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Journal of Chromatography A, 737 (1996) 101–107

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatographic determination of ^{14}C-labelled terbuthylazine and principal degradates in percolation water and soil extracts from leaching experiments

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Abstract

A new very porous polystyrene–divinylbenzene copolymer with large surface area as an adsorber for solid-phase extraction (SPE), was used for the analyses of terbuthylazine and principal degradates. It showed good recovery rates even for the dealkylated hydroxy metabolites. The polymer was treated similarly to a reversed-phase material, samples of 1000 ml showed no breakthrough for most of the terbuthylazine degradates. A detection limit of 0.1 μg/l could be reached even for the most polar compounds (de-*tert.*-butyldeethylterbuthylazine and de-*tert.*-butylhydroxyterbuthylazine) with recovery rates of 22 and 30%. Percolated water and soil extracts from column experiments, obtained with water and methanol, were analysed using the copolymer sorbent. Analyses were performed on a HPLC system with UV–Vis detection and a scintillation counter. The compounds were separated on a RP-8 column with an acetonitrile–phosphate-buffer gradient. In addition to terbuthylazine applied 2 months prior to extraction, deethyl- and hydroxyterbuthylazine were detected in the soil extracts; de-*tert.*-butylterbuthylazine was found only in traces.

Keywords: Soil; Environmental analysis; Triazines; Pesticides; Terbuthylazine

1. Introduction

The herbicide terbuthylazine (TA) has been used in Germany, mostly for maize protection, since 1990. It was principally introduced as a substitute for atrazine, which was banned because of frequent ground water pollution. The physical properties of TA indicate lower water solubility and stronger soil sorption as compared to atrazine [1]. Nevertheless, TA has already been detected in ground water in Germany [2]. Consequently, there is a need to investigate the leaching ability and transformation of TA.

There are many published methods for the analysis of *s*-triazines in water and soil, in particular for atrazine, but also for TA and dealkylated metabolites [3–5]. All these methods include a SPE process prior to chromatographic separation with gas chromatography or high performance liquid chromatography (HPLC). The most widely used adsorber is a C_{18} material, which is only suitable for *s*-triazines and less polar metabolites [5,6]. A variety of adsorber materials have been utilised in recent years, although routine analyses were seldom performed [7–11].

A non-ionic, highly porous polystyrene–divinylbenzene copolymer (Wofatit Y 77) with an extremely large surface area has been introduced recently [12,13]. The retention mechanism of chloro-

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0021-9673/96/$15.00
SSDI 0021-9673(95)01343-1

triazines with a similar copolymer was explained by Coquart et. al. [14] and Hennion and Pichon [15] through $\pi-\pi$ interactions between the aromatic structure of the sorbent and π-electrons of the analyte.

The aim of this work is to study the profile distribution of TA and principal degradates in a laboratory experiment with soil using the new adsorber polymer for SPE.

2. Experimental

2.1. Chemicals and materials

The soil was taken from the Ap-horizon of an agricultural soil; physical properties were: organic carbon 1.13%, clay 4%, silt 8%, sand 88%, pH 5.8 ($CaCl_2$).

LiChrolut EN (Merck, Darmstadt, Germany) cartridges with 200 mg polystyrene–divinylbenzene copolymer were used for solid-phase extraction. Physical properties of the adsorber were: surface area 1200 m^2/g, particle size 40–120 μm, pore volume 0.75 mg/l.

Water for HPLC and dilution steps was distilled using a distilling apparatus (Heraeus, Hanau, Germany).

Uniformly ^{14}C-ring labelled TA (Sigma, St. Louis, USA) was purified by thin-layer chromatography (>99.5%) and mixed with the formulation Gardoprim (Ciba Geigy, Switzerland).

Non-radioactive standards were purchased from Aldrich (Steinheim, Germany) and Ehrenstorfer (Augsburg, Germany)

2.2. Apparatus

Glass cylinders (100 mm×50 mm I.D.) filled with the disturbed soil were connected to a suction pump (100 mbar) in order to determine transport and transformation pathways. The liquid chromatographic system consisted of a solvent pump (L 6200 Merck/Hitachi, Darmstadt, Germany) serially attached to a UV-detector (L 4250 Merck/Hitachi) and a radioactive monitor (LB 506 C-1 Berthold, Wildbad, Germany), respectively. A reversed-phase C_8 column (LiChrospher 100 RP-8, 5 μm, 250×4 mm Merck) was used for separation. Samples were filtered with 0.2-μm Anopore microcentrifugation tube filters (Whatman, Kent, UK). Radioactive measurements for ^{14}C determination were performed with a Tri Carb 1900 TR liquid scintillation analyser (Canberra Packard, Germany). Soil samples were oxidized after organic extraction using a Tri Carb 306 oxidizer (Canberra Packard).

