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Complete List of Authors:	García-Alonso, Susana; CIEMAT, Chemistry Division (Department of Technology) Gómez-López, Luis Vicente; CIEMAT, Chemistry Division (Department of Technology) Pérez-Pastor, Rosa María; CIEMAT, Chemistry Division (Department of Technology)

Optimizing a simple procedure to determine organochlorine compounds in sediment samples: practical considerations

Susana García-Alonso*, Luis Vicente Gómez-López, Rosa María Pérez-Pastor

Eighteen organochlorine compounds were determined in contaminated sediments using small amounts of sample (0.25 g) and solvent (1 mL, dichloromethane). This involved the optimization and evaluation of a quick vortex assisted extraction. Target compounds included pentachlorobenzene, hexachlorocyclohexanes (α, ß, γ), hexachlorobenzene, DDE/DDD/DDT (o,p' and p,p' isomers) and the PCBs nº 28, 52, 101, 118, 138, 153 and 180. All involved assays were accomplished analyzing real contaminated samples, without previous spiking and using gas chromatography with mass detector (GC/MS). As interesting aspects, a light oven drying with subsequent ground and adding purchasable copper to remove sulfur were found to make more affordable the proposed procedure. In addition to describe on development of the analytical procedure, the assessment of result reliability was also included. In detail, the presence of possible bias and intermediate precision were estimated. The absence of bias in the analytical results was comprobed by Youden method. Regarding analytical variability, intermediate precision was estimated from the overall analyses of different real samples. Values were mainly between 6 and 15%, except to DDT isomers, DDE and HCH that ranged from 15 to 30%. The limits of detection (LODs) ranged from 0.1 to 0.7 ng mL^{-1}. This study describes a simple method to determine organochlorines in sediments and can be done with easy in routine laboratories.

Introduction

Environmental implications of organochlorine compounds (OCs) remain being of great interest. Spatial-temporal patterns of these pollutants are and will continue to be necessary due to their persistence and consequences on biota. There is a need for rapid and simple methods for monitoring in polluted areas.

Classical analytical procedures used to determine OCs in solid environmental samples are linked to different drawbacks. Regarding extraction step, they are laborious, expensive, time-consuming and require handling/disposal of large volumes of toxic extraction solvents [1]. If sophisticated and expensive instruments such as pressure solvent extraction (PSE) are demanded, the method is even less attractive for most laboratories. Among conventional extraction methods, agitation solid-liquid extraction has been also shown suitable for extraction of organochlorine compounds (OCs)[2]. These are generally based on the use of relatively large solvent/subsample sizes, although less expensive and simpler devices are necessary. Alternatively, low-cost methods with low environmental impact have been used in recent years [3]. With high potential for miniaturization, matrix solid phase dispersion (MSPD) has been also successfully applied for extraction of a wide range of compounds [4]. These methods include the sample disruption with a solid or dispersant support and then a solid phase extraction for cleanup. However, the use of small volumes/sample sizes without dispersant by a simple vortex assisted extraction (VAE) has been rarely reported. Recently, a study have addressed the determination of different pharmaceuticals in sewage sludge using 2 g of sample and 5 mL of extraction solvent [5]. The authors highlighted the need for grinding until obtain a fine powder, increasing the contact surface and allowing extraction with a reduced solvent volume. This step is crucial to reach reproducible results. Hence, the effect of drying/milling pre-treatment and matrix of sample on analytical results of organic compounds must be examined [6].

Regarding drawbacks linked to chromatographic analysis to determine OCs, factors such as polarity, volatility, moisture and susceptibility to interconversion among isomers for DDT/DDE/DDD family are key points to consider. In this way, solid-liquid extractions which apply high temperatures and/or pressures can cause matrix-enhanced degradation of thermolabile compounds such as p,p'-DDT [2, 7].

The breakdown of thermolabile DDT by the GC inlet must be also checked at regular intervals to control degradation. Matrix enhancement effect is easily caused by active sites in both GC column and mainly liner. Thus, a matrix-enhanced p,p'-DDT degradation at high temperatures in the GC inlet usually entails a

p,p'-DDT underestimation due to the formation of the more thermostable p,p'-DDD [8]. In opposite, a p,p'-DDT overestimation is also possible due to increases of the ratio p,p'-DDT/internal standard, using no labelled internal standards [7]. If these analytical limitations are not well identified and controlled throughout the set of analyses, serious errors and misunderstanding can be masked into the final interpretations on environmental degradation. Testing standards [9] and replacing GC liner can be, if not overcome, a choice to control these matrix effects.

In this paper, the optimization of an analytical method minimizing sample sizes (0.25 g) and solvent consumption (1 mL) to determine eighteen OCs in real contaminated sediments is described. The procedure involves a previous quartering with significantly higher amounts to get a representative sample. For optimization, the effect of drying treatment, type of agitation for extraction and the use of acid activated copper versus non activated copper for sulfur removal were investigated. The paper also includes how the performance was estimated determining intermediate precision and absence of significant constant bias in the results.

Experimental

Reagents and standards

Hexane and acetone for pesticide analysis grade were used for sample preparation from J.T. Baker (Deventer, Holland). Calibration check solutions were supplied by Dr. Ehrenstorfer (GmbH, Augsburg, Germany). Namely,

- Pentachlorobenzene (PeClBzn): 10 ng μL^{-1} in cyclohexane
- Hexachlorobenzene (HxClBzn): 10 ng μL^{-1} in cyclohexane
- Pesticide Mix 5: 2,4'-DDD,4,4'-DDD,2,4'-DDE,4,4'-DDE, 2,4'-DDT,4,4'-DDT, 10 ng μL^{-1} in cyclohexane
- Pesticide Mix 7: alpha-HCH, beta-HCH, gamma-HCH and delta-HCH, 10 ng μL^{-1} in cyclohexane
- PCBs Mix 3: PCB 28, PCB52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180, 10 ng μL^{-1} in acetone

As internal standard, *o*-Terphenyl (Supelco, Bellefonte, USA) was used. Certified reference material CNS391 (CRM) was also used, which contains pesticides and PCBs in a sediment of natural water.

Sample preparation

Different sediments were sampled (about 500 g) from an area of continental waters with previous industrial wastes. Samples were placed in amber glass bottles and kept cool under ice during transportation. Once delivered to laboratory, each sediment sample was thoroughly mixed following the referred to as quartering procedure [10]. Between 100 and 200 g of sample was blended and quartered to form a homogenized stock. To dry sub-samples, a gently oven dried (50 ºC) was initially applied for about 8 hours. Each dried sample was finally grounded in an agate mortar and stored at -5 ºC until analysis.

