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Analysis of Chemical Constituents of Volatiles and Petroleum Ether Extract from the Branch of *Zanthoxylum bungeanum* by GC-MS

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The essential volatiles were extracted by water steam distillation and the petroleum ether extract was extracted with alcohol and then petroleum ether from the branch of *Zanthoxylum bungeanum*. The chemical constituents of essential volatiles and petroleum ether extract were analyzed by GC-MS and their relative contents were calculated by area normalization method. 45 peaks were obtained and 34 components were identified from the volatiles and the major components were terpenes, alcohols and fats. As for petroleum ether extract, 60 peaks were obtained and 50 components were identified and the major components were organic acids, esters and alkanes.

Keywords: *Zanthoxylum bungeanum*, Volatiles, Petroleum ether, GC-MS.

INTRODUCTION

The genus *Zanthoxy lum L.* consists of about 250 species[1], which are mainly distributed in tropical and subtropical areas situated in Asia, America, Africa and Oceania[2-6]. In China, 45 species and 13 varieties are mainly distributed in south of the Yangtze river and southwest Provinces. Among them, 18 species are used as traditional Chinese medicine. The fruits, roots, branches, leaves of *Zanthoxy lum L.* are all medicine, which has been widely used for analgesia, anesthesia, anti-bacterial, pesticides and anti-tumor[7,8]. The plants of this genus are well known for containing several types of compounds, including volatile oil, alkaloids, amides, lignans, coumarins, fatty acids, flavones, terpenes, sterols and hydrocarbons[9-13]. Although many studies have shown the chemical constituents of *Zanthoxy lum L* extracted from the fruits or roots, there is little research on the compositions of branches. So the purpose of this study was to identify these compounds in the branches of *Zanthoxy lum L.* The gas chromatography-mass spectrometry (GC-MS) and computer spectrum search technique were used to identify the chemical composition of *Zanthoxylum bungeanum*. This study can provide test basis for the rational development and utilization of *Zanthoxylum resources*.

EXPERIMENTAL

The branches of *Zanthoxylum bungeanum* were collected from Hancheng city in the northwest of Shaanxi province in September, 2011 in China. The fresh branches were dried in the shade at room temperature.

Sample preparation: Dried branches of *Zanthoxylum bungeanum* were cut into pieces. The volatile was extracted from the branches of *Zanthoxylum* (0.75 kg) by water steam distillation, then dried to pale yellow liquid using Na_2SO_4. 1 mL volatile was diluted to 5 mL with ethanol. Furthermore, the branches of Zanthoxylum (0.75 kg) were extracted with alcohol and then petroleum ether to get the petroleum ether extract. 20 mg of petroleum ether extract was dissolved in 10 mL of petroleum ether. All the solutions were filtered through a 0.45 μm filter.

Apparatus and GC-MS condition: The compositions were analyzed by an ISQ 110953 GC-MS system (Thermo Fisher Corp, USA). The condition was shown in the Table-1. Retention times were utilized as primary criterion for the peaks identification. Using the mass spectrometer as chromatographic detector offered additional data (P/N: 274, 102, 74 Thermo Data system) for the identification of the separated compounds. The identification method was reference standard compounds and compared the compositions isolated with the recorded spectra in MS library.

RESULTS AND DISCUSSION

Total gas chromatograms of volatiles and petroleum ether extract from branches of *Zanthoxylum bungeanum* were shown in Figs. 1 and 2. The relative contents of chemical constituents

TABLE-1
SELECTED PARAMETERS OF GC-MS CONDITION

Parameter	Description	
Capillary column	GSBP-5MS (30 m × 0.32 mm, 0.32 µm film, 0.25 µm), poly (5 % diphenyl, 95 % dimethyl siloxane).	
Carrier gas	Helium	
Injection mode	Split	
Column flow	A: 2.1 mL/min	B: 1.4 mL/min
Split ratio	A: 50:1	B: 10:1
Injection temperature	250 °C	
Oven temperature progarm	A: Initial temperature was 80 °C (hold time 1 min) then ramped at 10 °C/min to 280 °C (hold time 2 min). B: Initial temperature was 80 °C then ramped at 3 °C/min to 260 °C (hold time 3 min), Again with ramped at 20 °C /min to 280 °C (hold time 3 min).	
Ionization energy	70 eV	
Scan range	A: 20-800 amu	B: 35-400 amu
Event time	A: 0.5 s	B: 0.2 s
Ionization source temperature	A: 200 °C	B: 260 °C
Transmission line temperature	A: 250 °C	B: 280 °C

