

Efficiency Evaluation of Extraction Methods for Analysis of OCPs and PCBs in Soils of Varying TOC

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Abstract

Organochlorine pesticides and polychlorinated biphenyls are toxic, carcinogenic, and have a high potential for bioaccumulation. Due to their stability, they are still considered an environmental problem even though the use of most of them has been phased out several decades ago. Soil is a matrix which can retain these contaminants to a great extent. This ability is often associated with the total organic carbon content (TOC). In order to judge the pollution status of soil and to make monitoring data more easily comparable a simple, yet robust extraction method is needed. Agitation solid-liquid-extraction is well suited for this purpose. However, the influence of TOC on the analyte recovery has to be known. For the presented study, 12 organochlorine pesticides and 7 polychlorinated biphenyls were spiked into four model soils with organic carbon contents between 1.6% - 13.3%. The matrices were extracted using solid-liquid extraction between 45 minutes and 16 hours. For comparison, all soils were also extracted using pressurised liquid extraction and Soxhlet extraction. After clean-up the extracts were measured using a gas chromatography-mass spectrometry (GC-MS) system. Statistical analysis of the results implied that the TOC content of the soils did not have significant influence on the extraction efficiency. A longer solid-liquid extraction time did not necessarily increase analyte recovery: Extraction for one hour resulted in 88% recovery while 16 hour extraction led to 89%. Thus, the efficiency of all the methods was comparable for all model soils. Additional investigations regarding GC liner performance highlighted the need for isotopically labelled standards during the analysis of thermolabile pesticides.

Keywords

POP, Soil Organic Matter, Solid-Liquid Extraction, Pressurised Liquid Extraction

1. Introduction

Persistent organic pollutants (POPs) are a group of chemicals which all share a resistance towards degradation by hydrolysis or photolysis, as well as a potential for long-range transport and bioaccumulation. Additionally, all of them pose a threat towards human health as they are toxic, carcinogenic, or act as endocrine disruptors [1]. These features render these substances chemicals of long-term global concern. As they fulfill all of the criteria mentioned [2] [3] [4], the United Nations Environment Programme (UNEP) strives to fully eliminate several organochlorine pesticides (OCPs) and the polychlorinated biphenyls (PCBs), restrict their use, or terminate their unintentional production. The compounds concerned are listed in the Annexes of the Stockholm Convention which entered into force in 2004. In order to confirm whether measures which are taken to lower the global pollutant concentration are effective a global monitoring plan (GMP) was implemented by the UNEP in 2007 [5]. Since soil has a high potential to retain non-polar organic xenobiotics like OCPs and PCBs it is one of the matrices monitored under the GMP. Because of the diverse functional groups present in humic substances, contaminants are bound to soil organics by various mechanisms [6] [7]. The fraction of pollutant bound to organic matter which cannot be extracted using a certain technique is referred to as the non-extractable residue (NER) [8]. There has been some discussion as to whether NER are of actual importance for monitoring, since bound contaminants are not readily bioavailable and mobile [9]. However, it has been shown that xenobiotics may be re-released from NER [10] [11] [12]. This implies that for an assessment of the pollution status of a certain soil a possible future liberation of contaminants also needs to be taken into account. Hence, an exhaustive extraction is preferred for pollutant monitoring. It has been suggested that the potential for formation of NER is governed by the total organic carbon (TOC) content of soils [13]. If this is the case, it might be helpful to use different extraction methods or to review analyte concentrations depending on the TOC content of the soil in question. Today, the most commonly used soil extraction techniques for subsequent analysis of OCPs and PCBs are pressurised liquid extraction (PLE), Soxhlet extraction, and agitation solid-liquid-extraction (SLE). The laboratories assigned to the GMP are free in their choice of method [5]. As SLE does not require costly and sophisticated equipment, it is often used in commercial laboratories. The dependence of its extraction efficiency on soil TOC content has however not been studied in detail. Additionally, there are no studies available which compare the extraction efficiency of SLE with more intense methods under the influence of varying TOC contents. However, an understanding of the comparability of extraction methods is required for the interpretation and comparison of monitoring data. Due to the complexity of the analytical procedure (extraction, clean up, measurement) and the specific nature of the analytes the determination of OCPs and PCBs is related with measurement uncertainties of 20% - 50% [14]. This makes a meaningful comparison exceedingly difficult. To overcome this problem the use of model soils for comparative studies is advisable as they offer sev-

eral advantages over naturally contaminated soil. First of all, when only two characterised raw materials for production of several model soils are used the number of degrees of freedom is greatly reduced. Additionally, a well characterised, commercially available reference soil can be used. Naturally contaminated soils which adhere to certain criteria are not easily obtained. The most dominant influencing factor which remains is the total organic carbon (TOC) content of the model soils. Besides, this has the additional benefit that the nature of the humic fraction stays the same for all model soils. Finally, when uncontaminated model soils are spiked, the spiked concentration is known and can be the base of all analyte recovery calculations. This way the absolute percentage of extracted analyte can be evaluated. If instead a reference method was used to determine what 100% recovery signifies, the study design would be more prone to overlook actual over- and underestimations.

The project presented in this paper aimed to compare the performance of SLE of varying duration to that of PLE and Soxhlet extraction in soils of differing TOC contents. Model soils prepared from a reference soil and a compost were spiked with 12 OCPs and 7 PCBs which were selected as representatives for the whole group of contaminants. The aspects taken into account for the choice of analytes were their ongoing use or production [15] [16], their presence in European soils [17], and the existence of well-established detection methods using gas chromatography [18].

2. Materials and Methods

2.1. Chemicals and Reagents

All solutions were prepared gravimetrically. For 12-point-calibration, appropriate volumes of Pesticide-Mix 17 ($10 \mu\text{g}\cdot\text{ml}^{-1}$ in cyclohexane) and PCB Mix 3 ($100 \mu\text{g}\cdot\text{ml}^{-1}$ in isooctane) were diluted using cyclohexane. As internal standard a mixture of PCB 30 and PCB 209 ($25.43 \mu\text{g}\cdot\text{g}^{-1}$ and $25.23 \mu\text{g}\cdot\text{g}^{-1}$ in cyclohexane) and as injection standard a solution of PCB 198 ($1.98 \mu\text{g}\cdot\text{g}^{-1}$ in cyclohexane) were used. For extraction, acetone and cyclohexane (picograde) were employed. All of the above were purchased from LGC Standards GmbH (Wesel, Germany). For testing the influence of the GC liner a mixture of ^{13}C -4,4'-DDT, ^{13}C 4,4' DDD, ^{13}C -4,4' DDE, ^{13}C - α -HCH, ^{13}C -HCB, and ^{13}C -dieldrin ($2 \mu\text{g}\cdot\text{mL}^{-1}$ each, in cyclohexane) prepared from solutions purchased from Cambridge Isotope Laboratories (Tewksbury, Massachusetts, USA) were used.