For testing, several drying treatments of sample before milling were compared. From a selected sediment sample, the following experiments were done:

- Untreated samples: 2 g of wet, as received, sub-samples were direct extracted using hexane/acetone mix. Assays were done in triplicate. Dichloromethane has a very low mixing capacity in the water cavities in untreated sediment matrix, so it was discarded for untreated sample extraction.
- Treated samples: between 100-150 g of subsample were dried and after grinded. Aliquots of 0.25 g of dried/grinded samples were then analyzed in triplicate. For the drying step, four different treatments were independently tested for comparison: a light oven drying (50 ºC), air dry during 24 h (~25 ºC) and freeze at -110 ºC (24 h with a previous frozen at -50 ºC, 24 h). Freeze drying was performed usng a ScanVac Coolsafe Touch 110-4 (from Lavogene A/S, Sweden).

Sample extraction

An aliquot of subsample was weighed into 10 mL closed glass tubes and 1 mL of dichloromethane was subsequently added. The tubes were vortex agitated for short time (30 seconds) using an automatic shaker, Vortex 1 with a touch function (IKA, Staufen, Germany). Extract is separated by a short and gentle centrifugation (2000 rpm, 3 min). One additional extraction is carried out to assure recovery. The final combined extracts were slowly concentrated in a drying step by a stream of nitrogen and changed solvent to hexane (0.5 mL).

As procedure to remove sulfur, about 0.3 g of copper powder (three mini-spatula points) was added to the final extract. Vortex was then applied for at least 30 minutes. The last centrifugation is not necessary because supernatant/copper powder layer is quickly and well separated. A time of 20 minutes can be estimated as necessary for extraction of four subsamples.

Sonication for 20 minutes combined with vortex agitation (US-VAE) was compared against a single VAE. An ultrasonic bath (Ultrasound-H, JP Selecta, Abrera/Barcelona, Spain) with 40 kHz of frequency and 400 W of power was used for sonication extraction.

GC/MS analysis

Chromatographic analyses were done applying experimental conditions included in previous work [11]. Briefly, an Agilent 7890B chromatograph with automatic sampler 7693, coupled to an Agilent 5977A mass spectrometer was employed. The column was a HP-5MS (30 m x 0.25 mm x 0.25 µm) operating at a flow rate of 1 mL min^{-1}. Splitless injected (split valve closed for 40 s) at 200 ºC. liner used was ultra-inert and without glasswool. The oven temperature was programmed from 100 ºC (held for 4 min) to 250 ºC at 8 ºC min^{-1} and then to 290 ºC at 8 ºC min^{-1}. Helium was used as carrier gas at 1 mL min^{-1}. MS detector operated in electronic impact mode and selected ion mode. Seven point calibration curves were prepared with standards, covering the linear concentration range from 1 ng mL^{-1} to 1000 ng mL^{-1}. Analyte concentration has to be into the scope of the calibration range of standards

Results and discussion

Analysis of standards: estimating basic quality parameters

Different standard solutions prepared in absence of matrix sample were analyzed for evaluating the calibration curves.

Calibration parameters for determination were obtained in the range of 1-1000 ng mL^{-1} with regression coefficients above 0.995. A wide linearity range in which chromatographic signal was lineal with concentration level was obtained. This is advantageous to quantify the wide concentration levels of target compounds presents in sediment samples. For a best fit between analytical response/concentration, concentration range of calibration standards to use was assigned according to the analyte response. Hence, analyte concentration had to be in the middle of the concentration range of calibration standards. Regarding repeatability, values of variation coefficient below 5% were deduced from analysis of 4 standard solutions (100 ng mL^{-1}).

Instrumental limits of detection (LOD) and quantification (LOQ) were calculated from analyses of five solutions containing low concentrations. They were assigned as the concentration corresponding to three and ten times the standard deviation of analytical response, respectively. LOD ranged from 0.14 to 0.72 ng mL^{-1} and LOQ corresponded to 0.27-1.4 ng mL^{-1}.

In relation to the strongly temperature-dependence for degradation of DDT isomers during GC/MS analysis, the presence of very small or minimum amounts of sulfur in the final extract was determining. This component in sediment matrix combined with high temperatures in GC-liner greatly accelerates DDT degradation, as follows below. The analysis of standards containing DDT isomers was periodically carried out for comparison of analytical response. In general, GC-liner had to be replaced after a maximum of 75-100 injections.

Analysis of real samples: Optimizing and evaluating the proposed method

Dichloromethane as solvent and vortex as agitation mode for extraction

One of our priority tasks was to remove elemental sulfur in the final extracts. The presence of sulfur has decisive drawbacks, such as the evident formation of active sites to adsorb and/or promote breakdown of thermolabile analytes. Consequently, life of capillary column/GC liner is reduced and reproducibility of results gets orse.
In the first set of experiments, dichloromethane was compared to hexane/acetone mix as extraction solvents manly to try reducing the sulfur co-extraction. The use of acetone as extracting solvent is linked to high levels of co-extracted impurities [12]. Although the chromatograms showed also sulfur band, a clear reduction was observed if compared to those from using hexane/acetone solvent. This is very interesting because it decreases the sulfur load in the extract and therefore the copper amount needed for cleaning. Moreover, extracts were cleaner.
Regarding extraction technique, the enhancement using sonication is attributed to the cavitation bubble collapse in the solvent which improves the mass transfer from solvent/sample interactions. In the case of agitation extraction, the formation of fine droplets during vortex agitation lead to increases interfacial area and facilitates analyte transfer[13]. Considering this, in the second set of experiments sonication for 20 minutes combined with vortex extraction (US-VAE) was compared against a single vortex extraction. In detail, trials were carried out by triplicate using samples of certified reference material (CNS 391) and a sediment sample for comparison of analytical results.

Table 1 –

As can be seen in table 1, similar results were obtained for both the CNS 391 and sediment sample. DDT isomers were better recovered with vortex agitation that those which employed US. These results could be explained on the basis of thermal degradation of DDT during sonication extraction [14, 15]. In fact, increases of bath temperature during ultrasonic process are produced as a result of energy conversion [13]. We choose to accomplish sediment extraction applying vortex agitation and dichloromethane as solvent. The duration of the analysis, laborious process and quantity of needed solvent/subsample are significantly reduced for routine analysis.

Sulfur removal

Different methods for removing elemental sulfur during extraction step or from the final solvent extracts have been reported [16]. Among them, the use of copper powder is an affordable method of treatment, which can be applied in different ways. One of the most recommended is the EPA protocol, which implies the preparation of activated copper after an acid pretreatment [17]. Initially, our experiments were accomplished using copper powder with previous acid pretreatment (10%). Nitric and hydrochloride acids were both tested. After treatment, acid solution was discarded and copper was rinsed with dionized water to remove all traces of acid. Acetone and hexane were sequentially used and then a stream of nitrogen was applied. Attempts were achieved by adding from one to three spatula points of copper powder with acid pretreatment to the extract during vortex/sonication extraction. This procedure was actually laborious and it took a lot of time.
We then set out to carry out attempts by adding copper powder without any cleaning pretreatment. The simplest way would be its addition to the final extract. Method using copper without acid pretreatment gave the best results. No chromatographic peak of sulfur was only obtained when the mix *final extract/copper* was vortex agitated at least 30 minutes. This agrees with Riis et al [18], who also recommend agitation extract combined to purchasable copper, without any pretreatment. The direct use of copper powder without pretreatment is very interesting because greatly simplify the extraction step.

