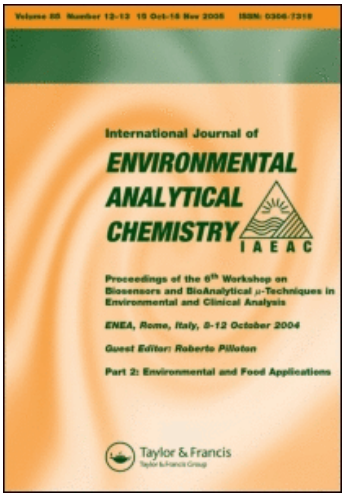

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Multi-residue Analysis of 66 Biocides in River Water, River Sediment and Suspended Solids Samples by Gas Chromatography-Mass Spectrometry

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MULTI-RESIDUE ANALYSIS OF 66 BIOCIDES IN RIVER WATER, RIVER SEDIMENT AND SUSPENDED SOLIDS SAMPLES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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A multi-residue method for the screening and analysis of 66 common pesticides from hydrological samples, including sediment, suspended solids and water is presented. The investigated pesticides belong to the following chemical classes: polychlorinated organic compounds, triazine- and chloroacetanilide herbicides, organophosphorous insecticides and miscellaneous. The method includes fluidized-bed and microwave-assisted extraction for solid samples and solid-phase extraction on C_{18}-cartridges for water samples, followed by a combined purification–separation step on adsorption chromatography using open silica gel columns. Two fractions were eluted separating the 66 analytes into the non-polar and the more polar compounds. All analytes were identified and quantified by gas chromatography coupled with mass spectrometry in selected ion monitoring mode. The method was characterized by recovery experiments and statistical methods and finally applied to environmental river samples during a one-year monitoring program. This method allowed the screening and measurement of the contaminants in all parts of Liao-He and Yangtse rivers (Eastern China) at levels as low as 0.07 ng/L resp. 0.7 ng/L for hexachlorobenzene, with a precision better than 20%.

Keywords: Multi-residue analysis; Water; Sediments; Suspended solids; Organochlorine pesticides; Organophosphorous pesticides; Triazines; Liao-He and Yangtse Rivers (China)

INTRODUCTION

Currently several hundred pesticides of different chemical nature are widely used for agricultural purposes throughout the world. Some are substitutes for the organochlorine compounds which were banned after evidence of their toxicity, persistence and bioaccumulation in the environment had become available [1]. Due to their persistence, the banned organochlorine pesticides, such as DDT or lindane, are still frequently detected even nowadays. Further organochlorine pollutants are environmental emissions from industrial activities, like polychlorinated biphenyls (PCBs) and

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octachlorostyrene (OCS). Owing to the persistence paired with high water solubility, the triazine herbicides and some organophosphorous insecticides are routinely detected in surface and ground waters [2–4]. Additionally, a wide group of organochlorine biocides are found to act as endocrine disrupters, so called environmental estrogens [5], even if they are less potent than natural estrogens, they bioaccumulate owing to their stability and may effect human health.

The contamination of the hydrological system, including water, suspended solids and sediment, by organic pollutants is a matter of great ecological concern. Rivers are depositories of most effluent discharges, leacheates and diffuse agricultural run-off [6]. Shortage of drinking water leads to utilization of river water or at least bank filtrate as drinking water resource. Therefore, an inventory of the contamination with organic pollutants followed by a river water quality assessment is required. Recent research on the fate and transformation of biocides in the environment has pointed out the need for including more and more compounds into multi-residue analysis [7–9]. On the other hand, owing to the physical and chemical properties of the organic pollutants they are distributed between the solid and liquid parts of the hydrological system. On this account, sediments, suspended solids and river water has to be included into the analysis of the total pollutant load of river systems.

Lacorte *et al.* [10] reported a multi-residue method for the determination of 109 compounds after solid-phase extraction (SPE) from water samples without clean up and fractionation. Fillion and Thorp [11] published a gas chromatography-mass spectrometry (GC-MS) method operated in selected ion monitoring (SIM) mode in a single run for the determination of 191 pesticides. Both GC-methods need nearly 90 min for a single run and had been developed for water samples. Multi-residue analysis without clean up and/or fractionation is practicable as long as the samples contain rarely interfering matrix compounds. Common solid samples, like soils or sediments, are complex matrices, which contain plenty of non-polar and polar constituents for analytical interferences [12]. Especially the complex composition of the solid sample extracts with co-extracted non-target compounds like biogenic macromolecules, lipids and pigments requires an efficient sample clean up. Therefore, special attention has to be paid to the development and optimization of the extraction, clean up and fractionation procedures prior to GC-MS-determination. Methods, which are of universal applicability to all parts of the hydrological system – water, suspended solids and sediment – are of great interest for routine analysis and monitoring protocols.

The aims of this work were: To develop an efficient multi-residue method based on SPE for water samples, microwave-assisted extraction (MAE) for suspended solid samples and fluidized-bed extraction (FBE) for sediment samples, followed by a fractionation and clean up by means of adsorption chromatography and gas chromatography (GC) with electron-impact ionization mass spectrometry (EI-MS) in SIM mode for the determination of the selected biocides of the following compound classes: Organochlorine pesticides, organophosphorous pesticides, triazines, chloroacetanilides and miscellaneous. For further analysis two fractions of analytes are practicable, a non-polar fraction, containing PCBs, organochlorine pesticides and some organophosphorous insecticides with chlorophenyl functionality, and a more polar fraction, mainly containing chloroacetanilide and triazine herbicides, and organophosphorous insecticides. Further, this analytical method needed to be applied in practice for the monitoring of the selected biocides in river samples from Eastern China.

EXPERIMENTAL

Reagents and Chemicals

Acetone, *n*-hexane, methanol and dichloromethane were purchased from Promochem (Wesel, Germany, purity: Pico grade, for residue analysis). *n*-Hexane (purity: UniSolv, for organic trace analysis), sulfuric acid (97%, p.a.), hydrogen peroxide (35%, p.a.) and sodium sulphate anhydrous (purity: pro analysis) were obtained from Merck (Darmstadt, Germany). Hexamethyldisilazane (purity: 99%) was purchased from Fluka (Buchs, Switzerland).

Silica gel 60 (0.063–0.200 mm) was obtained from Merck (Darmstadt, Germany) and was activated at 105°C for at least 72 h and stored at the same temperature. Diatomite (filter agent) was acquired from Aldrich (Gillingham, UK).

