Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of DDT, DDD and DDE in sediment

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Abstract

Sediment containing incurred DDT, DDD and DDE residues was extracted using supercritical fluid (CO_2) extraction (SFE) and Soxhlet extraction. The two extraction techniques were compared. Samples were collected from the Pongolo floodplain in KwaZulu-Natal and the Letaba River in Mpumulanga, South Africa. The sediment samples were freeze-dried, sieved, extracted and analysed by GC-MS for their DDT, DDD and DDE content. Validation of the GC-MS method was done against a reference standard. The paired t-test and two-way analysis of variance (ANOVA) without replication were used for the statistical evaluation of the two extraction techniques. Results of the paired t-test and two-way ANOVA showed that there is no evidence that SFE differs significantly from Soxhlet extraction for the determination of the three compounds. SFE can be used to replace Soxhlet extraction for the extraction of DDT, DDD and DDE from sediment. The determined limits of detection (LODs) were lower than the calculated suggested EP-based SQC for DDT, DDD and DDE making GC-MS coupled to SFE a useful method for the determination of the three compounds in sediment.

Nomenclature

ANOVA	analysis of variance
BLMS	between-locations mean square
BMMS	between-methods mean square
CI	confidence interval
CL	confidence limits
CO_2	carbon dioxide
DCB	4,4-dichlorobiphenyl
DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)ethene
DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
DF	degrees of freedom
EP	equilibrium partitioning
GC	gas chromatograph
GC-MS	gas chromatograph/mass spectrometer
LOD	limit of detection
LOQ	limit of quantitation
MS	mass spectrometer
Ms	mean square
OCPs	organochlorine pesticides
OM	organic matter
PFTBA	perfluorotributylamine
RMS	random mean square
RSD	relative standard deviation
SC	supercritical
SF	supercritical fluid
SFE	supercritical fluid extraction
SOM	sediment organic matter
SOV	source of variance
SQC	sediment quality criteria/criterion
TBA	tetrabutylammonium
US EPA	Environmental Protection Agency of the United States of America

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Introduction

The Soxhlet extraction technique is a traditional technique for extracting OCPs from sediment. The disadvantages of Soxhlet extraction are:

- The use of large volumes of hazardous and flammable liquid organic solvents.
- Potential toxic emissions during extraction.
- The need for costly, high-purity solvents.
- It is non-selective.
- It is a laborious procedure.
- It is a time-consuming procedure.

Extraction procedures should be environmentally friendly and not add to the pollution problem. Soxhlet extraction does not meet this criterion because it generates large volumes of contaminated, hazardous solvents and emits toxic fumes. Recently clean techniques, such as SFE, for extracting OCPs (and other organics) from complex matrices, have been developed to the stage where they can be used routinely.

The success of programmes monitoring pollutants in aquatic systems is significantly dependent on the quality of analytical information, and hence an economical, practical and clean method is needed. The purpose of this study is to investigate, by comparison, the potential of SFE as an alternative to Soxhlet extraction of incurred DDT, DDD and DDE from sediment.

SFE

An SF is a substance which, under conditions above, but close to its critical point, can no longer be classified as either a liquid or a gas but shares the physical properties of both (Breet et al., 1996). Physically an SF behaves like a gas (diffusivity and viscosity) while chemically it retains liquid-like solvating characteristics (Phelps et al., 1996).

The choice of a substance to be used as an SC solvent depends on the polarity of the target analytes. Also important when

choosing a SF is the practical considerations, such as the temperature and pressure needed to push a substance into its critical region (Phelps et al., 1996). CO_2 becomes supercritical under rather mild conditions: above 31.1°C and 7 380 kPa. Moreover, it is readily available at low cost, it has a low toxicity and reactivity, and it provides a clean alternative to conventional liquid/solid extraction techniques. This makes $SC-CO_2$ the most widely used extraction fluid and it is an excellent solvent for extracting nonpolar analytes (Hills and Hill, 1993; Lee et al., 1993; Barnabas et al., 1994; Reimer and Suarez, 1995; Bøwadt et al., 1995 and Kikic et al., 1995). Although $SC-CO_2$ is a poor solvent for polar compounds, it can be modified into a more polar solvent by adding small amounts of acetone or methanol, and can thus be used to extract slightly polar compounds. $SC-CO_2$ can, by variation of temperature and pressure, assume the equivalent properties of a range of conventional solvents, from pentane to pyridine (Phelps et al., 1996) to suit different applications. This range also includes solvents such as benzene, toluene, carbon tetrachloride, and other chlorinated solvents (Phelps et al., 1996). Solubility parameters of some common solvents vs. SFs can be found in Phelps et al. (1996).