Figure 1 – Analysis of sediment sample with/without copper.

Drying pretreatment

Although some researchers have concluded that maintaining sediment in wet state is considered as the most suitable [3], a preparation step to homogenize, dry and mill the sediment sample is commonly recommended before analysis [10]. Regarding sediments of interest in this

work, the analysis of samples as received (wet) led to values of intermediate precision ranged from 25 up to 50% [11]. Sample heterogeneity such as grain size and contaminant distribution greatly affects reproducibility aspects. Moreover, sediment characteristics such as pH, organic carbon, sulfides, mineralogy will largely govern the migration of contaminants between particles and pore waters in the sediment [19]. However, drying and milling techniques are also linked to some analyte losses and alteration of physical structure of the sample matrix. In consequence, it is not easy to find the appropriate procedure for minimizing changes in sample integrity during its treatment.

Considering simplicity for sample treatment and instrumental availability in the laboratory as determining factors, the effect of a gentle treatment by applying a low oven temperature was investigated. Along with air drying, they are recommended treatments for the determination of most organic pollutants [20]. From a selected sediment sample, the analysis of air, freeze, light oven and no dried subsamples were compared (table 1 ESI, in supplementary material available online at https://doi.org/).

Results are shown in figure 2. As can be seen, mass fraction values from untreated samples were the lowest, while an increased mass fraction of practically all studied organochlorines was observed after air drying. This trend was more evident for PCBs. Freeze and air drying led to similar measured mass fractions.

Figure 2 –

In view of these results, we decided clarify about the significance of these mean differences. For data evaluation one way analysis of variance (ANOVA) and Kruskal Wallace were tested as recommended Beriro et al [6]. Firstly, Levene's test and Bartlett's test were used to verify homogeneity of variance across the data set. When homogeneous variances were found (p-value > 0.05 by either one of the two tests), one way analysis of variance (ANOVA) was then used. to test differences among mean values of each treatment. All means indicated significant differences (p-values < 0.05). Table 2 includes the results obtained for the data set.

Table 2 - –

Secondly, the goal was to find which treatment corresponded to these significant discrepancies. For this, Tukey's Honest Significant Differences (HSD) test was applied relating means of treatments. When non equal variances were found, Kruskal-Wallis test was applied to find differences among medians of treatment. Results for these tests showed which treatments indicated significant differences.

Figure 3 represents significant differences obtained between each pair of drying treatments (at 95% with p-value<0.05, for HSD and Kruskal-Wallis tests). The thickest line marked in some of the bars in figure 3 represents found values with statistical differences to facilitate interpretation of these results. The statistical differences between dried versus no dried samples were the results more noticeable after comparison. Results from light oven dried analysis indicated statistical differences for PeClBzn, PCB52 and p,p'-DDD when compared to freeze and air dried samples. Discrepancies reaching 30% for PeClBzn could be explained based on volatilization losses at 50 ºC. In fact, attempts accomplished by a light oven drying at 37 ºC (12 h) led to increases of PeClBzn values which reaches those measured after freeze and air drying.

Figure 3 –

Optimizing subsample size

In order to obtain reliable results from the analysis of very small size subsamples it is crucial to start from a sample size large enough to obtain a representative matrix. The next mixed, quartered, dried and grinded will allows minimizing subsample size necessary for analysis. This point is even more critical when the matrix of the sample to be analyzed has high heterogeneity characteristics. Such is the case of the type of sediment focused in this work.

To optimize and reduce the subsample size, while maintaining the absence of significant bias in the results, different experiments were done. Namely, results obtained from the analyses of two different masses of the sample deliver the same results [11] . A student's *t* test on the significance of the constant bias was applied:

$$t_i = \frac{\delta / \overline{x}_i}{u(\delta)_{\%}} \quad (1)$$

where δ denotes the constant bias (expressed in ng), $\overline{x}_i$ is the mean mass of quantified chlorinated compound (ng) and $u(\delta)_{\%}$ corresponds to the uncertainty contribution of constant bias (expressed as relative uncertainty).
On the one hand, constant bias was deduced after solving the next equation:

$$\frac{\overline{x}_i - \delta}{\overline{a}_i} = \frac{\overline{x}_j - \delta}{\overline{a}_j} \quad (2)$$

where δ denotes the constant bias (expressed in ng), $\overline{a}_i$ and $\overline{a}_j$ are the mean values of the two masses of the sub-samples for comparison, $\overline{x}_i$ and $\overline{x}_j$ are the mean masses of quantified chlorinated compound (ng).
Solving equation 1, constant bias can be finally calculated.
On the other hand, its uncertainty, $u(\delta)_{\%}$, was then obtained after applying error propagation law :

$$u(\delta)_{rel} = \frac{1}{(\overline{a}_i - \overline{a}_j)} \sqrt{\left(\overline{a}_j \frac{u(\overline{x}_i)}{\overline{x}_i}\right)^2 + \left(\overline{a}_i \frac{u(x_j)}{\overline{x}_j}\right)^2} \quad (3)$$

Constant bias was evaluated by testing sub-samples of 0.1 g, 0.25 g and 0.5 g. Analyses were done in triplicates (table 2 ESI, in supplementary material available online at https://doi.org/). The values of the constant bias (δ), its relative standard uncertainty ($u(\delta)_{rel}$) and the corresponding "t" values ($t_{0.1}$, $t_{0.25}$ and $t_{0.5}$) were calculated and compared to the corresponding tabulated Student statistic. When "t" was below 2.776 (n-2=4, probability α=0.05), no significant constant bias would be found. A value of "t" above indicated significant constant bias. The results were quite acceptable, indicating the robustness of the method in terms of using reduced aliquots of subsample for analysis. The absence of major constant bias associated with the results involves an important advantage. It must be noted that results obtained from analysis of samples as received concluded that subsamples lower than 1 g implied significant constant bias in results, so subsamples higher than 2 g were selected as the best choice to avoid the need for correction final results. [11]. From the proposed procedure, sizes of sub-sample of 0.1 g could be used without obtaining bias in the results. Only DDD isomers showed significant bias associated to results obtained from the analysis of 0.5 g subsamples. This can be explained on the basis of the high levels present in the sediment, which would correspond to having reached the limit of the scope of the proposed method. That is, there would be a need to modify the ratio of sample size versus volume size of extraction solvent.

