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Analysis and detection of some emerging organic pollutants: Water samples from the Mammoth Cave in Kentucky and Harpeth and Cumberland Rivers in Tennessee

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ABSTRACT

Water samples from environmental waters, namely the Mammoth Cave in Kentucky and the Harpeth and Cumberland Rivers in Tennessee were analyzed to determine the extent of organic pollutants. Tap water and deionized water samples were also collected and used as controls. Acid/base liquid/liquid extractions were performed on the water samples including the controls. The aqueous layers were discarded and the collected organic layers were stored temporarily at -20 ºC before analysis. The dried organic layers were analyzed using a Varian Saturn Gas Chromatograph - Mass Spectrometer (GC-MS). Chromatograms of sample peaks were analyzed according to retention time using the GC-MS's embedded library to determine some of the perspective compounds present. Detected compounds were classified as either nontoxic, mildly toxic or toxic to humans based on the available information from the literature. The results indicate the presence of several emerging organic pollutants which may compromise the quality of drinking water for populations who largely depend on them.

KEYWORDS: environmental waters, Mammoth Cave, Harpeth River, Cumberland River, acid/base liquid/liquid extraction, gas chromatograph mass spectrometer, toxic, organic pollutants

INTRODUCTION

A large number of organic compounds may enter the waste water system and, subsequently, have the potential to enter the food supply *via* sewage sludge. A previous study investigated the likely fate and behavior of well-established groups of organic contaminants [1] and identified priority compounds which could potentially accumulate in livestock following sludge application to pasture as tri-, tetra-, penta- and hexa-chlorobenzenes, tri-, tetra- and penta-chlorophenols, polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) [1-3]. There has been an increasing amount of interest in the likely fate of other 'new' or more recently studied groups of organic compounds in the environment; these include short and medium chain chlorinated paraffins, polybrominated ethers and polychlorinated naphthalenes [3]. This interest also extends to biologically active compounds, either synthetic or naturally occurring compounds which possess estrogenic activity (e.g. ethinyl estradiol, di-2-ethylhexyl phthalate) [3-5]. Pollution as a result of chemical industries is the most serious of all environmental problems and poses a major threat to the health and well-being of millions of people [6]. These compounds are toxic and have been classified as either mutagenic or carcinogenic [7-9]. Some of the main compounds found in the water supply near a chemical industrial site include phenols and polycyclic aromatic hydrocarbons (PAHs) [10, 11]. Phenols, if ingested, can cause serious damage to the respiratory system, but have no known carcinogenic effects. PAHs are organic contaminants that form as a result of the incomplete combustion of fossil fuels, soot, untreated tar, and mildly treated mineral oils used in or created by the aluminum and steel production processes and contain compounds composed of two or more fused benzene (aromatic)

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rings [11], which, according to the International Agency for Research on Cancer (IARC), have shown carcinogenic effects in humans [4, 6]. Because PAHs are present in occupational settings, they are considered as indirect evidence of some carcinogenic effects [4, 12-17].

Analyzing organic pollutants in water is a very tedious and difficult process. Organic components must be extracted from the water before they can be analyzed. The overall trends in analytical methods for water analysis include an increased use of solid-phase micro-extraction (SPME) [18, 19], the use of newly developed solid-phase extraction (SPE) sorbents for improved extraction [19], and an increased use of other reduced solvent extraction methods such as the recently developed single-drop micro-extraction (SDME) method and the stir bar sorptive extraction method [20]. SPME, SPE, SDME and stir bar sorptive extraction methods are effective when isolating a known organic compound that is potentially present in the water [18]. When there are no known compounds, the acid/base liquid/liquid extraction method has been used for years [21]. Acid/base liquid/liquid extraction separates the organic components from the aqueous components, and uses the organic layer for analysis [21]. Several methods can be used for analysis and detection of the organic compounds present in the organic layer. Liquid chromatography-mass spectrometry (LC/MS) has now become commonplace for analyzing organic pollutants such as pharmaceuticals, hormones, and endocrine disrupting compounds (EDCs) [22-25]. LC/MS has its drawbacks which include matrix effect, difficulty in separating highly polar analytes in the aqueous LC eluent, and the necessity for a tandem-MS which must be used in conjunction with LC/MS to achieve enhanced selectivity and sensitivity [22, 26-28]. These drawbacks have led many scientists to use gas chromatography-mass spectrometer (GC/MS) to analyze organic pollutants found in environmental water [29]. GC/MS has far fewer drawbacks, is more cost effective, and is able to detect more compounds present in environmental waters [22-25]. Extracting organic compounds out of water using acid/base liquid/liquid extraction and analyzing and detecting the compounds with GC/MS is the least expensive and most effective way of extracting and detecting these organic compounds.

In the present study, a liquid/liquid extraction (LLE) of organic pollutants from water samples [30] was used to determine the extent of pollution of water from the Mammoth Cave in Kentucky and the Harpeth and Cumberland Rivers in Tennessee and to shed more light on the quality and health aspects of the pollutants. The determination of the nature of the organic pollutants was accomplished by gas chromatography equipped with a flame ionization detector (FID) [20].

The above sites were selected for their diversity and complexity. The Mammoth Cave in central Kentucky is part of the longest known cave system in the world. The Mammoth Cave Area Biosphere Reserve represents a karst system characterized by complex underground water courses and a multilayered cave system with unique fauna and mineralization features [31, 32]. Of particular concern to the Mammoth Cave Area is the impact of agricultural, commercial, and residential land-use on ecosystems, especially with respect to the effects of groundwater pollution on cave biota [31, 32]. Tennessee’s Cumberland River is a wild river above the headwaters of Lake Cumberland. It is home to one of the largest waterfalls in the eastern United States, Cumberland Falls, which is 68 feet (21 m) high. Most of the river below Lake Cumberland’s Wolf Creek Dam is navigable because of several locks and dams. A 90-mile section of its Big South Fork is protected by the National Park Service as Big South Fork National River and Recreation Area [33, 34]. The Cumberland River is an important waterway in the Southern United States. At 688 miles (1,107 km) long, it starts in Harlan County in eastern Kentucky on the Cumberland Plateau, flows through southeastern Kentucky, crosses into northern Tennessee, and then curves back up into western Kentucky before draining into the Ohio River at Smithland, Kentucky [33, 34]. Many of the counties in the state of Tennessee receive their water supply from the river and recent data indicate that industrial pollution and man-made activities have a profound effect on the quality of water supplied to these counties. Finally the waters of Tennessee’s Harpeth River support a high diversity of wildlife, which can be viewed well from a canoe. Barred Owls, Belted Kingfishers, water snakes, and various mammals can be seen. The area can provide very exciting birding during spring and fall migration and, in spring, a rich floodplain of ample wildflowers for viewing and photography [35].

MATERIALS AND METHODS

Materials

Nanopure water was employed throughout. All reagents (methylene chloride, dichloromethane, ethyl acetate, hydrochloric acid 37% w/w) were of analytical grade (Sigma-Aldrich, St. Louis, MO). Anthracene used as an analytical reference standard was obtained from Sigma-Aldrich, St. Louis, MO. A stock solution of anthracene was prepared in methylene chloride at a concentration of 0.055%. Water samples were spiked with anthracene standard before extraction with the organic solvents.

