Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Electronic Supporting Information

Linking biodegradation kinetics, microbial composition and test temperature - testing 40 petroleum hydrocarbons using inocula collected in winter and summer

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Content:

ESI1: Method descriptions for inoculum characterization parameters.

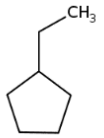
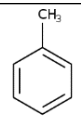
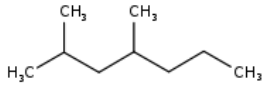
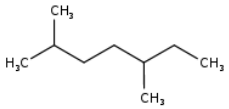
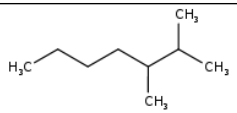
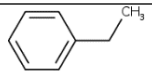
Onsite parameters: temperature (ºC), O_2 (mg/L), pH , and conductivity (µS/cm) was measured using a Hach HQ40D portable pH and dissolved oxygen meter.

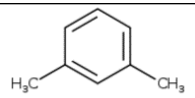
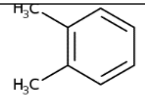
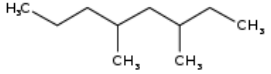
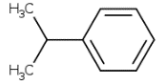
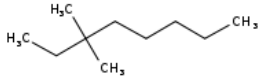
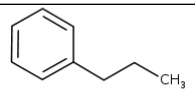
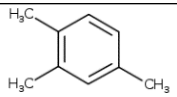
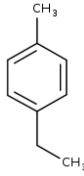
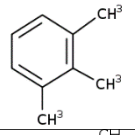
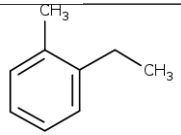
Colony forming units (CFU) was measured on R2A agar following the DS/EN ISO 8199 protocol (Dansk Standard 2018).

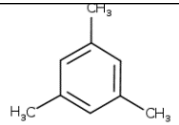
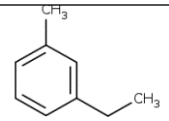
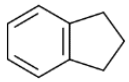
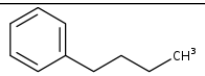
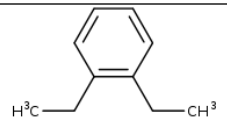
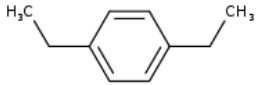
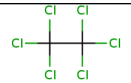
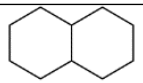
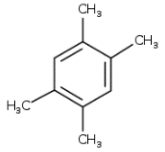
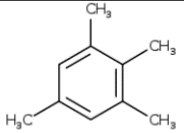
Total dissolved solids (TDS) (mg/L) and, total suspended solids (TSS) (mg/L) were analyzed following the DS/EN 872:2005 protocol (Dansk Standard, 2005), and non-volatile organic carbon (NVOC) (mg/L) was measured by internally method.

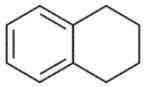
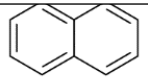
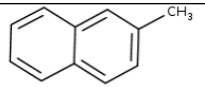
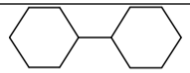
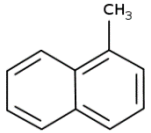
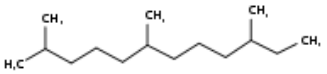
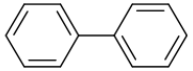
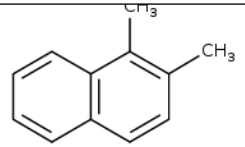
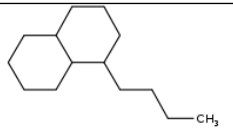
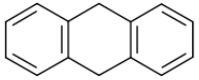
PO_4^{3-} (µg/L), NO^{3-} (µg/L), NH_3 (µg/L) and NO_x (µg/L) were analyzed using segmented flow analysis (San++ System, Skalar Analytical BV, The Netherlands.)

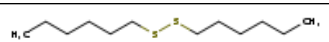
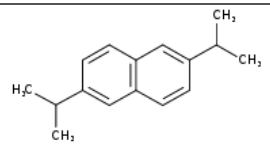
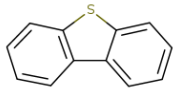
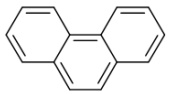
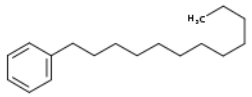
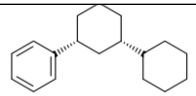
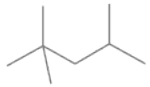
ESI2: Petroleum hydrocarbons tested, structure, retention time, quantifier, qualifier m/z's, air-water partitioning coefficients and initial concentrations.

#	Name	Cas#	Structure	Rt, mins	Quant. m/z	Qual. m/z	K_{aw},* L/L	Initial conc., µg/L**
1	Benzene	71-43-2		7.04	78	77	0.23	200
2	Ethylcyclopentane	1640-89-7		10.31	69	68	14	4
3	Toluene	108-88-3		12.14	91	92	0.28	100
4	2,4-Dimethylheptane	2213-23-2		15.34	43	85	170	0.2
5	2,5-Dimethylheptane	2216-30-0		16.09	57	43	170	0.2
6	2,3-Dimethylheptane	3074-71-3		17.22	43	84	18	0.2
7	Ethylbenzene	100-41-4		17.42	91	106	0.33	20
8	*p*-Xylene	106-42-3		17.88	91	106	0.29	20

9	*m*-Xylene	108-38-3		17.96	91	106	0.30	20
10	*o*-Xylene	95-47-6		19.01	91	106	0.22	20
11	3,5-Dimethyloctane	15869-93-9		20.43	57	43	220	0.06
12	Cumene	98-82-8		20.49	105	120	0.48	3
13	3,3-Dimethyloctane	4110-44-5		21.02	71	43	220	0.06
14	*n*-Propylbenzene	103-65-1		21.83	91	120	0.44	3
15	1,2,4-Trimethylbenzene	95-63-6		22.14	105	120	0.13	8
16	4-Ethyltoluene	622-96-8		22.28	105	120	0.21	4
17	1,2,3-Trimethylbenzene	526-73-8		22.50	105	120	0.18	-
18	1-Ethyl-2-methyltoluene	611-14-3		22.87	105	120	0.23	4

19	1,3,5-Trimethylbenzene	108-67-8		23.52	105	120	0.36	4
20	1-Ethyl-3-methyltoluene	620-14-4		24.60	105	120	0.36	4
21	Indane	496-11-7		25.14	117	118	0.01	8
22	*n*-Butylbenzene	104-51-8		25.91	91	92	0.66	0.9
23	1,2-Diethylbenzene	135-01-3		26.00	105	119	0.11	1
24	1,4-Diethylbenzene	105-05-5		25.99	119	105	0.31	1
25	Hexachloroethane	67-72-1		26.70	117	119	0.10	2[b]
26	Decalin	91-17-8		27.77	138	96	20	0.3
27	Durene	95-93-2		28.04	119	134	0.33	1
28	Isodurene	527-53-7		28.16	119	134	0.33	1

29	Tetralin	119-64-2		29.68	104	132	<0.01[a]	10
30	Naphthalene	91-20-3		30.45	128	102	0.02	8
31	2-Methylnaphthalene	91-57-6		33.80	142	141	0.02	3
32	Bicyclohexyl	92-51-3		34.16	82	166	4.0	0.09
33	1-Methylnaphthalene	90-12-0		34.27	142	141	0.02	2
34	2,6,10-Trimethyldodecane	3891-98-3		35.67	57	71	2700	-
35	Biphenyl	92-52-4		36.14	154	153	0.01	2
36	1,2-Dimethylnaphthalene	573-98-8		38.15	156	141	0.01	5
37	α- *n*- Butyldecalin	92369-80-7		38.49	137	81	<0.01[a]	-
38	9,10-Dihydroanthracene	613-31-0		43.45	180	179	0.01	5[b]

39	Dihexyl disulphide	10496-15-8		43.80	150	234	0.86	-
40	2,6-Diisopropylnaphthalene	24157-81-1		44.27	197	155	0.08	-
41	Dibenzothiophene	132-65-0		45.03	184	139	0.001	4
42	Phenanthrene	85-01-8		45.51	178	176	0.002	3 [b]
43	Dodecylbenzene	123-01-3		46.74	92	91	12	<0.001
44	3-Phenyl-1,1'-bi(cyclohexane)	33460-02-5		48.63	91	242	0.22	-
45	Iso-octane	540-84-1		-	-	-	-	0.01

*Calculated from K_H (20ºC) obtained from EpiSuite [1]. **Calculated from amount loaded into silicone and silicone water partition ratios found in the UFZ_LSER database: https://www.ufz.de/index.php?en=31698&contentonly=1&m=0&lserd_data[mvc]=Public/start. "-": no silicone water partition ratios were found in the database.

[a]Calculated from K_H (25ºC) obtained from EpiSuite[1].

[b]Calculated from a methanol silicone partition ratio of 3[2] and silicone water partition ratios found in the UFZ_LSER database.

ESI3: Microbial community analysis

Cell filtering

The inocula were filtered within 24 hrs of sampling the river water. All equipment was first sterilized with ethanol and cleaned by filtering ultrapure water. A 150 mL ultrapure water sample was filtered as a negative control, then triplicate 150 mL river water were filtered. The samples were filtered through a sterile cellulose nitrate filter (0.2 µm, diameter 47 mm) using vacuum suction. The filters were handled at all times with metal tweezers. After filtration, the filters were packed separately in aluminum foil and frozen to -80^{0}C. The filters were sent frozen to DNASense, Aalborg, Denmark for DNA extraction and microbial community analysis using 16S rRNA gene amplicon sequencing using Illumina MiSeq[3].