2.3. Soil treatment and sampling

^{14}C-Labelled TA mixed with the formulated herbicide was applied to the soil surface; the soil had been air-dried prior to filling the glass cylinders. The application rate was 1 kg/ha (in accordance with agricultural practice) and 2.25 μCi of radioactive material per glass cylinder. The soil surface was irrigated continuously with 0.01 *M* $CaCl_2$ solution at 1000 mm. Percolated water was collected in 50 ml portions. The leaching experiment was stopped 2 months after application and the soil cores were cut into layers (0–1 cm (A), 1–2 cm (B), 2–4 cm (C) and 4–8.5 cm (D)).

2.3.1. Water extraction

The air-dried and weighed soil (between ca. 30–150 g depending on the segments) was transferred into glass-stoppered Erlenmeyer flasks and bidistilled water was added (100 ml for horizon A and B, 200 ml for C and D). The mixture was agitated in a rotatory shaker for 24 h. After sedimentation for 8 h the supernatant was centrifuged (10 000 rpm for 20 min). The extraction method was then repeated.

2.3.2. Organic extraction

The water extracted soil was dried again at 50°C. The weighed soil was Soxhlet extracted with methanol–water (80:20, v/v) for 16 h. The extract was evaporated in a rotary evaporator to remove the methanol.

Liquid samples (1 ml) of the percolate and of each extraction step were taken and the radioactivity was determined. Aliquots of the extracted soil horizons were analysed for non-extractable ^{14}C-residues by combustion to $^{14}CO_2$.

2.4. SPE

The extraction cartridge was treated with 3 ml of methanol and 3 ml of water–methanol (99:1%, v/v). The sample solution (pH 7–7.5) was first sucked through a 70-μm filter and then through the adsorber cartridges at a flow-rate of 3–5 ml/min. The adsorber was washed with 10 ml of water and then dried with nitrogen for 20 min. The copolymer was eluted with 4×1 ml methanol, between elution steps the organic solvent was allowed to wet the adsorber for at least 1 min. The methanol was evaporated to dryness using a gentle stream of nitrogen and dissolved in 250 μl phosphate buffer–acetonitrile (95:5, v/v).

2.5. HPLC analysis

TA and its metabolites are weak bases with pK_a values principally in the range between 2 and 5. A phosphate-buffer (pH 7.2) was used in order to ensure separation of unprotonated compounds. Separation was performed with a gradient using two different solvents: (A) 100% acetonitrile, (B) 3 m*M* NaH_2PO_4/Na_2HPO_4 (1:2) solution in water. Chromatographic separation was carried out at a flow-rate of 1 ml/min and 20 μl injection. The solvent program started at 5% acetonitrile and was kept constant for 0.5 min. A linear gradient increased acetonitrile to 70% within 20 min. and was again constant for 5 min before returning to initial condition. Detection wavelengths for determination of the recovery rates were 210 nm, except for TA which was detected at 220 nm. Radioactive detection was carried out with a 150 μl-yttrium–glass cell.

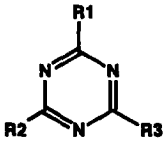
3. Results and discussion

TA is degraded in soil by chemical and microbial processes. According to Haefner [16] several degradates occur through hydrolysis and dealkylation. The chemical structures of the investigated analytes are listed in Table 1.