Water sample collection

A total of 36 samples, 12 from each river, were collected on site and in a radius of about 10 kilometers from each sample collection site. Water samples were collected in sterilized plastic containers (PVC 50 ml) after flushing out the tube wells (minimum 10 minutes) to get the fresh ground water, and the grab sampling method was followed in case wells. The containers were sealed and the samples were protected from direct sunlight during transportation. The water pH was determined by Systronics Digital pH meter standardized with buffer tablets [36, 37]. Samples were temporarily stored in a refrigerator before extraction of the organic pollutants.

Extraction of organic compounds from water samples

The liquid/liquid extraction procedure of organic pollutants from water samples was carried out as described in [30] with the following modifications. 21 ml of the sample, 5 ml of aqueous sodium bicarbonate and 30 ml of dichloromethane were added to a separating funnel. The solution was shaken several times to allow for complete mixing of the solvent and the sample and to allow the organic components to be extracted. The funnel was vented several times to release any gas build up during the extraction. Then 5 ml of 5% aqueous HCl solution was added to the aqueous layer while 5 ml of an aqueous 5% NaOH was added to the organic layer in the separating funnel and the solutions in the separate containers extracted (see Figure 1 - flowchart of the extraction procedure). Care was taken to allow for complete mixing and the separating funnel was vented to release any gas build up due to the extraction. The extractions with the respective solvents were carried out 4 times. Following the extraction, the organic layers were combined and dried by adding 1.5 g of magnesium sulfate (Mg_2SO_4) and filtered through Whatman filter paper #45 into a collection tube and stored at -80 ºC for further analysis using a Varian Saturn GC/MS equipped with a flame ionization detector (FID) [19, 20].

Extraction of control water samples - deionized water from Tennessee State University

Tap water and deionized water were used as controls and for comparison to the samples from the Cumberland River, which is the main drinking water source for the population in and around the Nashville metropolis. All control samples were extracted and analyzed as described under the section ‘Extraction of organic compounds from water samples’.

Chromatographic analyses

A Varian model 3800 GC was used, operating under the following conditions. Injection: Varian 1071 Injector operated in the splitless mode. Injector temperature: 280 ºC. Column: J&W fused silica DBS MS column, 30 m, with a 0.25-mm internal diameter and 0.25-µm film thickness. Carrier gas: He, 1 ml/min. Column oven: 60 ºC (0 min), 7 ºC/min to 130, 5 ºC/min to 200, 20 ºC/min to 350 (4 min) [19].

Mass spectrometer

The mass spectrometer was a Varian Saturn model 2000 ion trap system operated under the following conditions. Mass range: 45-450 µ. Scan rate: 0.81 sec/scan. Delay time: 3 min. Peak threshold: 2 counts. Background mass: 43 µ. Scan segments: 10-99/100-250/251-299/400-650. Tune factors: 100/160/140/35. Emission current: 15 µA. Multiplier gain: 10^5. Ion trap temperature: 150 ºC. Transfer line temperature: 350 ºC [19].

RESULTS

Identification of organic compounds from control tap and deionized water samples by GC/MS

Figures 2 and 3 show the profiles of the gas chromatograms (GC) of organic pollutants from water samples from tap and deionized water, respectively. The profiles seem to be the same,

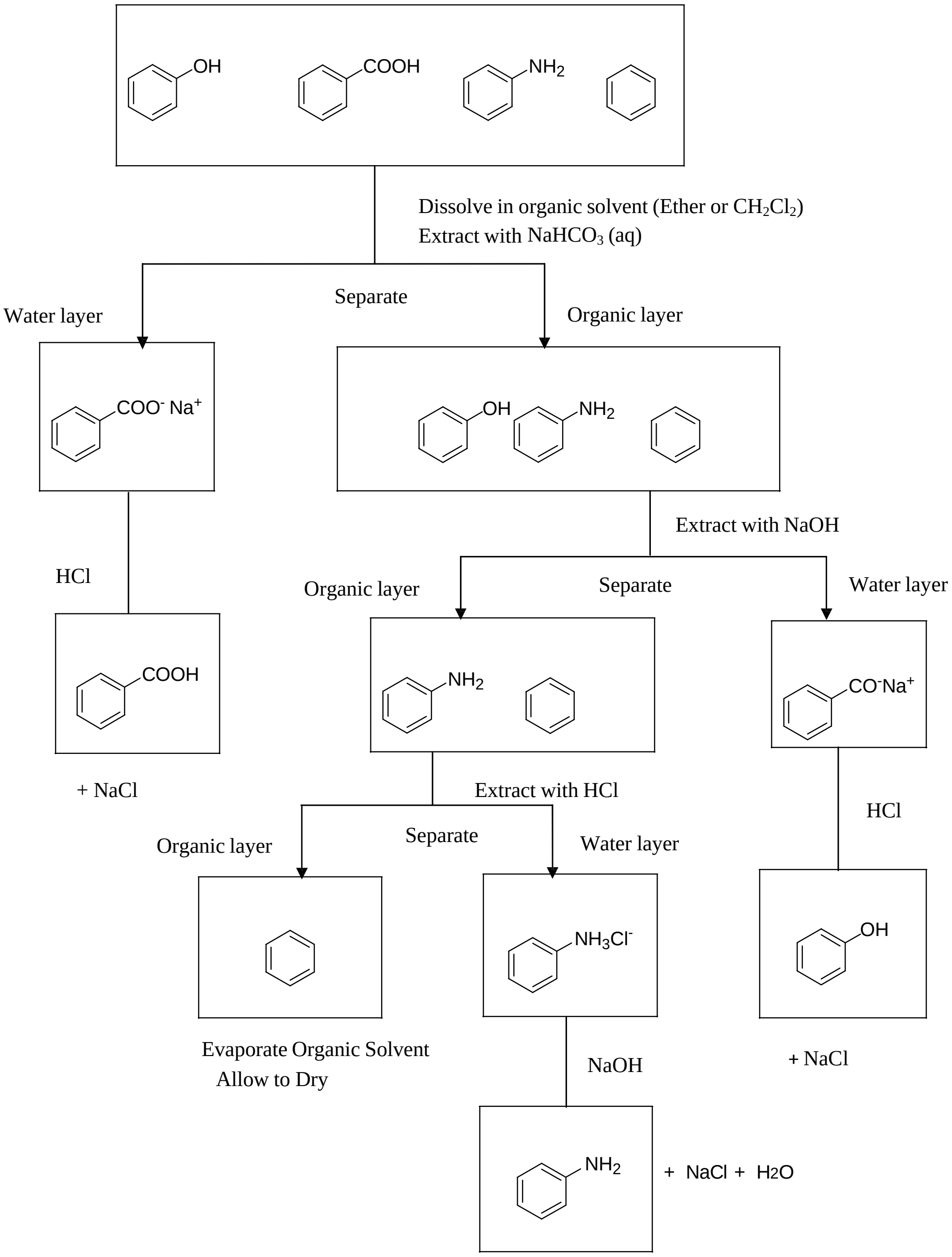


Figure 1. Outline of the extraction procedure [30].

indicating the presence of similar organic compounds in both water samples. However, it is interesting to note that the organic compounds detected are different. For example, the following were some of the major compounds detected in the tap water (Figure 2): 3-ethyl-3-methylheptane, 2-undecanethiol, 2-methyl, 3-hexadecyloxycarbonyl-5-(2-hydroxymethyl)-4-methylimidazolium ion, 7,9-di-terbutyl-1-oxaspirol[4,5] deca-6,9-dien-8-one, 4-trifluoroacetoxyte tradecene; 2-hexyl-1-octanol, trifluoracetroxypentadecane, 1,2-benzenedicarboxylic acid, butyl 8-methylnonly ester and bis(2-ethylhexyl)phthalate (Table 1). In the case of the deionized water (Figure 3) the following major compounds were detected: z-10-pentadecen 1-ol, 2-trifluoroacetoxy-pentadecane, 3-hexadecycloxycarbonyl-5-(2-hydroxymethyl)-4-methylimidazolium