DNA extraction, library preparation and sequencing & qPCR

For the Gudenaa Winter samples, all samples yielded >2 ng/µL (extract) and based on gel electrophoresis the majority of the DNA was >5000 bp, which is recommended for amplicon sequencing. Library preparation for the16S rRNA gene (V4 region) was successful for all samples and yielded between 15134 and 78711 reads after QC and bioinformatic processing. The blank samples revealed a much lower diversity than the rest of the samples, similar to the extraction negative and PCR negative.

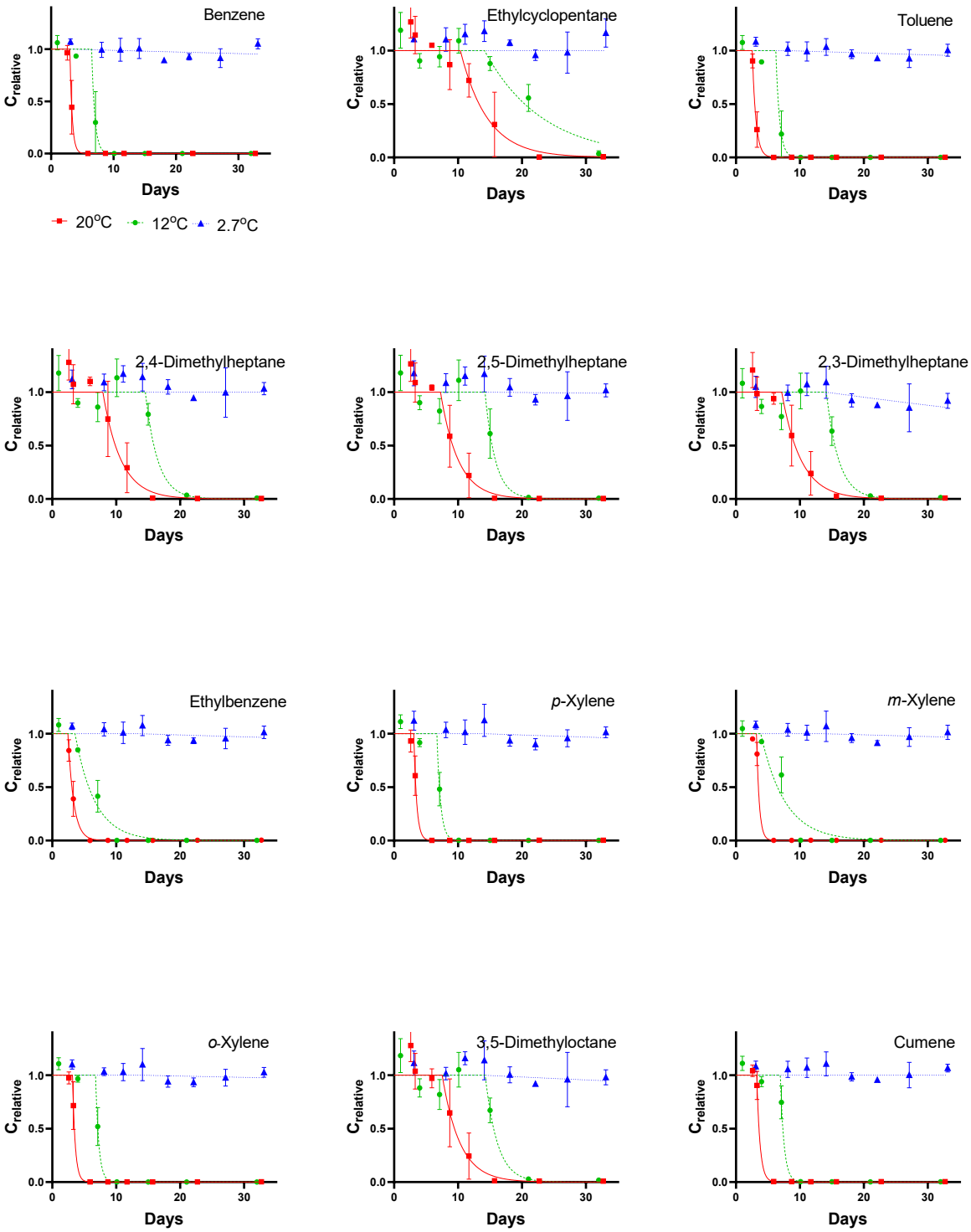
For the Gudenaa Summer samples, DNA extraction yielded concentrations <2 ng/µL (extract) for all samples except sample 12ºC, 15 days replicate 3. Gel electrophoresis showed that most of the extracted DNA was long fragments. Library preparation was successful for all samples and yielded between 13324 and 77892 reads after QC and bioinformatics processing. The blank samples revealed a much lower diversity than the rest of the samples, similar to the extraction negative and PCR negative.

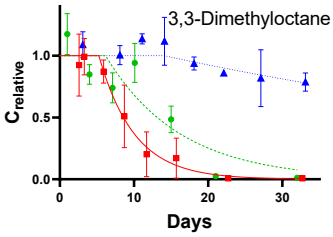
The abundance of 16S rRNA genes per ng of isolated DNA was estimated based on the broad-range qPCR probe and primer set [4]. A linearized plasmid containing the qPCR amplicon was used to create the standard curve. Briefly, the forward (5'-TCCTACGGGAGGCAGCAGT-3') and reverse (5'-GGACTACCAGGGTATCTAATCCTGTT-3') primers were used to amplify the qPCR amplicon of from E. coli MG1655 using the AccuPrime Pfx DNA polymerase (Thermo Scientific). The PCR was carried out according to the manufacturers recommendations.
The PCR product was purified on an E-gel Clone ell gel (Thermo Scientific) and cloned into the pCR4-TOPO vector using the TOPOTA CloningKit for Sequencing (Thermo Scientific) according to the manufactures recommendations. The obtained plasmid subsequently linearized with FastDigest NcoI (Fermentas), blunted using the Klenow fragment (Fermentas). The concentration of the amplicon stock was determined using the Qubit HS dsDNA assaykit (Life Technologies) and the copy number then calculated based on the molecular weight of the

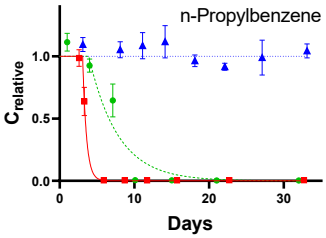
linerized plasmid. The amplicon stock was diluted to 108 copies/µL in 10 mM tris buffer (pH 8.5) and stored as aliquots at -18°C.

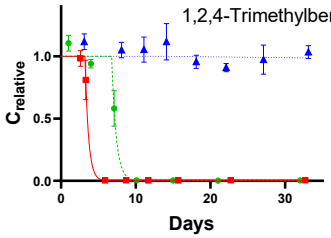
Sample qPCR measurements were done in technical duplicates using the Mx3005P qPCR system (Stratagene) and theEXPRESS qPCR Supermix (Life Technologies). Reactions of 24 µL were prepared according to manufacturer's instruction using 50 nM ROX, 500 nM of each primer, 200 nM hydrolysis probe (6-FAM)-5'-CGTATTACCGCGGCTGCTGGCAC- 3'-(BHQ-1) and 1 µL template DNA. The qPCR reaction conditions were as follows: UDG incubation (50°C, 2 min) and PCR activation (95 ◦C , 2 min) followed by 45 cycles of denaturation (95 ◦C , 15 s) and combined annealing and extension (60 ◦C , 1 min). Amplicon standards with concentrations ranging from 103-107 copies/µL were included for all qPCR runs and used for quantification. A clear logarithmic correlation was found between amplicon concentration and the Cq value ($R2 > 0.99$) and the efficiency of the qPCR was acceptable (> 90 %). All primers and probes were HPLC purified (DNA Technology, Denmark).

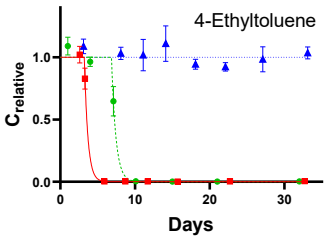
Figure ESI4. Biodegradation kinetics from all test temperatures (2.7, 12 and 20ºC). Modelled with first order decay with lag-phase.

