Comprehensive composition of Creosote using comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS)

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Abstract

Creosote is a distillation product of coal tar and is widely used as wood preservative for railway sleepers, utility poles and for other applications. Creosote can have potentially negative effects on the environment and many of the components are toxic. This study presents the analysis of a Creosote sample from a former wood impregnation plant located in the UK. The sample was analysed using two dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS) and a database of compounds that could be detected was produced. The GCxGG-TOFMS was capable of detecting 1505 individual compounds, which is far more than previous estimates for the number of compounds present within Creosote. Post extraction derivatization using BTSFA with 1% TMCS was employed to increase the potential number of compounds detected with 255 derivatized compounds detected, 231 of which would not have been detected without prior derivatization. Selected derivatized compounds were quantified with limits of detection ranging from

0.6mg/kg to 1.6mg/kg from a concentrated dense non-aqueous phase liquid (DNAPL). This work presents the first published full analysis of a Creosote using GCxGC-TOFMS combined with derivatization.

Keywords: Environmental Forensics, Creosote, GCxGC-TOFMS, Coal Tar, Derivatization

Introduction

Creosote is viscous distillation product of coal tar, with a density slightly higher than water (Giddings *et al*. 1985), and is widely used as a wood preservative (Mateus et al. 2008). It is still regularly used for the treatment of wooden railway sleepers. In the US 70% of all Creosote used is for the treatment of on railway sleepers and crossties and another 15-20% used for the treatment of utility poles and their cross arms (EPA, 2008). Coal tar Creosote is typically composed of approximate 85% polycyclic aromatic hydrocarbons; 10% phenolic compounds and 5% N-, O- and S- heterocycles (Mueller *et al*. 1989) although the overall composition may vary due to the production process, temperature and coal type used to produce the original coal tar (Johansen *et al*. 1997). The Creosote oil fraction of British coal tars ranged from 7% to 25% (Warne, 1913). Creosote can have negative effects on the environment as for example it can inhibit plant biomass accumulation (Marwood *et al*. 2003) and many of the compounds present within Creosote are toxic, carcinogenic and mutagenic.

When Creosote DNAPL (Dense Non Aqueous Phase Liquid) is spilled into the sub surface it will penetrate the water table due to it having a higher density than water and will continue its downward migration as a separate liquid (Johansen *et al*. 1998).

Within the vadose zone a portion of the volatile compounds will evaporate into the air phase, creating a gas phase contamination and infiltrating water can leach the soluble compounds present within Creosote (Johansen *et al*. 1998). Creosote within the groundwater zone will partially dissolve within the water, determined by the solubility of the individual compounds, and create a persistent long-term source of contamination. In 1978 fish in the Hersey River in Michigan USA were reported to have started tasting like "medicine" (Black. 1982). Investigation of the sediments at the bottom of the River revealed Creosote residue from a former wood preservation facility that had operated between 1902 and 1949. This demonstrated the ability of Creosote contamination to persist within the environment 20 years after plant closure and 4-5km downstream of the site (Sundström *et al*. 1986).

Polycyclic aromatic hydrocarbons (PAHs) form an important group of compounds that have been extensively studied as they persist within the environment. PAHs consist of fused aromatic rings, with their biochemical persistence arising from dense clouds of π-electrons on both sides of the ring structure (Wang *et al*. 2012). The hazards posed by PAHs can vary greatly with the number of fused rings. For example, the 4 and 5-ring PAHs have a strong tendency to be carcinogenic and/or mutagenic, while PAH's composed of 6 or more rings have substantial mutagenicity in human cells (Yu *et al*. 1998). The US EPA lists 16 parent PAHs on the list of priority pollutants. Alkylated PAHs are also important as they can contribute substantially to the toxicity of PAH mixtures, in some cases accounting for 80% of the toxic burden (Zeigler *et al*. 2012). In order to address this issue the EPA-34 was created which includes the original 16 EPA priority PAHs with alkylated PAHs included (Arp et al. 2011). It should be noted that due to the co-elution of the alkyl PAHs in GC the 34

PAH method actually represents several hundred individual alkylated PAH compounds (Hawthorne *et al*. 2006).

Heterocyclic compounds form an important group of compounds present within coal tars and coal tar derived liquids, such as Creosote. A heterocyclic compound is a compound that has at least two different elements as members of its ringed structure. Of particular interest in samples of coal tar, or coal tar derived liquids, are those containing oxygen (PAOH), sulfur (PASH) and nitrogen (PANH). The O, S and N heterocycles in tar are generally determined by the sulfur, oxygen and nitrogen content of the coal carbonized (McNeil. 1952) although with some temperature-dependent alteration (Gauchotte-Lindsay et al. 2012). Heterocyclic compounds are generally more water soluble than their PAH counterparts and therefore may be of particular interest when dealing with potential water source contamination from Creosote DNAPL.

The organic sulfur content of coal is determined by the original organic matter that formed the coal deposits and takes the form of aliphatic and aromatic thiols, sulfides, disulfides and heterocyclic combinations of thiophenes and dibenzothiophenes (Diez *et al*. 1994). Poly aromatic sulfur hydrocarbons (PASHs) are a group of sulfur containing compounds that are of particular environmental interest. PASHs exist in an even greater variety of structures compared to PAHs due to the presence of sulfur within the ring and with a larger number of alkylated isomers. PASHs in environmental samples can often be difficult to identify due to issues with separation (Mössner et al, 1999), however the use of GCxGC-TOFMS will reduce or potentially remove these issues.