Intermediate precision of results

The intermediate precision of the analytical results was investigated from the relative standard deviations deduced from the analysis of seven sediment samples and their corresponding mean and standard deviation values (table). They were pooled to obtain an estimate of intermediate precision (rsd_{pooled}). This mean relative standard deviation gives a very close value of the variability of the results because it's due to run to run variation of the overall analytical process. In detail, the study was carried out by applying the following equation:

$$rsd_{\text{pooled}} = \sqrt{\frac{\sum_{i=1}^{7}(3-1) \cdot rsd_{(i)}^2}{\sum_{i=1}^{7}(3-1)}} \quad (4)$$

In general, results of intermediate precision ranged from 6 to 27 %. If this is compared to values of variability deduced in the previous work (25-63%), the pooled relative standard deviation has been reduced by half for most of compounds (table 3 ESI, in supplementary material available online at https://doi.org/). For α/ß HCH and o,p'-

DDE isomers no better precision was obtained (25-30%). This may be explained by lower measured levels and matrix effects that characterize some of the different sediment samples analyzed.

The good homogenization of samples after milling produced, as it was expected, more reproducible measurements. But pursuing small sample sizes/reactive analyzed, it must be emphasized the need to obtain a fine powder after milling a large size of sample as to be representative. In opposite, milling have a destructive effect on the sediment structure which modify the own sediment matrix. Taking into account the great difficulty to determine selected analytes in this kind of so complex matrices, we choose to improve analytical precision against this handling effect.

Comparison with other methods

Key features of developed method and some of the more recently reported analytical methods are summarized and compared in table 3. General information about the type of studied sample, technique of pretreatment for drying and extraction are included.

The light oven for sample drying and vortex agitation extraction include some valuable advantages using significantly short extraction time (<1 min) and reduced solvent/subsample sizes. No similar analytical method has been found in literature for OC determination in soil and sediment samples.

It should be noted that traditional techniques such as Soxhlet are still used for extraction of compounds susceptible to volatilization losses or thermolabile such as some of the OCs of interest. Low recoveries of DDT isomers using Soxhlet as result of degradation during extraction cycles have been reported [2]. Differences on results for DDT isomers were also presented in this paper after comparison with sonication extraction. Likewise, in relation to the effect and need to control temperature during the analytical protocol, to point out the increases of pentachlorobenzene levels obtained after drying treatment of samples at 37 ºC.

Besides, the addition of copper powder after obtaining final extract and without need to be previously acid activated is easier to execute. No similar proposal has been found in literature either. However, this recommendation is in full agreement with those already raised a long time ago by Riis et al. [18].

According to the sample mass analyzed in each study, results on limits of detection (LOD) were in general of similar order.

Table 3 -

Conclusions

This paper proposes an affordable and simple method to determine typical target OCs by GC/MS in contaminated sediments. Among other features, the method is easy to execute using a sample mass of 0.25 g and 1 mL de dichloromethane by a brief vortex agitation. Practical considerations such as sulfur removal and soft temperature oven drying steps are carefully approached.

A quality assessment of analytical results by evaluating bias and intermediate precision from the analysis of routine sediment samples is addressed: intermediate precision of around 10-15% were obtained. These values have been reduced by half if compared with those obtained from analysis of untreated samples.

Finally, results involved in this paper were assessed from analysis of different real samples, with unknown composition and very complex matrix. This is rarely applied.