3.1. SPE method development

Initial studies were carried out to determine the recovery of TA and principal degradates. Four different volumes, between 250 and 1000 ml, of bidistilled water were spiked with 1 μg/l of each component and passed through an extraction cartridge. The recovery rates for the dealkylated compounds, shown in Fig. 1A, were ca. 100% with the exception of the completely dealkylated and very polar dealkylterbuthylazine (DEDTTA). Even the hydroxylated metabolites hydroxyterbuthylazine (OHTA) and deethylhydroxyterbuthylazine (DEOHTA) were well retained; de-*tert.*-butylhydroxyterbuthylazine (DTOHTA) showed an acceptable

Table 1
Chemical structures of terbuthylazine and degradates



Substances	Abbreviations	Substituents		
		R_1	R_2	R_3
Terbuthylazine	TA	Cl	NHC_2H_5	$NHCHC_3H_9$
Deethylterbuthylazine	DETA	Cl	NH_2	$NHCHC_3H_9$
De-*tert.*-butylterbuthylazine	DTTA	Cl	NHC_2H_5	NH_2
Dealkylterbuthylazine	DEDTTA	Cl	NH_2	NH_2
Hydroxyterbuthylazine	OHTA	OH	NHC_2H_5	$NHCHC_3H_9$
Deethylhydroxyterbuthylazine	DEOHTA	OH	NH_2	$NHCHC_3H_9$
De-*tert.*-butylhydroxyterbuthylazine	DTOHTA	OH	NHC_2H_5	NH_2

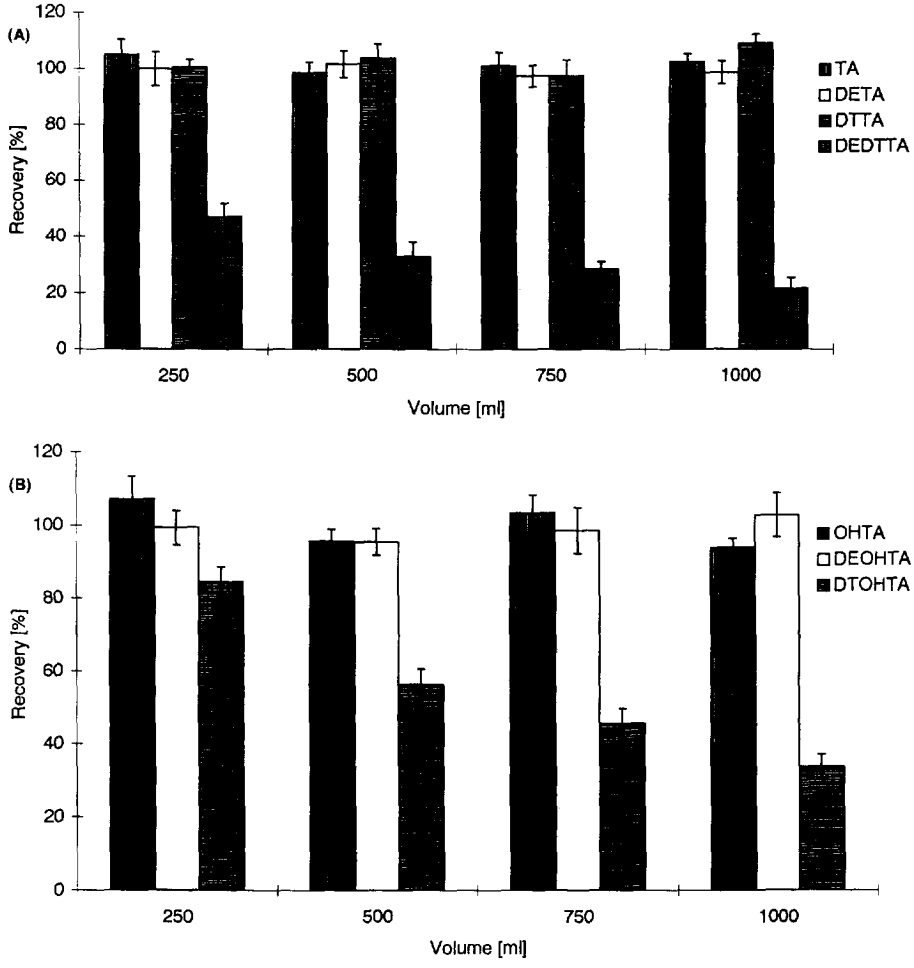


Fig. 1. (A) Recovery of terbuthylazine and dealkylated degradates added to bidistilled water. Bars and standard deviations are from three replicates (spike level 1 μg/l). (B) Recovery of terbuthylazine and hydroxylated degradates added to bidistilled water. Bars and standard deviations are from three replicates (spike level 1 μg/l).

value only for 250 ml sample volume, larger volumes decreased the recovery dramatically (Fig. 1B.).