C_{18}-SPE cartridges (Mega Bond Elut, 1 g) were purchased from Varian (Middleburg, The Netherlands). Glass-fibre filters (ID 47 mm) with a 0.7 μm pore size were obtained from Whatman (Maidstone, GB).

All analytical standard solutions or solid standard reference materials were supplied by Labor Dr. Ehrenstorfer (Augsburg, Germany, purity: > 98%). The compounds are listed in Tables I and II.

Samples

Method development for the solid sample analysis was performed using aliquots of the laboratory reference material S37 [13]. This sediment material was produced according to the general principles applicable for candidate reference material production except the final certification. The raw sediment material was sampled in a side arm of the Yangtse River System (PR China), situated down stream from Nanjing. The wet material was sucked to dryness using a large Büchner funnel (28 cm ID) and shipped to the Joint Research Center (JRC) Ispra (Italy) for further processing, such as drying, crushing, sieving and mixing. After a thorough homogeneity study of the bulk, the material was bottled in 50 g portions. During 7 months a simplified stability study was performed for selected polychlorinated organic compounds (PCOCs); the analytical data for these PCOCs were estimated by isotope dilution GC-MS.

A SPE-enrichment procedure of water samples was developed and optimized using spiked drinking water samples, processed in the same way as the environmental water samples from the Chinese rivers, performing the following steps: Conditioning of the SPE-cartridges, sample enrichment and drying of the sorption bed.

The sampling strategy and procedures for the monitoring program on the Chinese rivers are described in detail elsewhere [14]. Briefly, the sediment samples were taken using a specially designed crab sampler. The wet sample was passed through a 2-mm sieve, homogenized, filled into glass bottles and transported to the base laboratories in China. There, the sediment samples were sucked to dryness using a Büchner funnel; the filter cake was shipped to the JRC for further processing as described above. Water samples were taken at a depth of approximately 1 m by means of specially designed iron made bucket-like container (10 L). Aliquots of 1 L were filled into glass bottles. In the Chinese base laboratories the water samples were passed through glass-fibre filters. The filters were sucked to dryness and enclosed into headspace glass vials (22 mL) for shipping purposes. The resulting filtrate was extracted finally

TABLE I Fraction 1: Compounds in sequence of elution, time windows, retention time, selected ions, and estimated Limit of Detection (LOD), * internal standard

Window	*Start time* (min)	*No*	*Compounds*	*Retention time* (min)	*Target ion* (*m*/*z*)	*Qualifier ions* (*m*/*z*)	*LOD* (pg/μL)
1	0.00	1	Etridiazole	6.29	211	213/183	45.9
2	6.65	2	Chloroneb	6.73	191	193/206	9.2
3	6.80	3	Pentachlorobenzene	6.98	250	252/248	6.5
4	8.40	4	Trifluralin	8.52	306	264	14.6
5	8.65	5	Pentachlorotoluene*	8.75	229	227/231	–
6	8.85	6	α-HCH	9.01	219	181/183	15.4
7	9.10	7	Hexachlorobenzene	9.22	284	286/282	1.8
		8	Pentachloroanisole	9.33	280	282/265	7.3
8	9.50	9	β-HCH	9.69	219	181/183	16.2
		10	Lindan	9.88	219	181/183	24.0
		11	δ-HCH*	10.49	219	181/183	–
9	10.55	12	Chlorothalonil	10.62	266	264/268	8.8
10	10.95	13	PCB 28	11.32	256	258/186	2.4
11	11.45	14	Chlorpyriphos-methyl	11.52	286	288	14.6
12	11.65	15	Heptachlor	11.71	272	274/270	4.0
13	11.80	16	Fenchlorphos	11.88	285	287	9.8
14	12.10	17	PCB 52	12.17	292	290/294	3.4
		18	Aldrin	12.56	263	261/265	6.3
15	12.63	19	Chlorpyriphos	12.70	199	197/314	9.1
16	12.85	20	Trichloronate	13.00	297	299/269	11.8
17	13.20	21	Octachlorostyrene	13.39	380	378/382	2.1
18	13.50	22	Heptachlor epoxide	13.57	183	185/217/353	3.2
19	13.80	23	α-Chlordane	14.02	373	375/377	4.0
20	14.10	24	PCB 101	14.19	326	328/324	3.7
21	14.25	25	Endosulfan I	14.31	241	239/237	32.2
		26	γ-Chlordane	14.39	373	375/377	2.6
22	14.55	27	Prothiophos	14.73	309	311/267	16.1
23	14.80	28	*p*,*p*′-DDE	14.89	318	316	8.3
		29	Dieldrin	14.95	263	261/277	2.7
24	15.20	30	Endrin	15.51	263	265/281	7.5
25	15.65	31	Endosulfan II	15.74	241	265	45.0
26	15.85	32	*p*,*p*′-DDD	15.97	235	237	5.9
		33	*o*,*p*′-DDT	16.06	235	237	23.0
27	16.20	34	PCB 153	16.35	360	362/358	2.3
28	16.70	35	*p*,*p*′-DDT	17.00	235	237	11.7
		36	PCB 138	17.12	360	362	4.4
29	18.50	37	Methoxychlor	18.72	227	228	25.3
30	19.00	38	PCB 180	19.13	396	394/392	3.9
31	21.40	39	*cis*-Permethrin	21.77	183	184/163	8.1
		40	*trans*-Permethrin	22.10	183	184/163	21.0
32	23.00	41	PCB 209*	24.32	498	500/496	–

with pre-conditioned C_{18}-SPE cartridges, which were stored into polyethylene centrifugation containers (Greiner, Kremsmünster, Austria). After arrival of the samples in Europe, the sediment samples were stored in a dry and dark location outside the laboratory, the particulate samples and SPE-cartridges were stored in a refrigerator at +4°C.