[a]The GC-MS condition of the volatiles, [b]The GC-MS condition of the petroleum ether extract

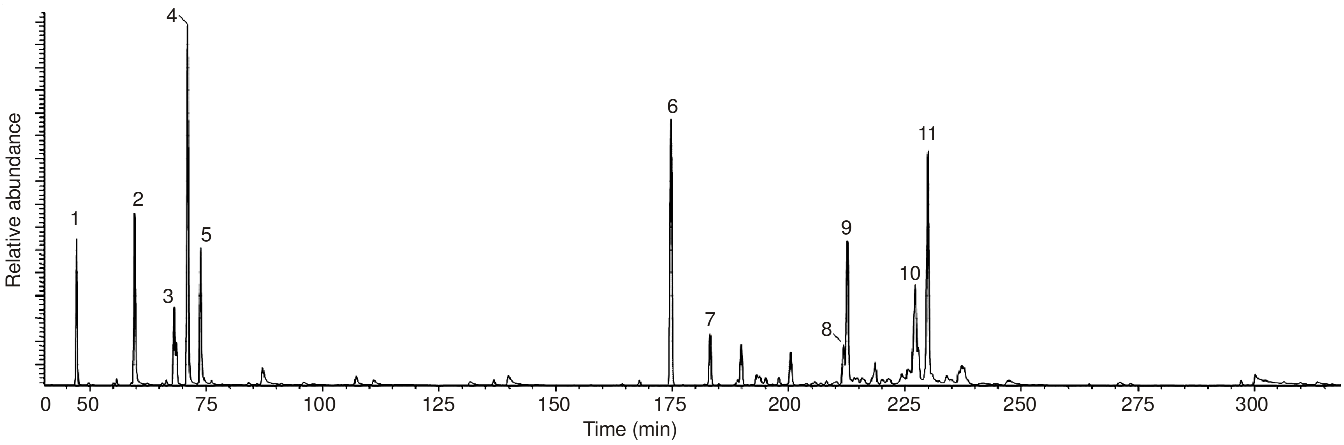

Fig. 1. GC-MS total ion chromatograms of volatiles; 1 = α-pinene, 2 = β-myrcene, 3 = β-phellandrene, 4 = β-*trans*-ocimene, 5 = β-*cis*-ocimene, 6 = caryophyllene, 7 = α-caryophyllene, 8 = (-)-spathulenol, 9 = (-)-caryophyllene oxide, 10 = torreyol, 11 = α-cadinol