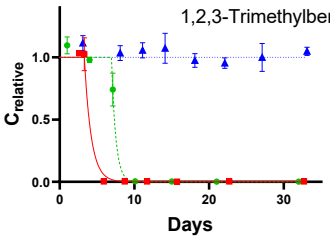


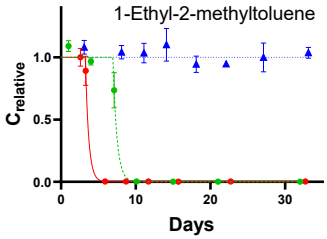

3,3-Dimethyloctane
C relative
1.0
0.5
0.0
0
10
20
30
Days

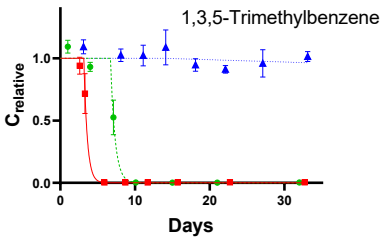

n-Propylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

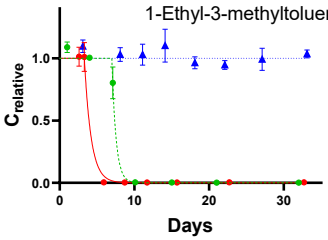

1,2,4-Trimethylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

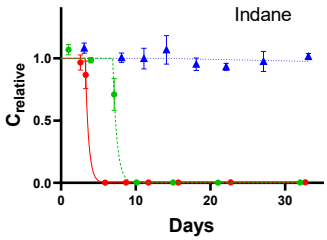

4-Ethyltoluene
C relative
1.0
0.5
0.0
0
10
20
30
Days

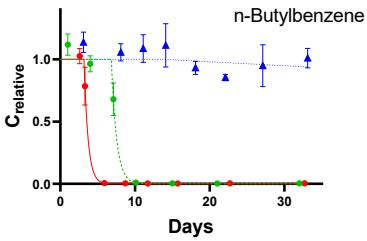

1,2,3-Trimethylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

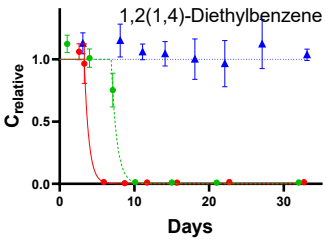

1-Ethyl-2-methyltoluene
C relative
1.0
0.5
0.0
0
10
20
30
Days

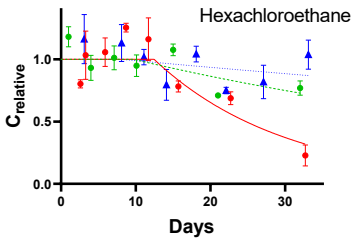

1,3,5-Trimethylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

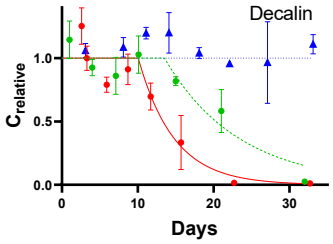

1-Ethyl-3-methyltoluene
C relative
1.0
0.5
0.0
0
10
20
30
Days

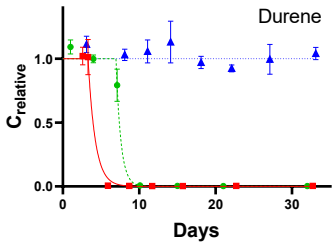

Indane
C relative
1.0
0.5
0.0
0
10
20
30
Days

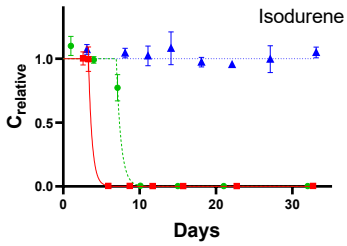

n-Butylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

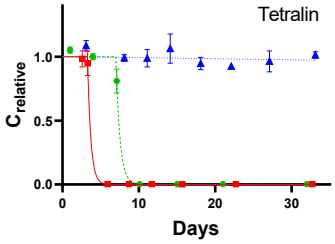

1,2(1,4)-Diethylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

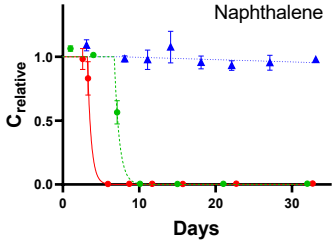

Hexachloroethane
C relative
1.0
0.5
0.0
0
10
20
30
Days

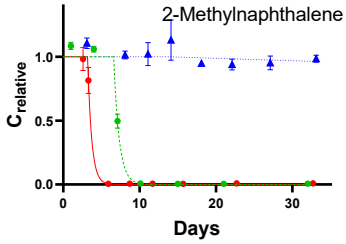

Decalin
C relative
1.0
0.5
0.0
0
10
20
30
Days

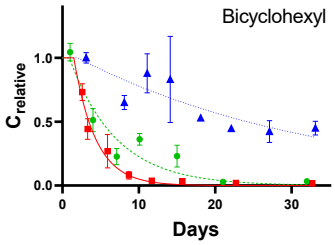

Durene
C relative
1.0
0.5
0.0
0
10