Sediment Sample Extraction

Sediment sample preparation was carried out by fluidized-bed extraction (FBE) using a fexIKA 200 control series extractor (Janke & Kunkel GmbH&Co.KG, IKA-Labortechnik, Staufen, Germany) [15,16]. With the standard configuration of this system, a potential for 4 simultaneous extractions is provided. A 10 g aliquot of

TABLE II Fraction 2: Compounds in sequence of elution, time windows, retention time, selected ions, and estimated Limit of Detection (LOD), * internal standard

Window	*Start time* (min)	*No*	*Compounds*	*Retention time* (min)	*Target ion* (*m*/*z*)	*Qualifier ions* (*m*/*z*)	*LOD* (pg/μL)
1	0.00	1	Mevinphos	7.63	127	192	10.7
2	12.80	2	Propachlor	13.02	120	176/169	6.4
3	13.40	3	Ethoprophos	13.83	158	126/139	5.0
4	14.00	4	Desisopropylatrazine	14.21	173	175/158	17.7
		5	Tributhylphosphate*	14.35	99	155	–
5	14.60	6	Desethylatrazine	14.75	172	174/187	14.8
6	17.40	7	Atraton	17.55	196	211/169	12.4
		8	Simazine	17.83	201	186/173	24.9
7	17.95	9	Prometon	18.13	210	225/168	10.6
		10	Atrazine	18.33	200	215/173	9.7
8	18.55	11	Propazine	18.74	214	229/172	10.4
9	19.00	12	Terbuthylazine	19.48	214	229/173	9.8
10	20.50	13	Diazinon	20.79	179	199	8.3
		14	Secbumeton	21.17	196	210	11.2
11	21.40	15	Sebuthylazine*	22.01	200	202/214	–
12	23.00	16	Propanil	23.38	161	163/217	18.2
13	23.80	17	Parathion-methyl	24.07	263	125	5.4
		18	Simetryn	24.38	213	170/155	11.0
14	24.45	19	Alachlor	24.56	188	160	6.3
		20	Ametryn	24.66	227	212/170	20.3
15	24.75	21	Prometryn	24.86	241	184/226	9.4
16	25.05	22	Terbutryn	25.25	226	241	10.4
17	26.80	23	Tetrachlorvinphos	26.94	329	331	8.9
18	27.10	24	Butiphos	27.26	169	202/170	11.4
19	27.50	25	Chlorobenzilate	27.63	251	253	4.6
		26	Fenthion	27.67	278	279	14.4
		27	Fensulfothion	27.68	293	308	8.3
20	28.00	28	Triphenylphosphate*	28.30	326	325	–
21	29.00	29	Azinphos-methyl	29.09	160	132	29.8
		30	Sulprofos	29.21	322	156	41.6
22	29.80	31	Coumaphos	30.04	362	226/364	45.8

sediment sample, mixed with 3 g diatomite to enhance the permeability of the solid bed, was weighted into each extraction tube, which was equipped with a fresh polytetrafluoroethylene-filter (47 mm ID, 10–20 μm pore size, fexIKA). The specimens were extracted with 50 mL of a mixture of *n*-hexane-acetone 4 + 1 (v/v) for 10 extraction cycles. Further FBE conditions were a pre-programmed heating temperature of 85°C for 8 min and a cooling temperature of 30°C [17]. Next, the raw extracts were transferred to 100 mL pear shaped flasks, 200 ng of each internal standard (PCB 209, pentachlorotoluene, delta-hexachlorocyclohexane, triphenylphosphate, tributhylphosphate and sebuthylazine) were added and finally, the extracts were concentrated by means of a rotary evaporator to about 1 mL.

Suspended Solids Sample Extraction

Extraction of suspended particle samples was carried out by microwave-assisted extraction (MAE) using a Multiwave from Paar Physica (Graz, Austria), a system described by Zischka *et al*. [18] for microwave-assisted wet digestion. Beside the special features of this system, the instrument was modified for extraction with organic solvents by installation of a solvent sensor as an additional safety mechanism. The headspace

vials containing the filters with the particulated matter were allowed to reach room temperature before opening, thus avoiding condensate formation. The filters were transferred into quartz extraction vessels and 25 mL *n*-hexane–acetone 1 + 1 (v/v) were added. In order to keep the filters in the fluid phase throughout the entire extraction period, a glass rod (approximately 50 × 3 mm) was put onto the upper surface of each filter. The vessels were closed with Teflon caps and the extraction was performed using the following conditions: 6 sample tray, extraction time: 30 min with ventilation level 1, initial power setting: 1000 W, maximum temperature: 130°C, maximum pressure: 70 bar, cooling time: 15 min with ventilation level 2. After extraction, the filters were removed and rinsed with small portions of fresh extraction solvent. To remove remaining particles the combined solutions of raw extract and washing solution were centrifuged (5 min, 90 g) and the extract was transferred to pear shaped flasks. 200 ng of each of the six internal standards (see above) were added and the raw extracts were concentrated by means of a rotary evaporator to approximately 1 mL. In order to remove remaining water from the sampling, the extracts were dried over 8 g anhydrous Na_2SO_4, rinsed several times with *n*-hexane and concentrated again to approximately 1 mL. Further purification was achieved by clean up on silica gel cartridges according to the procedure described below.

River Water Sample Preparation

SPE in general has now become the preferred method for carrying out simultaneously the extraction and concentration of many pesticides from aqueous samples [6,19]. SPE-C_{18}-cartridges have been chosen for the enrichment of contaminants contained in surface water samples from the both Chinese rivers Yangtse and Liao-He [14]. Remaining water traces from the sampling were first eliminated by centrifugation (15 min, 300 g) and second by eluting the analytes from the C_{18}-cartridges through a bed of 2 g anhydrous Na_2SO_4. Sample elution was carried out with 20 mL of a mixture of *n*-hexane–CH_2Cl_2–acetone 65 : 25 : 10 (v/v/v) into 50 mL pear shaped flasks. After elution, the cartridges were dried by a short flow of nitrogen at room temperature and the drying agent was washed several times with small amounts of fresh *n*-hexane. Finally, 200 ng of each internal standard were added to the eluates and the solutions were concentrated by means of a rotary evaporator to roughly 1 mL.