2.2. Preparation of Model Soils

Model soils were generated from compost (TOC = 16.1%, finished compost from green waste, dry fermentation), spiked com-post, and RefeSol 01 A [19] (TOC = 0.9%, silty sand) (Table 1). The raw materials were proven to be free of target analytes. TOC contents of the model soils were adjusted to represent the most common soil types in Germany [20]. The model soils were spiked to contamination levels derived from the Federal Soil Protection and Contaminated Sites Ordinance [21] using the chemicals compiled in Table 2.

Table 1. Components of model soils and resulting TOC.

m (spiked compost)/g	m (RefeSol 01-A)/g	m (compost)/g	TOC/% (measured value)
50	2274	76	1.6 ± 0.2
50	1654	696	5.0 ± 0.5
50	910	1440	9.2 ± 0.5
50	166	2184	13.3 ± 0.4

Table 2. Chemicals used during the study and spike level in the resulting model soils.

chemical	manufacturer	purity	spike level/ (mg/kg)	assigned analyte group
2,4'-DDT	LGC	99.8%	7.07	
4,4'-DDD	LGC	99.0%	8.38	
4,4'-DDE	Fluka	Pestanal	5.38	DDX
4,4'-DDT	Riedel de Haen	99.0%	10.28	
methoxychlor	Fluka	Pestanal	4.91	
aldrin	LGC	97.0%	3.17	
dieldrin	LGC	98.4%	2.54	
α -endosulfan (α -ES)	Riedel de Haen	99.0%	6.40	
hexachlorobenzene (HCB)	Riedel de Haen	99.0%	5.89	
α -hexachlorocyclohexane (α -HCH)	Riedel de Haen	98.0%	2.60	
β -hexachlorocyclohexane (β -HCH)	Riedel de Haen	98.4%	1.74	HCH
γ -hexachlorocyclohexane (γ -HCH)	LGC	99.9%	3.98	
PCB 28	LGC	99.0%	0.28	
PCB 52	LGC	99.5%	0.23	
PCB 101	LGC	99.0%	0.42	
PCB 118	LGC	99.5%	0.38	PCB
PCB 138	LGC	98.5%	0.24	
PCB 153	LGC	99.0%	0.17	
PCB 180	LGC	99.0%	0.27	

2.3. Spiking

OCPs and PCBs were dissolved in acetone (100 ml) and added to a slurry of compost (200 g, ≤ 2 mm) and acetone. The mixture was covered with aluminium foil and left to evaporate at room temperature for two weeks.

The spiked compost was divided into four parts of equal weight and blended with different amounts of unspiked compost and RefeSol 01 A to yield model soils of different total organic carbon content and the same OCP and PCB content (Table 1).

2.4. Total Organic Carbon

All measurements were performed by P. Hoelzmann (Freie Universität Berlin)

according to the method published by Vogel *et al.* [22]. The soil TOC content (Table 1) was determined by subtraction of total inorganic carbon from total carbon of the respective soil. In brief, the total carbon content of the samples was determined using combustion with pure oxygen at 950 °C and quantification of evolving carbon dioxide by infrared technology. For total inorganic carbon evaluation, samples were mixed with hot phosphoric acid and produced carbon dioxide was detected.

2.5. Material Homogeneity

The soils were mixed with a drum hoop mixer (ELTE 650/RGM, J. Engelsmann AG, Ludwigshafen, Germany) for 24 h and homogenised using the cross-riffling procedure (sample divider PT100, Retsch Technology GmbH, Haan, Germany). Of the resulting 56 bottles (content around 42 g soil each), four were chosen for inter- and intra-bottle homogeneity testing. Every bottle was analysed in triplicate using solid-liquid-extraction (SLE) in accordance with DIN 10382 (see section 2.7) and detection by GC-MS. Homogeneity was assessed using ANOVA.

2.6. Stability

During the project, stability of the materials was checked by analysing a control sample every week. For this purpose, 10 g of the soil with 9.2% TOC were extracted in accordance with DIN ISO 10382 (Table 3). The median recovery after 50 days was 93.9% and thus, materials were judged stable during the period of investigation.

2.7. Analysis

2.7.1. Extraction

PLE, Soxhlet extraction (Sox), as well as variations of SLE were evaluated. All glassware was rinsed with cyclohexane and acetone before experiments. For every analysis, 10 g of soil spiked with 500 µl of internal standard solution (concentration see 2.1) prior to extraction were used. Extraction parameters are summarised in Table 3.

Table 3. Overview of applied extraction methods.

Method	Solvent	Parameters
Pressurised liquid extraction (PLE)	acetone/cyclohexane 2/1 v/v	2 cycles, 100 °C, 140 bar, 10 min stationary phase
Soxhlet extraction (Sox)	acetone/cyclohexane 1/1 v/v (100 ml)	6 h
DIN 10382 (SLE)	1 x acetone (50 ml, 15 min), 2 x cyclohexane (50 ml, 15 min)	agitation at 300 min ⁻¹
Variants of SLE	1 x acetone (50 ml, 15 min), 1 x cyclohexane (50 ml), 1 x rinse with cyclohexane (50 ml)	length of second step varied between 1, 3, 6, or 16 h, agitation at 300 min ⁻¹

2.7.2. Pressurised Liquid Extraction (PLE)

A soil sample was weighed into a 22 ml PLE-cell and internal standard was added. Subsequently, the sample was extracted according to the procedure noted in **Table 3** using a Dionex ASE 200 (Thermo Scientific, Sunnyvale, CA, USA). The following procedure was carried out for all samples, regardless of the applied extraction technique: Obtained extracts were washed with deionised water (2×400 ml), dried over sodium sulfate, and concentrated to 5 ml using a Turbo Vap II (Biotage Concentration Workstation, Uppsala, Sweden). An aliquot of 100 μ l underwent the clean-up procedure.

2.7.3. Soxhlet Extraction (Sox)

Internal standard was added to a soil sample weighed into an extraction thimble (MN645, 33×80 mm id_{xh}, Macherey-Nagel GmbH & Co. KG, Düren, Germany). After placing the thimble in a Soxhlet apparatus the solvent mixture was added to the equipped 250 ml round-bottom flask. The solvent was then heated to reflux. Extraction parameters can be found in **Table 3**. After extraction was completed the obtained extract was treated as described for PLE.