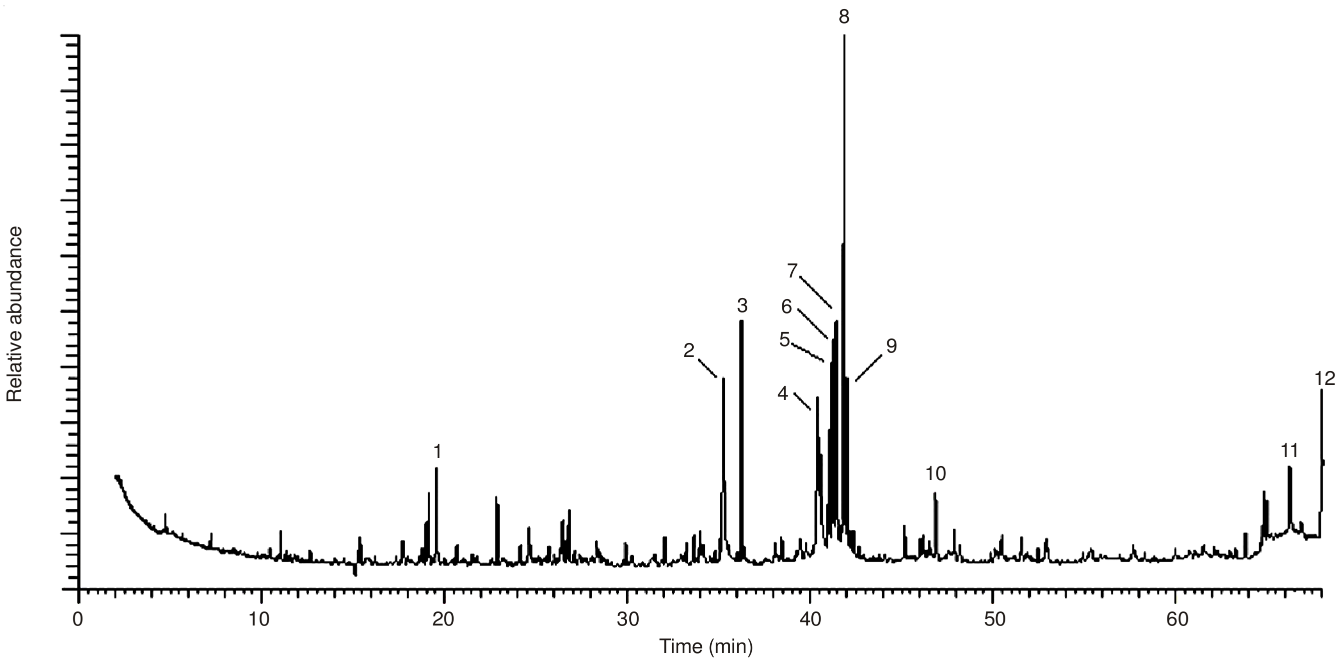

Fig. 2. GC-MS total ion chromatograms for petroleum ether extract part. 1 = 2,4-di-*tert*-butylphenol, 2 = *n*-hexadecanoic acid, 3 = hexadecanoic acid, ethyl ester, 4 = 2,3-dihydroxypropyl (9Z,12Z,15Z)-9,12,15-octadecatrienoate, 5 = (3Z,7E,10E)-trideca-3,7,10,12-tetraenoic acid methyl ester, 6 = mehyl 5,9-octadecadienoate, 7 = *cis*-5,8,11,14,17-eicosapentaenoic acid, 8 = doconexent, 9 = α-cbz-L-arginine, 10 = 2,4,6-*tri*-(*tert*-butyl)benzonitrile, 11 = campesterol, 12 = β-sitosterol

TABLE-2
MASS DATA OF 36 COMPOUNDS IDENTIFIED FROM VOLATILES

Peak No.	Compounds	T_R (min)	m.f.	m.w.	Area (%)
1	α-Pinene	4.717	$C_{10}H_{16}$	136	4.81
2	β-Pinene	5.575	$C_{10}H_{16}$	136	0.22
3	β-Myrcene	5.985	$C_{10}H_{16}$	136	6.74
4	Cymene	6.642	$C_{10}H_{14}$	134	0.18
5	β-Phellandrene	6.808	$C_{10}H_{16}$	136	2.91
6	1,5-Cyclooctadiene	6.858	$C_{10}H_{16}$	136	1.61
7	β-*trans*-Ocimene	7.092	$C_{10}H_{16}$	136	14.4
8	β-*cis*-Ocimene	7.375	$C_{10}H_{16}$	136	5.98
9	γ-Terpinene	7.608	$C_{10}H_{16}$	136	0.18
10	Linalyl alcohol	8.709	$C_{10}H_{18}$	138	0.46
11	Terpinene-4-ol	10.717	$C_{10}H_{18}$	138	0.46
12	Acetic acid, bornyl ester	13.675	$C_{12}H_{20}O_2$	196	0.24
13	Methyl nonyl ketone	13.983	$C_{11}H_{22}$	154	0.80
14	(-)-β-Elemene	16.800	$C_{15}H_{24}$	204	0.22
15	Caryophyllene	17.475	$C_{15}H_{24}$	204	12.4
16	α-Caryophyllene	18.308	$C_{15}H_{24}$	204	3.28
17	γ-Muurolene	18.892	$C_{15}H_{24}$	204	0.20
18	Tridecan-2-one	19.308	$C_{13}H_{26}$	182	0.61
19	(-)-α-Muurolene	19.500	$C_{15}H_{24}$	204	0.36
20	γ-Muurolene	19.792	$C_{15}H_{24}$	204	0.35
21	δ-Cadinene	20.042	$C_{15}H_{24}$	204	1.55
22	Germacra-1(10),4,7(11)-triene	20.808	$C_{15}H_{24}$	204	0.17
23	(-)-Spathulenol	21.175	$C_{15}H_{24}$	204	2.07
24	(-)-Caryophyllene oxide	21.258	$C_{15}H_{24}$	204	7.51
25	(-)-Globulol	21.408	$C_{15}H_{26}O$	222	0.41
26	Viridiflorol	21.575	$C_{15}H_{26}O$	222	0.40
27	Bicyclo[2.2.2]oct-2-ene,1,2,3,6-tetramethyl	21.633	$C_{12}H_{20}$	164	0.21
28	(-)-Caryophyllene oxide	21.858	$C_{15}H_{24}O$	220	1.64
29	4,7-Octadecadiynoic acid, methyl ester	22.008	$C_{19}H_{30}$	360	0.36
30	Widdrol	22.133	$C_{15}H_{26}O$	222	0.54
31	Torreyol	22.717	$C_{15}H_{26}O$	222	6.69
32	α-Copaene	22.783	$C_{15}H_{24}$	204	1.16
33	α-Cadinol	22.992	$C_{15}H_{26}O$	222	12.1
34	4-Bromo-1-naphthylamine	23.650	$C_{15}H_{24}O$	220	0.60
35	Sandaracopimaradiene	29.717	$C_{20}H_{32}$	272	0.24
36	Hexadecanoic acid	30.017	$C_{16}H_{32}O_2$	256	0.60