Finally we determined the recovery rate at a spiking level of 0.1 μg/l in tap water in order to meet the European Community (EC) directive on drinking water (Table 2).

3.2. Percolate and soil samples

Analyses of percolated water revealed only deethylterbuthylazine (DETA) in the range between 5 and 30 μg/l but revealed neither the herbicide itself nor other degradates.

Table 2
Recovery of terbuthylazine and degradates added to 1 l of tap water

Substances	Recovery [%]	Confidence interval (95%, $n=3$)
TA	101.5	±5.5
DETA	97.4	±4.7
DTTA	96.5	±3.8
DEDTTA	20.1	±13.1
OHTA	90.2	±4.0
DEOHTA	97.2	±5.6
DTOHTA	30.1	±14.0

Spike level 0.1 μg/l.

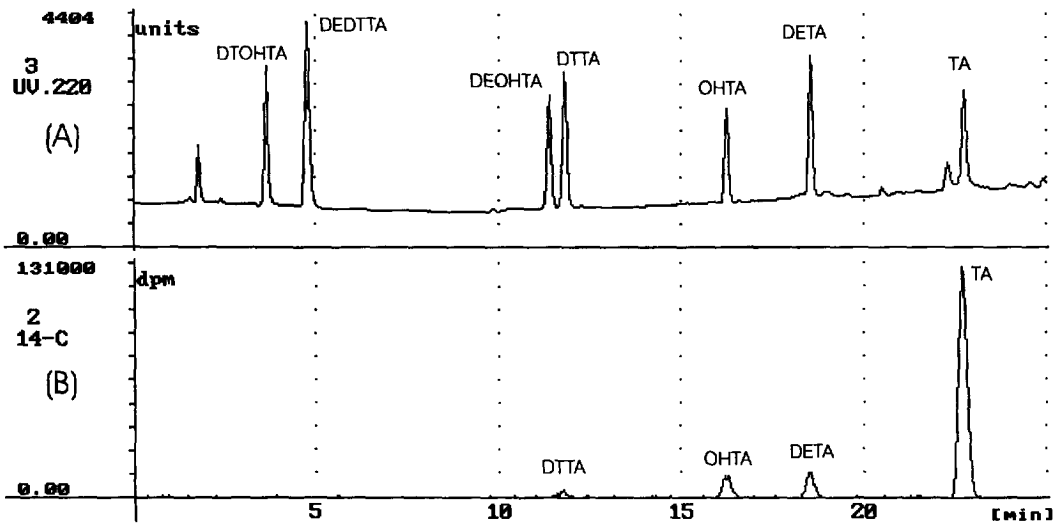


Fig. 2. (A) Standard mixture of non-radioactive terbuthylazine and degradates. (B) Typical chromatogram of a water extracted soil segment.

Chromatograms of a non-radioactive standard mixture and a typical sample are shown in Fig. 2A and B. The distribution pattern of the extracted metabolites dependent on soil depth is presented in Fig. 3A and B.

The ^{14}C distribution at the end of the leaching experiment, two months after the application, is shown in Table 3. The data distinguish between the total percolated amount, the water- and the organic extracts and the remaining "non-extractable" radioactivity, often referred to as bound residues.

Detection limits of soil residues were: (A) and (B): 3 μg/kg, (C): 1.5 μg/kg, (D): 0.6 μg/kg. These values were calculated from the detection limit of the HPLC radioactive monitor, according to DIN 32645 [17]. The fortifying level of the solid-phase extraction, assuming complete recovery, was taken into account; finally the values were projected to 1 kg of soil mass.

The amount of water-extractable TA was almost three times higher than the amount in the organic extract. Only a minor portion of the herbicide (11%) leached below 4 cm within two months. The distribution of DETA was found to be more homogeneous throughout the soil core, hence the leaching ability was greater. Similarly the major portion of DETA (94%) was found in the water extract, which indicates a lower K_D-value as compared to TA. Similar results were obtained by Brouwer et. al. [18] using atrazine.