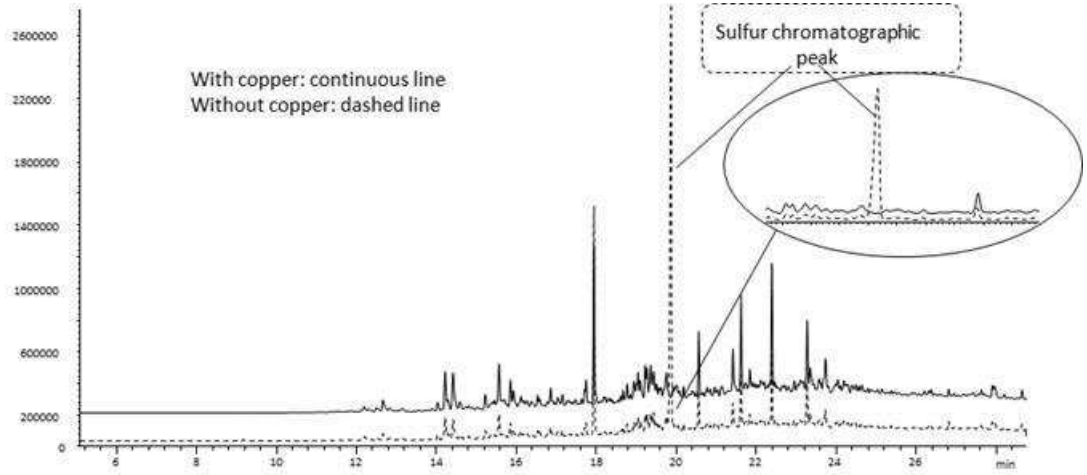
References

1. V. Andreu and Y. Picó, *TrAC Trends Anal. Chem.*, 2004, **23**, 772-789.

2. U. Mülow-Stollin, P. Lehnik-Habrink, S. Kluge, W. Bremser and C. Piechotta, *J. Environ. Protection*, 2017, **8 (06)**, 693-713.
3. D. Muir and E. Sverko, *Anal. Bioanal. Chem.*, 2006, **386**, 769-789.
4. S. A. Barker, *J. Biochem. Biophys. Methods*, 2007, **70**, 151-162.
5. M. B. R. Cerqueira, K. L. Soares, S. S. Caldas and E. G. Primel, *Chemosphere*, 2018, **211**, 875-883.
6. D. J. Beriro, C. H. Vane, M. R. Cave and C. P. Nathanail, *Chemosphere*, 2014, **111**, 396-404.
7. M. Gfrerer and E. Lankmayr, *J. Chromatogr. A*, 2005, **1072**, 117-125.
8. W. T. Foreman and P. M. Gates, *Environmental Science & Technology*, 1997, **31**, 905-910.
9. U. S. E. P. Agency., *available from https://www.epa.gov/sites/production/files/2015-10/documents/method_1699_2007.pdf*, 2007.
10. U. S. E. P. Agency, *SESDPROC-200-R3*, 2014, **Available from https://www.epa.gov/sites/production/.../Sediment-Sampling.pdf**, 1-23.
11. O. Pindado Jiménez, S. García Alonso and R. M. Pérez Pastor, *J. Anal. Methods Chem.*, 2017, **2017**, 13.
12. M. C. Vagi, A. S. Petsas, M. N. Kostopoulou, M. K. Karamanoli and T. D. Lekkas, *Desalination*, 2007, **210**, 146-156.
13. E. Psillakis, *TrAC Trends Anal. Chem.*, 2019, **113**, 332-339.
14. J. Evans, R. H. Kaake, M. Orr and M. Watwood, *J. Soil Contam.*, 1998, **7**, 589-597.
15. F. A. E.-T. Miguel de la Guardia, *John Wiley & Sons,*, 2019.
16. A. Mechlińska, L. Wolska, J. Namieśnik and L. Wolska, *TrAC Trends Anal. Chem.*, 2012, **31**, 129-133.
17. U. S. E. P. Agency, *Available from https://www.epa.gov/sites/production/files/2015-12/.../3500c.pdf*, 1996.
18. V. Riis and W. Babel, *Analyst*, 1999, **124**, 1771-1773.
19. S. L. Simpson and G. E. Batley, *CSIRO Publishing*, 2016, **available from https://www.researchgate.net/.../287218086_Sediment_Quality_A...**
20. R. B. Frank, F. Ulrich, D. M. Ian and G. Alex, *Sediment sampling, sample preparation, grain size corrections, and chemical criteria.*, McGraw Hill Professional, Access Engineering, 2002.
21. I. Haddaoui, O. Mahjoub, B. Mahjoub, A. Boujelben and G. Di Bella, *Chemosphere*, 2016, **146**, 195-205.
22. S. Zheng, B. Chen, X. Qiu, M. Chen, Z. Ma and X. Yu, *Chemosphere*, 2016, **144**, 1177-1192.
23. B. D. Skrbic, V. Marinkovic, I. Antic and A. P. Gegic, *Chemosphere*, 2017, **181**, 101-110.
24. H. Ahmed, M. Negussie and Ä. J. Jan, *J. Environ. Protection*, 2017, **8 (8)**, 867-883.
25. M. Y. Azis, L. Asia, A. Piram, B. Buchari, P. Doumenq and H. Setiyanto, *IOP Conf. Series: Journal of Physics. 4th International Seminar of Mathematics, Science and Computer Science Education*, 2018, **1013**.
26. N. B. Dirbaba, S. Li, H. J. Wu, X. Yan and J. Wang, *Plos One*, 2018, **13**, 20.
27. G. C. Adeyinka, B. Moodley, G. Birungi and P. Ndungu, *Environ. Monit. Assess.*, 2018, **190**, 621.
28. T. Gereslassie, A. Workineh, O. J. Atieno and J. Wang, *Int. J. Environ. Res. Public Health*, 2019, **16**, 18.
29. S. Mitra, S. Corsolini, K. Pozo, O. Audy, S. K. Sarkar and J. K. Biswas, *Chemosphere*, 2019, **221**, 154-165.
30. Y. W. Qiu, H.-L. Qiu, G. Zhang and J. Li, *Chemosphere*, 2019, **217**, 195-203.
31. T. X. Nguyen, B. T. Nguyen, H. T. T. Tran, T. T. Le, T. T. Trinh, T. T. Trinh, M. B. Tu, N. D. T. Cao and H. D. T. Vo, *Environ. Sci. Pollut. Res.*, 2019, **26**, 10756-10766.

Table 1 – US-VAE: sonication combined with vortex extraction, VAE: vortex extraction, Hx-ace: hexane/acetone mix, DCM: dichloromethane. Empty cells correspond to measured levels below detection limits.

	CNS391				Sediment		
	US-VAE		VAE	Certified	US-VAE		VAE
ng g^{-1}	Hx-ace	DCM	DCM		Hx-ace	DCM	DCM
PeClBzn					15±1.8	13.8±0.82	12.4±0.63
α-HCH	41±4.6	38±1.5	37±7.2	37	10±2.4	13±2.7	11±5.4
β-HCH	23±2.5	23±1.0	29±2.2	21	118±32	212±46	379±56
γ-HCH	13±1.5	12±1.0	12±2.6	10			
HxClBzn	41±3.5	36±2.1	34±8.6	37	118±20	147±14	140±14
o,p'-DDE	49±3.5	45±1.7	51.3±0.49	40	64±9.3	48±11	54±10
p,p'-DDE	23±1.5	22±0.60	25±1.2	19	149±12	178±18	204±32
o,p'-DDD	19±2.1	17±0.58	21±1.0	16	358±59	462±16	455±71
p,p'-DDD	11±1.5	11±0.60	17±1.5	14	471±76	542±20	531±89
o,p'-DDT	27±4.0	23±2.0	43±1.4	43	214±30	223±43	227±53
p,p'-DDT	5.3±1.2	5.7±0.55	15±3.2	10	2079±334	2270±701	2440±576
PCB-28	42±2.5	39±2.5	44±2.5	45	164±9.8	174±4.1	172±9.3
PCB-52	93±6.2	90±8.5	79±3.9	65	99±3.4	127±8.5	128±8.6
PCB-101	57±3.8	55±5.5	53±1.6	46	94±4.8	104±1.4	107±9.1
PCB-118	35±3.2	32±1.5	32.4±0.50	24	42±3.6	53±1.3	49±14
PCB-138	57±4.7	53±2.6	51.2±0.25	63	117±16	123±5.0	121±13
PCB-153	39±3.1	36±1.2	36.8±0.45	41	126±11	124±5.6	121±13
PCB-180	52±3.5	47±1.7	54±1.4	55	172±19	131±10	129±13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With copper: continuous line
Without copper: dashed line
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2200000
1800000
1400000
1000000
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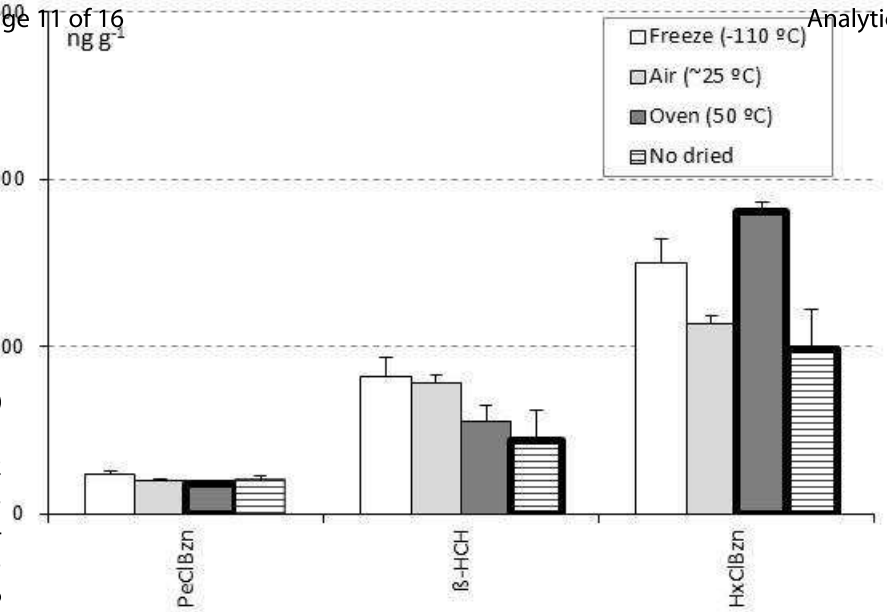
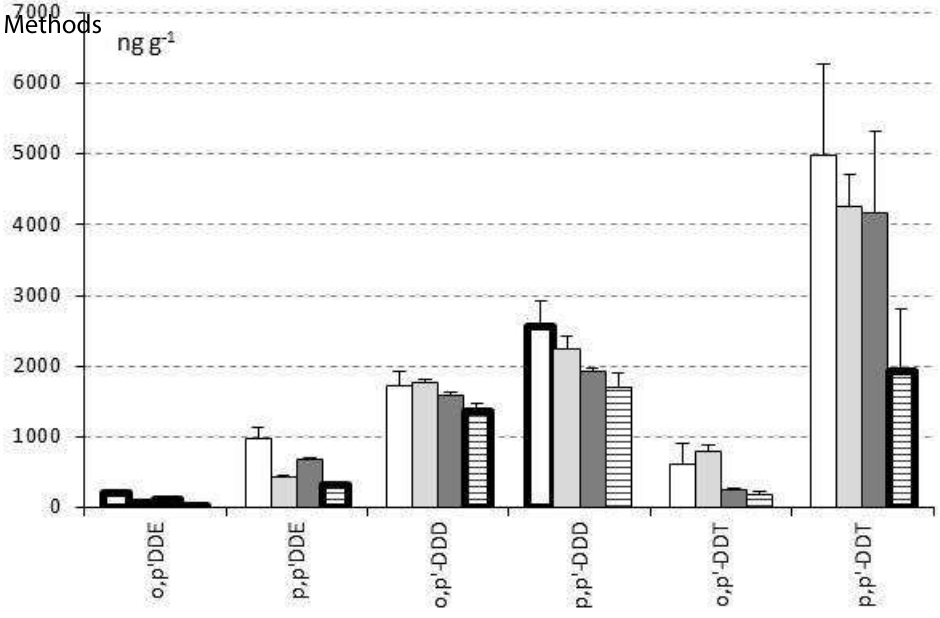
200000
0
6
8
10
12
14
16
18
20
22
24
26
min