More advantages of using $SC-CO_2$ are that the extracted analytes are collected in a concentrated form, because the CO_2 vapourises at ambient conditions. The solvent strength of $SC-CO_2$ can easily be altered by changing the density (pressure and temperature) and by adding modifiers. Extraction times are drastically reduced, because $SC-CO_2$ has superior mass transfer qualities. A drawback of SFE is that the initial cost of the equipment is high.

EP-based SQC

An important goal of the project was to develop a method that could meet the threshold levels of DDT, DDD and DDE suggested by the calculated EP-based SQC. For determining SQC for DDT, DDD and DDE the EP approach favoured by the US EPA (Adams et al., 1992) was used, because this method has been field validated for the compounds (Di Toro et al., 1991 and Webster and Ridgway, 1994). The EP approach, although not yet perfected and currently undergoing development by the US EPA, provides a mechanism whereby the degree of contamination and likely toxicity of sediment can be assessed (Webster and Ridgway, 1994).

Background

DDT is sprayed annually in the Pongolo floodplain in KwaZulu-Natal for mosquito control to protect the local population from malaria (Fig. 1) by e.g. spraying the inside of their dwellings (Bouwman et al., 1991). DDT enters the Pongolo floodplain via run-off from inter alia the sprayed dwellings.

Sorption of DDT to the sediment of the pans occurs through partitioning of DDT between the overlying water and the sediment. The sorption capacity of sediment is determined by the mass fraction of OM present in the sediment (Ames and Grulke, 1995). The SOM functions as a partition medium for the sorption of OCPs (Kile et al.,1995). Sediment of the pans can hence serve as a sink for OCPs but it can also serve as a source. Sediment is an important component of the aquatic ecosystem because of the niche it provides for benthic organisms. Many food-chain organisms spend a major portion of their lifecycle in or on aquatic sediment. Direct transfer of chemicals from sediment to organisms is considered to be a major route of exposure for many
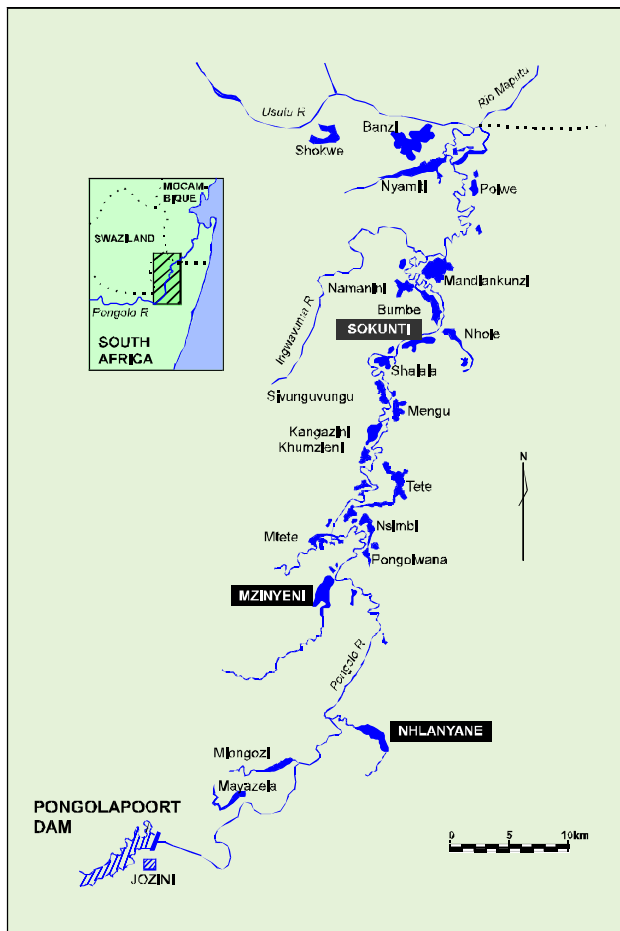

Figure 1
Sampling areas in the Pongolo Floodplain, KwaZulu-Natal, South Africa (Bouwman et al., 1990)

species (Adams et al., 1992). This provides a pathway for these chemicals to be consumed by higher aquatic life, animals, birds and humans.

Sediment extracts are analysed for DDT, DDE and DDD, because DDT degrades to DDD and/or DDE. Under aerobic (oxidative) conditions, DDE formation is favoured, while under anaerobic (reducing) conditions, DDD is favoured (Chau and Afghan, 1982). DDE is more persistent but normally less toxic than DDT. DDD is less persistent and less toxic than DDE (Bouwman, 1991).

Sampling area

Sediment samples were collected from three pans in the Pongolo floodplain in KwaZulu-Natal: Mzinyeni, Nhlanyane and Sokunti. A sediment sample was also collected at the Junction Weir in the Letaba River in Mpumalanga. The Junction Weir site was chosen because a study conducted by Heath (1994) showed that fish had accumulated DDT from their surroundings in the Letaba River.