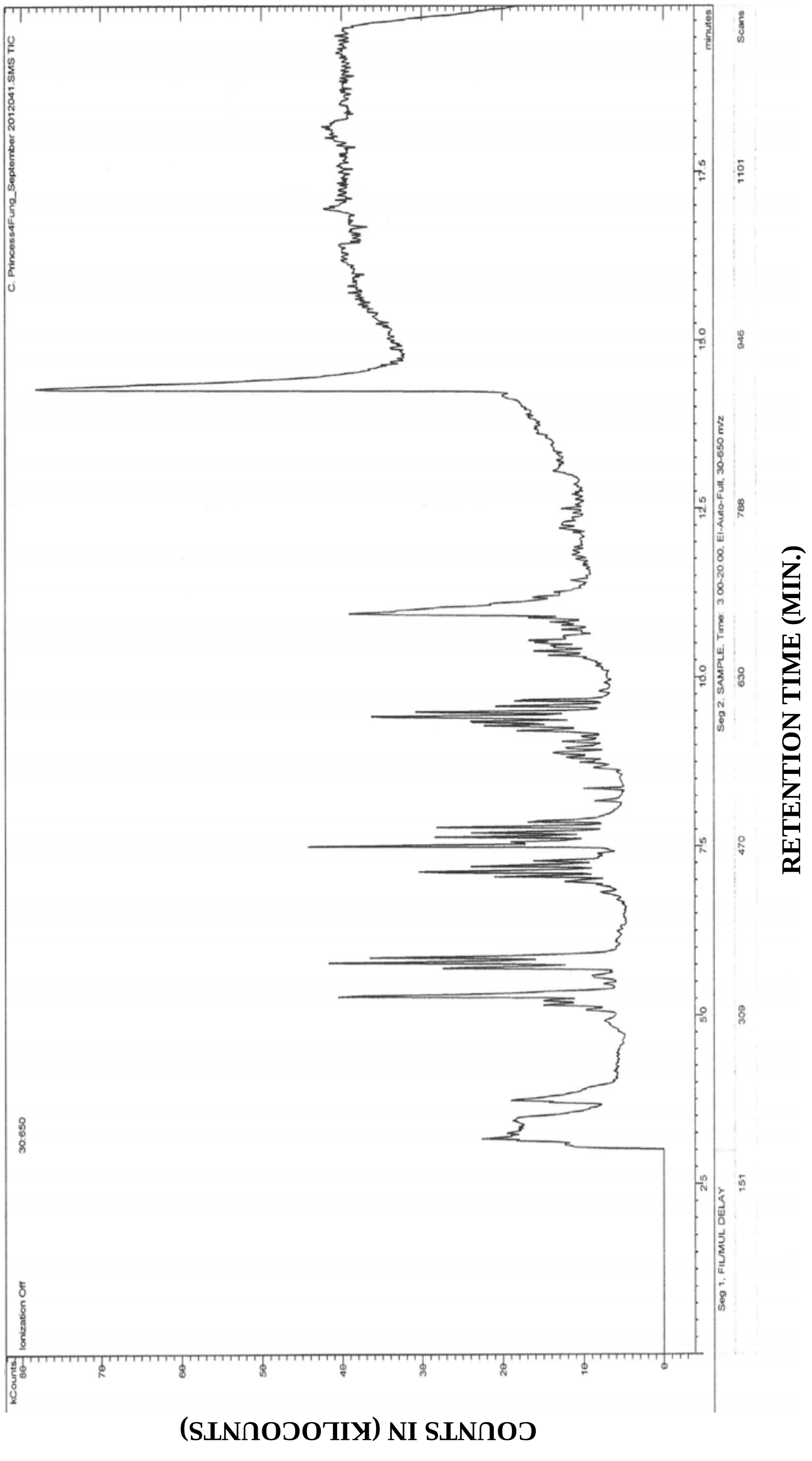


Figure 2. Chromatogram of organic pollutants in water from tap water.

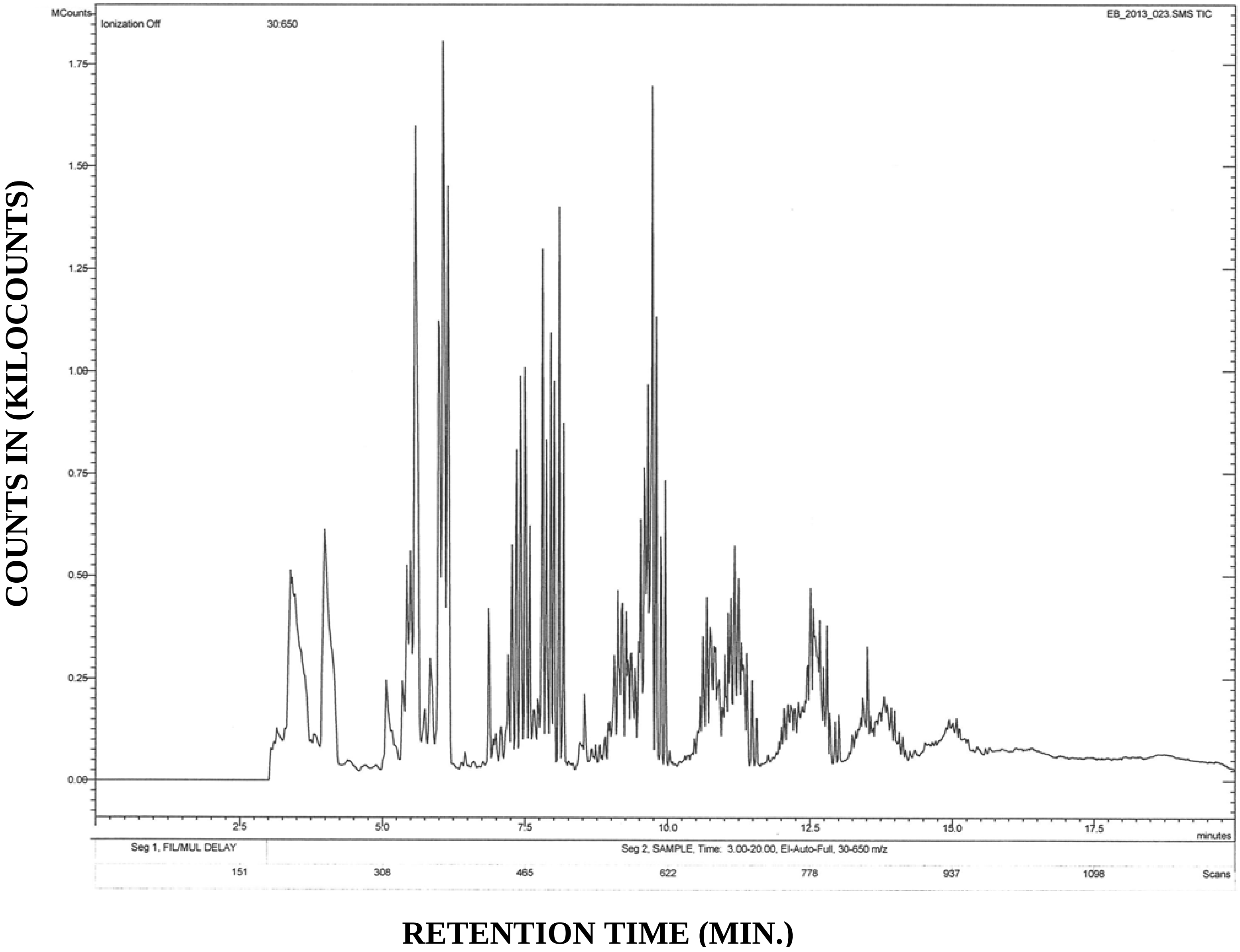


Figure 3. Chromatogram of organic pollutants in deionized water.