PANHs are another important group of heterocycles and are highly stable relative to neutral PAH's and can persist through severe thermal conditions and which makes them possible compounds of toxicological interest (Yu *et al*. 1999). The toxicity of aromatic compounds greatly depends on the structure and number of fused rings. The presence of nitrogen-containing substituents, such as nitro- and amino- functional groups can enhance toxicity by up to 100-fold (Yu *et al*. 1999). This means that whilst the nitrogen content of the parent coal may be low, the possible health effects from the presence of nitrogen containing polycyclic aromatic compounds (NPAC) should not be overlooked.

Oxygen containing compounds are of special concern as they can be toxic, mutagenic and carcinogenic and are more mobile within the environment than their parent PAHs, due to their increased solubility in water. This enhanced mobility increases the potential for exposure to hydroxylated PAHs in groundwater from sites contaminated with Creosote and also increase the risks to human and environmental receptors associated with the contaminant plume. Oxygen containing compounds also form an important diagnostic component within coal tars and of particular interest are the hydroxyl- and dihydroxy- PAH's (Shi et al. 2010).

Phenolic compounds form a major group of oxygen containing compounds in coal tar and brown coal derived liquids, of which the alkyl phenols dominate (Shi *et al*. 2010). High phenolic content is a major characteristic of low temperature coal tars (650ºC) and medium temperature coal tars (800ºC) (Shi *et al*. 2012). This means that the abundance of phenolic compounds within a tar could potentially be used to suggest

126 the production process used or the degree of exposure that the primary tar has had to
127 secondary degradation. This means the production process used to produce the crude
128 coal tar from which the Creosote is distilled will affect the overall composition of the
129 final Creosote produced.
130
131 Derivatization allows for a wider range of compounds to be detected within coal tar
132 (Gauchotte-Lindsay *et al*. 2012). The aim of using a derivatization method for GC is
133 to improve peak symmetry, resolution, selectivity and sensitivity of the target analytes
134 and improve their thermal stabilities (Segura *et al*. 1998). Derivatization can increase
135 the sensitivity of detection of a particular compound of interest by several orders of
136 magnitude (Parkinson. 2012) and so allow for more compounds to be identified
137 within a sample patterns that aid with structural identification.
138
139 Of particular concern when dealing with Creosote contaminated sites is the potential
140 for groundwater contamination and contamination of other marine environments.
141 Most environmental monitoring focuses on a small number of PAH compounds,
142 however in the case of Creosote contaminated water bodies substantial decreases in
143 PAH concentrations in groundwater due to remediation do not always significantly
144 reduce the ecotoxicity (Breedveld and Sparrevik. 2000). This implies that an
145 extended list of compounds should be considered when dealing with Creosote
146 contaminated sites and this demonstrates a vital need for a comprehensive database of
147 compounds found within Creosote. While lists of compounds present within Creosote
148 have been published previously such as the various lists found in Sundström *et al*.
149 1986, only a single paper used a GCxGC based method (Mateus *et al*. 2008), although
150 this paper only looked at the volatile compounds emitted from wood treated with

151 Creosote and did not analyse Creosote itself. Of the previously published lists none
152 are as comprehensive as the database presented within this study. This study presents
153 the first comprehensive database of compounds detected within a Creosote sample. It
154 provides the identification of several compounds, and groups of compounds, that may
155 be of concern to human health and of environmental interest beyond the small number
156 of PAHs that are often used.
157
158 **Materials and Methods**
159 *Methods:*
160 All solvents used were of analytical grade purchased from Fisher Scientific
161 (Loughborough, U.K.) and D_{10}-phenanthrene, D_8-naphthalene, D_{10}-fluorene, D_{10}-
162 fluoranthene and D_{10}-pyrene were purchased from Sigma-Aldrich (Gillingham, U.K.).
163 Quantification standards of phenol, p-cresol, 3,5-dimethylphenol, 2,4,6-
164 trimethylphenol, 1-naphthol, aniline, and 1-hydoxypyrene were purchased from
165 Sigma-Aldrich (Gillingham, U.K.). N,O-bis(trimethylsilyl)trifluoroacetamide
166 (BSTFA) with 1% trimethylchlorosilane (TMCS) was purchased from Sigma-Aldrich
167 (Gillingham, U.K.). The tar sample was sampled in was stored at 4^{o}C in the dark
168 prior to analysis.
169
170 Extraction was performed using an Accelerated Solvent Extraction system (ASE 350
171 Dionex, Camberley, UK) using 10 mL stainless steel extraction cells and a modified
172 version of the ASE method published in McGregor *et al*. 2011. Approximately 0.5g
173 of tar was mixed with an equal amount of diatomaceous earth and sodium sulfate
174 ($NaSO_4$) in a 1:1:1 ratio. Prior to extraction the samples were spiked with a recovery
175 standard. Extraction cells were lined with 2 Dionex glass fibre filter papers and

