleanup procedures. Table 1 and Fig. 2 shows the dioxin and furan's recoveries. The cleanup for PCBs with copper and sulphuric acid revealed identical recovery results.

### 2.6. Determination of PCDD/PCDFs and Co-PCBs

Procedures were done according to USEPA Method 1613 for PCDD/PCDFs and USEPA 1618 for PCBs. HRGC/HRMS (Agilent Technologies 6890 series coupled with a Micromass spectrometer AutoSpec ultima) was used. We achieved GC separation for TetraCDDs/CDFs to HexaCDDs/CDFs, for HeptaCDDs/CDFs to OctaCDDs/CDFs, and for TetraCBs to HeptaCBs, respectively, using a capillary column VF5MS (60 m × 0.25 mm i.d. × 0.25 μm; Varian, Mideelburg, The Netherlands) and a DB Dioxin column (60 m × 0.15 mm i.d. × 0.20 μm; J&W Scientific, Folsom, CA, USA).

Recoveries of the $^{13}C_{12}$-labelled extraction standards were good according to USEPA methods (1613 and 1688), which established the ranges 40–120% for labelled compounds and 80–120% for native compounds (observed value/certified value). Results are shown in Tables 1 and 2.

## 3. Results and discussion

Soxhlet extractions of CRM (DX-1 and BCR 529) were performed using *n*-hexane and toluene with and without copper and sulphuric acid. Tables 1 and 2 and Fig. 2 summarize the compound's recoveries. The $^{13}C_{12}$ recoveries are ratios of recovered $^{13}C_{12}$-labelled compound to the spiked $^{13}C_{12}$-labelled compound and recovery of native compounds are ratios of each isomer's concentration to the certified values. It is important to emphasize that certified values are determined using EPA Method 1613, this means Soxhlet extraction procedure using toluene as solvent. We have tried different solvents. Using *n*-hexane lower recoveries of PCDD/PCDFs and Co-PCBs (<80%) were achieved. Using toluene, a more polar solvent, better recoveries of both PCDD/PCDFs and Co-PCBs were produced. Our Soxhlet's results are in good agreement with those recently published [5].

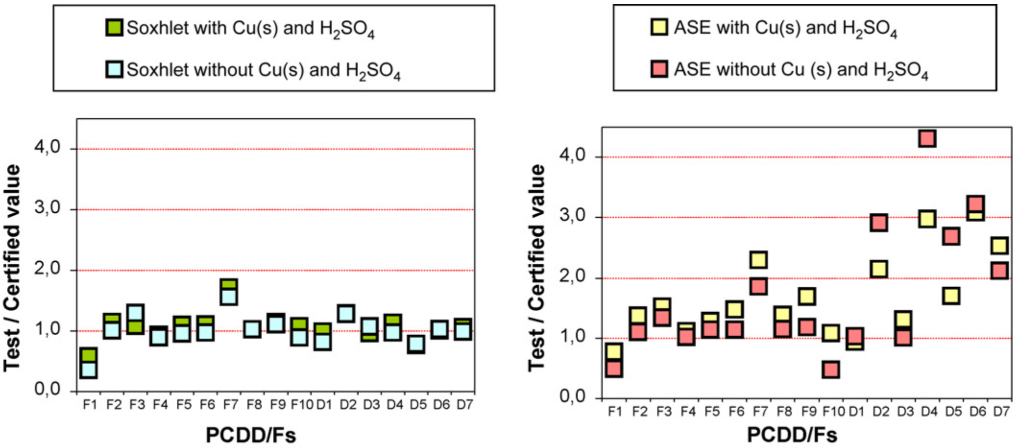

Fig. 2. Graphical visualization of cleanup effect on Soxhlet and ASE extraction procedures of PCDD/Fs from CRM DX-1: F1-F10, polychlorinated dibenzo-furans and D1-D7, polychlorinated dibenzo-dioxins (according to Table 1).