A description of the Pongolo floodplain can be found in Bouwman et al. (1990) (Fig. 1). A description of the Letaba River site can be found in Heath (1994).

Method validation

Early SFE methods were developed using spiked samples (Bøwadt et al., 1995). More recent studies emphasise the importance of the difference between the investigations on spiked samples and field samples (Van der Velde et al., 1994). SFE performed on contaminant containing real samples has shown SFE to be more difficult than initially indicated by the spiked samples. Addition of modifiers or stronger extraction conditions seem to be necessary to obtain SFE results which can be compared with conventional extraction techniques or with the values of certified reference materials (Van der Velde et al., 1994). Langenfeld et al. (1995) reported that great discrepancies exist between the SFE rates of spiked analytes and incurred analytes. This called for an approach in SFE where methods are developed directly on contaminated real sediment samples.

A new method or an adapted existing technique has to be validated to assure quality. In this study the GC-MS method for the determination of DDT, DDD and DDE was validated against reference standards using correlation and regression.

Materials and methods

Sediment samples were collected with a Peterson grab sampler at the study areas (Fig. 1). The pans were sampled in January 1995 and the river was sampled in September 1994. The samples were stored in 5 ℓ tins with lids and they were kept cool *en route* to the laboratory. Upon arrival at the laboratory, the samples were centrifuged to remove excess water and then they were frozen at -18°C prior to sample preparation.

The frozen samples were freeze-dried and sieved. The < 63 µm fractions of the sieved samples were homogenised by mixing thoroughly prior to SFE and Soxhlet extraction. It has been reported that DDT concentrates in the fine fraction (clay) for sediment with organic carbon > 0.1 % by mass (Karickhoff et al., 1979; Di Toro et al., 1991 and Kile et al., 1995). However, it is not practical to isolate the clay fraction (< 2 µm) for extraction purposes and therefore the < 63 µm particle size fraction, which includes both the silt and clay fractions, was used in this study. The % OM in the < 63 µm fraction of the sediment samples is given in Table 5. The % OM was determined by the Walkley-Black method (Hesse, 1972). Whole sediment samples from Junction Weir and Nhlanyane were characterised as clay, while the whole sediment samples from Mzinyeni and Sokunti were characterised as clay loam and fine sand, respectively. The < 63 µm particle size fraction comprised 89.7, 53.7, 96.7, and 3.9% for Junction Weir, Mzinyeni, Nhlanyane and Sokunti, respectively of each sediment sample. Extraction of each sample was done in quadruplicate (n = 4), and each extract was analysed in triplicate (n = 3), over a period of two months. Blank extractions were performed as well.

High purity solvents (Burdick & Jackson, Anatech, Midrand) analytical reagent pure chemicals (Merck, Midrand), certified neat standards (>99% purity, ChemService, Separations, Randburg), SFE grade liquid CO_2 (Fedgas, Johannesburg) and N_2 gas (99.9990%, Fedgas, Pretoria) were used during this study.

An Isco SFX 220 extractor module (Anatech, Midrand) with a temperature-controlled coaxially-heated restrictor with a reported flow rate of 2 mℓ/min liquid CO_2 at 34 475 kPa (5 000 psi), an SFX 200 controller and a model 260 D syringe pump were used for SFE. The sample cell was filled with 5 mm of Ottawa sand (20 to 30 mesh, Fisher Scientific, USA) 0.5 g copper powder (for the removal of sulphur), 2 g sediment sample, 5 µℓ acetone

(modifier) spiked onto the sediment, and Ottawa sand to fill the cell completely. The cell was pressurised to 41 370 kPa (6 000 psi) at 55°C with SC-CO_2 (density = 0.916 g/mℓ). The pressure was maintained for 15 min (static extraction), after which 30 mℓ of SC-CO_2 was pumped through the sample (41 370 kPa, 55°C) at a SF flow rate of 1.4 mℓ/min (dynamic extraction). SC-CO_2 was depressurised through the heated restrictor (55°C) into a glass tube containing 5 mℓ pre-cooled hexane and glass beads (3 mm O.D, N.T laboratory supplies, Midrand).

The Soxhlet extraction method used was based on the EPA Method 3540 (US EPA, 1986), sulphur was removed by using TBA sulphite reagent (US EPA, 1986), and the extracts were cleaned using Florisil cartridges (Varian, 1995). For the Mzinyeni, Nhlanyane and Sokunti samples 5 g of sediment was extracted, and for the Junction Weir sample 10 g of sediment was extracted.

All extracts were quantitatively transferred to a teflon-lined screw top vial and concentrated to 1 mℓ under nitrogen gas. An internal standard, 4,4-DCB was added to each extract. The extracts were stored at -18°C in the dark prior to analysis.