Sample Clean up and Fractionation

For a simultaneous determination of more than 70 analytes (66 analytes + 6 internal standards) out of one single sample special emphasis has to be given to the clean up procedure. For this purpose fractionation on silica gel cartridges was chosen. The cartridges were prepared by weighting in 1 g activated silica gel and 1 g Na_2SO_4 each into an empty 6 mL glass extraction cartridge (ID 8 mm), which was equipped with a polyethylene frit at its bottom. The solid bed was preconditioned with 40 mL *n*-hexane and packed by a stream of nitrogen. Next, the concentrated raw extract containing also the internal standards was pipetted onto the top of the column. After penetration of the sample volume into the column bed, fraction 1 was eluted with 10 mL *n*-hexane–CH_2Cl_2 (7 + 3, v/v) and collected in a 50 mL pear shaped flask. Thereafter, the receiving flask was changed and the remaining analytes (fraction 2) were eluted from the column with 10 mL CH_2Cl_2 and 12 mL acetone. Both fractions

TABLE III Temperature programs for both GC methods

	Initial temperature (°C)	*Rate* (°C min^{-1})	*Final temperature* (°C)	*Holding time* (min)
Fraction 1	70	–	70	0.5
	70	25	170	–
	170	4	190	–
	190	10	230	–
	230	4	270	–
	270	30	300	5
Fraction 2	80	–	80	0.3
	80	30	140	4
	140	2	175	–
	175	30	290	5

were concentrated by means of a rotary evaporator to approximately 1 mL, blown to dryness by a gentle stream of nitrogen, taken up in 250 µL benzene and transferred to micro-autosampler vials for further analysis.

Gas Chromatography

Analysis was carried out using an Hewlett-Packard HP6890 gas chromatograph equipped with an HP7683 autosampler and splitless injection of 2 µL into a split-splitless injector (Hewlett-Packard, Waldbronn, Germany) with a purge off for 0.5 min. The injector was equipped with a single tapered glass insert packed with a small amount of pesticide grade glass wool (Supelco, Bellefonte, USA) and thermostatted at 250°C. The capillary column used was an HP-5MS, 30 m × 250 µm ID and 0.25 µm film thickness. The carrier gas was helium (Air Liquide, Graz, Austria, 5.0) at a constant flow rate of 1.1 mL/min (fraction 1) respectively 1 mL/min (fraction 2). To achieve best peak separation two different GC-MS-methods were developed, one for each fraction. The GC oven programs for both methods are given in Table III. The gas chromatograph was coupled to an HP5973 mass selective detector operated in El and SIM mode using the time windows and m/z values as listed in Tables I and II. The interface temperature was maintained at 280°C.

The instrument was tuned daily with perfluorotributylamine (PFTBA) using the automatic tune facility of the G1034C Hewlett-Packard MS ChemStation.

RESULTS AND DISCUSSION

Extraction Efficiency

The extraction procedure is a critical step in the analytical cycle. Therefore, the extraction efficiencies of the both optimized extraction procedures FBE and MAE were verified in comparison to the standard Soxhlet extraction using the laboratory reference material S37. For definition of the standard reference procedure, the extraction parameters of the Soxhlet method were chosen according DIN 38414-20 [20]. In practice, 10 g reference sediment were transferred into an extraction thimble (MN 645, 30 × 100 mm, Macherey-Nagel, Düren, Germany), covered with preconditioned

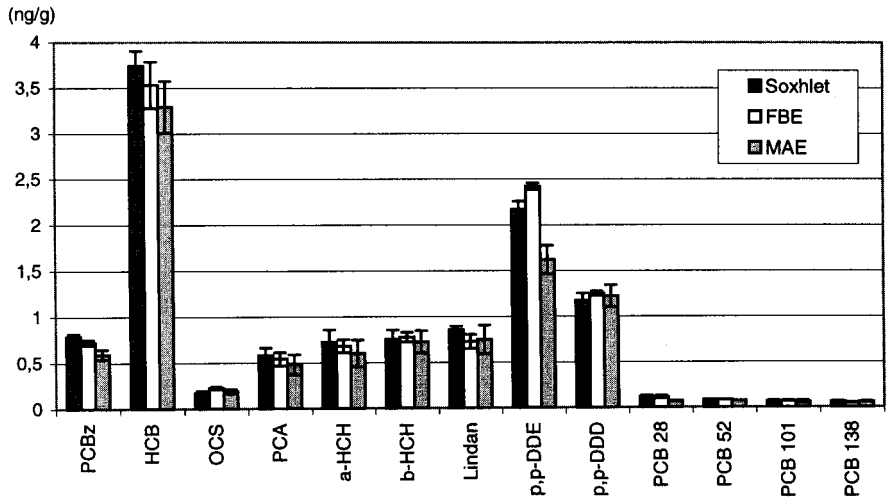

FIGURE 1 Concentrations of polychlorinated pesticides in reference sediment S37, values obtained by Soxhlet extraction, fluidized-bed extraction (FBE) and microwave-assisted extraction (MAE), $n=4$. (PCBz: Pentachlorobenzene, HCB: Hexachlorobenzene, OCS: Octachlorostyrene, PCA: Pentachloroanisole, a-HCH α-HCH, b-HCH: β-HCH).

(72 h at 300°C) glass wool and inserted into a 100 mL Soxhlet extractor. The samples were extracted under reflux with 150 mL of *n*-hexane–acetone 4 + 1 (v/v) for 24 h. Since extraction thimbles are a potential contamination source, they were pre-extracted before use under the same conditions as the samples. The further procedure was performed as described in Chapter Experimental. The results for some selected organochlorine biocides are presented in Fig. 1.

The values obtained by both alternative methods are comparable with that of Soxhlet extraction [21]. Especially, FBE shows excellent and precise results, obtained in 90 min, with 50 mL extraction solvent and with rather low cost equipment compared to other enhanced extraction techniques available. Therefore, the sediment extraction during the monitoring study was performed using FBE. On the other hand, FBE with its principle of a fluidized bed was not applicable to the suspended particle samples adsorbed on glass-fibre filters. Thus, the particulated matter samples were extracted by MAE, although the recoveries obtained by this method were some what lower than by FBE or Soxhlet extraction.

Recovery Experiments for SPE-enrichment

The sample enrichment procedure on C_{18}-SPE cartridges was controlled by systematic recovery experiments. For this purpose, 1 L glass bottles were pre-rinsed with acetone and two times with tap water and filled with 1 L tap water from Graz, Austria (pH = 6.5). 12 tap water samples were spiked with the analytes in the range of 100, 250, 500 and 1000 ng/L each concentration replicated three times. Prior to the extraction of the spiked samples, the SPE-cartridges were conditioned with 10 mL methanol and 2 mL deionized water by means of a glass vacuum filtration unit (Sartorius AG, Göttingen, Germany). Further sample pretreatment was performed following the procedure described in the Chapter Experimental. The mean recoveries as well

as the relative standard deviations (RSD) for all investigated biocides are listed in Table IV.