Table 2
Recoveries of PCDD/Fs and dioxin-like PCBs (Co-PCBs) from Certified Reference Materials BCR 529 using Soxhlet and PLE (3 cycles, 12.4 MPa and 150 °C) extractions

| PCDD/Fs and Co-PCBs isomers (IUPAC number) | BCR certificate value ($\mathrm{ng\,kg^{-1}}$) | Soxhlet ($n = 3$) | | | | PLE ($n = 3$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Concentration[a] ($\mathrm{ng\,kg^{-1}}$) | Observed/certified value (%) | RSD (%) | Recovery[b] (%) | Concentration[c] ($\mathrm{ng\,kg^{-1}}$) | Observed/certified value (%) | RSD (%) | Recovery[b] (%) |
| 2,3,7,8-TCDF (F1) | 78 ± 13 | – | – | – | – | – | – | – | – |
| 1,2,3,7,8-PeCDF (F2) | 140 ± 30 | 247 | 176 | 40 | 70 | 203 | 145 | 20 | 68 |
| 2,3,4,7,8-PeCDF (F3) | 360 ± 70 | 555 | 154 | 5.1 | 82 | 460 | 189 | 1.0 | 69 |
| 1,2,3,4,7,8-HxCDF (F4) | 3400 ± 500 | 4352 | 128 | 9.6 | 72 | 6218 | 198 | 18 | 71 |
| 1,2,3,6,7,8-HxCDF (F5) | 1090 ± 150 | 1184 | 109 | 11 | 74 | 1444 | 150 | 2.7 | 62 |
| 2,3,4,6,7,8-HxCDF (F6) | 370 ± 40 | 518 | 140 | 8.7 | 73 | 658 | 206 | 11 | 69 |
| 1,2,3,7,8,9-HxCDF (F7) | 22 ± 10 | – | – | 12 | – | – | – | 12 | – |
| 1,2,3,4,6,7,8-HpCDF (F8) | – | 13652 | – | 3.5 | – | 15171 | – | 14 | – |
| 1,2,3,4,7,8,9-HpCDF (F9) | – | 1984 | – | 2.9 | – | 2248 | – | 12 | – |
| OCDF (F10) | – | 60674 | – | 10 | – | 63556 | – | 16 | – |
| 2,3,7,8-TCDD (D1) | 4500 ± 600 | 3955 | 88 | 2.4 | 75 | 5507 | 122 | 1.8 | 69 |
| 1,2,3,7,8-PeCDD (D2) | 440 ± 50 | 666 | 151 | 12 | 80 | 3469 | 635 | 109 | 67 |
| 1,2,3,4,7,8-HxCDD (D3) | 1200 ± 300 | 1171 | 98 | 6.5 | 68 | 2058 | 163 | 54 | 71 |
| 1,2,3,6,7,8-HxCDD (D4) | 5400 ± 900 | 5041 | 93 | 7.2 | 68 | 7623 | 141 | 30 | 64 |
| 1,2,3,7,8,9-HxCDD (D5) | 3000 ± 400 | 2045 | 68 | 2.0 | – | 2964 | 99 | 46 | – |
| 1,2,3,4,6,7,8-HpCDD (D6) | – | 44920 | – | 8.4 | – | 59222 | – | 17 | – |
| OCDD (D7) | – | 245067 | – | 8.0 | – | 270657 | – | 10 | – |
| 3,4,4′,5-TCB (#81) | – | 782 | – | 3.1 | 39 | 2971 | – | 5.6 | 52 |
| 3,3′,4,4′-TCB (#77) | – | 21846 | – | 9.3 | 45 | 89640 | – | 26 | 65 |
| 2′,3,4,4′,5-PeCB (#123) | – | 3560 | – | 1.5 | 46 | 3301 | – | 8.0 | 97 |
| 2,3′,4,4′,5-PeCB (#118) | – | 29970 | – | 5.0 | 47 | 34366 | – | 8.5 | 96 |
| 2,3,4,4′,5-PeCB (#114) | – | 3728 | – | 11 | 44 | 4517 | – | 13 | 93 |
| 2,3,3′,4,4′-PeCB (#105) | – | 11727 | – | 0.8 | 42 | 14011 | – | 5.7 | 90 |
| 3,3′,4,4′,5-PeCB (#126) | – | 4089 | – | 1.4 | 54 | 6902 | – | 3.6 | 78 |
| 2,3′,4,4′,5,5′-HxCB (#167) | – | 25363 | – | 15 | 45 | 14374 | – | 4.6 | 82 |
| 2,3,3′,4,4′,5-HxCB (#156) | – | 19047 | – | 2.6 | 45 | 19763 | – | 4.8 | 79 |
| 2,3,3′,4,4′,5′-HxCB (#157) | – | 4899 | – | 5.8 | 45 | 3770 | – | 1.4 | 77 |
| 3,3′,4,4′,5,5′-HxCB (#169) | – | 554 | – | 1.1 | 58 | 569 | – | 10 | 67 |
| 2,3,3′,4,4′,5,5′-HpCB (#189) | – | 6124 | – | 4.0 | 44 | 6427 | – | 1.6 | 87 |

[a] Extraction solvent: toluene.