2.7.4. Solid-Liquid Extraction According to DIN ISO 10382

A soil sample, internal standard, and acetone (50 ml) were put into a 250 ml Erlenmeyer flask. After 15 min of agitation (300 min^{-1} , horizontal shaker HS501 digital, IKA Werke GmbH & Co. KG, Staufen, Germany) a portion of cyclohexane (50 ml) was added. Following an extraction period of another 15 min, the supernatant was decanted into a 1 L separating funnel. To the soil cyclohexane (50 ml) was added and it was extracted again for 15 min. Finally, the organic extracts were combined and treated further as explained for PLE.

2.7.5. SLE Variants

A soil sample was weighed into a 250 ml Erlenmeyer flask and internal standard as well as acetone (50 ml) were added. After agitation for 30 min cyclohexane (50 ml) was added and the mixture was extracted for 1, 3, 6 or 16 hours (**Table 3**). Subsequently, the supernatant was decanted into a 1 L separating funnel and the extract was washed as mentioned for PLE.

2.7.6. Clean-Up Procedure

Prior to use, aluminium oxide (neutral, activity Super I, J. T. Baker, Avantor Performance Materials B. V., Deventer, The Netherlands) was heated to 150°C for five hours and left to cool in a desiccator. Subsequently, the dried aluminium oxide was deactivated using 10 wt % water. After storing in the absence of air for one day it was ready to use.

For solid phase extraction clean-up, 2 g of deactivated aluminium oxide were weighed into a 6-ml glass cartridge equipped with a polyethylene frit. An aliquot of the extract was added to the dry aluminium oxide and eluted with 20 ml of cyclohexane. The cleaned extract was concentrated to 1 ml under nitrogen and transferred into a GC-vial. Prior to measurement, 100 μ l of injection standard (PCB 198) were added.

2.7.7. GC-MS Measurements

GC-MS analysis was completed using an Agilent GC 6890, MSD 5973N (Agilent Technologies, Waldbronn, Germany) with a HT 8 column (50 m, 0.22 mm i.d., 0.25 μm f.t.) (SGE Analytical Science, Ringwood, Australia). The carrier gas was helium (He 5.0) at a flowrate of 1 ml/min. The injector was split/splitless, 1 μl of sample was injected with a split flow 20 ml/min at 1 min. The oven was programmed with an initial hold of 1 min at 50 °C, followed by a ramp of 50 °C/min to 168 °C, a second ramp of 4 °C/min to 310 °C, and a final hold time of 15 min. The mass spectrometer parameters were EI 70 eV, selected ion mode (SIM), with a solvent delay of 6 minutes.

Instrument	Agilent GC 6890, MSD 5973N (Agilent Technologies, Waldbronn, Germany)
Carrier Gas	He 5.0, 1 ml/min
MSD Parameters	EI 70 eV, selected ion mode (SIM), solvent delay 6 min
Injection	split/splitless injector, 1 μl , split flow 20 ml/min @ 1 min
Liner	glass liner, baffled and deactivated (Gerstel GmbH & Co. KG, Mühlheim an der Ruhr, Germany)
Column	HT 8, 50 m, 0.22 mm inner diameter, 0.25 μm film thickness (SGE Analytical Science, Ringwood, Australia)
Oven Programme	1 min at 50 °C, with 50 °C/min to 168 °C, with 4 °C/min to 310 °C, 15 min at 310 °C
PTV	Gerstel KAS 4 (Gerstel GmbH & Co. KG, Mühlheim an der Ruhr, Germany)

2.8. Quality Assurance

Prior to the start of the extractions of the model soils, a certified reference material (ERM-007a) was analysed in accordance with DIN ISO 10382 to ensure adequate operation of methodology.

All extractions were carried out in quadruplicate. One control sample was analysed every week (section 2.2). The average standard deviation of compound concentrations in the control samples was 13.5% while the same value in the homogeneity studies was 8.1%.

The initial calibration used 12 points in the linear working range (0.01 - 5 μg). Two points were recalibrated during every measurement sequence. The measurement system performance was checked by evaluation of the area of the signal of PCB 198 (injection standard). The GC-liners were replaced in between runs or after a maximum of 100 injections. A blank value (solvent only) was measured after every sample run to ensure that carry-over was not significant.

2.9. Statistics

Normality, skewness, and kurtosis tests were run on the data. Outliers were identified using Grubbs' test. These data were informational only and outliers have been retained as a characteristic for the soil type or the method used. Basic

statistic values including mean, median, standard deviation of the mean, and median absolute deviation as the dispersion estimate for the median were calculated. Hierarchical cluster analysis versus the extraction method was carried out. Euclidian distances and Ward linkage were used.

The above was carried out for any analyte under consideration separately, for groups of chemically similar behaving analytes, and all results of the study (see correspondingly sections 3.1 to 3.3 of this paper). As long as different analyte levels had to be combined or compared, statistical analysis referred to normalised values, *i.e.* recovery rates obtained from normalisation against the spiked values.

Recovery rates were calculated using measurement results and the gravimetric data for spiking, treated as above, and displayed in graphs as shown (for the low-level TOC) under **Figure 2**.

Cluster analysis aimed at defining the best extraction procedure (assessed by the recovery rate) and included all analytes not (overwhelmingly) restricted (recoveries above 100% or clearly not normal data distribution). For the graph shown under **Figure 4**, analytes showing these peculiarities have been excluded, namely PCB 28, 52, 138; dieldrin, and methoxychlor.

3. Results and Discussion

In order to perform the data analysis in an extensive, yet comprehensible way a simplification approach was used. At first, the recovery of all analyte groups was evaluated separately in each soil and for each extraction method. As a next step, the results were judged independently of the different soil matrices. Finally, the data were reviewed for all analytes and matrices combined.

3.1. Analyte Group Specific Evaluation

Since the spiked PCBs vary only slightly in their structure, they were expected to behave quite similarly during extraction and clean-up. The OCPs however are a much more diverse group. They differ greatly in polarity, stability, and volatility [24]. Hence, uniform extraction behaviour was not expected and an evaluation focussing on the different substance classes was thought to be more helpful.