20
30
Days

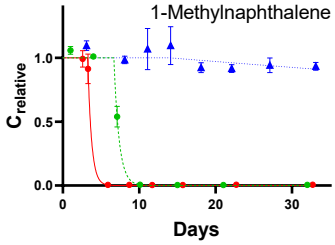

Isodurene
C relative
1.0
0.5
0.0
0
10
20
30
Days

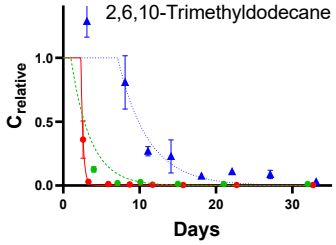

Tetralin
C relative
1.0
0.5
0.0
0
10
20
30
Days

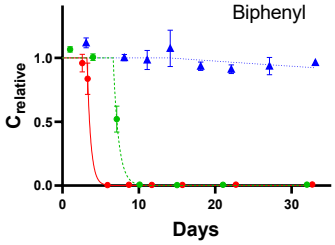

Naphthalene
C relative
1.0
0.5
0.0
0
10
20
30
Days

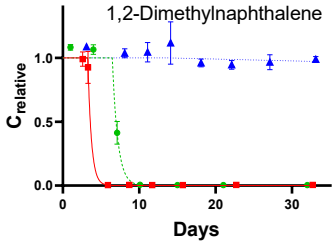

2-Methylnaphthalene
C relative
1.0
0.5
0.0
0
10
20
30
Days

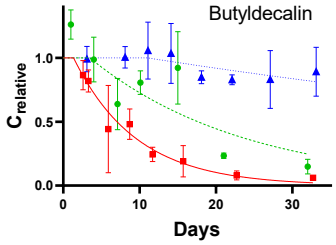

Bicyclohexyl
C relative
1.0
0.5
0.0
0
10
20
30
Days


1-Methylnaphthalene
C relative
1.0
0.5
0.0
0
10
20
30
Days


2,6,10-Trimethyldodecane
C relative
1.0
0.5
0.0
0
10
20
30
Days


Biphenyl
C relative
1.0
0.5
0.0
0
10
20
30
Days


1,2-Dimethylnaphthalene
C relative
1.0
0.5
0.0
0
10
20
30
Days


Butyldecalin
C relative
1.0
0.5
0.0
0
10
20
30
Days

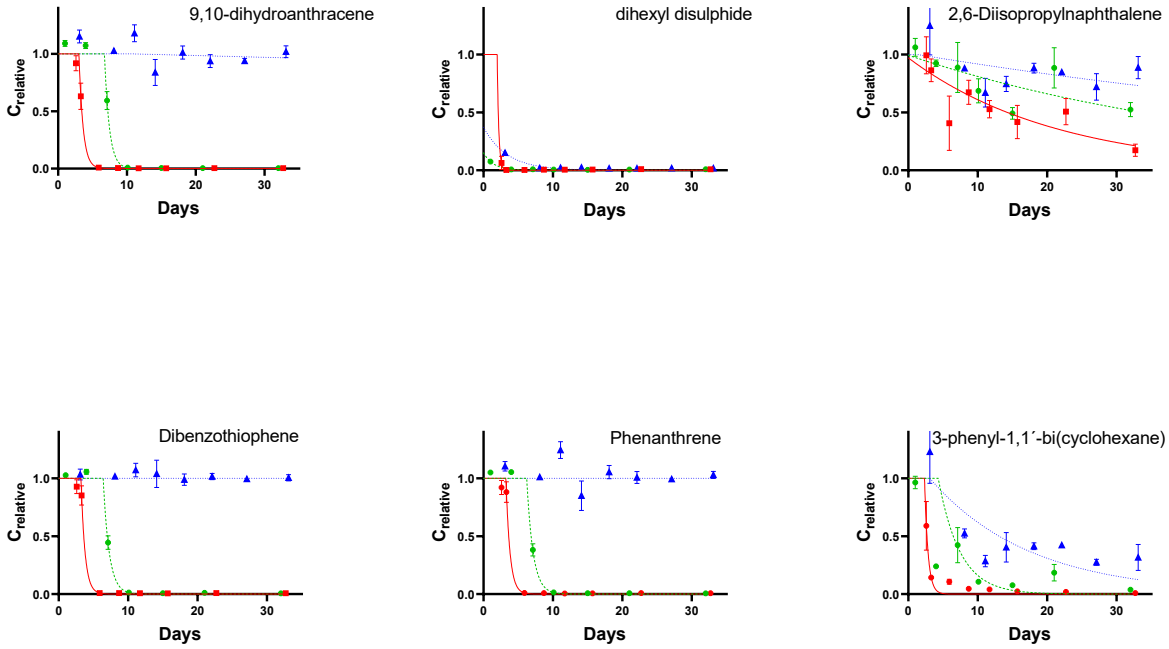
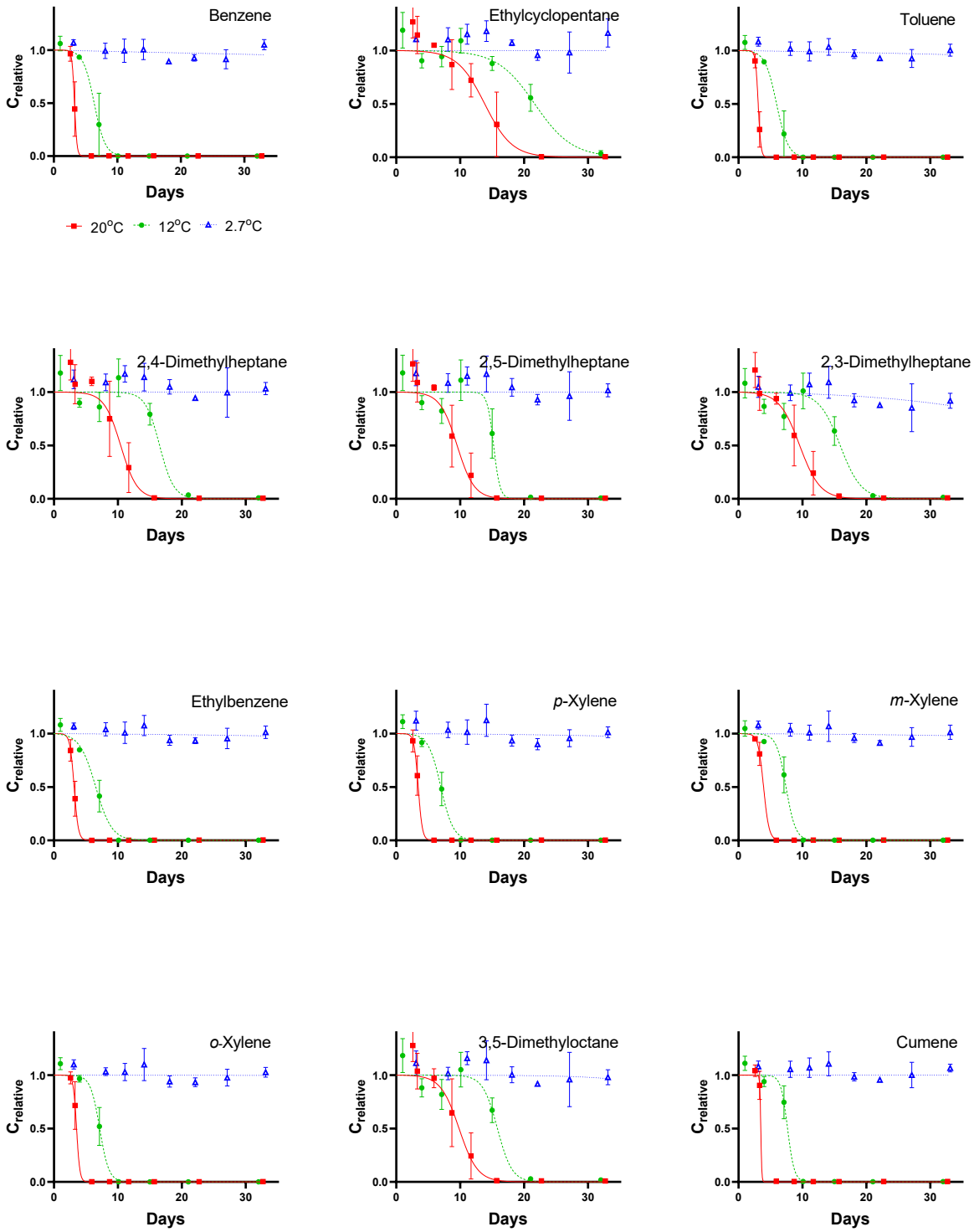
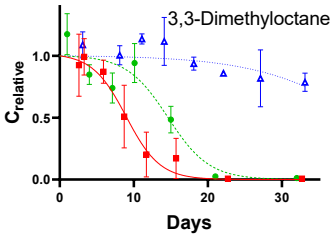
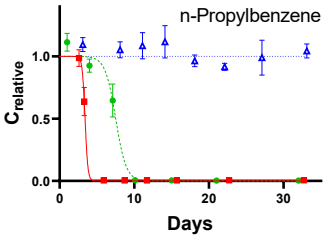


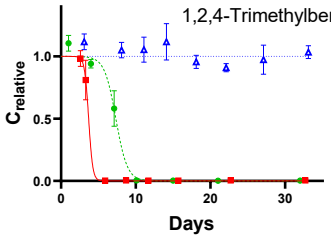
Figure ESI4: Biodegradation kinetics curves for incubation temperatures 2.7 (blue triangles & dotted line), 12 (green circles & dashed line) and 20ºC (red squares & solid line) obtained with Gudenaa Summer inoculum (sampling temperature 17ºC). Modelled with first order decay model with lag-phase (eq.2).

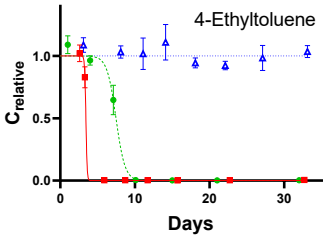
Figure ESI5: Biodegradation kinetics obtained from all test temperatures (2.7, 12 and 20ºC). Modelled with logistic model (eq.3).