ng g-1
Freeze (-110 ºC)
Air (~25 ºC)
Oven (50 ºC)
No dried
3000
500
0
PeClBzn
ß-HCH
HxClBzn

2A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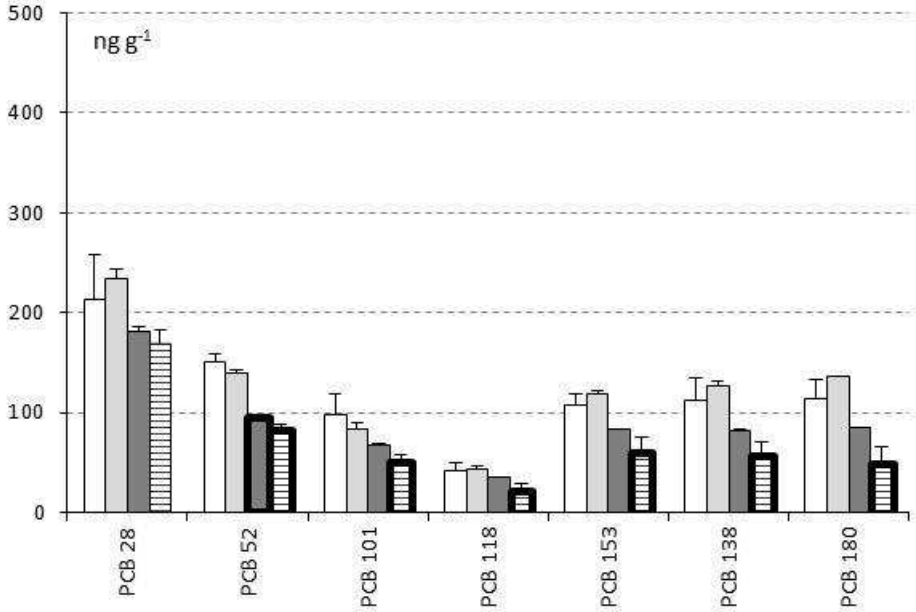
7000
6000
5000
4000
3000
2000
1000
0
o,p'DDE
p,p'DDE
o,p'-DDD
p,p'-DDD
o,p'-DDT
p,p'-DDT

2B


ng g-1
500
400
300
200
100
0
PCB 28
PCB 52
PCB 101
PCB 118
PCB 153
PCB 138
PCB 180

2C

Table 2 – Results obtained from ANOVA and Levene's test for comparison of drying methods. Critical p-value >0.05 is noted in bold and corresponded to no significant differences among means and equal variance across data set.

	Barlett test		**Levene's test**		**ANOVA**	
	F-statistic	**p-Value**	**F-statistic**	**p-Value**	**F-statistic**	**p-Value**
PeClBzn	**4.7**	**0.20**	4.1	0.0495	5.16	0.028
ß-HCH	**2.47**	**0.48**	2.7	**0.12**	7.2	0.012
HxClBzn	**4.71**	**0.19**	5.6	0.023	20	0.0004
o,p'DDE	7.88	0.049	2.7	**0.12**	442	0
p,p'DDE	9.61	0.022	9.4	0.0054		
o,p'-DDD	**4.65**	**0.2**	2	**0.2**	6.1	0.019
p,p'-DDD	**5.28**	**0.15**	2.6	**0.12**	7.7	0.0098
o,p'-DDT	10.53	0.015	10	0.0042		
p,p'-DDT	**1.69**	**0.64**	0.87	**0.5**	5.3	0.026
PCB 28	7.73	0.052	7.3	0.0092		
PCB 52	**3.01**	**0.39**	1.5	**0.28**	109	0
PCB 101	8.24	0.041	4.7	0.035		
PCB 118	**7.09**	**0.069**	5.1	0.03	9.8	0.0047
PCB 153	8.46	0.037	4.8	0.034		
PCB 138	10.1	0.018	4.3	0.045		
PCB 180	15.68	0.0013	6.2	0.017		