176 packed with 3g of silica gel 60 deactivated with 10% water. The sample mixture was
177 then loaded into the cells and excess diatomaceous earth was added until the cell was
178 well packed to ensure that there is no void space. Dichloromethane was used as the
179 extracting solvent for all extractions. ASE was performed at 100^{o}C and 10 MPa, using
180 one dynamic (7 min) and two static (5 min each) extractions. A flush volume of 150%
181 and purge time of 60 s was used. The extracts were concentrated to 1 mL using a
182 Büchi Syncore Analyst (Oldham, U.K). The extracts were then made up to exactly 10
183 mL using *n*-hexane. A 1 mL aliquot was then transferred to an auto sampler vial prior
184 to analysis and spiked with D_{10}-Phenanthrene. All samples were derivatized using
185 100ul of BSTFA with 1% TMCS placed in an oven at 70^{o}C for 1 hour.
186
187 GCxGC TOFMS analysis was performed using a Leco Pegasus 4D (St. Joseph,
188 Michigan) time of flight mass spectrometer, connected to an Agilent 7890A gas
189 chromatograph equipped with a LECO thermal modulator. The TOF ion source
190 temperature was 200 °C and the mass range 45 and 500u was scanned at a rate of 200
191 spectra/second. The detector voltage was set at 1700 V with an electron ionisation
192 voltage of 70 eV.
193
194 All standards and extracts were analysed with the following primary oven temperature
195 programme modified from McGregor *et al*. 2011: 60°C isotherm for 2 minute, then
196 ramp at 10°C/min to 110°C, then ramp at 3°C/min to 310 °C, and isothermal at 310°C
197 for 15 minutes. The secondary oven and modulator temperatures were programmed at
198 a 20 °C offset relative to the primary oven. The modulation period was 6 seconds with
199 a 1.3 second hot pulse time and a cool time of 1.7 seconds. The injection port
200 temperature was set to 250 °C and set to split injection with a split ratio of 50 and an

injection volume of 1µl. Helium was used as the carrier gas, with a flow rate of 1.0 mL/min.

The reversed polarity column set that was used comprised of a mid-polarity TR-50 MS supplied by Thermo Scientific (30 m × 0.25 mm i.d. × 0.25 µm film thickness) as the primary column and a non-polar Rtx-5SilMS supplied by Thames Restek (1.5 m × 0.25 mm i.d. m × 0.25 µm film thickness) as the secondary column, connected via a Thames Restek Press-tight connector.

The sample chromatogram was processed using Leco ChromaTOF software (Version 4.50.8.0) to search for, identify and align all peaks with a signal-to-noise ratio greater than 10.

Sample:

The sample was recovered using a Low Flow (US EPA. 2010) from a sump present on a former wood treatment facility, associated with a former tar distillery in the United Kingdom. The sample was collected within a glass bottle and stored at $4^{o}C$ prior to analysis. The sample has been previously included in the analysis by McGregor *et al*. 2011 and was shown to be highly weathered. The sample was also included in the multivariate statistics in McGregor *et al*. 2012.

Quality Control:

To ensure the analytical accuracy of the data produced strict quality control measures were used including: The use of reagent and procedural blanks, the use or a recovery standard containing D_8-naphthalene, D_{10}-fluorene, D_{10}-fluoranthene and D_{10}-pyrene

and the use of an injection standard containing D_{10}-phenanthrene. All recoveries fell within the range suggested by US EPA method 8800B of between 70% and 130% and all blanks were clean and free of contamination.

Compound Identification:

Compounds were identified using both their mass spectra, with a similarity of above 800 usually indicating that an acquired mass spectrum shows a good match with the library search (Lu *et al.* 2003*),* and logical order of elution, within both the horizontal and vertical phases. In the case of named isomers the isomers were identified using either, in the case of the EPA18 PAHs, previous runs of known standards or using retention time index order of elution information combined with an in house database of retention times. In cases were mass spectra were not present within the NIST database, which can be the case for some alkylated isomers, the compounds were identified using their molecular ions, as well as their logical order of elution. While classification systems have been developed for providing identification confidence such as that published in Schymanski *et al*. 2015, these have been developed for non-target screening of environmental samples. The use of mass spectra, logical order of elution and retention time index information presented within this study provides sufficient confidence for the correct identification of compounds. It should also be noted that the classification system developed in Schymanski *et al*. 2015 was specifically developed for electron spray ionization (ESI) mass specs, whereas electron impact (EI) was used to produce the data presented within this study which can often be performed with a spectral library (Schymanski *et al*. 2015).

Results and Discussion

Composition:

A sample previously identified as Creosote Oil, DNAPL011 (McGregor *et al*. 2011), obtained from a sump on a former wood treatment facility associated with a former tar distillery in the UK was analysed. Creosote is a distillation product of coal tar and is one of the most widely used wood preservative in the world (Mateus *et al*. 2008) and can contain up to 17% of the total composition as Phenolic compounds (Bedient *et al*. 1984). A total of 255 derivatized compounds, shown in table 1, were detected.

A total of 16 phenolic compounds were also detected that could not be derivatized due to steric hinderance. Steric hinderance is the process by which compounds that contain active hydrogen may not derivatized due to the hindrance of the derivatization reaction around the hydroxyl group. For example, the derivatization of a standard of 2,4,6-trimethyl phenol was attempted using BSTFA, but was found not to derivatize. This is likely due to the fact that no matter where the hydroxyl group falls within the ring it will always have a methyl group on either side protecting it from derivatization. As the number of alkyl groups increases the possible number of sterically hindered isomers will likely increase as well. As well as the derivatized compounds the sample also contains 134 Aliphatic compounds, 612 PAHs/Alkyl PAHs, 217 Sulfur containing PAHs, 129 Oxygen containing PAHs, 128 Nitrogen containing PAHs and 12 Mixed Heterocycles (e.g. containing both Oxygen and Sulfur). Both cyclo-S6 and cyclo-S8 sulfur were detected giving a total of 1505 individual compounds, a full list of compounds including retention times can be found in the supplementary information.