Table 1. Profile of some organic pollutants detected in tap water.

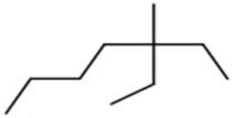
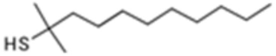
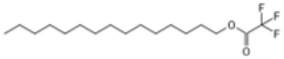
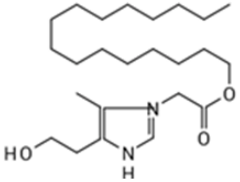
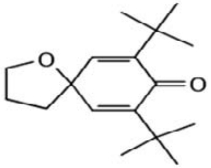
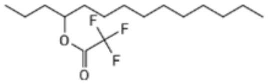
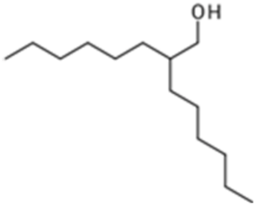
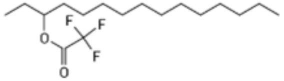
Analyzed sample	Retention time (Min.)	Compound present	Chemical formula	Molecular weight (MW)	Structure	Toxicity to humans
TSU. Tap water	3.138	3-Ethyl-3-methylheptane	$C_{10}H_{22}$	142		N/A
TSU. Tap water	3.765	2-Undecanethiol, 2 methyl	$C_{12}H_{26}S$	202		N/A
TSU. Tap water	5.715	Trifluoroacetic acid, n-heptadecyl ester	$C_{17}H_{31}F_3O_2$	324		N/A
TSU. Tap water	6.852	3-Hexadecyloxycarbonyl-5-(2-hydroxymethyl)-4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409		N/A
TSU. Tap water	7.496	7,9-Di-tertbutyl-1-oxaspiro[4,5]deca-6,9-dien-8-one	$C_{17}H_{26}O_2$	262		N/A
TSU. Tap water	7.855	4-Trifluoroacetoxytetradecene	$C_{16}H_{29}F_3O_2$	310		N/A
TSU. Tap water	8.856	2-Hexyl-1-octanol	$C_{14}H_{30}O$	214		Possible eye, skin & respiratory tract irritant.
TSU. Tap water	9.452	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324		N/A

Table 1 continued..

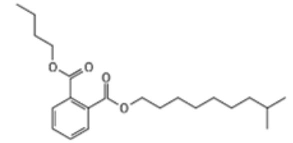
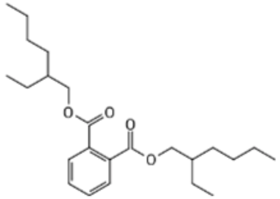
TSU. Tap water	11.032	1,2-Benzenedicarboxylic acid, butyl 8-methylnonly ester	$C_{22}H_{34}O_4$	362		N/A
TSU. Tap water	14.300	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390		Possible eye, skin & respiratory tract irritant. Endocrine disruptor, Teratogen. Obtain special instructions before use.

Table 2. Profile of some organic pollutants detected in deionized water.

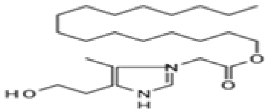
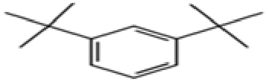
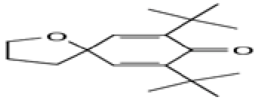
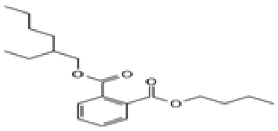
Analyzed sample	Retention time (Min.)	Compound present	Chemical formula	Molecular weight (MW)	Structure	Toxicity to humans
TSU. DI water	3.403	Z-10-Pentadecen-1-ol	$C_{15}H_{30}O$	226		N/A
TSU. DI water	3.718	2-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324		N/A
TSU. DI water	4.951	3-Hexadecyloxycarbonyl-5-(2-hydroxymethyl)-4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409		N/A
TSU. DI water	5.314	Benzene, 1,3-bis(1,1-dimethylethyl)	$C_{12}H_{22}$	190		Possible eye, skin & respiratory tract irritant.
TSU. DI water	5.765	Trifluoroacetic acid, n-heptadecylester	$C_{17}H_{31}F_3O_2$	324		N/A
TSU. DI water	7.484	7,9-Di-tertbutyl-1-oxaspiro[4,5]deca-6,9-dien-8-one	$C_{17}H_{26}O_2$	262		N/A
TSU. DI water	7.626	2-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324		N/A

Table 2 continued..

TSU. DI water	7.727	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324		N/A
TSU. DI water	10.308	4-Methyldocosane	$C_{23}H_{48}$	324		N/A
TSU. DI water	10.578	1-Docosanol	$C_{22}H_{46}O$	326		Possible eye, skin & respiratory tract irritant.
TSU. DI water	10.946	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$	334		Possible eye, skin & respiratory tract irritant. Endocrine disruptor, Teratogen. Obtain special instructions before use.

ion, benzene, 1,3-bis(1,1-dimethylethyl), trifluoroacetic acid, n-heptadecyl ester, 7,9-di-tertbutyl-1-oxaspirol [4,5]deca-6,9-dien-8-one, 2-trifluoroacetoxy pentadecane, 3-trifluoroacetoxypentadecane, 4-methyldocosane, 1-docosanol and 1,2-bezenedicarboxylic acid, butyl 2-ethylhexyl ester (Table 2). With regard to toxicity, 2-hexyl-1-octanol and bis(2-ethylhexyl)phthalate (Figure 2, Table 1) have been reported as possible eye, skin and respiratory irritants with bis(2-ethylhexyl) phthalate acting as a possible endocrine disruptor. With regard to deionized water samples, benzene, 1,3-bis(1,1-dimethylethyl) and 1-docosanol were identified as possible eye, skin and respiratory tract irritants. 1,2-bezenedicarboxylic acid, butyl 2-ethylhexyl ester was found to be an endocrine 2).

Identification of organic compounds from water samples from the Mammoth Cave by GC/MS

Figure 4 shows the profile of several possible organic compounds identified from the water samples from the Mammoth Cave and spiked with anthracene. The anthracene served as the internal standard and to ensure that the identified compounds truly came from the analyzed water samples and were not mere artifacts. As shown, the retention time of the anthracene was around 10.297 min with a molecular weight of 178. Compounds detected include phenols, fluorinated and chlorinated compounds, alkylated compounds, compounds found in prescription medications, and plastic derivatives which have been shown to be mildly toxic to humans based on median lethal dose (LD_{50}) (Table 3). Aromatic compounds such as benzene, 1,3-bis(1,1-dimethyl) with a retention time of 5.57 and long chain fatty acids and alcohols were observed and have been reported as possible eye, skin and respiratory tract irritants. Overall the molecular weights of compounds identified ranged from as low as 84 to as high as 466. The lowest compound detected had a molecular weight of 84 and was found to be methylene chloride, which has been suspected of causing cancer with a retention time of 3.04 min. The compound with the highest molecular weight was identified as heptafluorobutyric acid, n-octadecyl ester with a retention time of 9.65. Most of the compounds that were detected were identified as possible skin, eye or respiratory tract irritants.