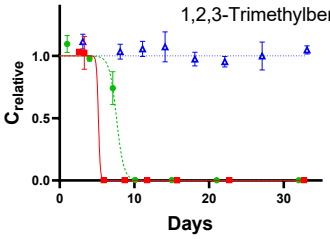


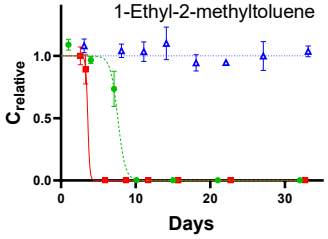

3,3-Dimethyloctane
C relative
1.0
0.5
0.0
0
10
20
30
Days

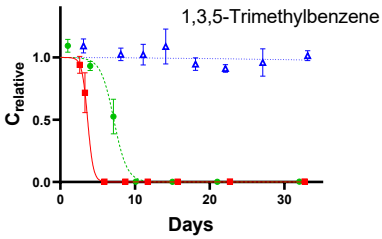

n-Propylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

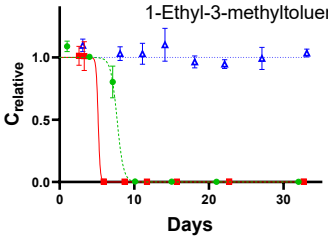

1,2,4-Trimethylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

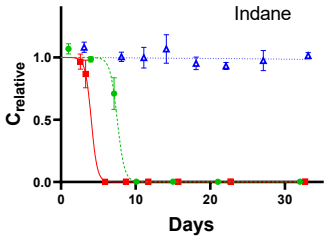

4-Ethyltoluene
C relative
1.0
0.5
0.0
0
10
20
30
Days

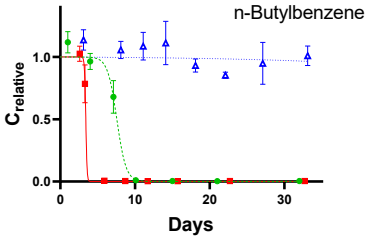

1,2,3-Trimethylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

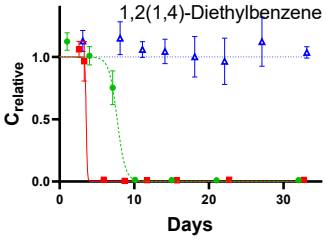

1-Ethyl-2-methyltoluene
C relative
1.0
0.5
0.0
0
10
20
30
Days

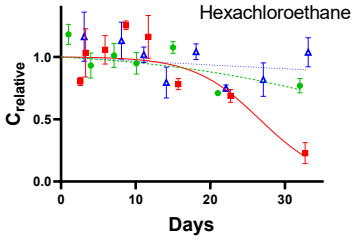

1,3,5-Trimethylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

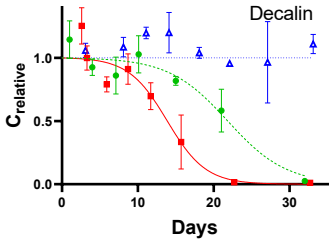

1-Ethyl-3-methyltoluene
C relative
1.0
0.5
0.0
0
10
20
30
Days

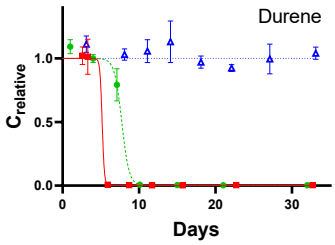

Indane
C relative
1.0
0.5
0.0
0
10
20
30
Days

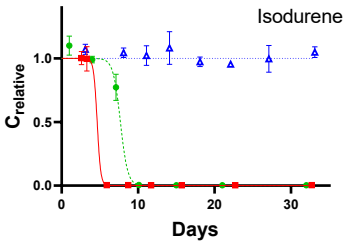

n-Butylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

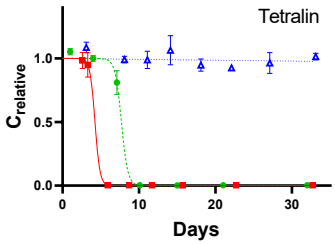

1,2(1,4)-Diethylbenzene
C relative
1.0
0.5
0.0
0
10
20
30
Days

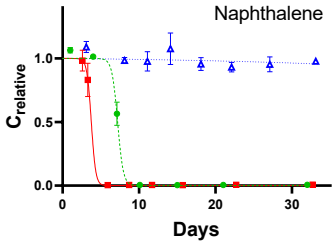

Hexachloroethane
C relative
1.0
0.5
0.0
0
10
20
30
Days

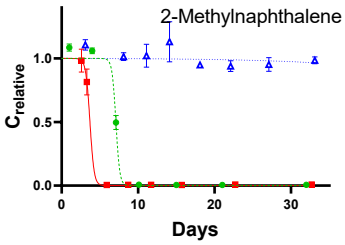

Decalin
C relative
1.0
0.5
0.0
0
10
20
30
Days

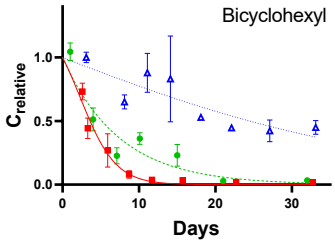

Durene
C relative
1.0
0.5
0.0
0
10
20
30
Days

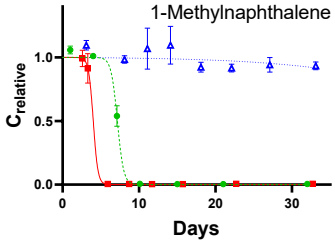

Isodurene
C relative
1.0
0.5
0.0
0
10
20
30
Days

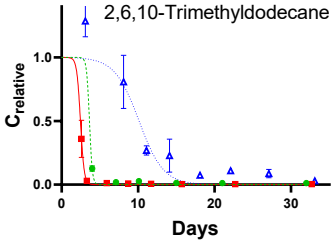

Tetralin
C relative
1.0
0.5
0.0
0
10
20
30
Days

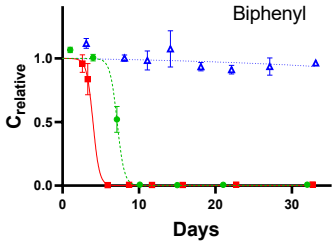

Naphthalene
C relative
1.0
0.5
0.0
0
10
20
30
Days

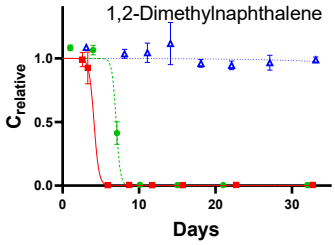

2-Methylnaphthalene
C relative
1.0
0.5
0.0
0
10
20
30
Days

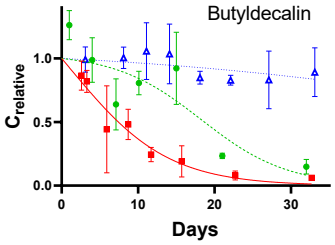

Bicyclohexyl
C relative
1.0
0.5
0.0
0
10
20
30
Days


1-Methylnaphthalene
C relative
1.0
0.5
0.0
0
10
20
30
Days


2,6,10-Trimethyldodecane
C relative
1.0
0.5
0.0
0
10
20
30
Days


Biphenyl
C relative
1.0
0.5
0.0
0
10
20
30
Days


1,2-Dimethylnaphthalene
C relative
1.0
0.5
0.0
0
10
20
30
Days


Butyldecalin
C relative
1.0
0.5
0.0
0
10
20
30
Days

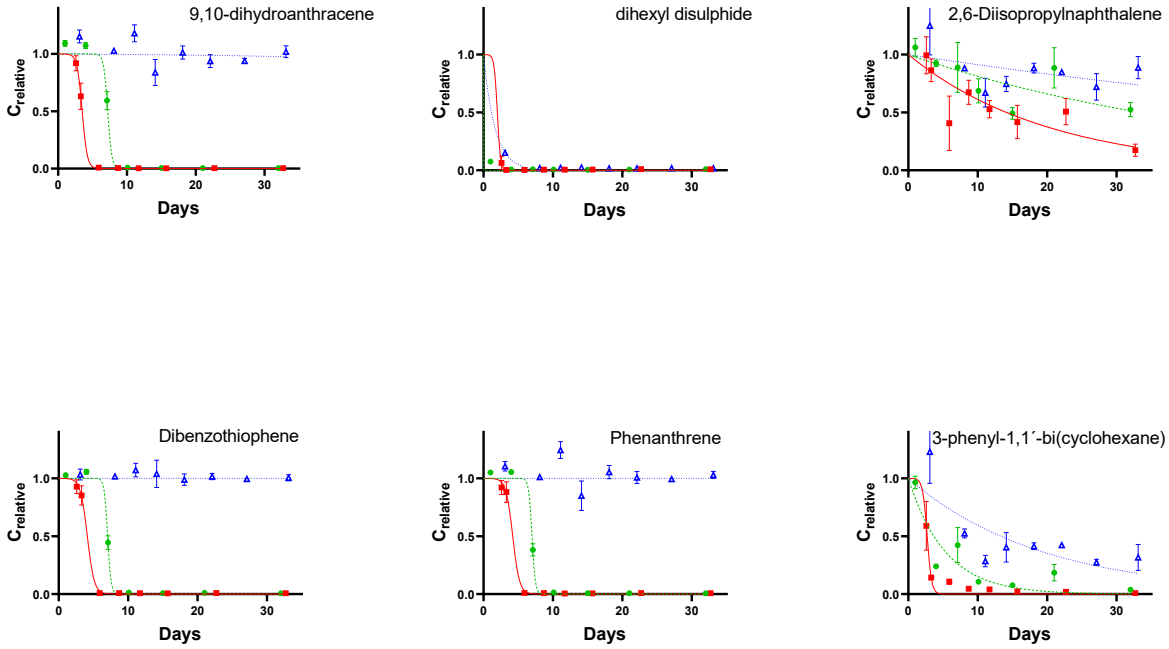


Figure ESI5: Biodegradation kinetics curves for incubation temperatures 2.7 (blue triangles & dotted line), 12 (green circles & dashed line) and 20ºC (red squares & solid line) obtained with Gudenaa Summer inoculum (sampling temperature 17ºC). Modelled with logistic model (eq.3).

Table ESI6: Table including all biodegradation kinetics parameters obtained with Gudenaa summer inoculum at 2.7 °C. First order model. Degradation time (DegT50 = T_{lag} + T½) is included for model fit, $R^2 > 0.7$. When $R^2 < 0.7$, DegT50 is provided as < measured time point after T½. Biodegradation rate (k) and half-life (T½) is provided for data set where $R^2 > 0.7$ and at least two measured points during the visual degradation phase on the curve. No deg.: no degradation observed during 28 days test period; lim.deg. limited degradation observed and incon.data: inconsistent data. Uncertainties on DegT50 are calculated as T_{lag} + 95% CI for T½ and are given in brackets. Parameters obtained with the first order decay model (eq.2). *Test substances with K_{aw}>1 at 20°C are included in italic.*

Gudenaa 17°C Test temperature 2.7°C													
			95% CI			**95% CI**			**95% CI**				Limits*
Compound	**R^2**	**T_{lag}, d**	**lower**	**upper**	**k, d^{-1}**	**lower**	**upper**	**t½, d**	**lower**	**Upper**	**DegT50, d**	Lower	Upper
Benzene											No deg.		
Ethylcyclopentane											*No deg.*		
Toluene											No deg.		
2,4-Dimethylheptane											*No deg.*		
2,5-Dimethylheptane											*No deg.*		
2,3- Dimethylheptane											*Lim.deg.*		
Ethylbenzene											No deg.		
p-Xylene											No deg.		
m-Xylene											No deg.		
o-Xylene											No deg.		
3,5-Dimethyloctane											*No deg.*		
Cumene											No deg.		
3,3-Dimethyloctane											*Lim.deg.*		
n-Propylbenzene											No deg.		
1,2,4-Trimethylbenzene											No deg.		
4-Ethyltoluene											No deg.		
1,2,3-Trimethylbenzene											No deg.		
1-Ethyl-2-methyltoluene											No deg.		
1,3,5-Trimethylbenzene											No deg.		
1-Ethyl-3-methyltoluene											No deg.		
Indane											No deg.		
n-butylbenzene											No deg.		
1,2(1,4)-Diethylbenzene											No deg.		
Hexachloroethane											No deg.		
Decalin											*No deg.*		

Gudenaa 17°C Test temperature 2.7°C													
			95% CI			95% CI			95% CI			Limits*	
Compound	**R^2**	**T_{lag}, d**	**lower**	**upper**	**k, d^{-1}**	**lower**	**upper**	**t½, d**	**lower**	**Upper**	**DegT50, d**	Lower	Upper
Durene											No deg.		
Isodurene											No deg.		
Tetralin											No deg.		
Naphthalene											No deg.		
2-Methylnaphthalene											No deg.		
Bicyclohexyl											*Incons.data*		
1-Methylnaphthalene											No deg.		
2,6,10-Trimethyldodecane	*0.83*	*7.1*	*3.7*	*8.8*	*0.254*	*0.12*	*0.571*	*2.73*	*1.2*	*8.5*	*9.95*	*8.34*	*12.88*
Biphenyl											No deg.		
1,2-dimethylnaphthalene											No deg.		
Butyldecalin											No deg.		
9,10-Dihydroanthracene											No deg.		
Dihexyl disulphide											No deg.		
2,6-Diisopropylnaphthalene											Lim.deg.		
Dibenzothiophene											No deg.		
Phenanthrene											No deg.		
3-Phenyl-1,1´-bi(cyclohexane)											Incon.data		