275

Compound	m/z	Formula	No of Isomers	Retention window (min:sec)
phenol	166	C_6H_6O	1	6.9, 1.505
cresols	180	C_7H_8O	3	8.1, 1.725 to 8.5, 1.785
C_2-phenol	194	$C_8H_{10}O$	6	9.2, 1.960 to 11.1, 2.130
C_3-phenol 1DB or indanol	206	$C_9H_{10}O$	2	15.0, 2.475 to 16.1, 2.454
C_3-phenol	208	$C_9H_{12}O$	11	10.0, 2.120 to 13.0, 2.385
napthalen-2-ol	216	$C_{10}H_8O$	1	22.6, 2.530
C_4-phenol 1DB or C1-indanol	220	$C_{10}H_{12}O$	11	15.3, 2.565 to 19.2, 2.615
hydroxybenzothiophene	222	C_8H_6OS	1	23.4, 2.430
C_4-phenol	222	$C_{10}H_{14}O$	16	11.3, 2.430 to 15.1, 2.745
C_1-naphthalenol	230	$C_{11}H_{10}O$	3	24.9, 2.700 to 26.9, 2.625
C_5-phenol 1DB or C_2-indanol	234	$C_{11}H_{14}O$	23	15.7, 2.730 to 22.8, 2.740
C_1-hydroxybenzothiophene	236	C_9H_8OS	6	25.0, 2.560 to 27.2, 2.545
C_5-phenol	236	$C_{11}H_{16}O$	18	13.5, 2.700 to 18.1, 2.995
o-biphenyol	242	$C_{12}H_{10}O$	1	23.4, 2.585
hydroxyacenaphthene	242	$C_{12}H_{10}O$	2	28.8, 2.540 to 30.1, 2.605
C_2-naphthalenol	244	$C_{12}H_{12}O$	8	26.8, 2.765 to 30.9, 2.720
C_6-phenol 2DB	246	$C_{12}H_{16}O$	5	24.1, 2.800 to 28.0, 2.740
C_6-phenol 1DB or C_3-indanol	248	$C_{12}H_{16}O$	17	17.7, 2.895 to 24.5, 2.855
C_6-phenol	250	$C_{12}H_{18}O$	7	17.1, 3.035 to 20.0, 3.155
hydroxyfluorenes	254	$C_{13}H_{10}O$	3	35.7, 2.525 to 37.3, 2.590
C_1-biphenylol	256	$C_{13}H_{12}O$	2	25.9, 2.650 to 26.5, 2.650
C_1-hydroxyacenaphthene*	256	$C_{13}H_{12}O$	9	30.7, 2.660 to 34.9, 2.650
C_3-naphthalenol	258	$C_{13}H_{14}O$	5	29.8, 2.825 to 32.0, 2.830
C_7-phenol 2DB	260	$C_{13}H_{16}O$	13	23.6, 2.955 to 29.4, 2.820
C_7-phenol 1DB or C_4-indanol	262	$C_{13}H_{18}O$	6	20.9, 2.990 to 25.3, 3.150
C_7-phenol	264	$C_{13}H_{20}O$	4	20.4, 3.220 to 23.2, 3.320
anthrol	266	$C_{14}H_{10}O$	3	43.2, 2.490 to 44.2, 2.565
C_1-hydroxyfluorene	268	$C_{14}H_{12}O$	8	37.8, 2.605 to 40.7, 2.655
C_2-biphenylol	270	$C_{14}H_{14}O$	11	28.2, 2.685 to 31.4, 2.760
C_2-hydroxyacenaphthene*	270	$C_{14}H_{14}O$	11	34.7, 2.680 to 38.3, 2.735
C_8-phenol 2DB	274	$C_{14}H_{18}O$	5	27.0, 3.010 to 29.5, 2.985
C_8-phenol 1DB or C_5-indanol	276	$C_{14}H_{20}O$	2	25.9, 3.130 to 26.6, 3.215
C_8-phenol	278	$C_{14}H_{22}O$	4	24.3, 3.335 to 27.8, 3.440
C_1-anthrol	280	$C_{14}H_{22}O$	4	45.1, 2.550 to 47.1, 2.550
C_3-biphenylol	284	$C_{15}H_{16}O$	8	29.2, 2.790 to 33.8, 2.660
C_3-hydroxyacenaphthene*	284	$C_{15}H_{16}O$	7	37.4, 2.825 to 40.9, 2.775
hydroxy-4-ring PAH	290	$C_{16}H_{10}O$	2	51.4, 2.445 to 51.6, 2.430
C_9-phenol	292	$C_{15}H_{24}O$	3	27.4, 3.465 to 29.6, 3.525
C_4-hydroxyacenaphthene*	298	$C_{16}H_{18}O$	3	39.8, 2.790 to 41.1, 2.760

276 Table 1: Total number of derivatized compounds in Creosote (DNAPL011) (DB =

277 Double Bond) * or Hydroxydibenzofuran isomers

278

279 *Derivatization:*

280 The expected predominant phenolic compounds present within coal tar Creosote are
281 phenol, o-cresol, m-cresol and p-cresol, which should make up 50% of the total
282 composition of pure Creosote (Mueller *et al*. 1989). However, the production process
283 and feedstock used to produce the coal tar affects the overall composition of the
284 distilled Creosote, for example the production of Phenols and alkyl Phenols is
285 significantly different between vertical and horizontal retort types (McGregor *et al*.
286 2011). The overall concentration of select derivatized compounds is shown in table 2.
287 The limits of detection for the method were calculated and ranged from 0.6mg/kg for
288 phenol to 1.6mg/kg for hydroxypyrene suggesting the majority of compounds
289 derivatized by the method would fall within this range in pure phase tar.