Identification of organic compounds from water samples from the Cumberland River by GC/MS

Figure 5 shows the profile of compounds identified by GC/MS from water samples from the Cumberland River spiked with anthracene as the internal standard. As shown, several compounds were identified from the library of compounds after the analysis. The molecular weights of the detected compounds ranged from 126 to 348. The profile of the compounds was, however, different from those identified in the Mammoth Cave sample. 2,4-dimethyl-1-heptane was identified as the compound with the lowest molecular weight of 126 at a retention time of 4.84 min. Possible eye, skin and respiratory irritants included the following: benzene, 1,3-bis(1,1-dimethylethyl), ethanone, 1-(9-anthracenyl) and dibutyl phthalate which in addition is a potent endocrine disruptor and teratogenic (Table 4).

Identification of organic compounds from water samples from the Harpeth River by GC/MS

Figure 6 is the chromatographic profile of organic compounds isolated from water samples from the Harpeth River after spiking with anthracene. As previously noted for the Mammoth Cave and the Cumberland River, several compounds were detected. Pentane, 3-ethyl-2-methyl pentane was noted to be the compound with the lowest molecular weight of 114 with a retention time of 3.92 min (Table 5). Several of the compounds were long chain hydrocarbons as well as derivatives of long chain hydrocarbons. 17-pentatriacontene, a possible eye, skin and respiratory tract irritant, was found to have the highest molecular weight of 490 with a retention time of 16.82. Most of the compounds present with no known toxicity to humans included the following: 3-ethyl-2-methyl pentane, 2,4-dimethyl-1-heptene, cyclopentene, 2,5-dimethylhaxane, 2,5-dihydroperoxide, 4,5-dipropyl, 2,2-dimethylpropionic acid, 2,6,7-trimethyl decane, trifluroacetic acid, hexyl ethyl ether, and 2,6,11-trimethyl dodecane, 1-docesene,1,2-benezenedicarboxylic acid (Table 5). These compounds were detected at different retention times.

DISCUSSION

A total of 40 water samples per location were collected on site from the Mammoth Cave in Kentucky and Tennessee’s Cumberland and Harpeth Rivers.

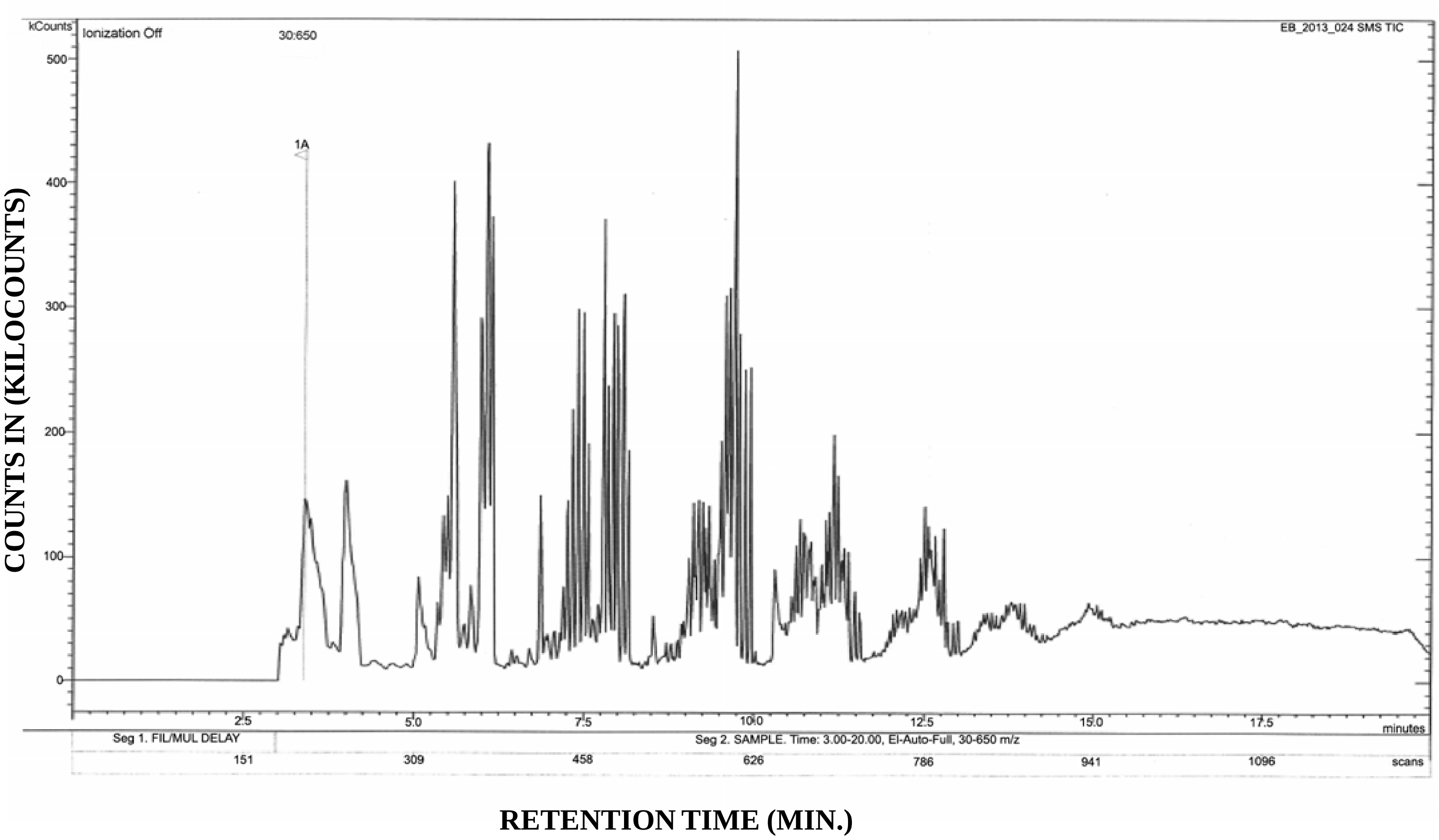


Figure 4. Chromatogram of organic pollutants in water samples from the Mammoth Cave spiked with anthracene.

Table 3. Profile of some organic pollutants detected in Mammoth Cave spiked with anthracene.