Table ESI7: *Table including all biodegradation kinetics parameters obtained with Gudenaa summer inoculum at 12 °C*. First order model. Degradation time (DegT50 = T_{lag} + T½) is included for model fit, $R^2 > 0.7$. When $R^2 < 0.7$, DegT50 is provided as < measured time point after T½. Biodegradation rate (k) and half-life (T½) is provided for data set where $R^2 > 0.7$ and at least two measured points during the visual degradation phase on the curve. No deg.: no degradation observed during 28 days test period; lim.deg. limited degradation observed and incon.data: inconsistent data. Uncertainties on DegT50 are calculated as T_{lag} + 95% CI for T½ and are given in brackets. Parameters obtained with the first order decay model. *Test substances with K_{aw}>1 at 20°C are included in italic.*

Gudenaa 17°C Test temperature 12°C													
			95% CI			**95% CI**			**95% CI**				Limits*
Compound	**R^2**	**T_{lag}, d**	**lower**	**upper**	**k, d^{-1}**	**lower**	**upper**	**t½, d**	**lower**	**Upper**	**DegT50, d**	Lower	Upper
Benzene	0.87	6.5	3.0	-	1.97	-	-	0.35	-	-	6.84	6.5	6.5
Ethylcyclopentane	*0.79*	*14.1*	*10.0*	-	*0.11*	*0.06*	*0.19*	*6.29*	*3.58*	*11.7*	*20.4*	*17.7*	*25.9*
Toluene	0.92	6.2	3.0	-	1.75	0.29	-	0.40	-	2.39	6.63	6.2	8.6
2,4-Dimethylheptane	*0.84*	*14.6*	*12.4*	-	*0.52*	*0.17*	-	*1.34*	-	*4.18*	*15.9*	*14.6*	*18.7*
2,5-Dimethylheptane	*0.79*	*14.2*	*9.5*	-	*0.62*	*0.11*	-	*1.12*	-	*6.09*	*15.3*	*14.2*	*20.3*
2,3- Dimethylheptane	*0.82*	*14.1*	*9.9*	-	*0.52*	*0.13*	-	*1.34*	-	*5.19*	*15.5*	*14.1*	*19.3*
Ethylbenzene	0.94	3.6	2.8	-	0.32	0.23	0.45	2.15	1.53	2.98	5.71	5.1	6.5
p-Xylene	0.95	6.7	5.0	-	1.77	0.41	-	0.39	-	1.71	7.08	6.7	8.4
m-Xylene	0.90	3.9	2.9	-	0.27	0.19	-	2.61	-	3.75	6.48	3.9	7.6
o-Xylene	0.95	6.8	5.3	-	1.98	0.42	-	0.35	-	1.63	7.12	6.8	8.4
3,5-Dimethyloctane	*0.82*	*14.2*	*10.3*	-	*0.53*	*0.14*	-	*1.32*	-	*5.11*	*15.6*	*14.2*	*19.4*
Cumene	0.95	6.9	6.4	-	1.74	0.56	-	0.40	-	1.24	7.33	6.9	8.2
3,3-Dimethyloctane	*0.73*	*6.2*	*0.4*	-	*0.10*	*0.05*	*0.17*	*6.96*	*4.11*	*14.3*	*13.1*	*10.3*	*20.5*
n-Propylbenzene	0.90	3.9	2.9	-	0.26	0.18	-	2.70	-	3.88	6.60	3.9	7.8
1,2,4-Trimethylbenzene	0.96	6.8	5.8	-	1.66	0.50	-	0.42	-	1.39	7.19	6.8	8.2
4-Ethyltoluene	0.97	6.8	6.2	-	1.71	0.58	-	0.41	-	1.19	7.25	6.8	8.0
1,2,3-Trimethylbenzene	0.97	6.9	6.5	-	1.77	0.62	-	0.39	-	1.13	7.32	6.9	8.1
1-Ethyl-2-methyltoluene	0.97	6.9	6.5	-	1.85	0.61	-	0.37	-	1.13	7.31	6.9	8.1
1,3,5-Trimethylbenzene	0.96	6.7	5.6	-	1.67	0.48	-	0.41	-	1.44	7.13	6.7	8.2
1-Ethyl-3-methyltoluene	0.97	7.0	6.7	-	1.79	0.67	-	0.39	-	1.03	7.36	7.0	8.0
Indane	0.97	6.9	6.5	-	1.84	0.65	-	0.38	-	1.07	7.29	6.9	8.0
n-butylbenzene	0.95	6.8	6.2	-	1.42	0.52	-	0.49	-	1.32	7.32	6.8	8.2
1,2(1,4)-Diethylbenzene	0.96	6.9	6.4	-	1.35	0.55	-	0.51	-	1.26	7.40	6.9	8.2
Hexachloroethane								Limited degradation					
Decalin	*0.73*	*13.5*	*8.4*	-	*0.10*	*0.05*	*0.19*	*6.79*	*3.62*	*13.8*	*20.3*	*17.1*	*27.3*

Gudenaa 17°C Test temperature 12°C													
		95% CI			95% CI			95% CI			Limits*		
Compound	**R^2**	**T_{lag}, d**	**lower**	**upper**	**k, d^{-1}**	**lower**	**upper**	**t½, d**	**lower**	**Upper**	**DegT50, d**	Lower	Upper
Durene	0.97	7.0	6.6	-	1.58	0.65	-	0.44	-	1.07	7.39	7.0	8.0
Isodurene	0.97	6.9	6.6	-	1.64	0.66	-	0.42	-	1.05	7.37	6.9	8.0
Tetralin	0.99	7.0	6.8	-	1.87	0.79	-	0.37	-	0.88	7.36	7.0	7.9
Naphthalene	0.98	6.7	6.2	-	1.56	0.66	-	0.45	-	1.05	7.18	6.7	7.8
2-Methylnaphthalene	0.99	6.6	5.9	-	1.41	0.63	-	0.49	-	1.10	7.10	6.6	7.7
Bicyclohexyl	*0.85*	*0.9*	*0*	*2.0*	*0.16*	*0.11*	*0.24*	*4.24*	*2.94*	*6.16*	*5.15*	*3.8*	*7.1*
1-Methylnaphthalene	0.99	6.7	6.1	-	1.48	0.66	-	0.47	-	1.05	7.15	6.7	7.7
2,6,10-Trimethyldodecane	*0.85*	*1.0*	*0.8*	-	*0.42*	*0.30*	-	*1.65*	-	*2.25*	*2.65*	*1.0*	*3.2*
Biphenyl	0.98	6.6	5.9	-	1.42	0.59	-	0.49	-	1.17	7.13	6.6	7.8
1,2-dimethylnaphthalene	0.98	6.5	5.2	-	1.41	0.50	-	0.49	-	1.39	6.96	6.5	7.9
Butyldecalin											>31		
9,10-Dihydroanthracene	0.98	6.7	6.2	-	1.42	0.62	-	0.49	-	1.11	7.22	6.7	7.8
Dihexyl disulphide	Invalid												
2,6-Diisopropylnaphthalene	Limited degradation												
Dibenzothiophene	0.99	6.4	5.8	-	1.16	0.63	-	0.60	-	1.10	7.00	6.4	7.5
Phenanthrene	0.99	6.2	5.3	-	1.10	0.57	-	0.63	-	1.21	6.86	6.2	7.4
3-Phenyl-1,1´-bi(cyclohexane)	0.90	1.0	0.9	-	0.72	0.44	-	0.96	-	1.58	1.96	1.0	2.6

Table ESI8: Table including all biodegradation kinetics parameters obtained with Gudenaa summer inoculum at 20 °C. First order model. Degradation time (DegT50 = T_{lag} + T½) is included for model fit, $R^2 > 0.7$. When $R^2 < 0.7$, DegT50 is provided as < measured time point after T½. Biodegradation rate (k) and half-life (T½) is provided for data set where $R^2 > 0.7$ and at least two measured points during the visual degradation phase on the curve. No deg.: no degradation observed during 28 days test period; lim.deg. limited degradation observed and incon.data: inconsistent data. Uncertainties on DegT50 are calculated as T_{lag} + 95% CI for T½ and are given in brackets. Parameters obtained with the first order decay model. *Test substances with K_{aw}>1 at 20ºC are included in italic.*