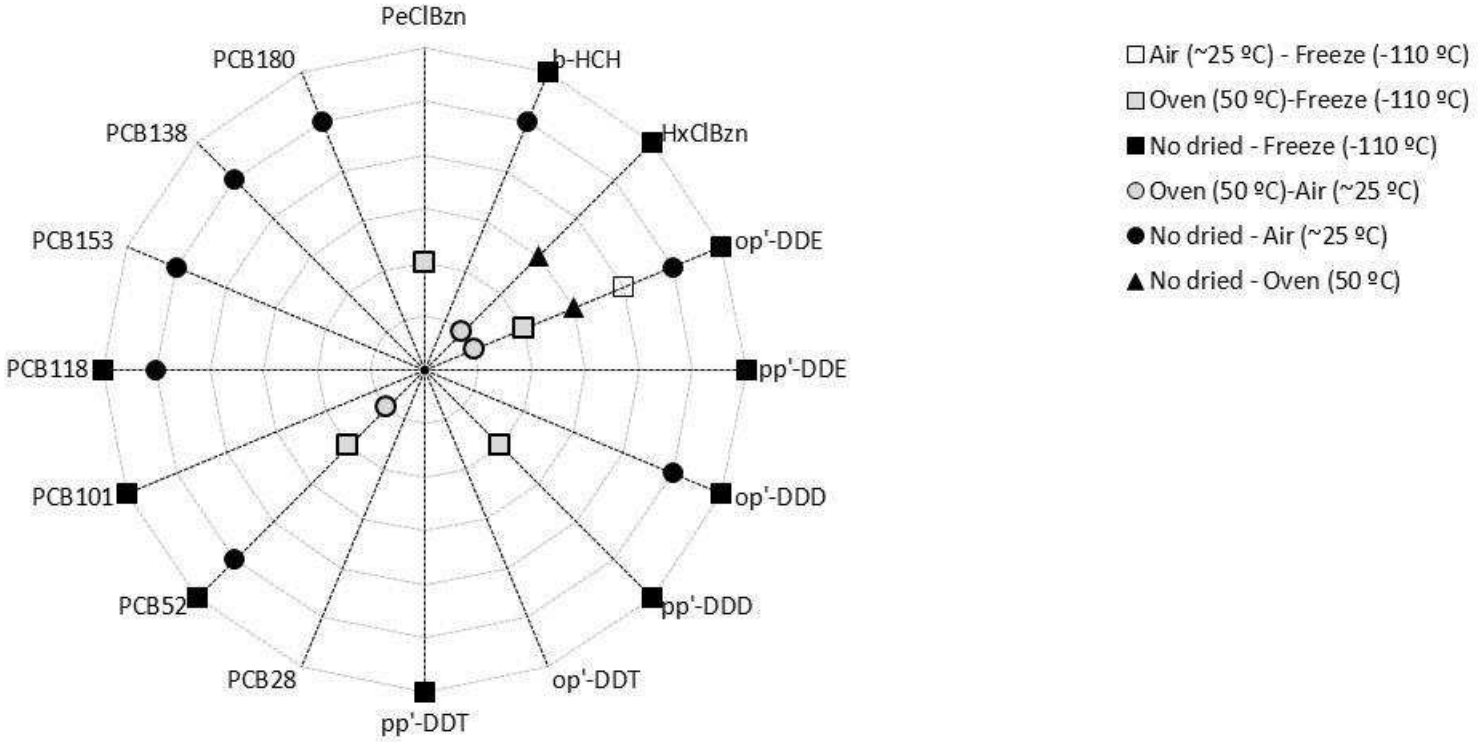

PeClBzn
b-HCH
HxClBzn
op'-DDE
pp'-DDE
op'-DDD
pp'-DDD
op'-DDT
pp'-DDT
PCB28
PCB52
PCB101
PCB118
PCB153
PCB138
PCB180
Air (~25 ºC) - Freeze (-110 ºC)
Oven (50 ºC)-Freeze (-110 ºC)
No dried - Freeze (-110 ºC)
Oven (50 ºC)-Air (~25 ºC)
No dried - Air (~25 ºC)
No dried - Oven (50 ºC)

Table 3 – Comparison with different analytical methods recently published (since 2016) on the determination of organochlorine pesticides by GC/MS in sediments (sed) and soils, analyzing real (R) or spiked (S) samples,. Abbreviates corresponded to: PSE: Pressurized Solvent Extraction; SX:soxhlet; SLE: Solid Liquid Extraction; cln: cleanup; can: adetonitrile; DCM: dichloromethane; Hx/ace: hexane-acetone; HP/ace: n-heptane-acetone; OCPs: organochlorine pesticides; USE: ultrasonic extraction; QQQ/MS: triple quadrupole mass detector; VA-: vortex assisted; MSPD: matrix solid phase dispersion

OCs	**Sample pretreatment**			**Extraction**			**GC**			
Number	**Type of sample**	**Drying**	**Mass (g)**	**Technique**	**Solvent (mL)**	**Copper** During extraction* After extraction**	**Injector (µL;tª)**	**Detector**	**LOD (ng g^{-1})**	**Ref**
34	Soil/R	Air/ ground&sieved	5	PSE	Hx (60)	-	PTV	MS/MS	0.1-2.5	21
10	Sed/R		10	USE	AcN	Activated*	Splitless (2; 250)	ECD/MS	0.006-0.01	22
19	Sed&soil	-	10	SLE/cln	Ace (50)	-	Splitless (1;PTV)	MS		3
11	Sed/R	-	2	VA-USE	Hx/ace (5)	-	Splitless (1;PTV)	MS	9-21	11
24	Soil/R	-	5	PSE	Hx/DCM (33)	-	Splitless (3;250)	MS	0.002-0.018	23
13	Soil/S	Chemical/ ground-sieved	2	PSE	Hp/ace (34)	-	On column (1)	µ-ECD	0.002-3.3	24
17	Sed/S	Freeze (-40 ºC, 48 h)/ground	10	PSE	Hp/ace (33)	HCl activated*	Pulsed splitless	QQQ/MS	0.25-2.5	25
24	Sed/R	Air/sieved	1	MSPD	DCM (15)	HCl activated (12 h)*	Splitless (1;,280)	ECD/MS	0.001-0.3	26
8	Sed/R	Air (5 days)/ sieved	20	USE	DCM (20)	Activated**	Splitless (1;,220)	MS	0.007-0.022	27
8	Soil/R	Freeze (-40 ºC, 48 h)/ground-sieved	1	MSPD	DCM (20)	Activated*	Splitless (1;270)	ECD	0.05	28
20	Sed/R	Oven (50ºC) &chemical	5	SX	DCM	-	Pulsed Splitless	MS	0.01-0.06	29
53	Sed/R	Freeze/ground	30	SX/cln	DCM	Activated*	Splitless (1;,250)	MS/MS	0.001	30
15	Sed/R	Air/chemical	10	SX/cln	Hx/ace (300)	Activated		ECD		31
18	Sed/R	Oven (37ºC, 12 h)/ground	0.25	VA	DCM (1)	Non activated**	Splitless (1;200)	MS	0.27-1.4	This work

Table 1ESI – Mass fractions (ng g^{-1}) obtained from the analyses of a selected sediment applying different drying treatments.