Extracts and standards were analysed by GC-MS, based on the EPA Method 8270 (US EPA, 1986). A Varian Star 3400 CX GC (SMM, Kyalami) equipped with Varian Saturn 3 mass spectrometric (ion trap) detection, and a Varian 8200 CX autosampler-injector were used for this study. Varian Saturn GC-MS Version 4.1 Analysis Software was used to control the GC-MS and the autosampler, to identify and confirm the presence of the analytes, and for peak integration. An HP-5 MS capillary column (30 m x 0.25 mm I.D., 0.25 µm coating) was used (HiPerformance Systems, Johannesburg). The GC oven temperature was programmed from 40°C (hold 4 min) to 270°C at 10°C/min. (hold 3 min). The total run-time was 30 min. The injector temperature was 300°C and the transfer line temperature was 290°C. Helium gas (99.9995 %) was used as the carrier gas at a flow rate of 1 mℓ/min. The column head pressure was 100 kPa. The septum purge flow rate was 2 mℓ/min and the splitter flow was 60 mℓ/min. Two microlitres of extracts and standards were injected in the splitless mode. The delay time before opening of the split valve was 1 min. Each of the extracts was injected in triplicate (n = 3). The injector speed was 10 µℓ/s. The sandwich injection mode was used. The MS was set to scan the mass range 35 to 500 m/z at 0.81 s/scan. The EI ionisation mode was used. Mass calibration was done with PFTBA (FC-43). Confirmation of identity of the analytes was done by comparing the sample mass spectrum with the mass spectra of the standards and computer generated library search routines. The ions used for quantitation of the analytes were 235 m/z for DDT and DDD, and 246 m/z for DDE. A multi-level calibration was performed using the internal standard method. Concentrations of the calibration standards (n_i = 1 and n_{tot} = 7) (n_i = number of injections per standard and n_{tot} = total number of standards) are given in Table 1. The concentration of the internal standard was 1 000 ng·µℓ^{-1}.

Statistical evaluation

Validation of the GC-MS method was done against a reference standard using correlation and regression to test linearity, accuracy and sensitivity. The results of the evaluation of the GC-MS calibration are summarised in Table 2 where the correlation coefficient (r), the slope (b), the intercept (a), the error (standard deviation) in the y-residual ($S_{y/x}$), the errors in the slope (S_b) and in the intercept (S_a), the 95 % CL of b and a, the 95 % CI for b and a, the LOD, and the LOQ are tabulated. The results of the validation of the GC-MS calibration are summarised in Table 3

TABLE 1
GC-MS CALIBRATION USED FOR SFE AND SOXHLET EXTRACTION QUANTITATION

Number of standard	DDT		DDD		DDE	
	x_i ng·µℓ⁻¹	y_i (Response)[1]	x_i ng·µℓ⁻¹	y (Response)[1]	x_i ng·µℓ⁻¹	y_i (Response)[1]
1	0	0	0	0	0	0
2	0.00152	0.0025	0.0015	0.0059	0.0015	0.0038
3	0.0152	0.0160	0.0135	0.0202	0.0125	0.0256
4	0.114	0.0650	0.1015	0.0728	0.0945	0.0489
5	0.532	0.3100	0.4745	0.3250	0.441	0.2038
6	1.52	1.0800	1.356	0.9809	1.26	0.5603
7	3.80	2.8100	3.39	2.5855	3.15	1.425

1 = Area compound/Area DCB (internal standard) for single injections of the standards

TABLE 2
RESULTS OF THE STATISTICAL EVALUATION OF THE GC-MS CALIBRATION USED FOR THE ANALYSIS OF THE < 63µm SEDIMENT PARTICLE SIZE FRACTIONS AFTER SFE AND SOXHLET EXTRACTION

Statistical calculations	DDT	DDD	DDE
Regression line: $y = bx + a$	y = 0.7400x - 0.0205	y = 0.7596x - 0.0091	y = 0.4492x + 0.0057
r^2	0.9990	0.9994	0.9998
$S_{y/x}$	0.03595	0.0261	0.0077
S_b	0.0104	0.0085	0.0027
95% CL for b	0.7400 ± 0.0266	0.7596 ± 0.0218	0.4492 ± 0.0069
95% CI for b	0.7134 < b < 0.7666	0.7378 < b < 0.7814	0.4423 < b < 0.4561
S_a	0.0162	0.0118	0.0035
95% CL for a	- 0.0205 ± 0.0416	- 0.0091 ± 0.0303	0.0057 ± 0.0089
95% CI for a	- 0.0621 < a < 0.0211	- 0.0394 < a < 0.0212	- 0.0032 < a < 0.0146
X_{LOD} (ng·µℓ⁻¹extract)	0.1451	0.1031	0.0514
X_{LOQ} (ng·µℓ⁻¹extract)	0.4838	0.3436	0.1714
X_{LOD}(mg·kg⁻¹sediment) - SFE - [1] Soxhlet - [2] Soxhlet	0.0726 0.0290 0.0145	0.0516 0.0206 0.0103	0.0257 0.0103 0.00514
X_{LOQ}(mg.kg⁻¹sediment) - SFE - [1] Soxhlet - [2] Soxhlet	0.2419 0.0968 0.0484	0.1718 0.0687 0.0344	0.0857 0.0343 0.0171