[b] $^{13}C$: labelled compounds.

[c] Extraction solvent: *n*-hexane.

Table 3
Comparison of Soxhlet and PLE extraction results with literature data [6]

| PCDD/Fs isomers | CRM DX-1 (sediment) | | CRM 0422 (forest soil) [6][a] | |
|---|---|---|---|---|
| | Soxhlet with toluene | PLE with *n*-hexane | Soxhlet with toluene | PLE with (1/1) *n*-hexane/acetone |
| | Observed/certified value (%) | Observed/certified value (%) | Observed/certified value (%) | Observed/certified value (%) |
| 2,3,7,8-TCDF (F1) | 58 | 77 | 103 | 77 |
| 1,2,3,7,8-PeCDF (F2) | 114 | 138 | 124 | 108 |
| 2,3,4,7,8-PeCDF (F3) | 109 | 152 | 114 | 83 |
| 1,2,3,4,7,8-HxCDF (F4) | 94 | 112 | 116 | 99 |
| 1,2,3,6,7,8-HxCDF (F5) | 110 | 128 | 104 | 89 |
| 2,3,4,6,7,8-HxCDF (F6) | 110 | 148 | 118 | 95 |
| 1,2,3,7,8,9-HxCDF (F7) | 172 | 230 | 107 | 87 |
| 1,2,3,4,6,7,8-HpCDF (F8) | 103 | 139 | 105 | 72 |
| 1,2,3,4,7,8,9-HpCDF (F9) | 115 | 169 | 114 | 107 |
| OCDF (F10) | 108 | 108 | 117 | 81 |
| 2,3,7,8-TCDD (D1) | 99 | 94 | 96 | 83 |
| 1,2,3,7,8-PeCDD (D2) | 129 | 215 | 109 | 86 |
| 1,2,3,4,7,8-HxCDD (D3) | 96 | 131 | 101 | 91 |
| 1,2,3,6,7,8-HxCDD (D4) | 114 | 297 | 105 | 81 |
| 1,2,3,7,8,9-HxCDD (D5) | 77 | 170 | 104 | 85 |
| 1,2,3,4,6,7,8-HpCDD (D6) | 101 | 308 | 124 | 96 |
| OCDD (D7) | 106 | 254 | 92 | 83 |

[a] Internationally CRM 422 corresponds to a fish muscle [7].