OHTA was detected at the highest concentrations among the metabolites. The average extractable amount of OHTA, in the range of 3 μg/kg–109 μg/kg, exceeded that of DETA by 88%. Winkelman and co-workers [19] reported similar results for atrazine, whereby highest concentrations of hydroxyatrazine were found to be about 500 μg/kg. This was partly due to the longer half-life of hydroxyatrazine as compared to atrazine and the other metabolites [20]. Raju et. al. [21] achieved equivalent results four years after application.

Comparison of the two different extraction methods demonstrated the strong adsorption of OHTA to soil particles. The concentration in the methanolic extract was even greater than in the water extract. In organic extracts hydroxyatrazine was detected in higher concentrations than the chlorinated transformation products [20].

De-*tert.*-butylterbuthylazine (DTTA) played only a minor role among the detected degradates. Only 1% of the applied herbicide was found to be metabolised to DTTA. This is comparable to measurements of atrazine and dealkylated metabolites in the unsaturated zone from Mills and Thurman [22].

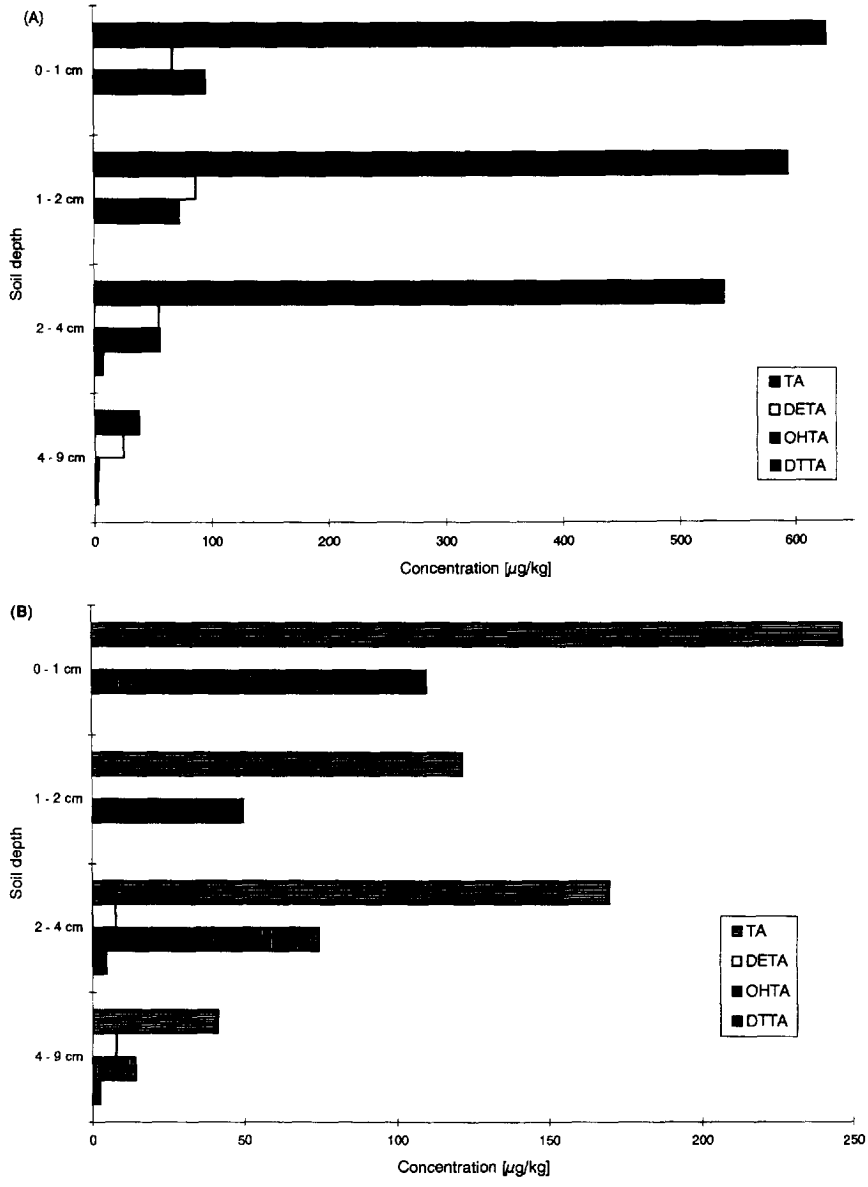


Fig. 3. (A) Profile distribution of water extractable residues of terbuthylazine and degradates in soil. ^{14}C-labelled terbuthylazine was applied on the surface of small columns two months prior to extraction. (Mean values of three replicates are shown). (B) Profile distribution of organic extractable residues of terbuthylazine and degradates in soil. ^{14}C-labelled terbuthylazine was applied on the surface of small columns two months prior to extraction. Mean values of three replicates are shown.