were calculated by area normalization method and the results were summarized in Tables 2 and 3.

Forty five peaks were obtained and 34 components were identified from the branches of *Zanthoxylum* by steam distillation, accounting for 95 % of the total of the volatiles (Fig. 1). According to the percentage contents of comparison, the main constituents were β-*trans*-ocimene(14.4 %), caryophyllene (12.4 %), α-cadinol (12.1 %), (-) -caryophyllene oxide (7.51 %), β-myrcene (6.74 %) and torreyol (6.69 %). The major compositions of the petroleum ether were the terpenes, including 21 kinds of chemical constituents, accounting for 66.1 % of the total compounds. The alcohols included 8 kinds of compositions, accounting for 23.2 %. Furthermore, the fats, ketones and silane acids were also detected in the volatiles (Table-2).

Sixty peaks were obtained and 50 components were identified from petroleum ether extract, accounting for 93.7 % of the total petroleum ether extract (Fig. 2). According to the percentage content of comparison, the main constituents were doconexent (15.5 %), *cis*-5,8,11,14,17-eicosapentaenoic acid (7.30 %), hexadecanoic acid, ethyl ester (6.95 %), methyl 5,9-octad ecadienoate (6.06 %) and *n*-hexadecanoic acid (5.84 %). The major constituents of the petroleum ether extract were the organic acids, including 6 kinds of components, accounting for 34.5 % of the total. The constituents of esters, including 11 kinds of components, accounting for 24.2 %. In addition, the petroleum ether extract also contained 18 kinds of alkanes, accounting for 21.3%, ketones and phenols (Table-3).

Conclusion

Previous experiments have discovered the physiological activity of the volatile oil. Such as, cadinol has anti-diarrheal effect and can inhibit intestinal fluid accumulation of cholera toxin-induced mice[14]. Caryophyllene can modulate immune function by increasing NK cell activity in tumor-bearing mice[15]. Pinene has obviously contact toxicity to aphis gossypii glover. Ocimene and caryophyllene are used to synthesize the spices[16]. All of these constituents of volatiles mentioned above can be extracted from the branches of *Zanthoxylum bungeanum* and have high contents. As for petroleum ether extract, this kind