b = slope
a = intercept
r = correlation coefficient
$S_{y/x}$ = error (standard deviation) in the y - residual
S_b = error in the slope
CL = confidence level
CI = confidence interval
S_a = error in the intercept
LOD = limit of detection
LOQ = limit of quantitation
[1] 5g sediment (method for extraction of Mzinyeni, Nhlanyane and Sokunti)
[2] 10g sediment (method for extraction of Junction Weir)

TABLE 3
RESULTS OF THE VALIDATION OF THE ACCURACY AND PRECISION OF THE GC-MS METHOD FOR THE DETERMINATION OF DDT, DDD AND DDE AFTER SFE AND SOXHLET EXTRACTION

Statistical calculations	DDT	DDD	DDE
[1]μ (ng·$\mu\ell^{-1}$)	0.530	0.475	0.440
m	1	1	1
y_0	0.3120	0.3280	0.2040
x_0	0.4495	0.4438	0.4415
Sx_0	0.0522	0.0365	0.0212
95 % CL of x_0	0.4495 ± 0.1343	0.4438 ± 0.0938	0.4415 ± 0.0544
95 % CI of x_0	$0.3152 < x_0 < 0.5838$	$0.3500 < x_0 < 0.5376$	$0.3871 < x_0 < 0.4959$
% RSD	11.61	8.22	4.80

[1]   Standard reference sample
m = number of readings
y_0 = response
x_0 = concentration determined from the regression line
Sx_0 = error in x_0

TABLE 4
SUMMARY OF THE RESULTS OF THE DETERMINATION OF DDT, DDD AND DDE IN THE SEDIMENT SAMPLES (< 63 µm) AFTER SFE AND SOXHLET EXTRACTION

Area	Technique	DDT				DDD				DDE			
		m	Xo (mg·kg⁻¹)	S_{XO}	RSD (%)	m	Xo (mg·kg⁻¹)	S_{XO}	RSD (%)	m	Xo (mg·kg⁻¹)	S_{XO}	RSD (%)
Junction Weir	Soxhlet	[1]4	0.0087	0.0032	37.0	15	0.0120	0.0019	15.67	13	0.0235	0.0009	3.62
	SFE	12	0.0396	0.0127	32.1	12	0.0392	0.0098	25.03	11	0.0487	0.0046	9.35
Mzinyeni	Soxhlet	11	0.3200	0.0051	1.6	11	0.3740	0.0045	1.29	11	0.3460	0.0021	0.60
	SFE	12	1.3090	0.0172	1.31	12	0.6210	0.0093	1.50	12	0.3165	0.0041	1.30
Nhlanyane	Soxhlet	[1]2	0.0187	0.0080	42.99	[1]5	0.0392	0.0046	11.71	10	0.0592	0.0017	2.97
	SFE	10	0.1762	0.0125	7.07	9	0.0474	0.0102	21.52	9	0.0437	0.0047	10.77
Sokunti	Soxhlet	[1]6	0.0568	0.0056	9.89	12	0.0750	0.0037	4.88	11	0.1272	0.0017	1.31
	SFE	9	0.4838	0.0122	2.52	9	0.1820	0.0097	5.33	9	0.1517	0.0045	2.97

m   = number of readings
[1]   Values of m low, because the analytes were not detected.
x_0 = concentration determined from the regression line
Sx_0 = error in x_0
RSD = relative standard deviation

where the response (y_0), the concentration (x_0), the error in the determined concentration (Sx_0), the 95% CL of x_0, the 95% CI of x_0, and the %RSD of the reference standard are given.

The mean of all readings of the samples was taken except those readings that were identified as outliers by using the Dixon Q-test (Miller, 1993). The analyte concentrations in the extracts were determined in ng·$\mu\ell^{-1}$. The results were converted to µg analyte per kilogram dry mass. Quantitation was performed using linear regression. The results are summarised in Table 4 where the mean concentration x_0, Sx_0, and the %RSD are given.

It was assumed that the analytical results conform to a normal distribution.

EP-based suggested SQC were calculated using EPA acute criteria, partition coefficients and OM, and are summarised in Table 5. The comparison of the suggested SQC, chemical contents of the sediment, and the LOD and LOQ of the SFE method are given in Table 6.

The statistical evaluation of the two methods was done by using the paired t-test and two-way ANOVA without replication (Tables 7 to 9).