290

Retention time (min:sec)	Compound	Conc mg/kg	LOD mg/kg	Retention time (min:sec)	Compound	Conc mg/kg	LOD mg/kg
6.9, 1.505	phenol	38	0.6	10.3, 2.100	C_2-phenol	313	0.8
8.1, 1.725	o-cresol	278	0.8	10.6, 2.140	C_2-phenol	227	0.8
8.3, 1.750	m-cresol	181	0.8	11.1, 2.130	C_2-phenol	165	0.8
8.5, 1.785	p-cresol	112	0.8	22.6, 2.530	napthalen-2-ol	426	0.9
9.2, 1.960	ethyl phenol	206	0.8	51.4, 2.445	hydroxy 4-ring PAH a	47	1.6
9.5, 2.015	C_2-phenol	612	0.8	51.6, 2.430	hydroxy 4-ring PAH b	40	1.6
9.9, 2.060	3,5-dimethyl phenol	1958	0.8				

291

292 Table 2: Concentration of selected derivatized compounds in Creosote (DNAPL011).

293

294 The relative concentrations of phenol, o-cresol, m-cresol and p-cresol found within
295 the samples are low with only 38 mg/kg of phenol and a combined concentration of
296 571 mg/kg for the 3 cresol isomers. The most dominant phenolic compound found in
297 DNAPL011 was 3,5-dimethyl phenol, which would be expected to make up 7.5% of

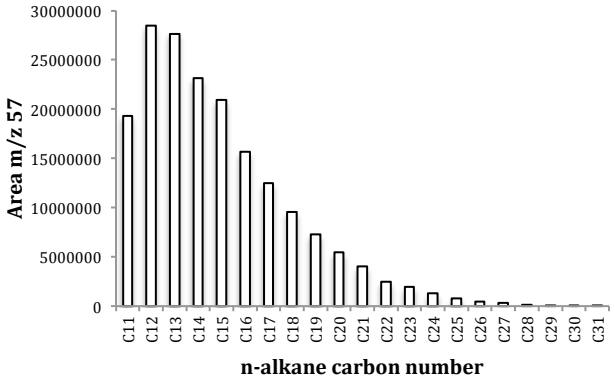
298 the predominant phenolic compounds (Mueller at al. 1989), and is present in a
299 concentration of 1958 mg/kg. Since the sample has been previously shown to be
300 heavily weathered (McGregor *et al*. 2011) one possible explanation for the low
301 concentrations of Phenol and Cresols is their aqueous solubility, although volatility
302 may also play a role through volatilization into the air surrounding the sump.
303
304 p-Cresol, which is present at a concentration of 112 mg/kg is the most toxic of the
305 cresol isomers with a 5 to 10-fold concentration of either o-cresol or m-cresol being
306 needed to observe the same degree of toxicity as p-cresol (Thompson *et al*. 1994).
307 This means that although p-cresol has the lowest concentration of the cresol isomers it
308 would have the environmental highest risk associated with it. p-Cresol and phenol
309 also have the ability to change bacterial membrane lipid structure, increasing the
310 degree of saturation of the lipids, as the phenols alter the cell membrane permeability
311 and increase their fluidity (Keweloh *et al*. 1991).
312
313 The environment effects of the cresols do not only extend to their direct toxicity.
314 Creosote is a complex mixture of compounds and interactions between these
315 compounds are important when considering the overall risk associated with a
316 contaminated site. Low concentrations of o-cresol can increase the carcinogenicity of
317 benz(a)pyrene, whereas high concentrations can inhibit the carcinogenic effect
318 (Yanysheva *et al*. 1993). p-Cresol can be utilized by bacteria as a sole carbon and
319 energy source (Yu and Loh 2002) and the presence of p-cresol can inhibit the
320 degradation of carbazole with incomplete degradation of carbazole at p-cresol
321 concentrations above 20mg/L and complete removal of carbazole can only occur
322 when p-cresol concentrations are below 10mg/L (Yu and Loh 2002). When

concentrations of p-cresol are higher than 120mg/L carbazole degradation is completely inhibited. This means that the concentrations of p-cresol are important as they will affect degradation of other compounds present within the sample. p-Cresol also has the ability to inhibit the degradation of phenanthrene (Millete *et al*,.1995) and Phenol (Kar *et al*. 1997). Due to the concentrations of p-cresol this suggests that biodegradation of carbazole is unlikely to take place within the sump itself, although it may take place within the environment around the sump.

Among the other Phenolic compounds detected the octyl (C_8) and nonyl (C_9) phenols may be of particular interested from an UK/European perspective. Both octyl and nonyl Phenols are included in directive 2008/105/EC due to the fact they are potential endocrine disruptors. Octyl and nonyl phenols are also persistent within the environment, moderately bio accumulate and are extremely toxic to aquatic organisms. In total 4 C_8 phenols were detected (as well as 2 C_8 phenols with 1 double bond and 5 with 2 double bonds) and 3 C_9 phenols were detected within the sample. No literature could be found reporting the presence of octyl or nonyl phenols within Creosote or coal tars. One possible reason for the lack of literature reporting octyl and nonyl phenols within coal tar, or coal tar distillates, is that the compounds were only detected due to derivatization and derivatization techniques have not commonly been applied to coal tar. Another possible reason is that the octyl and nonyl phenols both boil within the range of Creosote and so may be enriched during the distillation process and therefore become detectable. Octyl and nonyl phenols may be present in other forms of coal tar, or coal tar distillate, in trace amounts and are not detected due to being present below the limits of detection of these compounds.