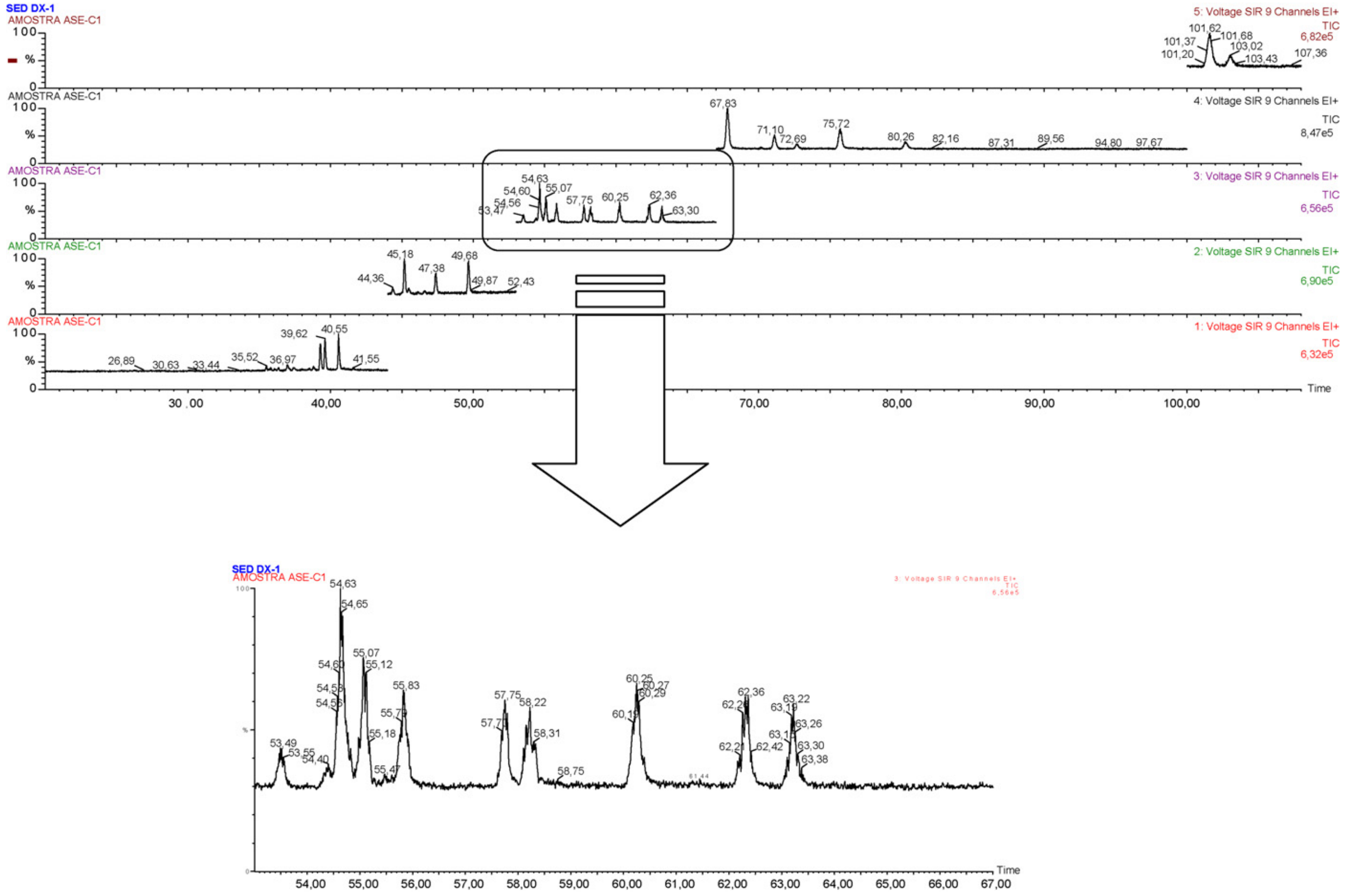

Fig. 3. *n*-Hexane ASE extraction chromatogram from BCR DX-1 sample, using DB-Dioxin column. The specific congeners of PCDDs are shown. Zoom area shows the Hx(6)CDD/Fs.

PLE extractions were done using the same extraction solvents as those used for Soxhlet extractions. Different conditions were tried till the ASE optimal conditions were achieved: three (3) cycles, $P = 12.4$ MPa, $T = 150\,^{\circ}$C. As the solvent recoveries were comparable, due to safety reasons *n*-hexane was considered to be our choice. Recovery results are summarized in Tables 1 and 2. Contrary to our expectations based on the results obtained with Soxhlet methodology, PLE extractions using toluene produced similar recoveries as those using *n*-hexane. During the cleaning step the sample matrix is washed with *n*-hexane. The extraction with *n*-hexane seems to guarantee the uniformity of the process. PLE extraction using *n*-hexane, at described conditions, exhibited the highest "native" recoveries of PCDDs ever reported. These recoveries are comparable to those recently obtained with more polar solvents at different operation conditions by Kiguchi et al. [5,6] (Table 3). The RSDs of both PCDD/PCDFs and Co-PCBs for the PLE extraction using 3 cycles are low. The good recoveries and the low RSDs of PCDD/PCDFs and Co-PCBs may indicate that, at these conditions, exists a strong analyte–matrix interaction.

Some researchers [5,6] have mentioned that several cycles are necessary to achieve satisfactory extraction of PCDD/PCDFs and Co-PCBs from environmental samples. Our results support those findings, proving that PLE using 3 cycles, 12.4 MPa and 150 °C, produce the best native analyte's recoveries already reported. However, Table 3 also shows that using these conditions no mixed solvents are necessary for good extraction of more tightly bounded PCDFs and Co-PCBs, being the PCDDs increasingly recovered from certified reference materials. The Z-score values for PCDD/Fs and Co-PCBs were also determined (data not shown). This parameter is considered to evaluate the performance of the method during the laboratories' accreditation or inter-laboratorial assays and during proficiency testing schemes. For PCDDs, using ASE extraction, the Z-score values were consistently high in all samples, revealing that recovery values are better than those obtained with Soxhlet extraction. As PLE extraction applies temperature and pressure to accelerate extraction processes, the effect was particularly improved with PCDDs using three cycles, showing a good efficiency of *n*-hexane extraction.