TABLE-3
MASS DATA OF 36 COMPOUNDS IDENTIFIED FROM PETROLEUM ETHER EXTRACT PART

Peak No.	Compounds	T_R (min)	m.f.	m.w.	Area (%)
1	Hexadecane	11.02	$C_{16}H_{34}$	226	0.71
2	2-Butanone,1-[(1,1-dimethylethyl)amino]-3,3-dimethyl	15.23	$C_{10}H_{21}NO$	171	0.75
3	Tetradecane	15.38	$C_{14}H_{30}$	198	0.94
4	2,6,10,15-Tetramethylheptadecane	17.72	$C_{21}H_{44}$	296	0.89
5	11-Pentan-3-ylhenicosane	18.99	$C_{26}H_{54}$	366	3.50
		26.67			
		26.80			
6	2,6,10-Trimethyltetradecane	19.14	$C_{17}H_{36}$	240	1.95
7	2,4-Di-*tert*-butylphenol	19.56	$C_{14}H_{22}O$	206	2.54
8	5,14-Dibutyloctadecane	22.86	$C_{26}H_{54}$	366	1.94
9	3-Ethyl-5-(2-ethylbutyl)octadecane	24.15	$C_{26}H_{54}$	366	0.69
10	Paromomycin	24.62	$C_{15}H_{26}O$	222	1.16
11	9-Hexylheptadecane	25.73	$C_{23}H_{48}$	324	0.66
12	Eicosane	26.46	$C_{20}H_{42}$	282	1.81
13	Heneicosane	28.32	$C_{21}H_{44}$	296	1.44
		33.23			
14	5,8-Diethyldodecane	29.92	$C_{16}H_{34}$	226	0.69
15	9-Hexylheptadecane	30.24	$C_{23}H_{48}$	324	1.14
		42.37			
16	7,8-Epoxylanostan-11-ol, 3-acetoxy-	31.39	$C_{32}H_{54}O_4$	502	0.55
17	Phthalicacid butyl undecyl ester	32.04	$C_{23}H_{36}O_4$	376	0.96
18	7,9-Di-*tert*-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-Dione	33.62	$C_{17}H_{24}O_3$	276	0.86
19	2,4-Dimethylicosane	33.97	$C_{22}H_{46}$	310	0.97
20	1,2-Benzenedicarboxylic acid, butyl octyl ester	35.07	$C_{20}H_{30}O_4$	334	0.49
21	*n*-Hexadecanoic acid	35.25	$C_{16}H_{32}O_2$	256	5.84
22	Ethyl 9-hexadecenoate	35.53	$C_{18}H_{34}O_2$	282	0.30
23	Hexadecanoic acid, ethyl ester	36.25	$C_{18}H_{36}O_2$	284	6.95
24	Erucic acid	38.07	$C_{22}H_{42}O_2$	338	0.71
25	Ethyl 14-methyl-hexadecanoate	38.44	$C_{19}H_{38}O_2$	298	0.81
26	1,1-Didodecoxyhexadecane	39.45	$C_{40}H_{82}O_2$	594	0.70
27	1,3,5-Trimethyl-2-octadecylcyclohexane	39.77	$C_{27}H_{54}$	378	0.49
28	2,3-Dihydroxypropyl (9Z,12Z,15Z)- 9,12,15-Octadecatrienoate	40.54	$C_{21}H_{36}O_4$	352	3.66
29	(3Z,7E,10E)-trideca-3,7,10,12-tetraenoic acid Methyl ester	41.01	$C_{14}H_{20}O_2$	220	3.41
30	Methyl 5,9-octadecadienoate	41.21	$C_{19}H_{34}O_2$	294	6.06
31	*cis*-5,8,11,14,17-Eicosapentaenoic acid	41.40	$C_{20}H_{30}O_2$	302	7.30
32	Minaprine	41.61	$C_{19}H_{36}O_3$	312	0.48
33	Doconexent	41.85	$C_{22}H_{32}O_2$	328	15.5
34	Nalpha-cbz-L-arginine	42.03	$C_{14}H_{20}N_4O_4$	308	4.78
35	Eicosanoic acid	42.23	$C_{23}H_{48}$	324	0.50
36	Pentacosane	45.17	$C_{25}H_{52}$	352	1.06
37	Ethyl iso-allocholate	46.52	$C_{26}H_{44}O_5$	436	0.67
38	2,4,6-Tri-(*tert*-butyl)benzonitrile	46.86	$C_{19}H_{29}N$	271	2.19
49	Heneicosane	47.87	$C_{21}H_{44}$	296	0.88
40	9-Oximino-2,7-diethoxyfluorene	48.19	$C_{17}H_{17}NO_3$	283	0.55
41	Tetratetracontane	50.47	$C_{44}H_{90}$	618	0.79
42	2-Butyryl-3-{[2-(5-methoxy-2-methyl-1H-indol-3-yl)ethyl]amino}-2-cyclohexen-1-one	51.55	$C_{22}H_{28}N_2O_3$	368	0.79
43	Docosanoic acid, ethyl ester	52.89	$C_{24}H_{48}O_2$	368	0.59
44	Ethyl tetracosanoate	57.66	$C_{26}H_{52}O_2$	396	0.54
45	24α-Methylcholesterol	61.53	$C_{30}H_{50}O_2$	442	0.44
46	Stigmasta-5,22-dien-3 β-ol	63.81	$C_{31}H_{50}O_2$	454	1.42
47	Sesamin	64.80	$C_{20}H_{18}O_6$	354	1.55
48	1,4-*Bis*(1,3-benzodioxole-5-yl)tetrahydro-1*H*,3*H*-furo[3,4-c]furan	64.97	$C_{20}H_{18}O_6$	354	1.22
49	Campesterol	66.23	$C_{28}H_{48}O$	400	2.27
50	β-Sitosterol	67.99	$C_{29}H_{50}O$	414	3.01