348 The sample was also run under the same GCxGC conditions without the use of
349 derivatization with 24 phenolic compounds, excluding sterically hindered compounds
350 detected in both samples, detected. The compounds detected were phenol, the 3
351 cresol isomers, 5 C_2-phenol, 5 C_3-phenol, 3 C_4-phenol, 2 C_1-naphthalenol, 4 C_5-
352 phenol and 1 C_6-phenol isomers. This clearly demonstrates that derivatization of the
353 sample allowed for the detection of 231 compounds that would have otherwise not
354 been detected, including the octyl and nonyl phenols.
355
356 *Aliphatic:*
357 Alkyl-cyclohexanes are compounds that are associated with being derived from
358 petrogenic sources (Saber *et al*. 2006) and can be used for differentiation of fuel-types
359 from petrogenic sources (Kaplan *et al*. 1997). Alkyl-cyclohexanes were detected
360 within the Creosote sample with an alkyl range between C_4 and C_{18}. This suggests
361 that there is a petrogenic element in the sample. One possibility is that the crude tar
362 from which the Creosote was distilled, may have contained an element of Carbureted
363 Water Gas (CWG) tar. The CWG was a process used at gasworks to produce a gas
364 relatively quickly from hot coke injected with steam and then enriched with oil
365 (Thomas, 2014). CWG tar was often mixed with coal tar to enable its sale to tar
366 distillers. This was because CWG tar had a higher water content (due to the emulsions
367 it would form) and contained less compounds of value to distillers making it of little
368 or no commercial value (Lunge, 1916). Mateus *et al*. 2008 published a qualitative
369 analysis of the volatile fraction of Creosote-treated railway sleepers using GCxGC-
370 TOFMS and detected a total of 314 compounds including alkyl-cyclohexanes. This
371 suggests that alkyl-cyclohexanes may form a part of Creosote oil, although it could

372 also be from petrogenic contamination of the samples. Of the 314 volatile compounds
373 detected by Mateus *et al*. 2008 212 were detected within DNAPL011.
374
375 A wide range of other aliphatic compounds were also detected within the samples
376 including n-alkanes from C_{11} to C_{31}, Pristane and Phytane, and 36 branched alkanes
377 between C_{11} and C_{24}. A large number of alkyl-cyclopentanes and alkyl-cyclopentenes
378 were also detected within the sample ranging from C_5-cyclopentene to C_7-
379 cyclopentane. The overall distribution of the n-alkanes is shown in figure 1 and
380 shows that the C_{12} and C_{13} n-alkanes dominate with a decreasing trend within
381 increasing carbon area.



382
383 Figure 1 – n-alkane distribution Creosote tar sample (DNAPL011)
384
385 *PAHs:*
386 The single largest class of compounds present within the samples were the PAHs and
387 alkyl PAHs. Of the EPA34 PAHs, 32 out of the 34 groups of compounds were
388 detected within the sample. As the EPA34 list actually contains many hundreds of
389 individual compounds a total of 168 individual compounds were detected with the
390 majority being alkylated isomers. Only C_4-chrysene and C_4-phenanthrene, from the
391 EPA34, were not detected. The lowest molecular weight PAH detected was styrene

392 (C_8H_8) with the highest molecular weight compound being a dibenzopyrene isomer
393 ($C_{24}H_{14}$). The vast majority of the PAHs detected within the sample are in the form of
394 alkylated isomers. The concentration of the EPA16 PAHs in the sample have
395 previously been published in McGregor *et al*. 2011 and showed that Naphthalene and
396 Phenanthrene had the highest concentrations.
397
398 *Heterocycles:*
399 Of the mixed heterocycles detected within the Creosote sample the most common
400 were thienobenzofurans ($C_{10}H_6OS$), 6 of which were detected, and have not
401 previously been reported in the literature. Dimethylbenzoxazole (C_9H_9NO) was also
402 detected within the sample and has not previously been reported in coal tar or coal tar
403 distillates. Thieno[2,3-c]pyridine (C_7H_5NS) has been previously reported in
404 Anthracene oil (Burchill *et al*. 1982) and azadibenzothiophenes ($C_{11}H_7NS$), of which
405 3 were detected, have been previously reported in Anthracene oil (Burchill *et al*.
406 1982) and solvent refined coal heavy distillate SRCII (Nishioka *et al*. 1985), although
407 none of the mixed heterocycles have been previously reported in Creosote. Elemental
408 Sulfur can also be found within Creosote (Sundstrom *et al*. 1986) and is found within
409 the Creosote sample in the form of *cyclo*-hexasulfur (S_6) and *cyclo*-octasulfur (S_8).
410
411 PANHs form an important group of compounds of interest with DNAPL011
412 containing PANHs ranging from dimethyl pyridine (C_7H_9N) to 4H-
413 benzo[def]naphtho[2,3-b]carbazole ($C_{22}H_{13}N$). A large number of alkyl PANH
414 isomers are present with the largest group being dimethyl carbazole with a total of 9
415 isomers. Of the 128 PANHs present within the sample 79 are alkylated isomers.
416 Only a single compound containing more than 1 nitrogen was detected in the form of