3.1.1. Dependence on Soil Type

Several studies have confirmed the assumption that the soil TOC content has a major influence on the extractability of organic pollutants [23] [25] [26]. Therefore it was expected that the analyte recoveries from the model soil with 1.6% TOC would surpass those from the soil with 13.3% TOC, independently of the employed extraction method. **Figure 1(a)** shows the recovery of HCB from the different soils. The extraction methods which were used are represented as grey columns which are grouped in such a way as to illustrate their affiliation to a certain model soil. The recoveries for HCB lie between ($62\% \pm 12\%$) (1.6% TOC, 6 h agitation SLE) and ($107\% \pm 7\%$) (5.0% TOC, PLE). Concerning pesticide analysis, these values are generally considered acceptable [14] [27]. The highest mean recovery values are achieved for the soils with 5.0% und 9.2% TOC. How-

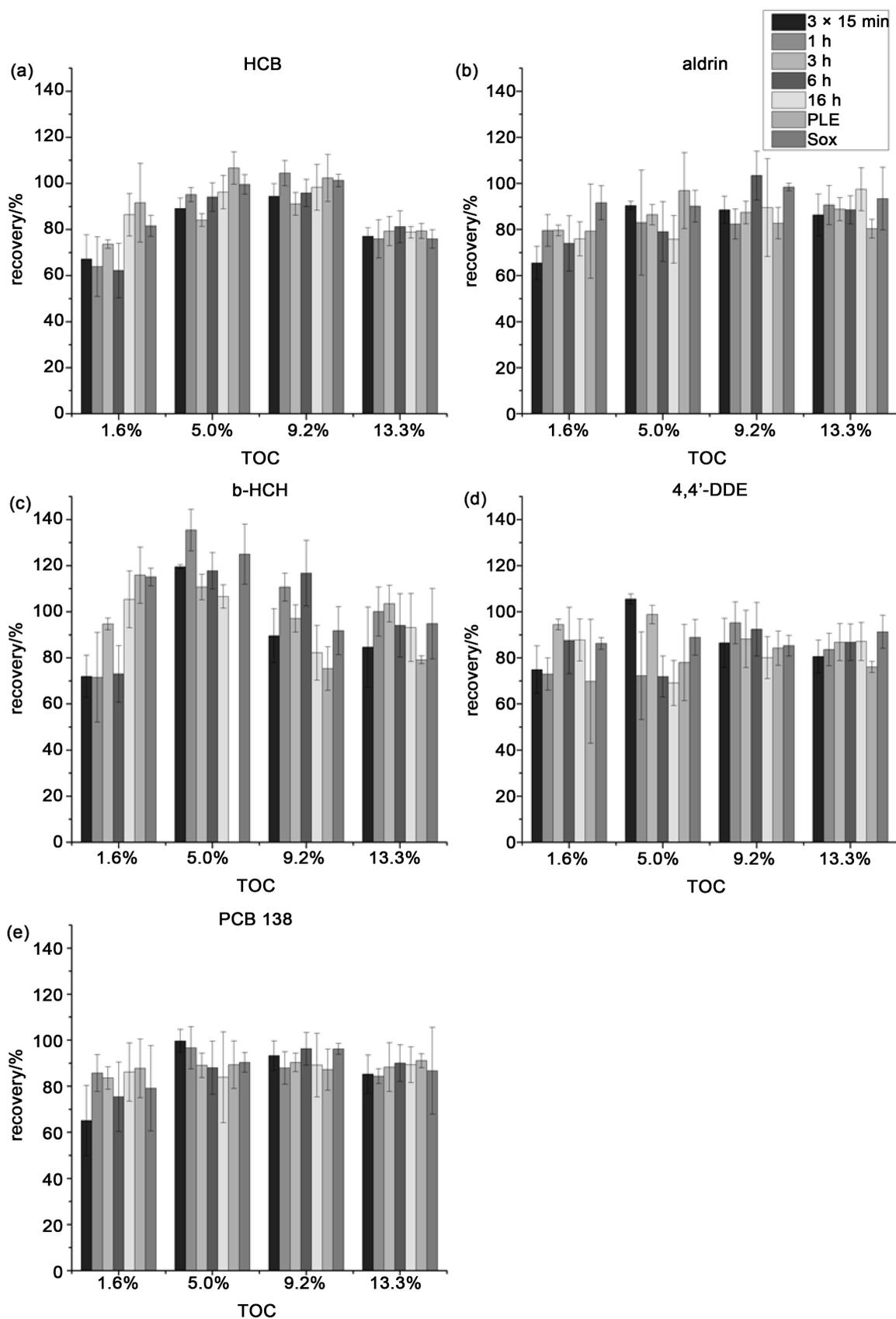


Figure 1. Recovery of the analytes (a) HCB, (b) aldrin, (c) β -HCH, (d) 4,4'-DDE, and (e) PCB 138 in % of spiked concentration. The error bars indicate the standard deviation ($n = 4$).

ever, the mean values and standard deviations calculated for each separate extraction method are most similar for the soil of highest TOC and they differ the most for the soil with low TOC. In the latter case prolonged extraction time and more drastic conditions (PLE, Soxhlet extraction) lead to higher HCB recovery. Nevertheless, none of these variations are statistically significant and no general rule can be deduced. Of all the analytes which were evaluated the standard deviations are always lowest for HCB as it can be very well measured using a GC MS system. This is also the reason why HCB is sold as a compound on which to judge system performance.

Concerning the analyte aldrin (**Figure 1(b)**), the results are fairly similar. Overall, a minimum recovery of ($65\% \pm 7\%$) (1.6% TOC, 3×15 min agitation SLE) and maximum of ($103\% \pm 11\%$) (9.2% TOC, 6 h agitation SLE) were achieved. The mean extraction efficiencies do however not differ as much as for HCB. Regardless, the differences between extraction methods are also not statistically significant. In the case of aldrin it has to be noted that it is known to degrade into its epoxy analogue dieldrin very quickly [28] [29]. As a comparison with the values obtained for dieldrin recovery showed (data not displayed) it is possible that part of the aldrin has already been converted. This is also supported by comparing median values of all recoveries for aldrin and dieldrin. The median of aldrin (87%) is lower than the median for Dieldrin (107%).

In the case of the HCH group (**Figure 1(c)**) findings do not differ much from those described for the other analytes. It is however noticeable that the standard deviations are higher (up to 19%) and the recoveries are the lowest in case of the model soil with 1.6% TOC content (compare HCB). For this matrix slightly better recoveries are achieved as well when the SLE time is longer or a more intense method like Soxhlet extraction or PLE is used.

As a representative of the group of DDT, its metabolites, and related compounds (henceforth called DDX), the graph for 4,4' DDE is shown (**Figure 1(d)**). Recovery rates range from ($69\% \pm 10\%$) (5.0%, 16 h agitation SLE) to ($106\% \pm 2\%$) (5.0% TOC, 3×15 min agitation SLE). A clear dependency of recovery on soil type cannot be determined. 4,4' DDE is the most stable of the DDT metabolites. For the other DDX, high overestimations were found in some cases (>140%, data not considered).