of chemical constituent is often applied to chemical production. Such as, hexadecanoic acid ethyl ester is used in lubricant. Erucic acid is used to manufacture the artificial fiber[17]. The identification of the chemical constituents of volatiles and petroleum ether extract from the branch of Zanthoxylum bungeanu would contribute to making full use of herb resource.

REFERENCES

1. J. Hu, W.D. Zhang, Y.H. Shen, C. Zhang, L. Xu, R.-H. Liu, B. Wang and X.-K. Xu, *Biochem. Syst. Ecol*., **35**, 114 (2007).
2. D. Geng, D.X. Li and Y. Shi, *Chin. J. Nat. Med*., **7**, 274 (2009).
3. T. Hatano, K. Inada, T. Ogawa, H. Ito and T. Yoshida, *Phytochemistry*, **65**, 2599 (2004).

4. K. Hashimoto, K. Satoh, Y. Kase, A. Ishige, M. Kubo, H. Sasaki, S. Nishikawa, S. Kurosawa, K. Yakabi and T. Nakamura, *Planta Med*., **67**, 179 (2001).
5. Quanbo Xiong, S. Dawen, H. Yamamoto and M. Mizuno, *Phytochemistry*, **46**, 1123 (1997).
6. C.-T. Chang, S.-L. Doong, I.-L. Tsai and I.-S. Chen, *Phytochemistry*, **45**, 1419 (1997).
7. S. Wine-show, T. Ian-Lih, T. Che-Ming and C. Ih-Sheng, *Phytochemistry*, **36**, 213 (1994).
8. K. Jang, Y.H. Chang, D.-D. Kim, K.-B. Oh, U. Oh and J. Shin, *Arch. Pharm. Res*., **31**, 569 (2008).
9. A.P.K. Nissanka, V. Karunaratne, B.M.R. Bandara, V. Kumar, T. Nakanishi, M. Nishi, A. Inada, L.M.V. Tillekeratne, D.S.A. Wijesundara and A.A.L. Gunatilaka, *Phytochemistry*, **56**, 857 (2001).
10. I. Chen, T. Chen and W. Lin, *Phytochemistry*, **52**, 357 (1999).
11. Y. Kashiwada, C. Ito, H. Katagiri, I. Mase, K. Komatsu, T. Namba and Y. Ikeshiro, *Phytochemistry*, **44**, 1125 (1997).
12. S.L. da Silva, P.M.S. Figueredo and T. Yano, *Pharm. Biol*., **44**, 657 (2006).
13. S.L. Da Silva, P.M.S. Figueiredo and T. Yano, *Acta Amazon*., **37**, 281 (2007).
14. C.B. Duschatzky, A.N. Martinez, N.V. Almeida and S.L. Bonivardo, *J. Essent. Oil Res.*, **16**, 626 (2004).
15. T. Ashitani, A.K. Borg-Karlson, K. Fujita and S. Nagahama, *Nat. Prod. Res.*, **22**, 495 (2008).
16. W.N. Setzer, *J. Essent. Oil Bear Pl*., **10**, 475 (2007).
17. P.P. Fu, S.-H. Cheng, L. Coop, Q. Xia, S.J. Culp, W.H. Tolleson, W.G. Wamer and P.C. Howard, *J. Environ. Sci. Health C*, **21**, 165 (2003).