417 biphenyldicarbonitrile ($C_{14}H_8N_2$), which is not heterocyclic and contains two nitrile
418 groups. The vast majority of PANHs detected within the sample are in the form of
419 nitrogen containing heterocycles, however several compounds that have nitrogen
420 containing functional groups were also detected. Compounds detected that contain
421 functional nitrogen include 1-naphthalenecarbonitrile and 2-naphthalenecarbonitrile
422 ($C_{11}H_7N$), as well as their alkylated isomers, which contain nitrogen in the form of a
423 nitrile group.
424
425 A wide range of PASHs were detected ranging from C_2-thiophene (C_6H_8S) to a
426 napthobenzodithiophene isomer ($C_{18}H_{10}S_2$). Napthobenzodithiophene isomer is one
427 of 7 Sulfur compounds present within the sample which contains 2 Sulfur atoms
428 within the ring as well as thieno[2,3-b]thiophene ($C_6H_4S_2$), 3 benzodithiophenes
429 ($C_{10}H_6S_2$), and 2 benzo[b]thieno[3,2-b]benzo[b]thiophene ($C_{14}H_8S_2$) isomers. C_2-
430 Thiophene is the lowest molecular weight PASH that can be detected using the GC
431 method and so it is possible that more volatile, and lower molecular weight, PASHs
432 are present within the sample but are undetectable. Due to the presence of Sulfur
433 within the ring a large number of alkylated PASHs exist. Of the 217 PASHs detected
434 166 are in the form of alkylated isomers. C_4-Benzothiophene ($C_{12}H_{14}S$) and C_2-
435 dibenzothiophene ($C_{14}H_{12}S$) form the largest groups of isomers with 14 compounds
436 present in each group. Of the PASHs detected alkyl-benzothiophenes and alkyl-
437 dibenzothiophenes both form the largest groups with 94 compounds and 47 in each
438 group. Only two 2-ring parent PASHs were detected within the sample,
439 benzo[b]thiophene and 2-benzothiophene (C_8H_8S), meaning that the largest individual
440 group of compounds is likely to be alky-benzothiophenes as the alkyl-
441 dibenzothiophene group does not differentiate between the 3-ring parent PASH

isomers. Of the 3-ring parent PASHs 4 were detected including dibenzothiophene ($C_{12}H_8S$). Naphtha[1,2-b]thiophene was also detected and is the only 3-ring PASH that has been shown to be mutagenic (Jacob. 1990). A total of seven 4-ring parent PASHs were detected including phenanthro[3,4-b]thiophene ($C_{16}H_{10}S$). Phenanthro[3,4-b]thiophene is the most mutagenic PASH (Jacob. 1990) with phenanthro[4,3-b]thiophene showing a much lower mutagenicity indicating that the position of the Sulfur plays a key role in the biological effect of the compound (Jacob. 1990).

PAOHs form an important group of compounds present within coal tar and coal tar distillates and includes all heterocyclic oxygen containing compounds as well as non-heterocyclic oxygen containing compounds such as acetophenone (C_8H_8O), for the purposes of this study hydroxylated compounds are classified within their own group. A total of 129 PAOHs are present within the Creosote sample ranging from benzofuran (C_8H_6O) to dinapthofuran isomers ($C_{20}H_{12}O$). Of the 129 PAOHs detected 105 are in the form of Heterocycles with alkyl isomers again dominating, as well as 3 benzobisbenzofuran isomers ($C_{18}H_{10}O_2$) containing 2 oxygen atoms within the ring. Of the remaining 24 compounds the majority are in the form of aromatic ketones such as anthrone ($C_{14}H_{10}O$) and 4H-cyclopenta[def]phenanthren-4-one ($C_{15}H_8O$), 1 coumarin in the form of xanthone ($C_{14}H_{12}O$) and 2 quinones in the form of 9,10-anthracenedione ($C_{14}H_8O_2$) and 5,12-naphthacenedione ($C_{18}H_{10}O_2$) both of which have been previously reported in coal tar (Benhabib *et al*. 2010).

Fluorenone ($C_{13}H_8O$) has also previously been reported in coal tar (Benhabib *et al*. 2010) and could be produced during the pyrolysis process, however, it can also be

467 produced during the metabolism of fluorene (Grifoll et al. 1992) and fluoranthene
468 (Kelley et al. 1993) so it is possible it may have been produced, or a portion of it
469 produced, during microbial degradation of the tar. Fluorenone can also be produced
470 by the oxidation of fluorene (Korfmacher et al. 1980). Eriksson *et al*. 2000 reported
471 increases in the concentrations of both fluorenone and 4H-
472 cyclopenta[def]phenanthren-4-one during the Creosote contaminated soils.
473 Wischmann and Steinhart. (1997) also reported increases in the concentrations of
474 fluorenone and 9,10-antracenedione during the degradation of a coal tar oil, it is
475 reportedly used as a wood-preservative so likely to be Creosote, in soil. 9,10-
476 Antracenedione has been reported to have potential negative environmental effects as
477 it inhibits the growth of duckweed (Mallakin *et al*. 1999) and has around 31 times
478 higher aqueous solubility than anthracene, although it is still has a relatively low
479 water solubility of 1.4mg/kg H_2O at 25^{o}C. The detection of these compounds
480 suggests the possibility for bacterial activity within the sample.
481
482 *Toxicity:*
483 PAHs account for up to 85% of pure Creosote but only account for around 13% of the
484 total toxicity in Creosote contaminated groundwater (Hartnik *et al*. 2007). 80% of the
485 toxicity can be attributed to methylated benzenes, phenolic compounds and N-
486 heterocyclic with up to 26% of the total toxicity coming from the alkylated quinolines
487 (Hartnik et al. 2007), which dominated the most toxic fraction analysed by Hartnik *et*
488 *al*. 2007. A total of 20 alkylated quinolines were detected within our sample with 4
489 methyl quinolines, 8 dimethyl quinolines and 8 trimethyl quinolines, in addition to
490 this a total of 106 other PANHs were also detected. The toxicity of dimethyl
491 quinolines can span over two orders of magnitude and is affected by the relative