ng g^{-1}	Freeze drying (-110 ºC) & milled			Air drying (~25 ºC) & milled			Light oven drying (50 ºC) & milled			No dry, as received		
PeClbzn	115	110	128	96	100	107	87	91	90	95	93	118
α-HCH	82	111	127	83	66	75	37	34	38	44	40	80
ß-HCH	368	474	390	419	379	379	234	261	331	164	180	323
γ-HCH	12.8	22	24	29	38	35	8	13	6	26	29	40
HxClbzn	794	664	788	556	548	596	874	906	930	426	432	629
o,p'-DDE	205	195	217	60	48	51	109	107	111	31	29	31
p,p'-DDE	1142	864	895	432	400	451	651	659	700	302	317	343
o,p'-DDD	1932	1686	1506	1748	1719	1827	1539	1595	1630	1235	1364	1469
p,p'-DDD	2881	2629	2134	2417	2077	2240	1876	1966	1940	1502	1755	1871
o,p'-DDT	762	790	284	738	741	885	219	266	221	156	198	221
p,p'-DDT	6152	5234	3596	3800	4703	4250	3032	5344	4149	1194	1625	2920
PCB 28	264	193	183	229	225	246	176	181	186	161	164	184
PCB 52	142	152	158	139	137	144	92	93	96	76	81	89
PCB 101	104	115	76	89	84	76	65	67	68	46	50	58
PCB 118	50	38	39	42	40	47	35	35	36	12	25	27
PCB 153	116	94	111	118	114	122	82	83	84	48	55	77
PCB 138	137	93	108	124	123	132	81	82	83	48	53	72
PCB 180	135	99	109	135	135	137	84	84	85	37	43	67

Table 2ESI – Parameters deduced for evaluation of possible constant bias of GC/MS analysis. The measured mass, expressed as ng and "t" statistical corresponded to 0.1, 0.25 and 0.6 g (x_1, x_2 and x_3, respectively). The constant bias is noted as b_{cte} (in ng) and its relative uncertainty contribution is noted as $u(b_{cte})$. A value of "t" noted bold implies significant constant bias

	Sub-sample$_1$	Sub-sample$_2$	Sub-sample$_3$	b_{cte}		$u(b_{cte})$ %		$t(b_{cte})$	
(ng)	$x_{1=0.1}$	$x_{2=0.25}$	$x_{3=0.6}$	x_1-x_2	x_2-x_3	x_1-x_2	x_2-x_3	x_1-x_2	x_2-x_3
Pentachlorobenzene	13±1.0	23±0.41	57±3.1	4.0	3.0	0.15	0.051	2.07	2.57
α-HCH	4.0±1.9	8.0±1.2	22±14	0.34	2.09	0.90	0.54	0.094	0.48
β-HCH	29±3.3	67±13	199±51	4.1	30	0.27	0.38	0.51	1.17
γ-HCH	2.1±0.29	2.9±0.72	7.6±2.3	1.3	0.51	0.34	0.48	1.79	0.36
Hexachlorobenzene	124±12	239±15	557±79	23	5.0	0.19	0.15	0.97	0.14
o,p'-DDE	14±3.4	24±5.5	86±50	4.0	21	0.52	0.58	0.57	1.51
p,p'-DDE	122±38	201±20	548±116	52	55	0.59	0.23	0.72	1.17
o,p'-DDD	342±68	712±19	1191±19	12	**361**	0.38	0.047	0.59	**11**
p,p'-DDD	338±98	635±20	1789±148	73	**214**	0.52	0.081	0.70	**4.1**
o,p'-DDT	29±6.0	50±12	141±499	10	18	0.44	0.57	0.81	0.63
p,p'-DDT	482±188	703±159	1751±12	**285**	68	0.76	0.45	0.78	0.22
PCB-28	24±2.6	51±2.9	121±5.2	0.53	0.23	0.21	0.10	0.10	0.043
PCB-52	13±2.1	26±1.2	64±3.1	1.0	2.1	0.31	0.090	0.26	0.89
PCB-101	8.9±1.2	18±1.5	45±1.8	0.50	1.2	0.26	0.14	0.21	0.45
PCB-118	3.7±0.69	8.8±1.2	22±1.4	0.72	0.84	0.37	0.25	0.52	0.39
PCB-138	10±1.0	22±2.5	55±4.5	0.25	2.8	0.21	0.21	0.12	0.62
PCB-153	10±1.0	22±2.7	57±5.2	0.23	3.4	0.21	0.22	0.10	0.69
PCB-180	8.3±0.48	19±2.5	53±13	0.87	6.6	0.16	0.29	0.66	1.2

Table 3 ESI – Intermediate precision estimated from analysis of sediment samples with different mass fraction levels of target compounds. Empty cells correspond to measured levels below detection limits.

(ng g^{-1})	**sediment 1**	**sediment 2**	**sediment 3**	**sediment 4**	**sediment 5**	**sediment 6**	**sediment 7**	**sediment 8**	**Intermediate precision %**	
									This work	Previous [11]
PeClBzn	15.0±0.93	156±1.9	172±20	201±16	97±5.2	80±2.5	123±18	12.4±0.63	8.5	*43*
α-HCH	5.5±0.34	6.6±1.8	20±5.2	412±101	34±16	27±5.5	145±21	11±5.4	29	31
β-HCH			140±38	896±64	328±85	559±76	346±26	379±56	15	36
γ-HCH	1.85±0.057	1.5±0.31	4±1.4	146±42	15±6.6	11±1.8	47±7.7		30	25
HxclBzn	614±188	2656±33	711±112	896±83	964±128	388±15	1032±143	140±14	15	39
o,p'-DDE	24±1.9	21.8±0.31	646±89	68±10	145±81	250±8.1	193±50	54±10	24	23
p,p'-DDE	218±5.6	155±8.1	1221±91	973±65	938±204	1461±76	1150±110	204±32	11	24
o,p'-DDD	237±11	273±5.3	1064±174	1465±113	1864±219	1915±219	1875±146	455±71	10	23
p,p'-DDD	382±16	482±11	1715±124	1969±199	2634±535	2394±100	2731±278	531±89	11	36
o,p'-DDT	78±23	63.5±0.50	217±36	182±33	315±137	223±14	417±152	227±53	27	*54*
p,p'-DDT	487±52	467±102	2267±159	2851±379	3429±1216	3054±272	6822±3113	2440±576	26	*63*
PCB-28	15.2±0.39	35±2.6	153±11	192±7.1	205±11	189±3.1	241±22	172±9.3	6.0	27
PCB-52	10.7±0.25	16.4±0.21	60±1.9	73±6.4	104±7.4	98±6.2	106±11	128±8.6	6.9	24
PCB-101	12.5±0.51	18.1±0.57	58±2.7	85±21	74±4.9	66±4.6	78±3.9	107±9.1	10	30
PCB-118	5.87±0.052	8.05±0.035	27±2.1	85±21	74±4.9	30±2.7	43±2.2	49±14	14	24
PCB-138	20.2±0.48	29.5±0.67	87±10	91±8.4	92±11	85±13	107±5.9	121±13	9.6	37
PCB-153	20.9±0.34	28±1.2	88±6.5	92±8.5	97±13	81±12	114±2.7	121±13	8.8	32
PCB-180	18.8±0.23	38±3.4	108±11	86±12	85±22	79±18	115±3.7	129±13	14	33