The results for organophosphorous and organochlorine pesticides are in good agreement with previously reported recoveries [22–24], even the rather low recovery for Mevinphos (50.1%) and Fenthion (54.4%) [25,26]. The recoveries for the more polar triazine and chloroacetanilide herbicides are comparable to values reported by Dupas *et al*. [27] and Barceló and Hennion [6], including also the very low recoveries for the polar atrazine metabolites desethyl- (56.4%) and desisopropylatrazine (20.1%). Owing to the low recoveries of the atrazine metabolites, sample enrichment on C_{18}-SPE-cartridges for these analytes is critical. The metabolite contents obtained by this method are more informal than quantitative. Alternative SPE-materials are already available; examples are porous graphitic carbon (PGC) or styrene divinylbenzene (SDB) polymer, respectively. Barceló and Hennion [6], Quintana *et al*. [28] as well as Thurman and Mills [29] reported recoveries around 100% for both atrazine metabolites using PGC or SDB cartridges. Decreased recoveries obtained for the HCHs and the chlorinated cyclodienes, could be attributed to losses during concentration and evaporation of the solvent extract.

TABLE IV SPE-recoveries (mean ± standard deviation) and relative standard deviation (RSD) for all 66 investigated biocides

Compounds	*Recovery* (%)	*RSD* (%)	*Compounds*	*Recovery* (%)	*RSD* (%)
Alachlor	98.3 ± 6.4	6.5	Lindan	78.3 ± 0.8	1.1
Aldrin	77.8 ± 0.4	0.5	Methoxychlor	84.1 ± 5.2	6.2
α-Chlordan	93.4 ± 4.4	4.7	Mevinphos	50.1 ± 7.6	15.2
α-HCH	79.7 ± 0.6	0.7	Octachlorostyrene	83.5 ± 1.2	1.4
Ametryn	88.5 ± 2.0	2.2	*o,p′*-DDT	78.1 ± 4.8	6.2
Atraton	98.2 ± 2.2	2.2	Parathion-methyl	107.6 ± 7.4	6.8
Atrazine	86.9 ± 1.9	2.2	PCB 28	82.9 ± 4.2	5.1
Azinphos-methyl	101.4 ± 11.9	11.7	PCB 52	84.9 ± 6.4	7.5
β-HCH	78.8 ± 3.4	4.3	PCB 101	84.8 ± 8.1	9.6
Butiphos	83.4 ± 11.9	14.3	PCB 138	84.4 ± 5.3	6.3
Chlorobenzilate	91.1 ± 11.2	12.3	PCB 153	83.6 ± 4.6	5.5
Chloroneb	85.3 ± 7.1	8.4	PCB 180	80.2 ± 3.5	4.3
Chlorothalonil	94.5 ± 7.6	8.1	Pentachloranisole	86.8 ± 1.4	1.8
Chlorpyriphos	94.9 ± 12.0	12.6	Pentachlorbenzene	85.0 ± 3.2	3.8
Chlorpyriphos-methyl	99.0 ± 12.5	12.6	*p,p′*-DDD	83.7 ± 1.5	1.8
cis-Permethrin	92.5 ± 5.3	5.8	*p,p′*-DDE	88.4 ± 4.1	4.7
Coumaphos	95.8 ± 21.6	22.5	*p,p′*-DDT	77.8 ± 4.2	5.4
Desethylatrazine	56.4 ± 0.3	0.5	Prometon	86.7 ± 1.5	1.7
Desisopropylatrazine	20.1 ± 1.5	7.6	Prometryn	88.0 ± 3.8	4.3
Diazinon	85.0 ± 12.3	14.5	Propachlor	98.4 ± 4.6	4.7
Dieldrin	62.7 ± 0.9	1.4	Propanil	90.4 ± 6.4	7.1
Endosulfan I	80.5 ± 0.3	0.4	Propazine	85.8 ± 2.1	2.4
Endosulfan II	49.6 ± 0.4	0.9	Prothiophos	81.6 ± 11.1	13.6
Endrin	73.9 ± 1.0	1.4	Secbumeton	95.2 ± 2.5	2.6
Ethoprophos	102.2 ± 4.3	4.2	Simazine	90.3 ± 1.6	1.8
Etridiazole	89.6 ± 8.5	9.5	Simetryn	93.8 ± 5.9	6.3
Fenchlorphos	103.8 ± 9.2	8.9	Sulprofos	94.4 ± 11.6	12.2
Fensulfothion	89.3 ± 4.3	4.8	Terbuthylazine	87.1 ± 2.6	3.0
Fenthion	54.4 ± 9.8	18.0	Terbutryn	88.5 ± 2.5	2.8
γ-Chlordane	94.6 ± 5.4	5.7	Tetrachlorvinphos	94.0 ± 11.9	12.6
Heptachlor	85.8 ± 1.3	1.7	*trans*-Permethrin	90.3 ± 4.6	5.1
Heptachlor epoxide	74.7 ± 2.3	3.1	Trichloronat	100.1 ± 16.4	16.4
Hexachlorobenzene	83.8 ± 3.1	3.8	Trifluralin	98.2 ± 7.5	7.7

Clean up and Fractionation

The first aim of the clean up step was to remove non-target co-extracted materials like biogenic macromolecules, lipids and pigments, which may interfere with the final determination and quantitation of the compounds of interest. The second objective was to decrease the number of analytes for simultaneous determination by the GC-MS measurement. Adsorption chromatography on open silica columns allows the removal of lipids and other organic material from environmental extracts and the separation of the groups of analytes into appropriate fractions for analysis.

In order to test the efficiency of the clean up procedure, extract solutions spiked with the investigated biocides in the range of 0.1–1 ng/μL were given up on top of the column and then eluted according to the scheme described above. The clean up was performed with 12 replicates and showed acceptable recoveries ranging between 89 and 104% for the high-molecular-mass compounds in fraction 1. Again, lower recoveries were obtained for the more volatile biocides, presumably due to losses during solvent evaporation.

Besides the recovery of the clean up procedure, also the performance of the fractionation was tested. It is essential to have a clear separation of the two fractions, so that the main part of each analyte is found in one of the both fractions. Therefore, each fraction was measured with both GC-MS-methods. The separation was better than 95% for all analytes, except dieldrin, which was found to be 85% in the first fraction. Chromatograms of the fractionation for the both fractions are shown in Fig. 2 from a spiked sediment extract solution.