492 position of the nitrogen within the ring as well as the relative positions of the methyl
493 groups to the nitrogen (Birkholz *et al*. 1990). Of the other compounds detected within
494 the most toxic fraction in Hartnik *et al*. 2007 acridine and 2-benzothiophene were also
495 detected within our Creosote sample. A total of 71 alkylated benzenes were detected
496 within the sample with 3 C_3-, 10 C_4-, 16 C_5-, 21 C_6-, 11 C_7- and 10 C_8-Benzenes
497 detected several of which may contribute to the overall toxicity of the Creosote.
498
499 While in general PANH compounds are present in lower concentrations than their
500 non-substituted PAH-analogues their higher water solubility leads to a higher
501 bioavailability and potential toxic effects (Neuwoehner *et al*. 2009) and low molecular
502 weight PANHs can account for up to 70% of the water-soluble fraction of Creosote
503 (Padma *et al*. 1998). For example Quinoline has a water solubility of 60,000mg/L
504 whereas naphthalene has a solubility of 30mg/L. Acridine and quinoline, both of
505 which were detected within DNAPL011, have toxic and teratogenic effects at
506 sufficiently low concentrations to make them potential environmental hazards (Davis
507 *et al*. 1981). The environmental impacts of these compounds may be greater than
508 their reported LC50 values because of sub lethal effects such as decreased growth rate
509 that may render surviving organisms incapable of coping with environmental stress
510 (Davis *et al*. 1981).
511
512 *Forensics:*
513 Since Creosote is a distillation fraction of coal tar covering the ranges 200^{o}C-400^{o}C
514 (McNeil. 1952), the presence of compounds that boil below 200^{o}C, such as styrene
515 (C_8H_8), and compounds that boil well above 400^{o}C, such as coronene ($C_{24}H_{12}$),
516 suggests that the Creosote is not in the form of pure distillate and has been blended

517 with another form of tar, most likely in the form of CWG tar. The presence of these
518 compounds may also suggest when the CWG tar was added to the blend as if it was
519 added before distillation styrene and coronene should not be distilled from the
520 resulting tar mix.
521
522 McNeil. 1952 states that Creosote derived from vertical retort (VR) tars contain 25%
523 tar acids (Phenolics) and 60-65% PAHs, with the majority containing one or more
524 methyl substituent groups. McNeill. 1952 also states that in contrast coke oven (CO)
525 and horizontal retort (HR) tars contain no more than 10% phenolics and generally
526 90% PAHs with a considerable proportion containing no substituent groups. It should
527 also be noted that while HR and CO produced Creosotes do differ from those
528 produced from VR tars the constituents of the Creosote do not vary only the relative
529 amounts and distribution (McNeil. 1952). Coke oven tars fall loosely into two
530 categories, those produced at low temperatures (<700^{o}C) such as Coalite coke and
531 those produced at higher temperatures (>700^{o}C) (Hamper, 2006). This also applies to
532 horizontal retort tars as early horizontal retorts operated at lower temperatures of
533 around 600^{o}C (Harkins *et al*. 1988) with later designs being capable of operating in
534 excess of 1000^{o}C (Butterfield. 1904). Low temperature coke oven tars and low
535 temperature horizontal retort tars would both contain phenolic compounds in greater
536 degree than the high temperature processes of the same type (Hamper, 2006). While
537 McNeil. (1952) does not state if the horizontal retort or coke oven tars are from a
538 higher temperature or low temperature process it is most likely to be a high
539 temperature process due to the compositions listed.
540

One of the most important differences given in McNeill. (1952) is that VR derived Creosote contains a much higher tar acid content than CO and HR tars mainly in the form of high-boiling water-insoluble compounds which are not likely to be leached out by weathering. While the paper does not directly state what these compounds would be, Woolfolk *et al*. 1950 defines these high boiling compounds as those that boil above the Xylenol (C_2-phenol) range. The presence of a large number of Phenolic compounds that boil above C_2-phenol, with 258 of the 271 phenolic compounds (including sterically hindered phenolics) detected within the sample boiling above the C_2-phenol range, suggests that the Creosote was derived primarily from a VR tar.

The large database of compounds that the GCxGC can produce is also important from a legal forensics standpoint. Polluter pays forms the basis of environmental regulation in many European countries and the USA, for example within the European Environmental Liability Directive 2004/35/EC. In complex sites where multiple possible sources of contamination are present, increasing the potential number of unique compounds that can be identified increases the chances of establishing exactly which process the contamination has originated from. This means that the use of GCxGC greatly increases the forensic potential of a sample, with the use of the derivatization further increasing the capability of the method.

Conclusion

The use of GCxGC-TOFMS allowed for the resolution and detection of 1505 individual compounds within a sample of Creosote and the use of derivatization allowed for 231 compounds to be detected than would be detected without

566 derivatization. A large number of potential compounds of environmental interest
567 were detected including octyl and nonyl phenols, which have not previously been
568 reported in coal tar, or coal tar distillates. The GCxGC analysis was able to
569 determine that the Creosote was likely produced from a Vertical Retort tar due to the
570 presence of high boiling phenols, many of which would not have been detected
571 without the use of derivatization. The GCxGC analysis was also able to detect the
572 presence of petrogenic compounds, such as alkyl cyclohexanes, that were likely added
573 into the tar prior to distillation. The use of GCxGC for the analysis of environmental
574 samples increases the potential number of compounds detected within a sample
575 without the need for any length separation methods and will likely increase with
576 importance in the future.
577

578 **Acknowledgements**

579 We thank the Scottish Funding Council (SFC) Glasgow Research Partnership in
580 Engineering, the University of Strathclyde, WSP Parsons Brinckerhoff and National
581 Grid property for funding support. The authors declare no competing financial
582 interests.

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