Some authors, using different conditions from the ones that are reported in this work, have already demonstrated that PLE is equivalent to classical extraction methods such as Soxhlet [3]. This work proves that using optimized conditions, PLE has higher native dioxin-like compounds' recoveries than Soxhlet methodology. As organic solvents required to extract solid samples using Soxhlet method, represent a large source of waste in the environmental analysis laboratory, PLE proved to be an important alternative extraction procedure for PCDDs and PCDFs from environmental samples.

Figs. 3 and 4 show the DB-Dioxin column chromatograms. It is possible to visualize the comparison of the native recovery of

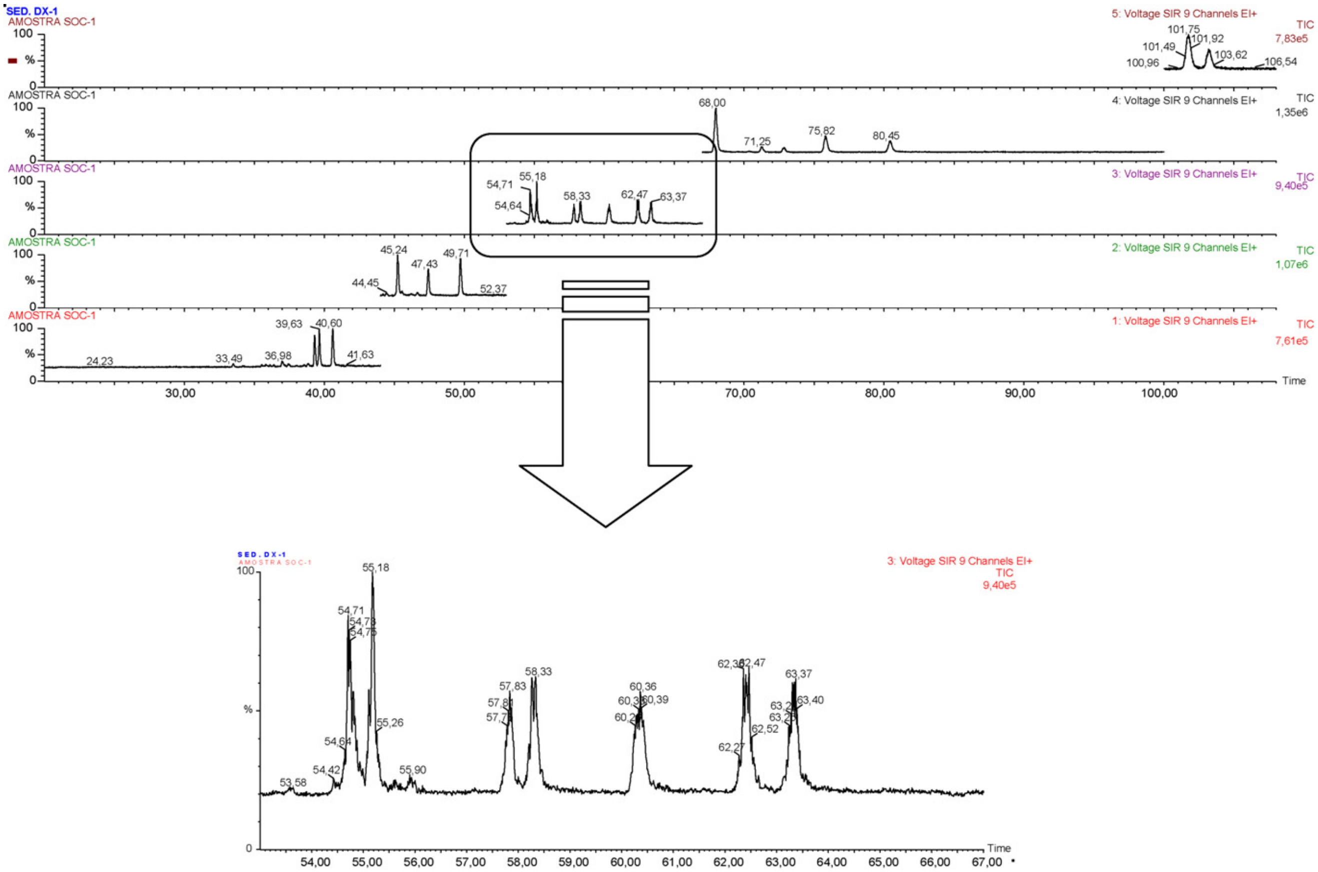

Fig. 4. Toluene Soxhlet extraction chromatogram from BCR DX-1 sample, using DB-Dioxin column. The specific congeners of PCDDs are shown. Zoom area shows the Hx(6)CDD/Fs.

Table 4
Individual WHO–TEF values for risk assessment and native recoveries (WHO–TEQ values) of PCDD/Fs and Co-PCBs using Soxhlet and PLE extraction for two real environmental samples: sediment and forest soil

| PCDD/Fs isomers | WHO–TEF[a] | Sediment (TOC = 2.1%) | | Forest soil (TOC = 13.8) | |
|---|---|---|---|---|---|
| | | Soxhlet with toluene (ng WHO–TEQ kg$^{-1}$) | PLE with *n*-hexane (ng WHO–TEQ kg$^{-1}$) | Soxhlet with toluene (ng WHO–TEQ kg$^{-1}$) | PLE with *n*-hexane (ng WHO–TEQ kg$^{-1}$) |
| 2,3,7,8-TCDF (F1) | 0.1 | 0.02[b] | 0.02[b] | 6.4 | 6.1 |
| 1,2,3,7,8-PeCDF (F2) | 0.05 | 0.03[b] | 0.03[b] | 3.0 | 3.6 |
| 2,3,4,7,8-PeCDF (F3) | 0.5 | 0.25[b] | 1.3[b] | 63 | 69 |
| 1,2,3,4,7,8-HxCDF (F4) | 0.1 | 0.38 | 0.34 | 11 | 14 |
| 1,2,3,6,7,8-HxCDF (F5) | 0.1 | 0.08[b] | 0.24[b] | 9.7 | 15 |
| 2,3,4,6,7,8-HxCDF (F6) | 0.1 | 0.09[b] | 0.24[b] | 14 | 21 |