TABLE 5
SUGGESTED SEDIMENT CRITERIA CALCULATED USING EPA ACUTE CRITERIA, PARTITION COEFFICIENTS AND FRACTION ORGANIC MATERIAL

Compound	[1] EPA water quality criteria ($\mu g \cdot \ell^{-1}$)	[4]K_{oc} ($\ell \cdot kg^{-1}$)	% OM			
			[5]2.13	[6]2.81	[7]3.21	[8]3.83
			[9]Suggested SQC mg·kg^{-1} dry sediment			
DDT	1.1	160 000	3.749	4.946	5.650	6.741
DDD	0.06 [2]	180 000	0.230	0.303	0.347	0.414
DDE	1.05 [3]	99 000	2.214	2.921	3.337	3.981

[1]   Fresh water-acute toxicity to aquatic organisms (health) (Meyer and Barclay, 1990 and US EPA, 1994).

[2 and 3]   Interim values based on the no observable effects limit (NOEL)(Meyer and Barclay, 1990 and US EPA, 1994)

[4]   Webster and Ridgway, 1994

[5]   Junction Weir

[6]   Mzinyeni

[7]   Sokunti

[8]   Nhlanyane

[9]   SQC were calculated using the EP approach (Di Toro et al., 1991, Adams et al., 1992 and Webster and Ridgway, 1994):

SQC   =   WQC x K_{oc} x f_{oc} where
WQC   =   water quality criterion
K_{oc} =   organic carbon normalised partition coefficient
f_{oc} =   fraction organic carbon in the sediment
Unit conversion: ÷ 1000

TABLE 6
COMPARISON OF THE SQC, THE DDT, DDD AND DDE CONTENTS IN THE SAMPLES, AND THE LOD AND LOQ OF THE METHOD

	Junction Weir	Mzinyeni	Nhlanyane	Sokunti
SQC$_{DDT}$ (mg·kg^{-1} sediment)	3.749	4.946	6.741	5.650
SQC$_{DDD}$ (mg·kg^{-1} sediment)	0.230	0.303	0.414	0.347
SQC$_{DDE}$ (mg·kg^{-1} sediment)	2.214	2.921	3.981	3.337
*DDT (mg·kg^{-1} sediment)	<LOD	1.309	<LOQ	0.4838
*DDD (mg·kg^{-1} sediment)	<LOQ	0.621	<LOD	0.1820
*DDE (mg·kg^{-1} sediment)	<LOQ	0.3165	<LOQ	0.1517
*LOD of the method (mg·kg^{-1} sediment)	0.0726 - DDT 0.0516 - DDD 0.0257 - DDE			
*LOQ of the method (mg·kg^{-1} sediment)	0.2419 - DDT 0.1718 - DDD 0.0857 - DDE			
*Determined by SFE and GC-MS				

Results and discussion

Validation of the GC-MS method

The results for the statistical evaluation of the GC-MS method are shown in Table 2. The r-values are excellent, indicating almost perfect linear correlation for the three compounds. The $S_{y/x}$, S_a and S_b appear to be relatively small and satisfactory for the three compounds. The LODs are relatively high (0.1451 ng DDT.$\mu\ell^{-1}$, 0.1031 ng DDD.$\mu\ell^{-1}$ and 0.0514 ng DDE.$\mu\ell^{-1}$) and could perhaps be improved by analysing more standards close to the blank concentration. This would also improve $S_{y/x}$ and S_a for the three compounds. The results for the validation of the accuracy and precision of the GC-MS method for the determination of DDT, DDD and DDE are shown in Table 3. Since $\mu = 0.530$ ngDDT.$\mu\ell^{-1}$ falls in the 95 % CI of x_0, the method is validated for accuracy. Since the value of x_0 is very low the precision of x_0, namely Sx_0 (0.0522), is acceptable and likewise the RSD of 11.61% is acceptable. For DDD, $\mu = 0.475$ ngDDD.$\mu\ell^{-1}$ falls in the 95% CI of x_0, the method is validated for accuracy. Both Sx_0 and the RSD are small, and the method is validated for precision. For DDE, since the $\mu = 0.440$ ngDDE.$\mu\ell^{-1}$ falls in the 95% CI of x_0, the method is validated for accuracy. Since both Sx_0 and the RSD are small, the method is validated for precision.

From the results in Table 6 it is evident that the SFE-GC-MS method has the sensitivity to determine the threshold concentrations suggested by the SQC; because the LODs and LOQs of the method for DDT, DDD and DDE respectively are lower than the corresponding SQCs. The chemical concentrations in the sediment samples were all below the suggested SQC, except for the DDD content in the Mzinyeni sediment which is higher than the suggested SQC.