4. Conclusion

A new styrol–divinylbenzene copolymer was introduced for the SPE of TA and degradates. A method was established, which allows the preconcentration of TA from aqueous matrices and methanolic extracts from soil. The sorbent fulfils the requirements for the detection level of 0.1 μg/l in accordance with the European Community (EC) directive on drinking water, even for the more polar metabolites, such as hydroxy- and dealkylterbuthylazine.

Table 3
Distribution of ^{14}C two months after application to soil surface

% Applied	% Recovered			
Total ^{14}C	Percolate	Water extractable	Organic extractable	Nonextractable
98.3	3.5	51.7	28.0	15.1

Mean values of three replicated leaching experiments.

Small cylinders packed with agricultural soil were used to determine transport and transformation pathways of TA. The soil cores were extracted with water followed by methanol 2 months after the application of ^{14}C-labelled TA. As a result, TA was mostly detected within the first 4 cm. DETA and OHTA were found throughout the soil, the latter was measured at the highest concentration among the degradates. DTTA was detected only at trace levels below 2 cm.

Acknowledgements

We would like to thank A. Attar for the purification of ^{14}C-labelled TA.

References

[1] N. Burkhard and J.A. Guth, Pestic. Sci., 12 (1981) 45–52.
[2] C. Skark and N. Zullei-Seibert, Vom Wasser, 82 (1994) 91–105.
[3] H.-R. Buser, Environ. Sci. Technol., 24 (1990) 1049–1058.
[4] C.D. Adams and S. Randtke, J. Environ. Qual., 20 (1991) 540–547.
[5] E.M. Thurman, M. Meyer, M. Pomes, C.A. Perry and A.P. Schwab, Anal. Chem., 62. (1990) 2043–2048.
[6] T.R. Steinheimer and M.G. Ondrus, U.S. Geological Survey, Water Resources Investigations, Report 89-4193; U.S. GPO: Washington, DC, 1990a; 19 pp.
[7] T.R. Steinheimer, J. Agric. Food Chem., 41 (1993) 588–595.
[8] R.N. Lerch, and W.W. Donald, J. Agric. Food Chem., 42 (1994) 922–927.
[9] V. Pacakova, K. Stulik and M. Prihoda, J. Chromatogr., 442 (1988) 147–155.
[10] Q. Wenheng, N.A. Schultz, J.D. Stuart, J.C. Hogan and A.S. Mason, J. Liq. Chromatogr., 14 (1991) 1367–1392.
[11] M. Berg, S.R. Mueller and R.P. Schwarzenbach, Anal. Chem., 67 (1995) 1860–1865.
[12] W. Dedek, L. Weil and L. Feistel, Vom Wasser, 78 (1992) 155–164.
[13] B. Edelmann, W. Dedek, L. Weil and R. Niessner, Fresenius J. Anal. Chem., 343 (1992) 148–149.
[14] V. Coquart, P. Garcia-Camacho and M.-C. Hennion, Int. J. Environ. Anal. Chem., 52 (1993) 99–112.
[15] M.-C. Hennion and V. Pichon, Environ. Sci. Technol., 28 (1994) 567A–583A.
[16] M. Haefner, Schriftenr. Verein WaBoLu, 79 (1988) 261–294.
[17] Deutsches Institut für Normung, Nachweis- und Bestimmungsgrenzen, (1990), Entwurf DIN 32645.
[18] W.W.M. Brouwer, J.J.T.I. Boesten and W.G. Siegers, Weed Research, 30 (1990) 123–128.
[19] D.A. Winkelmann and S.J. Klaine, Environ. Toxicol. Chem., 10 (1991) 335–345.
[20] D.A. Winkelmann and S.J. Klaine, Environ. Toxicol. Chem., 10 (1991) 347–354.
[21] G.S. Raju, J.A. Millette and S.U. Khan, Chemosphere, 26 (1993) 1429–1442.
[22] M.S. Mills and E.M. Thurman, Environ. Sci. Technol., 28 (1994) 600–605.