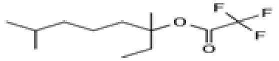
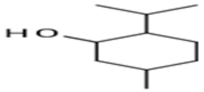
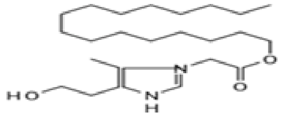
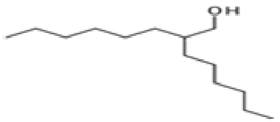
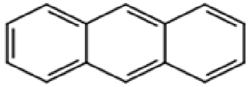
Analyzed sample	Retention time (Min.)	Compound present	Chemical formula	Molecular weight (MW)	Structure	Toxicity to humans
MW-25 Cresole	3.042	Methylene chloride	CH_2Cl_2	84		Possible eye, skin & respiratory tract irritant. Suspected of causing cancer.
MW-25 Cresole	3.729	1,8-Nonadien-3-ol	$C_9H_{16}O$	140		N/A
MW-25 Cresole	3.994	2,6-Dimethyl-6-trifluoroacetoxyoctane	$C_{12}H_{21}F_3O_2$	254		N/A
MW-25 Cresole	5.071	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1.alpha.,2.beta.,5.alpha.)	$C_{10}H_{20}O$	156		Possible skin & respiratory irritant. Serious eye irritant.
MW-25 Cresole	5.576	Benzene, 1,3-bis(1,1-dimethylethyl)	$C_{12}H_{22}$	190		Possible eye, skin & respiratory tract irritant.
MW-25 Cresole	5.751	3-Hexadecyloxycarbonyl-5-(2-hydroxymethyl)- 4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409		N/A
MW-25 Cresole	6.070	4-Trifluoroacetoxyhexadecane	$C_{18}H_{33}F_3O_2$	338		N/A
MW-25 Cresole	6.150	Trifluoroacetic acid, n-hepadecylester	$C_{17}H_{31}F_3O_2$	324		N/A
MW-25 Cresole	6.312	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324		N/A
MW-25 Cresole	7.434	2-Hexyl-1-octanol	$C_{14}H_{30}O$	214		Possible eye, skin & respiratory tract irritant.
MW-25 Cresole	7.513	Heptadecane, 2,6-dimethyl	$C_{19}H_{40}$	268		N/A
MW-25 Cresole	7.818	Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206		Skin, serious eye & respiratory tract irritant. Specific target organ toxicity.

Table 3 continued..

MW-25 Cresole	8.004	Isotridecanol	$C_{13}H_{28}O$	200		Skin irritant; low acute toxicity by oral, dermal & inhalation routes of exposure.
MW-25 Cresole	9.127	1-Decanol, 2-hexyl	$C_{16}H_{34}O$	242		Possible eye, skin & respiratory tract irritant.
MW-25 Cresole	9.652	Heptafluorobutyric acid, n-octadecylester	$C_{22}H_{37}F_7O_2$	466		N/A
MW-25 Cresole	10.297	Anthracene*	$C_{14}H_{10}$	178		Carcinogen-specific target organ toxicity; skin, serious eye & respiratory tract irritant.

*: means that Anthracene was added as an internal standard

Table 4. Profile of some organic pollutants detected in the Cumberland River spiked with anthracene.

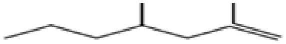
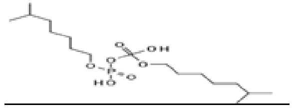
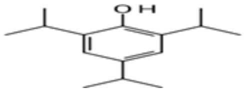
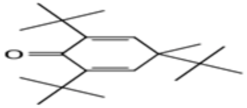
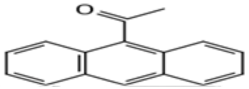
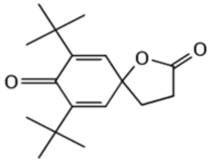
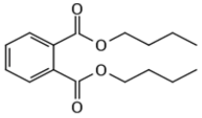
Analyzed sample	**Retention time (Min.)**	**Compound present**	**Chemical formula**	**Molecular weight (MW)**	**Structure**	**Toxicity to humans**
Pond in front of the steam plant	4.844	2,4-Dimethyle-1-heptene	C_9H_{18}	126		N/A
Pond in front of the steam plant	4.844	Diphosphoric acid diisoocty ester	$C_{16}H_{36}O_7P_2$	402		N/A
Pond in front of the steam plant	7.059	Decane, 2,6,7-trimethyl	$C_{13}H_{28}$	184		N/A
Pond in front of the steam plant	9.652	Benzene, 1,3-bis(1,1-dimethylethyl)	$C_{14}H_{22}$	190		Possible eye, skin & respiratory tract irritant.
Pond in front of the steam plant	10.192	Isotridecanol	$C_{13}H_{28}O$	200		Skin irritant; low acute toxicity by oral, dermal & inhalation routes of exposure.
Pond in front of the steam plant	11.568	4-Methyldocosane	$C_{23}H_{48}$	324		N/A

Table 4 continued..

Pond in front of the steam plant	11.965	Phenol,2,4,6-tris(1-methylethyl)	$C_{15}H_{24}O$	220		N/A
Pond in front of the steam plant	11.965	2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one	$C_{19}H_{32}O$	276		N/A
Pond in front of the steam plant	11.965	Ethanone, 1-(9-anthracenyl)	$C_{16}H_{12}O$	220		Possible eye, skin & respiratory tract irritant.
Pond in front of the steam plant	12.329	3-Eicosene, (E)	$C_{20}H_{40}$	280		Possible eye, skin & respiratory tract irritant.
Pond in front of the steam plant	13.817	Trifluoroacetic acid, n-heptadecylester	$C_{17}H_{31}F_3O_2$	324		N/A
Pond in front of the steam plant	14.428	Antracene*	$C_{14}H_{10}$	178		Carcinogen-specific target organ toxicity; skin & serious eye irritant.
Pond in front of the steam plant	15.007	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276		N/A
Pond in front of the steam plant	15.323	Dibutyl phthalate	$C_{16}H_{22}O_4$	278		Possible eye, skin & respiratory tract irritant. Endocrine disruptor, Teratogen. Obtain special instructions before use.
Pond in front of the steam plant	15.499	Acetic acid, chloro-, octadecylester	$C_{20}H_{39}ClO_2$	346		N/A
Pond in front of the steam plant	16.605	1-Heneicosyl formate	$C_{22}H_{44}O_2$	340		N/A