Addition of Internal Standards

In general, internal standards are added in solution, while the native analytes are in contact with the solid sample matrix of real world samples for a long time, often resulting in a stronger binding to the matrix. As a result of this different behavior, the extraction efficiency can neither be monitored exactly by use of internal standards nor by the calibration procedure. Therefore, the internal standards were added directly after extraction or elution. Thus, they are in solution as well as the analytes of interest and are useful to compensate for losses during extract concentration and clean up.

GC Determination Instrumental Set-up

An important detail of the instrumental set-up is the preparation of the injector inlet. After not more than 50 injections the single taper glass insert was cleaned over-night in a bath of a 1 : 1 mixture of sulfuric acid and hydrogen peroxide. Afterwards the glass insert was rinsed several times with tap water, deionized water and acetone, dried and heated in an oven to 300°C for at least 24 h. After cooling down to room temperature, the glass insert was deactivated in hexamethyldisilazane for 30 min and then heated in an oven to 160°C for 1 h to remove the excess of reagent. After cooling down, the single taper liner was packed with a small amount of pesticide grade glass wool, deactivated again with hexamethyldisilazane for 30 min and heated to 160°C for 1 h and finally reinstalled into the GC-injector. This careful cleaning of the glass insert is mandatory for the separation of the triazines; otherwise adsorption phenomena, peak tailing and degradation occur as reported by Loos and Niessner [30]. On the other

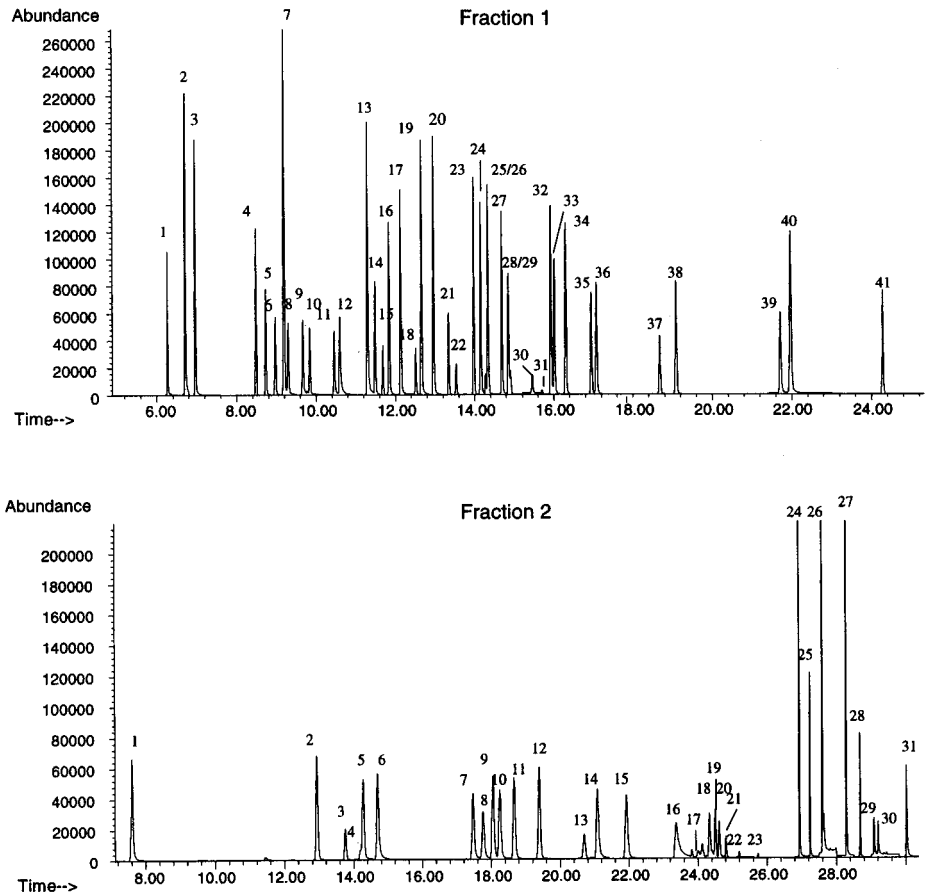

FIGURE 2 GC-MS chromatograms of fraction 1 and 2 obtained from a clean up recovery experiment (200 pg/μL). The numbers refer to the pesticides listed in Tables I (fraction 1) and II (fraction 2).

hand it is well known, that a matrix-enhanced dehydrohalogenation of DDT and some polychlorinated cyclodienes occurs in a 'dirty' GC-inlet [31]. Therefore, after GC-MS-measurement of 10 real samples pesticide standard solutions prepared without the corresponding metabolites were measured and the breakdown-rates were calculated. Breakdown-rates higher than 15% were attributed to a 'dirty' GC-inlet – the glass insert was cleaned. These phenomena can be observed using packed as well as unpacked glass inserts, while packed glass inserts produce much better peak shapes due to a faster evaporation of the injected solution.

Calibration

Calibration was accomplished by internal standardization at 7 concentration levels spanning the range from 5 pg/μL to 1.1 ng/μL. For fraction 1, pentachlorotoluene was chosen as the internal standard for the compounds eluting up to PCB 153 (see Table I), δ-hexachlorocyclohexane (δ-HCH) was the internal standard for lindane and its α- and β-congeners and PCB 209 was selected for the remaining retained

analytes. Again, 3 internal standards were chosen for the second fraction: Sebuthylazine was used as internal standard for all triazine and chloroacetanilide herbicides, tributhylphosphate and triphenylphosphate were chosen for the remaining compounds following EPA Method 8141A [32] and a Restek Application Guide [33]. Chromatographic peak areas were fitted by linear regression. The corresponding correlation coefficients for four replicates ranged from 0.997 to 0.999.

Limit of Detection

Four series of standards were measured for the determination of the first order graphs and the limit of detection (LOD). Calculation was performed with the MS-Excel Macro Validata Version 3.02.54ger (Wegscheider-Rohrer-Neuböck, Leoben, Austria) at the 95% confidence interval, following the Eurochem/CITAC Guide [34]. The LOD values for all target compounds are listed in Tables I and II The residual mass-charge-ratios were used for the qualitative interpretation of the mass spectra: They were allowed to vary up to 20% from a standard value. Larger deviations were attributed to inadequate peak purity and the data were not further used for quantification.