This instance is illustrated in **Figure 2** in which the recoveries for all analytes in the low-level TOC model soil can be found. Concerning the 3×15 minute and 1 hour SLE, the recovery values of most analytes are below 1. This is the generally expected case, as the total analyte content may not be available for extraction due to formation of NER. With a longer extraction period (16 hours) or the use of Soxhlet extraction the apparent recovery of the DDX increases drastically to levels above 100%. This increase is attributed to analyte decomposition [30] and matrix enhancement effects [31] in the GC liner. This is reasonable as a prolonged extraction time or the use of an intense extraction method like Soxhlet also promotes the extraction of unwanted matrix. This matter is further discussed in Section 3.3.

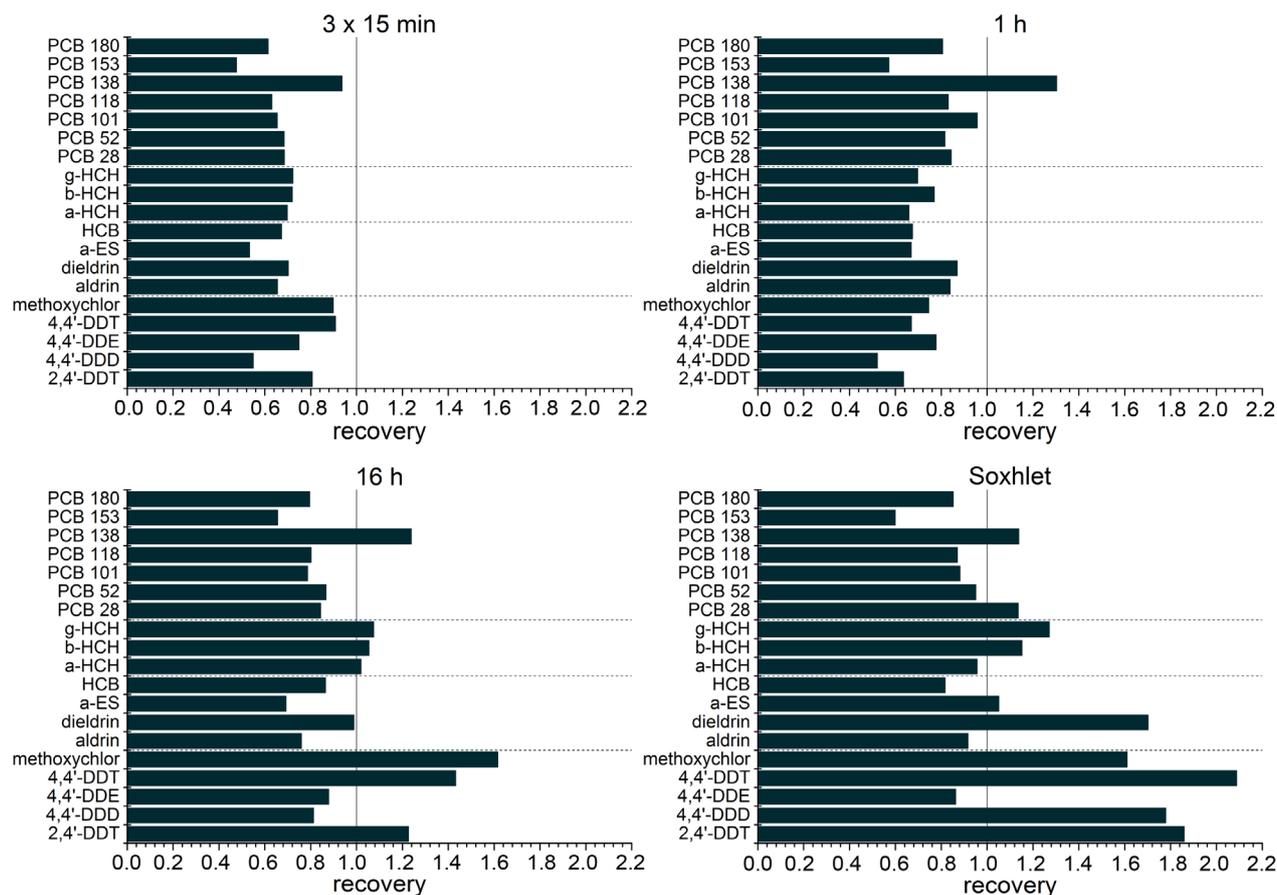


Figure 2. Recoveries as assessed for the measurands under consideration in the low-level TOC soil using SLE with 3×15 min, 1 h, 16 h and Soxhlet extraction. All “explainable” values are below the unity line (recovery = 1), all unexpected above.

Judging from the data obtained during this study PCBs do not seem to be a point of concern during SLE from soils. The data for PCB 138 are shown in **Figure 1(e)**. A connection between longer agitation times and higher recoveries cannot be found in this case.

Considering all the results, it can be concluded that the analyte recovery is not significantly dependent on the TOC content of the model soils used in this study. No relationship can be observed. Of course, fluctuations in recovery cannot solely be attributed to this soil property [32]. Regardless, it was unexpected how little impact the TOC content seemed to have. It has been discussed before that the type of organic matter which makes up the TOC content has a greater impact on NER formation and extractability of pollutants [33]. Concerning OCPs and PCBs especially the aromaticity of soil organic matter should have an influence on analyte recovery [34]. A detailed characterisation of the soil organic matter in the model soils could lead to some insight.

3.1.2. Dependence on Extraction Method

The first approach towards data analysis showed that analyte recovery is in general not dependent on the model soil TOC. Therefore, to find out if the extraction time and method have an effect on the substance recovery the data were