Statistical evaluation

The non-parametric method, Kolmogorov-Smirnov two-sample test, was used to determine whether the analytical results obtained with the two methods may reasonably have the same distribution. The calculated significance values for DDT(0.6994), DDD(0.6994) and DDE(0.9996) were above the critical value (0.05) at the 95% confidence level; and therefore the analytical results for SFE and Soxhlet extraction came from the same population

Statistical evaluation using the paired t-test

Statistical hypotheses for the paired t-test. H_0:$\mu_d = 0$ and any difference in the results of the two methods is random and not significant. H_1:$\mu_d \neq 0$ and the results of the two methods for DDT, DDD and DDE extraction differ significantly.

The d-values for DDT and DDD tend to be biased (they are all negative) which is statistically not desirable (Tables 7(a) and 8(a)). For DDE, the d-values are not biased (there are two negatives and two positives) which is statistically desirable (Table 9(a)). Since the $t_{calc} < t_{crit}$ (Tables 7(b) to 9(b)), H_0 cannot be rejected and therefore Soxhlet and SFE gave results for DDT,

DDT: STATISTICAL EVALUATION OF THE SFE AND SOXHLET EXTRACTIONS

TABLE 7(a)
STATISTICAL EVALUATION WITH THE THE PAIRED T-TEST

Location	Soxhlet extraction (mg·kg⁻¹)	SFE (mg·kg⁻¹)	d
Junction Weir	0.0087	0.0396	-0.0309
Mzinyeni	0.3200	1.3090	-0.9890
Nhalnyane	0.0187	0.1762	-0.1575
Sokunti	0.0568	0.4838	-0.4270

TABLE 7(b)
STATISTICAL QUANTITATION OF THE PAIRED T-TEST

n	Σ_d	$\bar{x}_d$	S_d	DF	t_{calc}	t_{crit}
4	-1.6044	-0.4011	0.4253	3	1.89	3.18

TABLE 7(c)
STATISTICAL EVALUATION USING THE TWO-WAY ANOVA WITHOUT REPLICATION

Source of variation (SOV)	Sum of squares (SOQ)	DF	Mean square (Ms)	F_{calc}	F_{crit}
Between-methods	0.3182	1	0.3182	3.49	10.13
Between-locations	0.7691	3	0.2564	2.81	9.28
Residual/random	0.2736	3	0.0912		
Total	1.3608	7			

DDD and DDE that do not differ significantly. SFE can therefore be used to replace Soxhlet extraction as a method for extraction of DDT, DDD and DDE from sediment.

Statistical evaluation using the two-way ANOVA

Tables 7(c) to 9(c) show the results of the statistical evaluation using the two-way ANOVA without replication. In the comparison of the BMMS and RMS for DDT, DDD and DDE, H_0 cannot be rejected, because $F_{calc} < F_{crit}$; and Soxhlet extraction and SFE gave results for the extraction of DDT, DDD and DDE that do not differ significantly. SFE can therefore be used to replace Soxhlet extraction as a method for the extraction of DDT, DDD and DDE from sediment. In Tables 10(a) and (b) the random variances, the between-methods variances and the between-locations variances for DDT, DDD and DDE are determined and compared. The between-method variance is smaller than the random experimental variance (Table 10(a)), and therefore the between-method variance is not significant. Since σ_T^2/σ_0^2 is smaller than one [$(\sigma_T^2/\sigma_0^2)<1$] for DDT, DDD and DDE; it implies that the between-method variances are not significant for the three compounds and SFE and Soxhlet extraction gave results that do not differ significantly. Soxhlet extraction can therefore be replaced by SFE. For DDT $\sigma_b^2/\sigma_0^2 < 1$, implying that the DDT concentrations in the four locations do not differ significantly. For DDD

DDD: STATISTICAL EVALUATION OF THE SFE AND SOXHLET EXTRACTIONS

TABLE 8(a)
STATISTICAL EVALUATION WITH THE PAIRED T-TEST

Location	Soxhlet extraction (mg·kg^{-1})	SFE (mg·kg^{-1})	d
Junction Weir	0.0120	0.0392	-0.0272
Mzinyeni	0.3740	0.6210	-0.2470
Nhalnyane	0.0392	0.0474	-0.0082
Sokunti	0.0750	0.1820	-0.1070

TABLE 8(b)
STATISTICAL QUANTITATION OF THE PAIRED T-TEST

n	Σ_d	$\bar{x}_d$	S_d	DF	t_{calc}	t_{crit}
4	-0.3894	-0.0974	0.1086	3	1.79	3.18

TABLE 8(c)
STATISTICAL EVALUATION USING THE TWO-WAY ANOVA WITHOUT REPLICATION

SOV	SOQ	DF	Ms	F_{calc}	F_{crit}
Between-methods	0.0190	1	0.0190	3.21	10.13
Between-locations	0.2917	3	0.0972	16.50	9.28
Residual/random	0.0177	3	0.0059		
Total	0.3283	7			