Gudenaa 17°C Test temperature 20°C													
			95% CI			**95% CI**			**95% CI**			Limits*	
Compound	**R^2**	**T_{lag}, d**	**lower**	**upper**	**k, d^{-1}**	**lower**	**upper**	**t½, d**	**lower**	**Upper**	**DegT50, d**	Lower	Upper
Benzene	0.86	3.00	-	-	2.70	-	-	0.25	-	-	3.25	-	-
Ethylcyclopentane	*0.74*	*10.36*	*4.43*	-	*0.24*	*0.07*	-	*2.93*	-	*9.40*	*13.29*	*10.36*	*19.76*
Toluene	0.92	2.54	2.44	-	1.78	1.22	2.62	0.39	0.26	0.57	2.93	2.81	3.11
2,4-Dimethylheptane	*0.77*	*7.91*	*4.68*	-	*0.35*	*0.13*	-	*1.96*	-	*5.43*	*9.87*	*7.91*	*13.34*
2,5-Dimethylheptane	*0.80*	*7.25*	*4.13*	-	*0.36*	*0.14*	-	*1.91*	-	*4.93*	*9.16*	*7.25*	*12.18*
2,3- Dimethylheptane	*0.80*	*7.15*	*3.27*	-	*0.33*	*0.13*	-	*2.08*	-	*5.53*	*9.23*	*7.15*	*12.68*
Ethylbenzene	0.90	2.45	2.20	2.57	1.11	0.70	1.68	0.62	0.41	0.99	3.07	2.86	3.44
p-Xylene	0.91	3.08	2.27	-	2.27	0.49	-	0.31	-	1.41	3.39	3.08	4.49
m-Xylene	0.98	3.21	3.00	-	2.36	0.83	-	0.29	-	0.83	3.50	3.21	4.04
o-Xylene	0.91	3.16	2.49	-	2.39	0.53	-	0.29	-	1.32	3.45	3.16	4.48
3,5-Dimethyloctane	*0.79*	*7.52*	*3.67*	*8.63*	*0.36*	*0.13*	-	*1.92*	-	*5.49*	*9.44*	*7.52*	*13.00*
Cumene	0.97	3.25	3.10	-	1.98	0.78	-	0.35	-	0.89	3.60	3.25	4.14
3,3-Dimethyloctane	*0.73*	*5.26*	*1.48*	-	*0.21*	*0.09*	-	*3.31*	-	*7.31*	*8.57*	*5.26*	*12.58*
n-Propylbenzene	0.97	3.07	2.57	-	1.98	0.68	-	0.35	-	1.01	3.42	3.07	4.09
1,2,4-Trimethylbenzene	0.95	3.20	2.90	-	2.14	0.68	-	0.32	-	1.02	3.53	3.20	4.22
4-Ethyltoluene	0.98	3.21	3.04	-	2.11	0.86	-	0.33	-	0.81	3.54	3.21	4.02
1,2,3-Trimethylbenzene	0.00	3.30	3.23	-	1.59	0.80	-	0.44	-	0.87	3.74	-	-
1-Ethyl-2-methyltoluene	0.97	3.25	3.09	-	2.25	0.80	-	0.31	-	0.86	3.56	3.25	4.11
1,3,5-Trimethylbenzene	0.94	3.15	2.65	-	2.18	0.62	-	0.32	-	1.12	3.47	3.15	4.26
1-Ethyl-3-methyltoluene	0.97	3.30	3.22	-	1.60	0.79	-	0.43	-	0.87	3.73	3.30	4.13
Indane	0.97	3.24	3.07	-	2.28	0.81	-	0.30	-	0.85	3.54	3.24	4.09
n-butylbenzene	0.96	3.17	2.85	-	1.84	0.67	-	0.38	-	1.03	3.55	3.17	4.20
1,2(1,4)-Diethylbenzene	0.96	3.28	3.14	-	1.62	0.71	-	0.43	-	0.98	3.71	3.28	4.26
Hexachloroethane	0.64	12.45	8.48	22.07	0.06	0.03	0.11	12.30	6.30	22.58	24.75	18.75	35.03

Gudenaa 17°C Test temperature 20°C													
			95% CI			95% CI			95% CI			Limits*	
Compound	**R^2**	**T_{lag}, d**	**lower**	**upper**	**k, d^{-1}**	**lower**	**upper**	**t½, d**	**lower**	**Upper**	**DegT50, d**	Lower	Upper
Decalin	*0.81*	*10.06*	*5.95*	*11.54*	*0.21*	*0.09*	*0.57*	*3.26*	*1.23*	*7.63*	*13.32*	*11.29*	*17.69*
Durene	0.97	3.30	3.21	-	1.51	0.75	-	0.46	-	0.93	3.76	3.30	4.18
Isodurene	0.98	3.30	3.24	-	2.09	0.92	-	0.33	-	0.75	3.63	3.30	4.05
Tetralin	0.98	3.28	3.19	-	2.29	0.89	-	0.30	-	0.78	3.58	3.28	4.06
Naphthalene	0.96	3.21	2.97	-	2.03	0.72	-	0.34	-	0.96	3.55	3.21	4.17
2-Methylnaphthalene	0.97	3.19	2.97	-	1.90	0.76	-	0.37	-	0.92	3.56	3.19	4.11
Bicyclohexyl	0.90	1.49	0.58	2.07	0.35	0.24	0.56	1.97	1.23	2.89	3.47	2.73	4.38
1-Methylnaphthalene	0.97	3.26	3.13	-	2.03	0.82	-	0.34	-	0.85	3.60	3.26	4.10
2,6,10-Trimethyldodecane	*0.71*	*2.31*	*1.81*	-	*3.55*	*1.45*	-	*0.20*	-	*0.48*	*2.51*	*2.31*	*2.79*
Biphenyl	0.97	3.22	3.00	-	2.09	0.76	-	0.33	-	0.92	3.55	3.22	4.13
1,2-dimethylnaphthalene	0.97	3.26	3.14	-	2.11	0.81	-	0.33	-	0.86	3.59	3.26	4.12
Butyldecalin	0.76	1.36	-1.33	2.82	0.12	0.08	0.19	5.69	3.72	8.85	7.05	5.08	10.21
9,10-Dihydroanthracene	0.96	3.02	2.36	-	1.66	0.54	-	0.42	-	1.29	3.44	3.02	4.31
Dihexyl disulphide	Incons.data												
2,6-Diisopropylnaphthalene	Incons.data												
Dibenzothiophene	0.98	3.21	3.06	-	1.73	0.82	-	0.40	-	0.84	3.61	3.21	4.05
Phenanthrene	0.98	3.23	3.09	-	1.71	0.81	-	0.40	-	0.85	3.63	3.23	4.08
3-Phenyl-1,1´-bi(cyclohexane)	0.91	1.39	0.83	1.77	1.82	1.28	2.61	0.38	0.27	0.54	1.77	1.65	1.93

Table ESI9: Biodegradation kinetics parameters obtained by the logistic model for the Gudenaa Summer sample tested at 12 and 20ºC. Compounds with K_{aw}>1 are included in italic.

Gudenaa	**12ºC**		**20ºC**	
Compound	**R^2**	**$DegT_{50}$**	**R^2**	**$DegT_{50}$**
Benzene	0.88	6.4	0.86	3.3
Ethylcyclopentane	*0.80*	*21.6*	*0.75*	*13.8*
Toluene	0.92	6.0	0.92	3.1
2,4-Dimethylheptane	*0.84*	*16.5*	*0.77*	*10.4*
2,5-Dimethylheptane	*0.79*		*0.80*	*9.5*
2,3- Dimethylheptane	*0.82*	*15.9*	*0.80*	*9.5*
Ethylbenzene	0.95	6.5	0.90	3.2
p-Xylene	0.95	7.0	0.91	3.4
m-Xylene	0.95	7.4	0.98	3.9
o-Xylene	0.95	7.2	0.91	3.5
3,5-Dimethyloctane	*0.82*	*15.8*	*0.78*	*9.8*
Cumene	0.95	7.7	0.97	
3,3-Dimethyloctane	*0.80*	*14.5*	*0.72*	*8.8*
n-Propylbenzene	0.96	7.5	0.97	3.4
1,2,4-Trimethylbenzene	0.96	7.3	0.95	3.7
4-Ethyltoluene	0.97	7.5	0.98	
1,2,3-Trimethylbenzene	0.97	7.6	0.98	
1-Ethyl-2-methyltoluene	0.97	7.6	0.97	
1,3,5-Trimethylbenzene	0.96	7.2	0.94	3.7
1-Ethyl-3-methyltoluene	0.97		0.97	
Indane	0.97		0.97	4.1
n-butylbenzene	0.95	7.6	0.96	
1,2(1,4)-Diethylbenzene	0.96	7.7	0.96	
Hexachloroethane				
Decalin	*0.76*	*21.6*	*0.82*	*13.8*
Durene	0.97	7.7	0.97	
Isodurene	0.97	7.7	0.98	4.6
Tetralin	0.99		0.98	4.2
Naphthalene	0.98		0.96	3.8
2-Methylnaphthalene	0.99		0.97	3.7
Bicyclohexyl	*0.83*		*0.89*	*2.3*
1-Methylnaphthalene	0.99		0.97	4.0
2,6,10-Trimethyldodecane	*0.87*		*0.71*	*2.5*
Biphenyl	0.98		0.97	4.0
1,2-dimethylnaphthalene	0.98		0.97	4.1
Butyldecalin			0.75	1.3
9,10-Dihydroanthracene	0.98		0.96	3.5
Dihexyl disulphide				
2,6-Diisopropylnaphthalene				
Dibenzothiophene	0.99		0.98	4.1
Phenanthrene	0.99		0.98	4.2
3-Phenyl-1,1´-bi(cyclohexane)	0.72		0.71	1.6

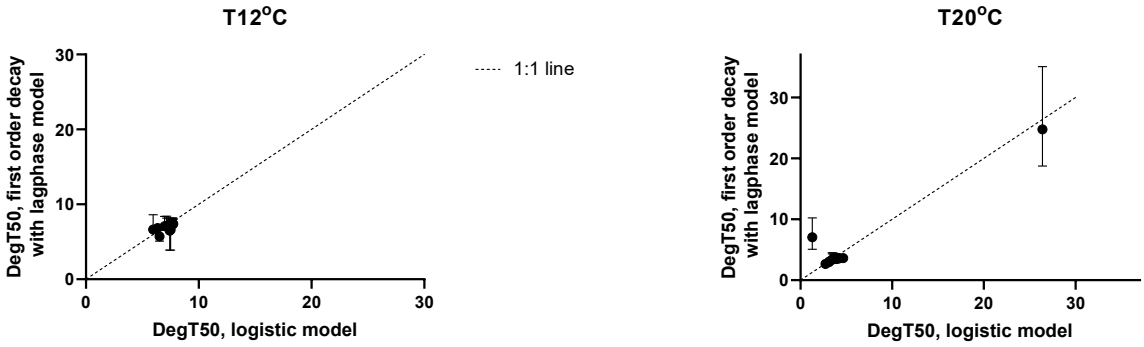


Figure ESI10: $DegT_{50}$ obtained with the first order model with lag-phase vs $DegT_{50}$ obtained with the logistic model.