| 1,2,3,7,8,9-HxCDF (F7) | 0.1 | 0.13[b] | 0.13[b] | 3.8 | 4.1 |
| 1,2,3,4,6,7,8-HpCDF (F8) | 0.01 | 0.12 | 0.20 | 4.9 | 6.2 |
| 1,2,3,4,7,8,9-HpCDF (F9) | 0.01 | 0.012[b] | 0.012[b] | 0.54 | 0.36 |
| OCDF (F10) | 0.0001 | 0.0044 | 0.0063 | 0.051 | 0.019 |
| 2,3,7,8-TCDD (D1) | 1 | 0.5[b] | 0.5[b] | 6.8 | 10 |
| 1,2,3,7,8-PeCDD (D2) | 1 | 1.2[b] | 2.5[b] | 43 | 35 |
| 1,2,3,4,7,8-HxCDD (D3) | 0.1 | 0.06[b] | 0.06[b] | 2.2 | 2.6 |
| 1,2,3,6,7,8-HxCDD (D4) | 0.1 | 0.11[b] | 0.11[b] | 3.7 | 4.9 |
| 1,2,3,7,8,9-HxCDD (D5) | 0.1 | 0.13[b] | 0.13[b] | 2.8 | 2.7 |
| 1,2,3,4,6,7,8-HpCDD (D6) | 0.01 | 1.12 | 1.39 | 2.2 | 3.3 |
| OCDD (D7) | 0.0001 | 0.111 | 0.155 | 0.073 | 0.11 |
| | Total | 4.35 | 7.31 | 176 | 196 |
| Co-PCBs isomers (IUPAC number) | WHO–TEF[a] | Soxhlet with Toluene (ng WHO–TEQ kg$^{-1}$) | PLE with *n*-Hexane (ng WHO–TEQ kg$^{-1}$) | Soxhlet with Toluene (ng WHO–TEQ kg$^{-1}$) | PLE with *n*-Hexane (ng WHO–TEQ kg$^{-1}$) |
| 3,4,4′,5-TCB (#81) | 0.0001 | 0.00039 | 0.00019 | 0.010 | 0.0086 |
| 3,3′,4,4′-TCB (#77) | 0.0001 | 0.0037 | 0.0019 | 0.18 | 0.18 |
| 2′,3,4,4′,5-PeCB (#123) | 0.0001 | 0.00002[b] | 0.00004[b] | 0.024 | 0.090 |
| 2,3′,4,4′,5-PeCB (#118) | 0.0001 | 0.0225 | 0.0129 | 0.57 | 0.59 |
| 2,3,4,4′,5-PeCB (#114) | 0.0005 | 0.0002[b] | 0.0003[b] | 0.14 | 0.18 |
| 2,3,3′,4,4′-PeCB (#105) | 0.0001 | 0.0088 | 0.0038 | 0.19 | 0.27 |
| 3,3′,4,4′,5-PeCB (#126) | 0.1 | 0.34 | 0.29 | 83 | 76 |
| 2,3′,4,4′,5,5′-HxCB (#167) | 0.00001 | 0.00032 | 0.00012 | 0.046 | 0.041 |
| 2,3,3′,4,4′,5-HxCB (#156) | 0.0005 | 0.034 | 0.0125 | 1.2 | 1.4 |
| 2,3,3′,4,4′,5′-HxCB (#157) | 0.0005 | 0.0001[b] | 0.0034 | 0.29 | 0.28 |
| 3,3′,4,4′,5,5′-HxCB (#169) | 0.01 | 0.001[b] | 0.001[b] | 1.2 | 1.4 |
| 2,3,3′,4,4′,5,5′-HpCB (#189) | 0.0001 | 0.00001[b] | 0.00001[b] | 0.068 | 0.072 |
| | Total | 0.41 | 0.33 | 87 | 81 |