*: means that Anthracene was added as an internal standard

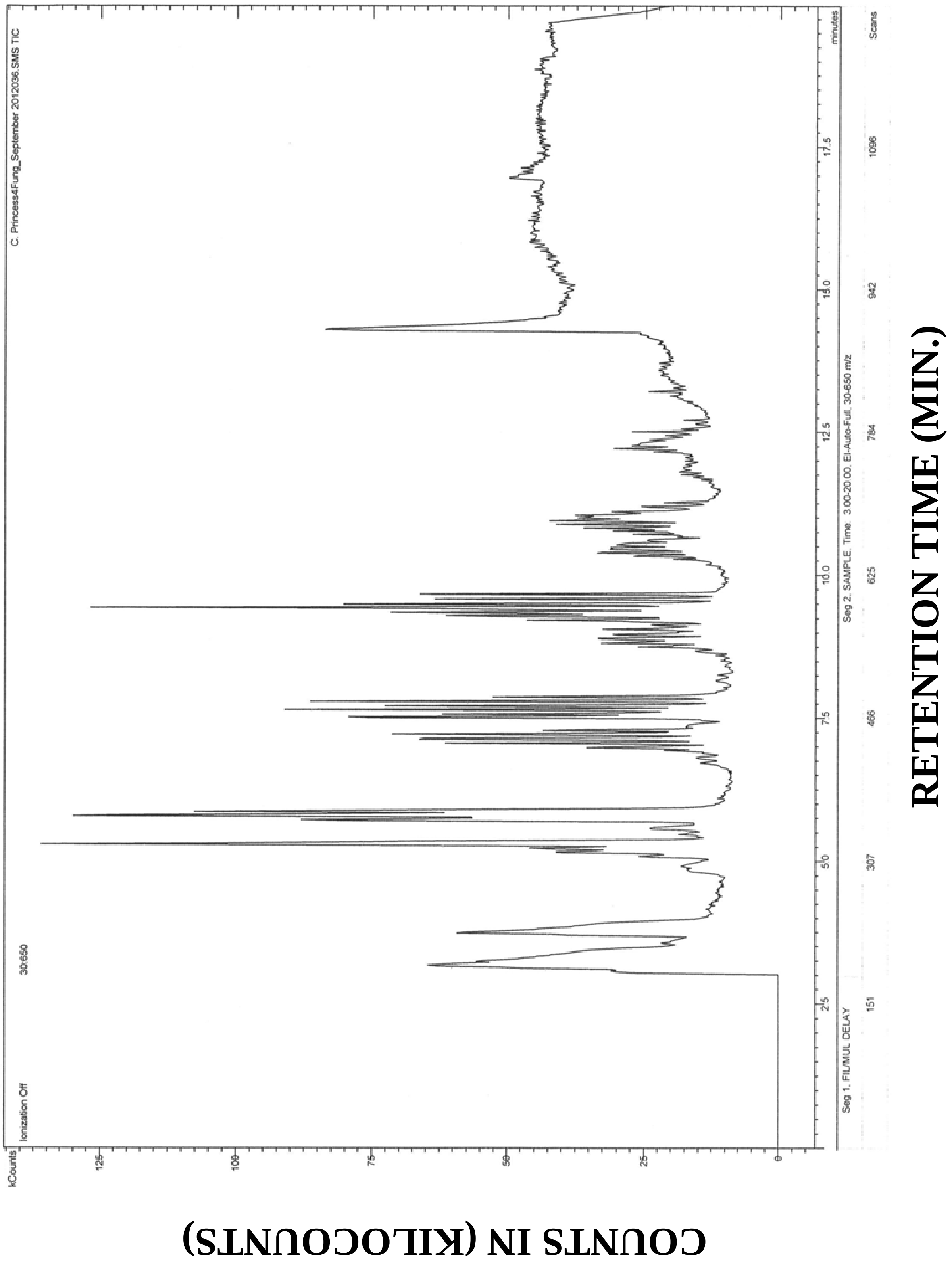


Figure 5. Chromatogram of organic pollutants in water samples from the Cumberland River spiked with anthracene.

Table 5. Profile of some organic pollutants detected in the Harpeth River spiked with anthracene.

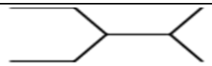
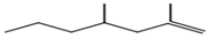
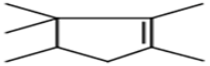
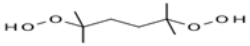
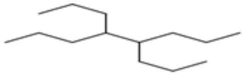
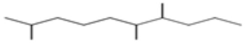
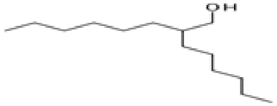
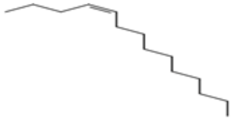
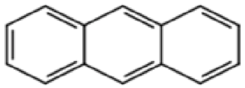
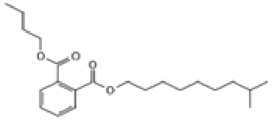
Analyzed sample	Retention time (Min.)	Compound present	Chemical formula	Molecular weight (MW)	Structure	Toxicity to humans
Harpeth River State Park Hwy 70, Kingston Springs.	3.927	Pentane, 3-ethyl-2-methyl	C_8H_{18}	114		N/A
Harpeth River State Park Hwy 70, Kingston Springs.	4.630	unknown	unknown	unknown	unknown	unknown
Harpeth River State Park Hwy 70, Kingston Springs.	4.895	2,4-Dimethyl-1-Heptene	C_9H_{18}	126		N/A
Harpeth River State Park Hwy 70, Kingston Springs.	5.924	Cyclopentene, 1,2,3,3,4-pentamethyl	$C_{10}H_{18}$	138		N/A
Harpeth River State Park Hwy 70, Kingston Springs.	6.128	2,5-Dimethylhexane-2,5-dihydroperoxide	$C_8H_{18}O_4$	178		N/A
Harpeth River State Park Hwy 70	6.424	Octane,4,5-dipropyl	$C_{14}H_{30}$	198		N/A
Harpeth River State Park Hwy 70	6.629	unknown	unknown	unknown	unknown	unknown
Harpeth River State Park Hwy 70	6.896	2,2-Dimethylpropioni cacid, decyl ester	$C_{15}H_{30}O_2$	242		N/A
Harpeth River State Park Hwy 70	7.040	Decane, 2,6,7-trimethyl	$C_{13}H_{28}$	184		N/A
Harpeth River State Park Hwy 70	7.541	Hydroxylamine, O-decyl	$C_{10}H_{23}NO$	173		Skin, eye & respiratory tract irritant. Specific target organ toxicity.
Harpeth River State Park Hwy 70	8.103	Trifluoroacetic acid, n-tetradecyl ester	$C_{16}H_{29}F_3O_2$	310		N/A
Harpeth River State Park Hwy 70	8.305	unknown	unknown	unknown	unknown	unknown
Harpeth River State Park Hwy 70	8.629	Hexyl octyl ether	$C_{14}H_{30}O$	214		N/A

Table 5 continued..

Harpeth River State Park Hwy 70	9.098	Nonadecane	$C_{19}H_{40}$	268		Possible eye, skin & respiratory tract irritant.
Harpeth River State Park Hwy 70	9.397	Dodecane, 2,6,11-trimethyl	$C_{15}H_{32}$	212		N/A
Harpeth River State Park Hwy 70	9.554	2-Hexyl-1-octanol	$C_{14}H_{30}O$	214		Possible eye, skin & respiratory tract irritant.
Harpeth River State Park Hwy 70	9.918	4-Tetradecane, (Z)	$C_{14}H_{28}$	196		N/A
Harpeth River State Park Hwy70	9.965	unknown	unknown	unknown	unknown	unknown
Harpeth River State Park Hwy 70	10.155	1-Docesene	$C_{22}H_{44}$	308		N/A
Harpeth River State Park Hwy 70	12.194	Isotridecanol	$C_{13}H_{28}O$	200		Skin irritant; low acute toxicity by oral, dermal & inhalation routes of exposure.
Harpeth River State Park Hwy 70	12.774	unknown	unknown	unknown	unknown	unknown
Harpeth River State Park Hwy 70	13.917	unknown	unknown	unknown	unknown	unknown
Harpeth River State Park Hwy 70	14.463	Anthracene	$C_{14}H_{10}$	178		Carcinogen-specific target organ toxicity; skin, serious eye & respiratory tract irritant.
Harpeth River State Park Hwy 70	15.301	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	$C_{22}H_{34}O_4$	362		N/A
Harpeth River State Park Hwy 70	16.824	17-Pentatriacontene	$C_{35}H_{70}$	490		Possible eye, skin & respiratory tract irritant.