DDE: STATISTICAL EVALUATION OF THE SFE AND SOXHLET EXTRACTIONS

TABLE 9(a)
STATISTICAL EVALUATION WITH THE PAIRED T-TEST

Location	Soxhlet extraction (mg·kg^{-1})	SFE (mg·kg^{-1})	d
Junction Weir	0.0235	0.0487	-0.0252
Mzinyeni	0.3460	0.3165	0.0295
Nhalnyane	0.0592	0.0437	0.0155
Sokunti	0.1272	0.1517	-0.0245

TABLE 9(b)
STATISTICAL QUANTITATION OF THE PAIRED T-TEST

n	Σ_d	$\bar{x}_d$	S_d	DF	t_{calc}	t_{crit}
4	-0.0047	-0.0012	-0.0279	3	0.085	3.18

and DDE, $\sigma_b^2/\sigma_0^2 > 1$ and therefore the DDD and DDE concentrations at the four locations differ significantly (See also Tables 8(c) and 9(c) where $F_{calc} > F_{crit}$ for between locations).

It has been shown that both SFE and Soxhlet extraction can be used to extract DDT, DDD and DDE from sediment. However, SFE benefits are:

- For individual samples, SFE drastically reduced the methodology time from the 16 h it took to perform Soxhlet extraction to the 30 min needed for SFE.
- Sample preparation steps were reduced, as for SFE the sulphur was removed, with the use of Cu powder, from the sample inside the extraction cell whilst the extraction was being carried out.
- No clean-up of the extracts was necessary.
- A large fume-hooded laboratory space and expensive rigorous glassware set-up were required for Soxhlet extraction.

In contrast SFE required little space, and was easy and safe to perform:

- SFE was virtually solvent-free, whereas 500 mℓ of solvent was used for each Soxhlet extraction.
- SFE can be automated and coupled to a GC-MS.

Conclusions

The paired t-test and the two-way ANOVA without replication showed that the results of the two extraction techniques do not differ significantly. The between-method variance for SFE and Soxhlet extraction is smaller than the random experimental variance for DDT, DDD and DDE, confirming that the between-method variance for SFE and Soxhlet extraction is statistically not significant and SFE can replace Soxhlet extraction for the extraction of DDT, DDD and DDE from sediment.

If the DDT contents of the four locations are compared, the between-location variance is smaller than the random variance and the DDT contents of the four locations therefore do not differ significantly. If the DDD and the DDE contents of the four locations are compared, the between-location variance is greater than the random variance and the DDD and DDE contents of the four locations therefore differ significantly.

The LOD of the SFE method was lower than the suggested EP-based SQC, making SFE coupled to GC-MS a useful method for the determination of the three compounds in sediment.

SFE showed several advantages to Soxhlet extraction. The liquid solvent had poor mass transfer qualities compared to SFE and thus

TABLE 9(c)
STATISTICAL EVALUATION USING THE TWO-WAY ANOVA WITHOUT REPLICATION

SOV	SOQ	DF	Ms	F_{calc}	F_{crit}
Between-methods	0.0000	1	0.0000	0.000	10.13
Between-locations	0.1104	3	0.0368	94.37	9.28
Residual/random	0.0012	3	0.0004		
Total	0.1116	7			

TABLE 10(a)
RESULTS OF THE QUANTITATION OF THE RANDOM VARIANCE, THE BETWEEN-METHODS VARIANCE AND THE BETWEEN LOCATIONS VARIANCE FOR DDT, DDD AND DDE

Statistical calculations	DDT	DDD	DDE
σ_0^2	0.0912	0.0059	0.0004
σ_b^2	0.0826	0.0456	0.0182
σ_T^2	0.0568	0.0033	0.0000

σ_0^2 = Random (experimental) variance
σ_b^2 = Between - location variance
σ_T^2 = Between - method variance
σ_b^2 and σ_b^2 were calculated from the equations
BLMS = $\sigma_0^2 + c\sigma_b^2$
BMMS = $\sigma_0^2 + r\sigma_T^2$ where
c = number of columns
r = number of rows

TABLE 10 (b)
COMPARISON OF THE RATIOS OF σ_B^2 TO σ_0^2 AND σ_T^2 TO σ_0^2 FOR DDT, DDD AND DDE

Statistical calculations	DDT	DDD	DDE
$\dfrac{\sigma_b^2}{\sigma_0^2}$	0.91	7.73	45.0
$\dfrac{\sigma_T^2}{\sigma_0^2}$	0.62	0.56	0.00

resulted in longer extraction times when using the liquid than when using SC-CO$_2$. World-wide the directives are for clean extraction procedures. The use of SC-CO$_2$ is considered to be more environmentally friendly than the organic solvent used. SFE has been validated as a technique to extract DDT, DDD and DDE from sediment.

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