Since the contribution of the uncertainty of the measurement was taken into account by calculating the LODs according to the calibration method, the LODs are rather high. This fact has already been reported by a Spanish group [35], statistically calculated limit of detections are 3–20 times higher than applying the conventional signal/noise ratio 3 : 1 method. Nevertheless, the statistical method should be used since it is based on mathematical data instead of subjective estimation, as has been reported by Lacorte *et al.* [36] and Barceló *et al.* [37].

Application to Environmental Samples

Once the various steps and procedures of the protocol had been characterized, the entire method was applied to environmental samples within the framework of a shared-cost action supported by funding from the European Commission. During a one-year monitoring program on the Eastern Chinese rivers Liao-He and Yangtse, an evaluation of the presence of polychlorinated pollutants and a screening for some additional polar pesticides was performed [17]. The spatial and seasonal variations, distributions and dynamics of the biocides in both rivers were investigated. During this study, the participating laboratories analyzed the samples by their own methods, using their own calibration solutions and measurements. The number of biocides analyzed by the individual laboratories consisted of a limited set of analytes, only PCOCs were determined. Inter-comparison of the collected results for these substances showed good agreement between the laboratories and their methods.

The results of the monitoring campaign are reported in detail elsewhere [38–42]. Briefly, 19 analytes from the investigated 66 pesticides could be detected. Beside the target analytes – polychlorinated organic compounds (PCOCs) – which were found in moderate concentrations, larger amounts of triazines were found in the water of the Liao-He River. In Table V typical concentration ranges for the target analytes in both investigated rivers are summarized. Analytes which are not listed in this table were not detected above the calculated limit of detection during the whole monitoring campaign.

The Liao-He region in Eastern China has traditionally a strong agricultural background, resulting in high concentrations of detected triazines and HCH-congeners.

TABLE V Concentration range for positive determined target analytes in the Eastern Chinese Rivers Liao-He and Yangtse (–: all values lower than the estimated limit of detection)

Compounds	*Liao-He sediments* (ng/g)	*Particulates* (ng/L)	*Water* (ng/L)	*Yangtse sediments* (ng/g)	*Particulates* (ng/L)	*Water* (ng/L)
Pentachlorobenzene	–	–	–	0.2–1.4	–	–
Hexachlorobenzene	< 0.1–0.2	< 0.5–1.2	< 0.5–0.8	0.3–4.9	< 0.5–0.8	< 0.5–0.8
Octachlorostyrene	–	–	–	< 0.1–0.2	–	–
Pentachloroanisole	–	–	–	< 0.2–1.3	–	–
α-HCH	< 0.2–1.8	–	< 2.4–83.18	< 0.2–0.9	–	–
β-HCH	< 0.2–1.4	< 2.1–4.9	< 2.1–17.8	< 0.2–1.2	–	–
Lindan	< 0.5–6.5	–	< 4.7–5.8	< 0.5–1.1	–	–
o,p′-DDT	–	–	–	< 0.3–0.6	–	–
p,p′-DDT	–	–	–	< 0.8–1.8	–	–
p,p′-DDE	< 0.1–1.5	< 0.9–7.9	< 0.9–1.6	0.4–2.3	–	–
p,p′-DDD	< 0.5–0.7	–	–	< 0.5–1.4	–	–
Atrazine	< 2.4–3.3	< 2.4–11.5	56.1–1370.3	–	–	< 2.4–5.6
Desethylatrazine	–	–	19.2– 135.9	–	–	–
Desisopropylatrazine	–	–	< 4.4–47.7	–	–	–
Prometryn	–	–	< 3.2–6.2	–	–	< 3.2–10.3
Propazine	–	–	< 2.6–47.3	–	–	–
Simetryn	–	–	< 6.0–16.5	–	–	–
Parathion-methyl	–	–	–	–	–	< 4.7–26.3
Alachlor	–	–	< 1.7–13.6	–	–	< 1.7–3.9

Due to the actual use of the pesticides, the highest triazine values – up to 1.4 µg/L – were measured in late spring, while the highest HCH-concentrations were detected in late autumn. The atrazine values in the sediment and particulate matter samples are correlated with the values of the aqueous phase: High concentrations in water imply high values in the solid samples, although atrazine is highly water-soluble. The measured concentrations of the three HCH-isomers are indicating a relatively fresh application of a technical quality lindane (65–70% α-HCH, 7–10% β- and 14–15%. γ-isomer). Other pesticides are not affecting the river water of the Liao-He. The Yangtse River, known as third largest river of the world, has a reported discharge rate of 25.000 m^3/s [43]. Due to the low load with particulated matter, only hexachlorobenzene was detected in concentrations up to 0.8 ng/L. In the corresponding river water samples some pesticides could be found during the late spring and summer in rather low concentrations. Owing to the high discharge rate, even low concentrations may cause a considerable mass transport. In the sediment samples several PCOCs were detected in concentrations up to 5 ng/g.

CONCLUSIONS

A multi-residue analytical method based on GC-MS measurement has been developed and characterized for the screening analysis of 66 common pesticides. This protocol includes the extraction of solids by FBE and MAE, and SPE on C_{18}-cartridges of water samples. Next, the extracts were cleaned and separated by adsorption chromatography on an open column into two fractions, the first containing the non-polar compounds and the second mainly the polar triazines, chloroacetanilide herbicides and organophosphorous insecticides. Finally, the contaminants are identified and quantified by GC-MS. This procedure enables the determination of a considerable variety

of ecologically relevant compounds in hydrological systems, including sediment, suspended solids and river water, at concentrations as low as 0.07 ng/g (0.7 ng/L) for hexachlorobenzene up to 1.15 ng/g (11.5 ng/L) for etridiazole. The limits of detection were calculated according the general roles of statistics. The precision of the method is better than 20% depending on the contamination level. The participation in an international monitoring program has proven the accuracy and applicability of this method for an analytical characterization of contaminated environmental samples.