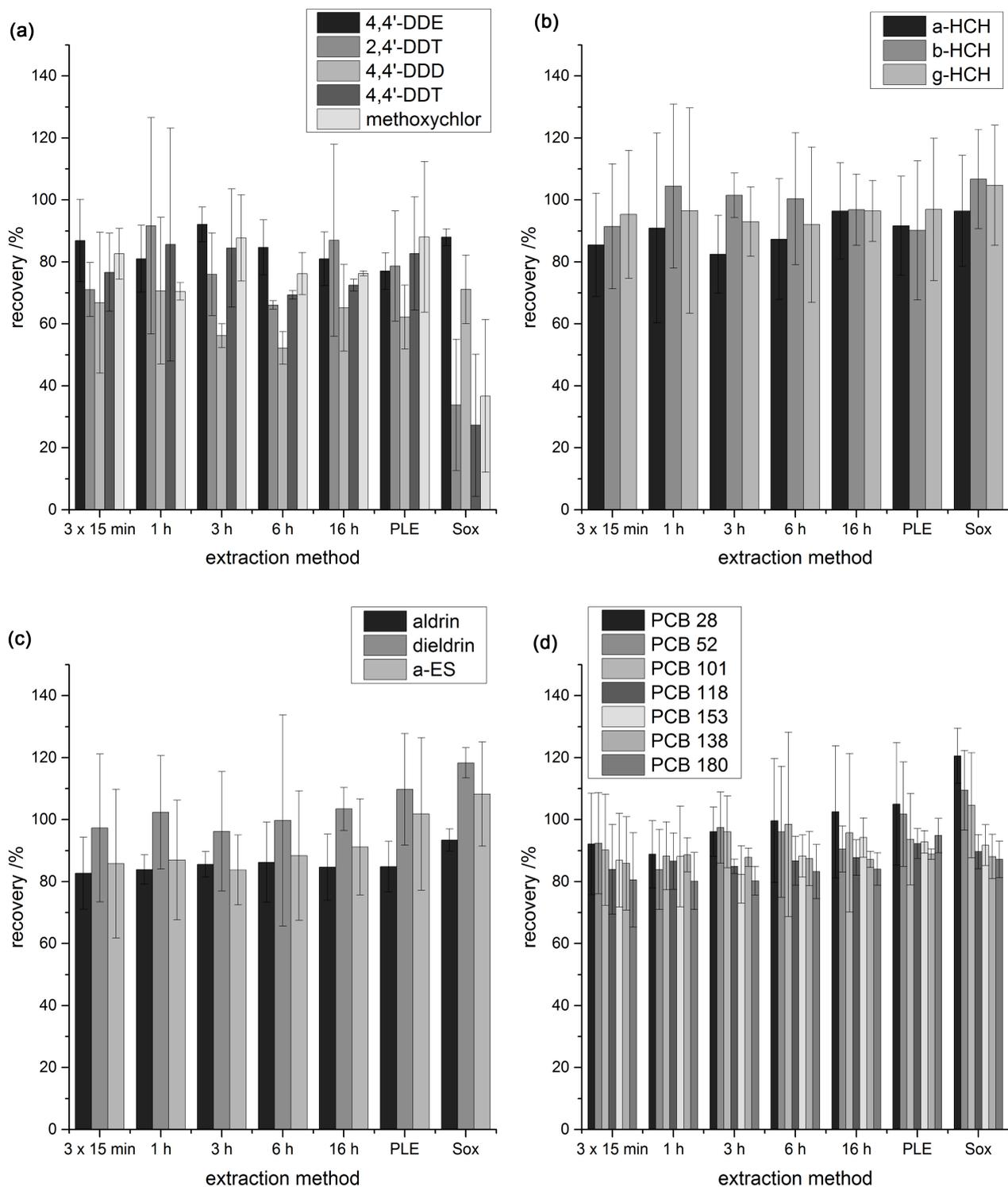


Figure 3. Recovery of (a) DDX, (b) HCH, (c) aldrin, dieldrin, and α -ES, and (d) PCB in % of spiked concentration using different extraction techniques. The error bars indicate the standard deviation ($n = 4$).

re-evaluated independent of the amount of soil organic matter. **Figure 3(a)** shows the mean recovery over all soils of the DDX for different extraction techniques. A clear trend is not immediately recognisable. For SLE, the recoveries lie between $(52\% \pm 5\%)$ (4,4' DDD, 6 h agitation SLE) and $(92\% \pm 6\%)$ (4,4' DDE, 3

h agitation SLE). The degradation products of DDT, 4,4' DDE and 4,4' DDD, generally show acceptable recovery for all extraction methods. With recoveries as low as 27% of the spiked concentration, the use of Soxhlet is not suitable for extraction of 2,4' DDT, 4,4' DDT, and methoxychlor. This can be associated with the thermolability of these analytes which leads to degradation during the extraction cycles [35]. The comparably low recovery of DDX using Soxhlet has already been noted by several researchers [36]. Surprisingly, Soxhlet extraction is widely used for these analytes. The standard deviations for all DDX except 4,4' DDE are relatively high, up to 38%. This can be attributed to the condition of the GC liner and is discussed in Section 3.3.

The extraction of the HCH (**Figure 3(b)**), aldrin, dieldrin, and α -endosulfan (α ES) (**Figure 3(c)**) is possible with all employed extraction methods. Recoveries range from $(83\% \pm 12\%)$ (aldrin, 3×15 min SLE) to $(118\% \pm 5\%)$ (dieldrin, Soxhlet) with acceptable standard deviations. Yet, it is to be noticed that the mean value of dieldrin is higher than that of aldrin for all experiments. A possible explanation for this effect might be the conversion of aldrin into the more stable dieldrin in soils (see above) [28]. In literature there are already some studies available which deal with a comparison of OCP extraction from soils using different techniques. Most of them focus on Soxhlet, PLE, ultrasonic-assisted extraction, and microwave-assisted extraction, which are not found to differ much in extraction efficiency [37]. Our study is able to show that SLE also performs satisfactory.

Concerning the PCBs (**Figure 3(d)**) a review of the data does not give a most favourable extraction method, either. Admittedly, the mean recovery of the PCBs with less chlorine substituents seems to increase slightly when the agitation time is longer. Since PCBs are considered very thermostable, Soxhlet is also applicable and yield satisfying results. Previously published studies have also shown that results from Soxhlet extraction, PLE, and microwave-assisted extraction are usually comparable for PCBs [38] [39] [40]. In the standard EN 16167 [41] which describes the extraction of PCBs from sludge, treated biowaste, and soil the duration of the SLE is required to be 12 hours. The results of our study do however demonstrate that such a long extraction is not necessary for the matrix soil.

In summary, no preferred extraction method for any analyte group could be identified. The results of this study do not lead to the assumption that prolonged SLE time leads to better recovery. This is supported by cluster analysis as shown in **Figure 4**. There are clearly two groups differing from each other. The second group encompasses all extractions of the 13.3% TOC soil. As for the other soils, no clear assignment to one group is evident. The same holds for the different extraction methods. Hence, an extraction method with superior performance cannot be identified.