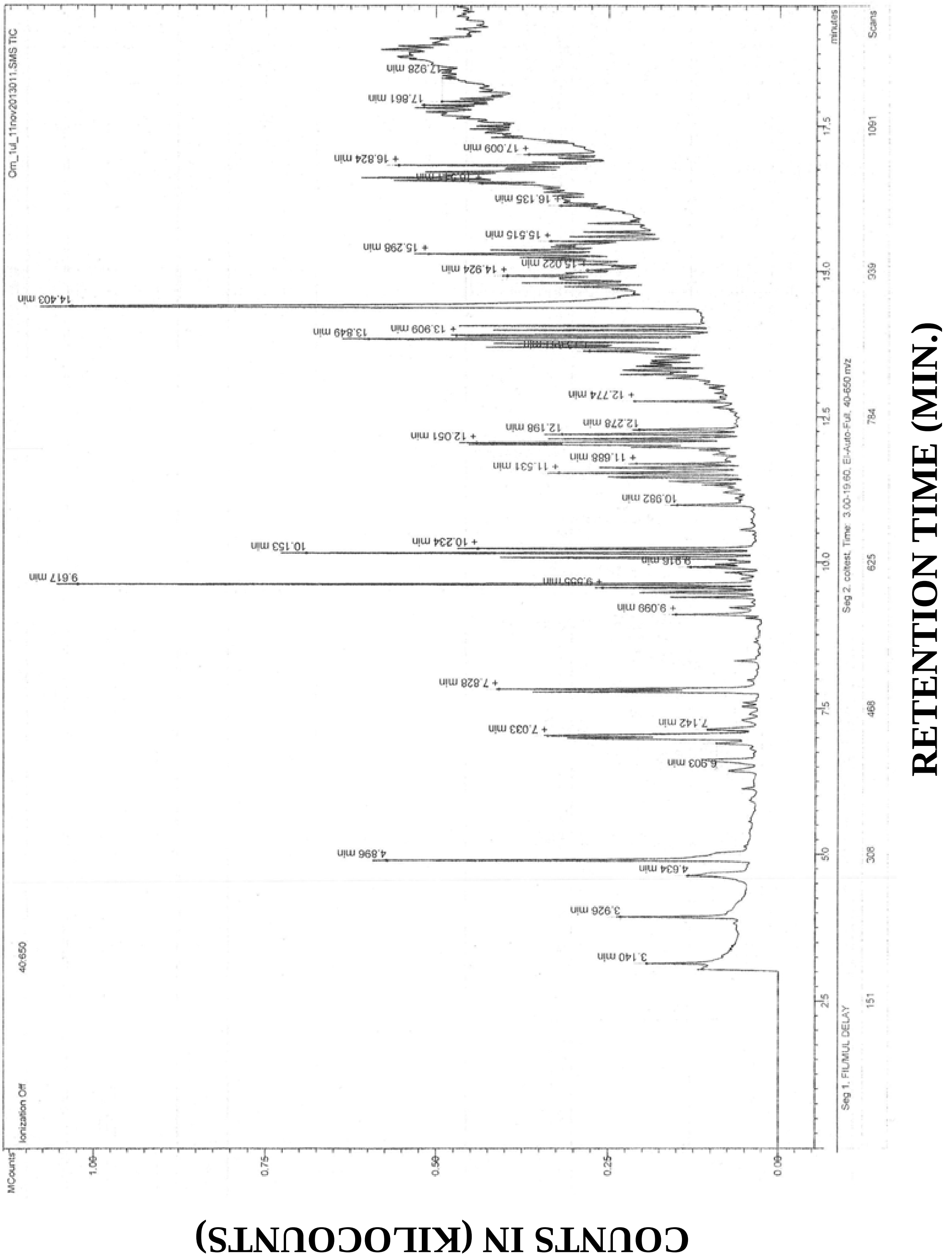


Figure 6. Chromatogram of organic pollutants in water samples from the Harpeth River spiked with anthracene.

They were analyzed for organic pollutants and PAHs in our laboratory over a period of 1 year, using the techniques of acid/base/liquid/liquid extraction and detection by GC/MS. The extents of organic pollutants or contaminants in these samples were then compared to the control water samples. Figures 2 and 3 show the profiles of organic pollutants from tap and deionized water respectively. As indicated under the results section, several organic compounds were detected, indicating the presence of these compounds even after water treatment with all the quality control measures. Though levels of the detected compounds were low in comparison to the Mammoth Cave, Cumberland and Harpeth Rivers, it is interesting to note that compounds such as 2-hexyl-1-octanol and bis(2-ethylhexyl)phthalate (Figure 2) as detected in the tap water have been reported as possible eye, skin and respiratory irritants and possible endocrine disruptors, respectively [38, 39]. In the case of the deionized water samples, 1,3-bis(1,1-dimethylethyl) benzene and 1-docosanol have been reported as possible eye, skin and respiratory tract irritants with 1,2-bezenedicarboxylic acid and butyl 2-ethylhexyl ester being teratogenic and an endocrine disruptor, respectively (Figure 3).

These findings may suggest that most of the above compounds are able to escape or resist current water treatment protocols and establish presence in the drinking water [40]. Thus, additional and more efficient and effective treatment protocols may be needed to be put in place to eliminate some of these toxic molecules.

With regard to the three locations, the water samples were highly contaminated with long chain hydrocarbons including aldehydes, ketones, esters and several aromatic compounds (Figures 4-6). The presence of these compounds may indicate that development is quickly transforming the landscape from forests and pastures to parking lots, streets and rooftops, causing rain to rush off the land instead of soaking into the ground [41]. It has also been found that storm water runoff is polluted, causes flooding, and erodes streams and riverbanks. Also as more land is converted from pastures and forests to pavement and rooftops, rain no longer soaks into the ground to percolate gradually to nearby streams and creeks [41, 42]. Instead, it immediately flows into storm drains and rushes into delicate creeks at high speed, washing away banks and vegetation. This causes both pollution and increased flooding in developed areas. About one-third of the Harpeth River watershed is located in one of the fastest growing regions in the country - Williamson County, Tennessee. Rapid development, certain agricultural activities, and some poorly functioning sewage systems may explain the levels of organic pollutants as detected (Figure 6) [43]. The levels of organic pollutants in the Cumberland River compared to that of the Harpeth River were heavy and almost identical. These findings are not surprising since the Harpeth River of middle Tennessee is a tributary to the Cumberland River and contains over 1,000 miles of streams which are subject to frequent minor flooding and major flooding every few decades [43, 44]. The most recent major flood, which occurred in May 2010, might have increased the organic burden and, for that matter, pollutants in both rivers. As reported, we have also observed the presence of toluene, derivatives of acetone and other long chain and modified fatty acids. These findings have been reported elsewhere and have been classified as known hazardous compounds and toxic. In fact, the above problem of organic pollutants in both rivers was realized in early 2007 and was addressed by the TDEC and the Egyptian Lacquer Manufacturing Company, which was also a source of such toxic compounds [45]. The levels of organic pollutants from the Mammoth Cave, though different from those of the Cumberland and Harpeth Rivers, were also highly contaminated with compounds such as derivatives of plastics, fatty acids and long chain hydrocarbons. These findings are in accordance with the fact that the Mammoth Cave Area Biosphere Reserve represents a karst system characterized by complex underground water courses and a multilayered cave system with unique fauna and mineralization features [38, 39]. Thus, the diversity of organic pollutants as detected may be due to the impact of agricultural, commercial, and residential land use on ecosystems, especially with respect to the effects of groundwater pollution on cave biota [46, 47].

In conclusion, acid/base liquid/liquid-GC/MS extraction is an effective method for detecting organic compounds in and extracting them from water and other contaminated sites. It is also important to mention that the GC/MS may have some drawbacks since the resolution of the retention time peaks due to the presence of many compounds, making it nearly impossible to identify every compound. The addition of LC/MS in tandem with GC/MS could be the most effective method of identifying all present compounds. Identifying these compounds is very important to enhance research efforts with regard to their health effects. Educating the public on the presence of hazardous and toxic materials in the aquatic ecosystem and the importance of their impact provides an awareness that could help reduce the presence of these contaminants.

CONCLUSION

Several compounds were detected and classified as either nontoxic, mildly toxic or toxic to humans based on the available information from the literature. The results indicate the presence of several emerging organic pollutants which may compromise the quality of drinking water for populations who largely depend on them.

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CONFLICT OF INTEREST STATEMENT

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