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References

[1] D. Barceló, *Analyst*, **116**, 681–689 (1991).
[2] J.-J. Yu, *Water Res*., **36**, 1095–1101 (2002).
[3] T.A. Albanis, D.G. Hela, T.M. Sakellarides and I.K. Konstantinou, *J. Chromatogr. A*, **823**, 59–71 (1998).
[4] R.D. Dean, G. Wade and I.J. Barnabas, *J. Chromatogr. A*, **733**, 295–335 (1996).
[5] S.A. Ahmed, *Toxicology*, **150**, 191–206 (2000).
[6] D. Barceló and M.-C. Hennion, *Trace Determination of Pesticides and their Degradation Products in Water*. Elsevier Publisher, Amsterdam, The Netherlands (1997).
[7] V. Pichon, *Analusis Magazine*, **26**, M91–M98 (1998).
[8] V. Pichon, C. Cau dit Coumes, L. Chen and M.-C. Hennion, *Int. J. Environ. Anal. Chem*., **65**, 11–25 (1996).
[9] H. Sabik, R. Jeannot and B. Rondeau, *J. Chromatogr. A*, **885**, 217–236 (2000).
[10] S. Lacorte, I. Guiffard, D. Fraisse and D. Barceló, *Anal. Chem*., **72**, 1430–1440 (2000).
[11] J. Fillion and J. Thorp, *Hewlett-Packard Application Note 228–348* November (1995).
[12] C.F. Poole and S.K. Poole, *Anal. Commun*., **33**, 11H–14H (1996).
[13] B.M. Gawlik, D. Martens, B. Henkelmann, K.-W. Schramm, A. Kettrup and H. Muntau, *Fresenius J. Anal. Chem*., **367**, 232–224 (2000).
[14] B.M. Gawlik, C. Sun, X. Quan, D. Martens and B. Platzer, In: B.M. Gawlik, B. Platzer, H. Muntau, (Eds.), *Monitoring of Chlorinated and Other Organic Trace Compounds in Selected Stretches of the Liao and Yangtse River* (*China*), pp. 35–48. European Commission EUR-Report 19531 EN, Ispra, Italy (2000).
[15] H. Siegel, *GIT Fachz. Lab*., **41**, 86–87 (1997).
[16] M. Gfrerer, M. Stadlober, B.M. Gawlik, T. Wenzl and E. Lankmayr, *Chromatographia*, **53**, 442–446 (2001).
[17] M. Gfrerer, T. Wenzl, E. Lankmayr, G. Knapp, C. Sun and X. Quan, In: B.M. Gawlik, B. Plazer and H. Muntau, (Eds.), *Monitoring of Chlorinated and Other Organic Trace Compounds in Selected Stretches of the Liao and Yangtse River* (*China*), pp. 63–83. European Commission EUR-Report 19531 EN, Ispra, Italy (2000).
[18] M. Zischka, P. Kettisch, A. Schalk and G. Knapp, *Fresenius J. Anal. Chem*., **361**, 90–95 (1998).
[19] M.C. Bruzzoniti, C. Sarzanini and E. Mentasti, *J. Chromatogr. A*, **902**, 289–309 (2000).
[20] DIN 38414-20: *Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Schlamm and Sedimente* (1996).
[21] D. Martens, M. Gfrerer, T. Wenzl, A. Zhang, B.M. Gawlik, K.-W. Schramm, E. Lankmayr and A. Kettrup, *Anal. Bioanal. Chem*., **372**, 562–568 (2002).
[22] I. Tolosa, J.W. Readman and L.D. Mee, *J. Chromatogr. A*, **725**, 93–106 (1996).
[23] E. Viana, M.J. Redondo, G. Font and J.C. Moltó, *J. Chromatogr. A*, **733**, 267–274 (1996).
[24] S. Bengtsson and Å. Ramberg, *J. Chrom. Sci*., **33**, 554–556 (1995).
[25] C. Molina, P. Grasso, E. Benfenati and D. Barceló, *J. Chromatogr. A*, **737**, 47–58 (1996).
[26] D. Lambropoulou, T. Sakellarides and T. Albanis, *Fresenius J. Anal. Chem*., **368**, 616–623 (2000).
[27] S. Dupas, P. Scribe and J.F. Dubernet, *J. Chromatogr. A*, **737**, 117–126 (1996).
[28] J. Quintana,l. Martí and F. Ventura, *J. Chromatogr. A*, **938**, 3–13 (2000).

[29] S.M. Thurman and M.S. Mills, *Solid Phase Extraction – Priciples and Practice*. Wiley, New York, USA (1998).
[30] R. Loos and R. Niessner, *J. Chromatogr. A*, **835**, 217–229 (1999).
[31] W.T. Foreman and P.M. Gates, *Environ. Sci. Technol.*, **31**, 905–910 (1997).
[32] EPA Method 8141A, United States Environmental Protection Agency, Revision 1 (1994).
[33] D. Smith, *Restek-Advantage*, **3–5** (1997).
[34] Eurochem/CITAC Guide: *Quantifying Uncertainty in Analytical Measurement*, 2nd Edn. (2000)
[35] C. De la Colina, A. Peña, M.D. Mingorance and F. Sánchez Rasero, *J. Chromatogr. A*, **733**, 275–281 (1996).
[36] S. Lacorte, C. Molina and D. Barceló, *Anal. Chim. Acta*, **281**, 71–84 (1993).
[37] D. Barceló, S. Chiron, S. Lacorte, S. Martinez, E. Martinez, J.S. Salau and M.-C. Hennion, *Trends Anal. Chem.*, **13**(9), 352–361 (1994).
[38] Y. Dong, C. Sun, S. Xu, J. Dai, J. Fen, X. Jiang and L. Wang, *Bull. Environ. Contam. Toxicol.*, **64**, 383–389 (2000).
[39] X. Jiang, D. Martens, K.-W. Schramm, A. Kettrup, S. Xu and L.Wang, *Chemosphere*, **41**, 901–905 (2000).
[40] S. Xu, X. Jiang, Y. Dong, C. Sun, J. Feng, L. Wang, D. Martens and B. M. Gawlik, *Chemosphere*, **41**, 27–33 (2000).
[41] B.M. Gawlik, B. Platzer and H. Muntau (Eds.), *Freshwater Contamination in China – Current Topics of Research*. European Commission EUR 19770 EN, Office for Official Publications of the European Communities, Luxembourg (2001).
[42] M. Gfrerer, D. Martens, B.M. Gawlik, T. Wenzl, A. Zhang, Q. Xie, C. Sun, J. Chen, B. Platzer, E. Lankmayr and A. Kettrup, *Chemosphere*, **47**, 455–466 (2002).
[43] G. Wei-Bin, *SCOPE/UNEP Sonderband*, **58**, 539–557 (1985).