3.2. Evaluation for Total Analyte Spectrum

Ideally, OCPs and PCBs should be extracted together with one suitable method. All in all, the review of the experimental data so far has led to the assumption that the recovery of all analytes is more or less the same independent of model

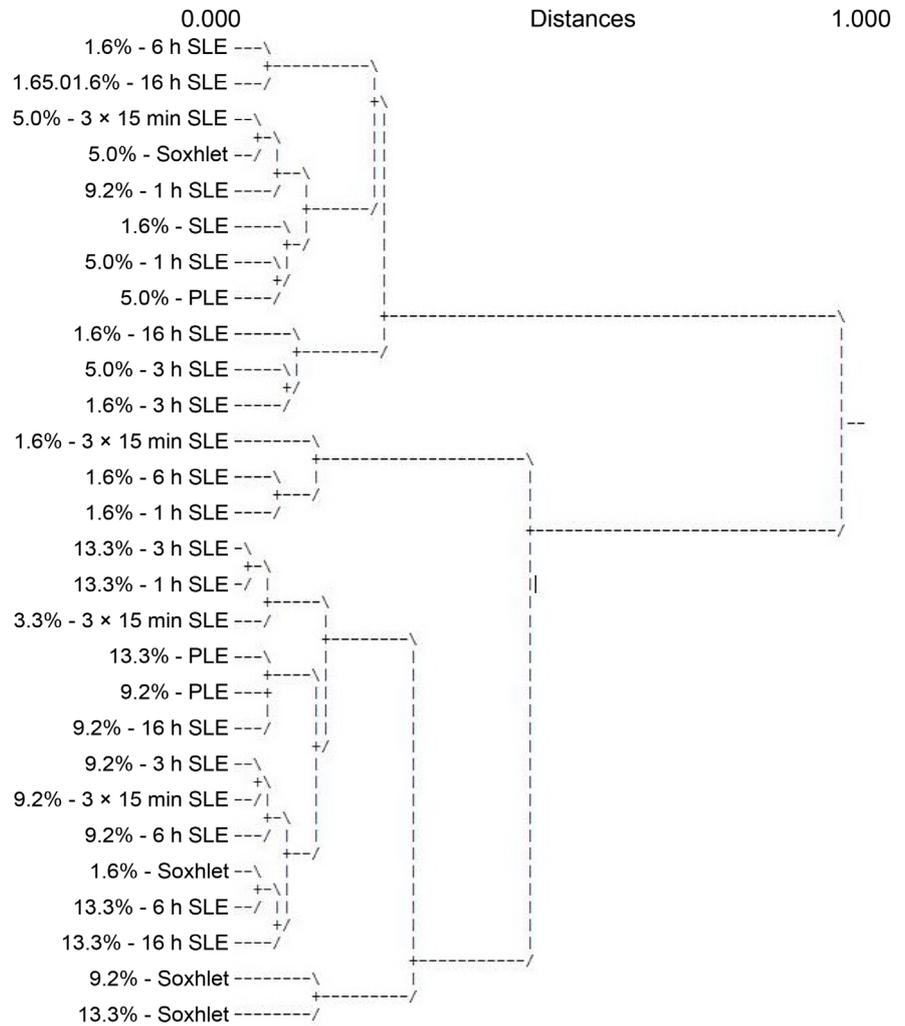


Figure 4. Hierarchical clustering of all data obtained sorted by extraction method (Euclidean distance, Ward linkage).

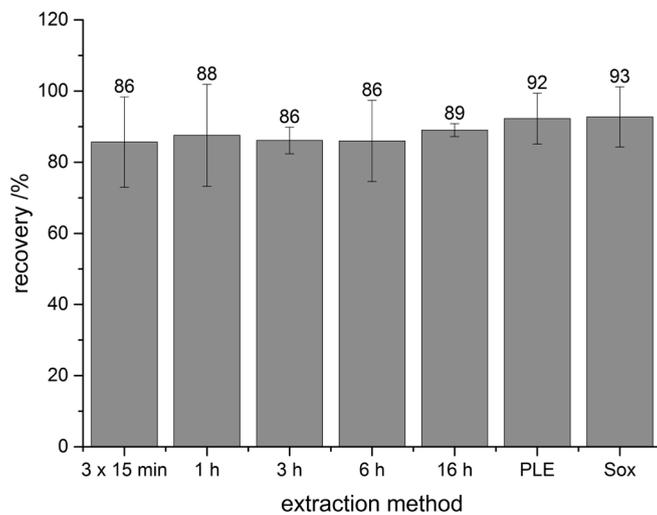


Figure 5. Recovery of all analytes (in % of spiked concentration) from soils of varying TOC. The error bars indicate the standard deviation over all analytes (n = 19) and extractions (n = 4).

soil TOC content and applied extraction technique. For this reason, the data set was re-evaluated using the mean recoveries of all analytes. To verify the previous results, **Figure 5** shows the recoveries for all analytes in dependence of model soil TOC. Generally, all extraction methods give more or less the same results, independent of the soil type. Concerning the soil with the lowest TOC however, prolonged agitation and the use of other extraction techniques lead to higher mean recoveries by about 20%. This has also been noted for single analytes, as described in the previous section. When taking into account the standard deviations which are in the range of 10–20%, the t-test at a 95% confidence level does however give as a result that none of these findings differ significantly. Therefore, it is possible to neglect classification of the data altogether and handle them as a single population. **Figure 6** illustrates this approach. The differences in the performance of certain extraction techniques are greatly diminished when all analytes and all model soils are considered simultaneously. Recoveries range from 86% (3 × 15 min) to 93% (PLE, Sox). The standard deviations exceed the differences between the means by far. Hence, the performance of all extraction methods can be seen as equivalent. The more intense extraction techniques do not lead to a significantly better analyte recovery than SLE. However, they do offer some advantages (**Table 4**). An analysis done with PLE or Soxhlet requires less solvent and the washing step is facilitated as no matrix particles get into the solvent phase as may be the case during decanting from an Erlenmeyer flask. PLE is also faster and can be automated. Additionally, the manual work load is lower. Nevertheless, PLE and Soxhlet should preferably be used for the extraction of thermolabile analytes in combination with labelled internal standards (see Sections 3.1 and 3.3). Additionally, they call for trained personnel, whereas SLE is relatively easy to execute.