[a] Individual WHO–TEF values for risk assessment [11] are based on the conclusions of the World Health Organization meeting in Stockholm, Sweden, 15–18 June 1997.

[b] The concentration of compounds are below DL or QL.

the specific congeners, according to Tables 1 and 2. Respecting the total of the isomers, it can be seen that PLE is essentially identical to Soxhlet method. Respecting congeners, it was possible to clearly find more isomers in the HxCDD/Fs congener group, using PLE extraction.

Individual WHO–TEF values are mentioned in Table 4. Table 4 also shows WHO–TEQ values of different magnitude for two real samples, sediment and forest soil. The sediment sample exhibited low levels of dioxins/furan and Co-PCBs. The majority of the compounds in this sediment are below the detection limit (DL) and quantification limit (QL) of the method. However, the forest soil sample showed considerable amounts of dioxins/furans and Co-PCBs.

According to literature [9,10] the optimal value of dioxins/furans and Co-PCBs for a soil should be below 5 ng WHO–TEQ kg$^{-1}$. Values greater than 100 ng WHO–TEQ kg$^{-1}$ are considered relatively high and reduces the possibility of using the soil, due to contamination and level of toxicity. The possible reason for the high values of this sample could be related with the fact that this specific soil belongs to a place used has a trust for electrical components industry (condensers and other electrical components which use oils).

## 4. Conclusions

As conclusion, PLE, when used at optimal conditions, is a good alternative extraction technique for the simultaneous and quantitative analysis of PCDD/PCDFs and dioxin-like PCBs (Co-PCBs) in solid environmental samples. The average exposure of the European population (8–21 pg/kg body weight) is occasionally higher than the total tolerable weekly intake for dioxins and dioxin-like PCBs [8]. Consequently, there is a driving force from the European Commission to decrease the overall intake. An important tool to achieve this goal is to increase monitoring (Commission Directive 2002/69/EC). PLE methodology, in this work, proved to be a very good alternative, faster and sustainable analytical methodology for the simultaneous determination of PCDDs, PCDFs and Co-PCBs in environmental samples. The method allowed the simultaneous determination of 29 organohalogen compounds in one single extraction, and can be applied to other kind of matrices, e.g. food. Having the best cost-efficiency ratio, may improve the control and provide more information about the occurrence of dioxins and the levels of toxicity and thereby may contribute to increase human health.

## Acknowledgments

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