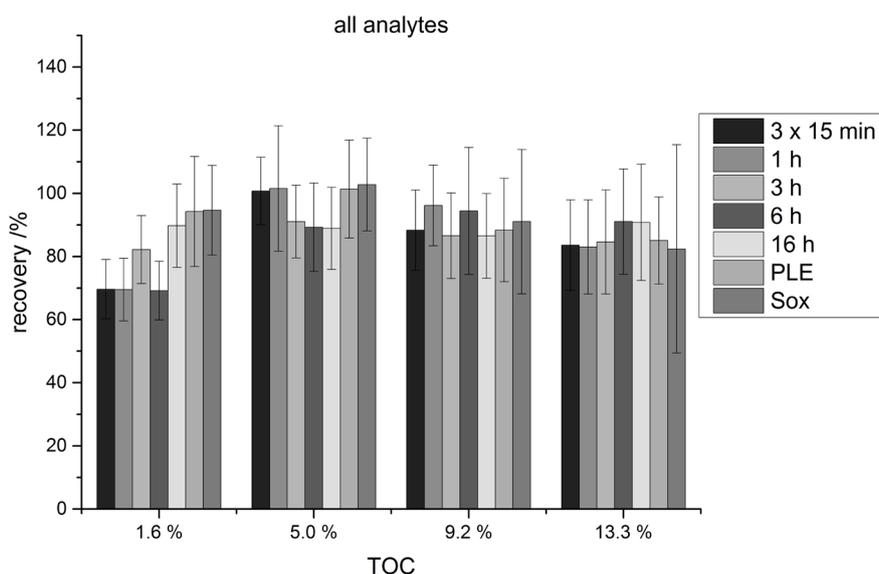


Figure 6. Recovery of all analytes (in % of spiked concentration) using different extraction techniques. The error bars indicate the standard deviation over all analytes ($n = 19$) and extractions ($n = 4$).

Table 4. Advantages and disadvantages of SLE, PLE, and Soxhlet.

	SLE	PLE	Soxhlet
Compounds	Suitable for all tested OCPs and PCBs	Suitable for all tested OCPs and PCBs	Only suitable for thermolabile analytes in combination with labelled internal standards
Complexity of technique	Easy extraction principle, washing step potentially problematic	Trained personnel necessary, washing step uncomplicated	Trained personnel necessary, washing step uncomplicated
Amount of solvent per sample	150 ml	22 ml (PLE)	100 ml (Sox)
Time consumption (without clean-up)	~1.5 h	~0.5 h/sample	min. ~6 h (Sox)

3.3. Use of Internal Standards and GC Liners

Undoubtedly, the GC liner has a great influence on the reproducibility of analytical results. Its inertness is of decisive importance, as analytes may adsorb to active sites in the inlet system and are thus lost to analysis [31]. When matrix contaminated samples are injected, high boiling residues accumulate in the liner [42]. These are known to promote the breakdown of several OCPs, for example endrin and DDT, thereby leading to underestimations [43] [44] [45]. This phenomenon has been used to judge the performance of inert liners and capillary columns [46] [47] [48].

In this study, an unexpected reverse effect was noticed. In several cases the analyte content was greatly overestimated (>140%) for the thermolabile DDX and dieldrin. To gain insight into this problem, a soil extract of the campaign was additionally spiked with ^{13}C -labelled standards and injected 100 times, starting with a new liner. The results for the thermolabile 4,4' DDT and the thermostable 4,4' DDE are shown in **Figure 7**. For both analytes the ratio of the native and ^{13}C -labelled substance remain more or less the same for all injections. However, when PCB 209 is employed as internal standard it can be noticed that after 100 injections the ratio of 4,4' DDT/PCB 209 has increased by a factor of around 1.5, even though the area of PCB 209 does not decrease. This is not the case for 4,4' DDE: The performance of the labelled standard and PCB 209 is comparable.

Unfortunately, a definite cause of the problem could not be found. However, such an effect is often observed in food analysis where highly matrix-contaminated samples are analysed [31]. This matrix enhancement effect is supposedly caused by active sites in the liner and on the GC column. When a calibration solution in a clean solvent is injected, the analytes may adsorb to these sites leading to signal intensity loss. If a matrix containing sample is injected the matrix components compete with the analytes for adsorption sites, leading to a higher signal intensity and often better peak shape [49]. To overcome this

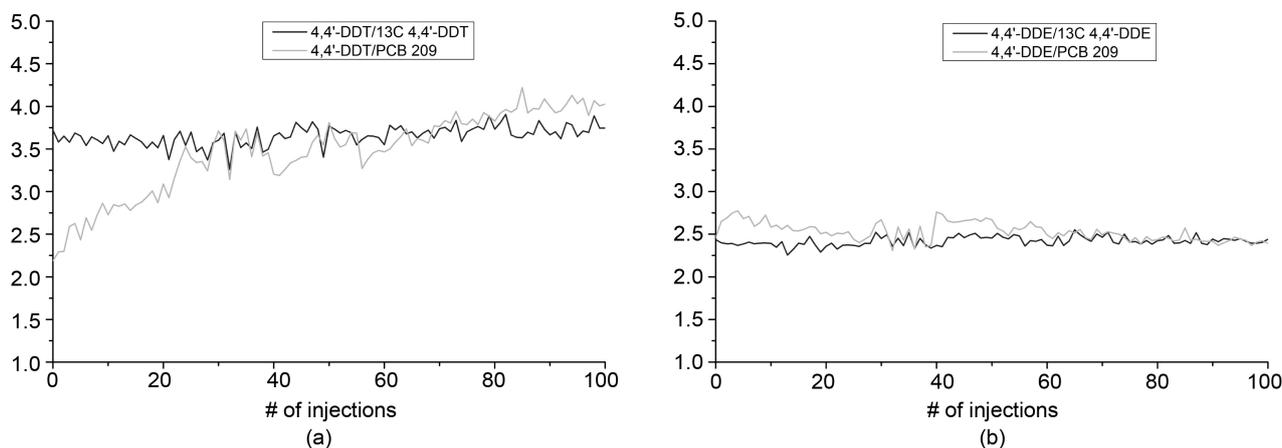


Figure 7. Ratios of the area of 4,4'-DDT and 4,4'-DDE versus the ¹³C-labelled substances and PCB 209 as internal standard over the course of 100 injections.

problem, it is advisable to use for example matrix calibration, analyte protectants, or labelled standards [50] [51]. The latter may be a simple solution, but high financial cost is associated with this approach. In this study, it could be shown that the use of labelled standards for critical analytes helps to counter-balance changing conditions in the injection system.

4. Conclusion

Based on the data obtained during this study, no relation between the TOC content of the model soils and analyte recovery could be identified. Additionally, no clear correlation between the duration of SLE and recovery of analytes could be found. For PLE and Soxhlet extraction, analyte recoveries are comparable to those of SLE. However, the analysis of thermolabile pesticides is only appropriate when used in combination with ¹³C-labelled standards. When taking into account the typically high standard deviations, SLE with short extraction times are also suitable for extraction of PCBs and OCPs from soil. To overcome matrix effects during GC MS measurements it is preferred to use isotopically labelled standards. However, for certain analytes, e.g. PCBs, other internal standards may be applicable.

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