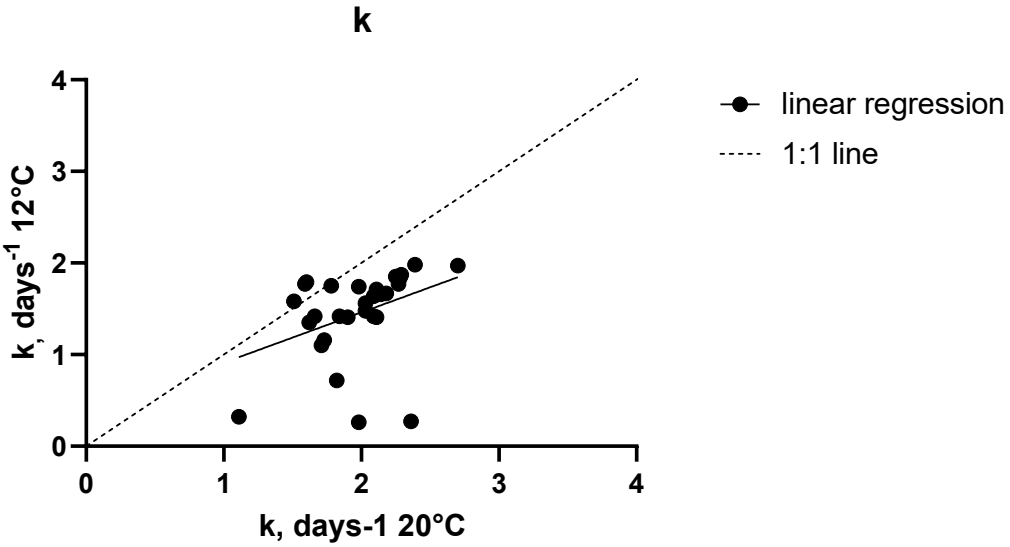


Figure ESI11: k at 12ºC plotted against T_{lag} and k obtained at 20ºC. Data from first order model.

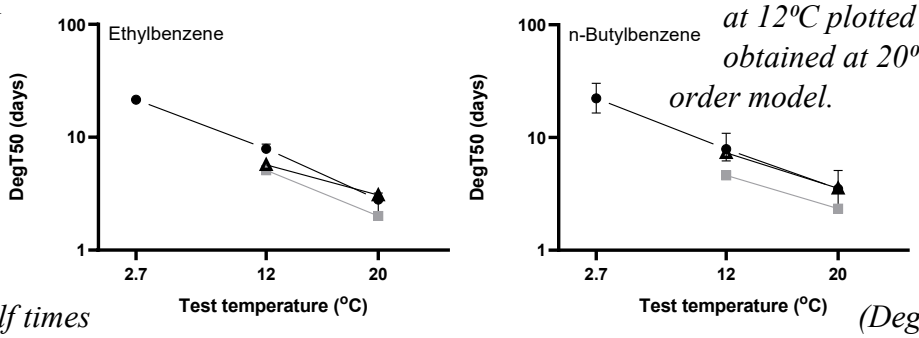


Figure ESI12: Degradation half times ($DegT_{50}$) (days) of selected compounds versus test temperature

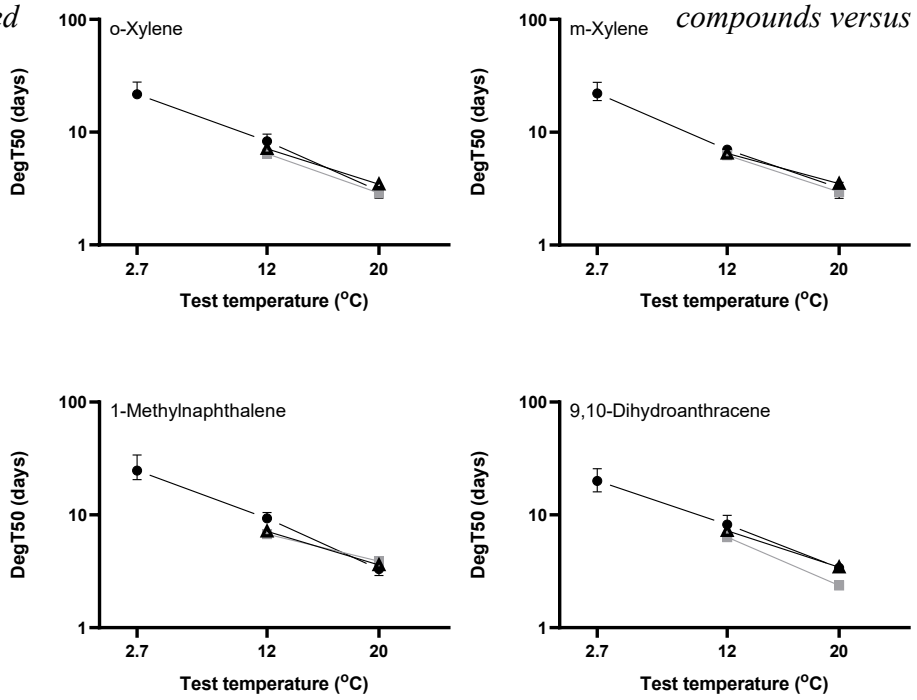


Figure ESI12: Degradation half times ($DegT_{50}$) (days) of selected compounds versus test temperature for Gudenaa Winter (closed circles),[5] Gudenaa Summer (open triangles) and Danube inoculum (grey squares).[5] Agreement with Arrhenius is indicated by a linear relationship (semi-log plot).

Table ESI13: qPCR data for Gudenaa Winter inoculum, and during incubation (D15 = day 15)

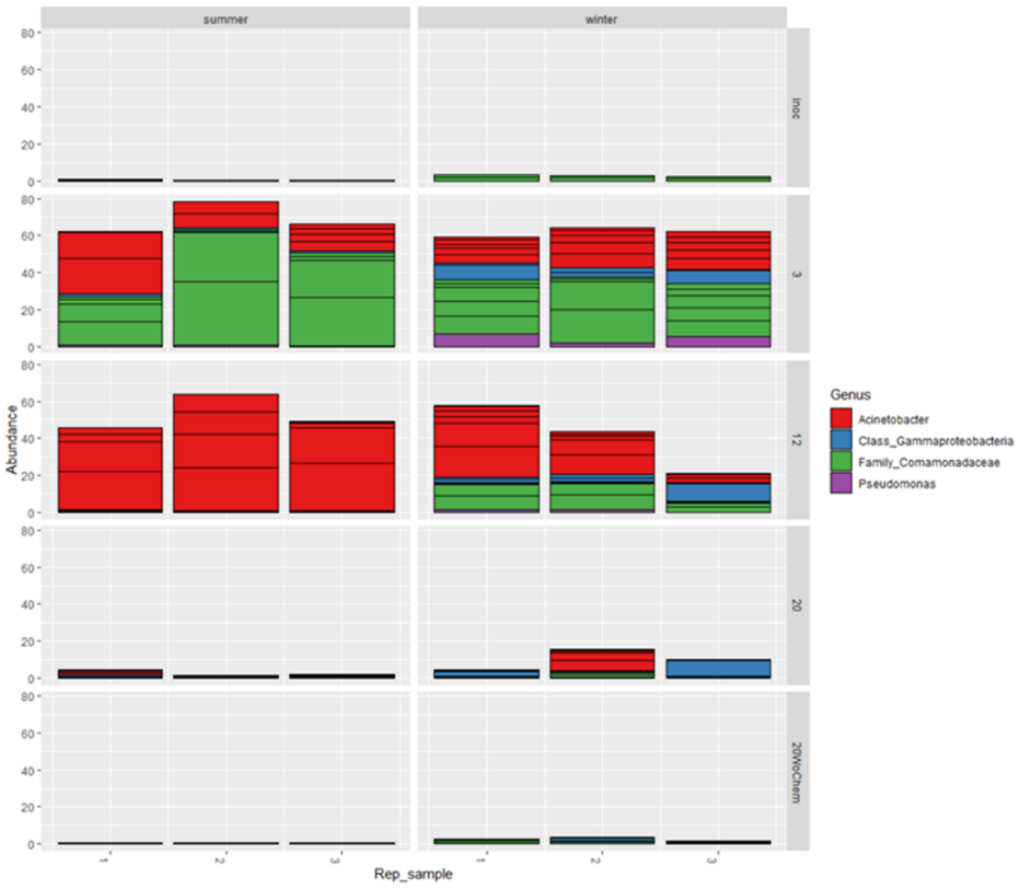
Sample ID	Extracted DNA Conc. [ng/uL]	Prokaryotic concentration estimated from 16S rRNA qPCR [copies/uL]	Number of Reads after sequencing, QC	Obs. number of ASVs	Faith's Phylogenetic Diversity
Inoculum_1	3.9	1945000	16624	183	34.5
Inoculum_2	4.1	2813000	29353	256	47.8
Inoculum_3	3.9	1845500	38230	355	66.6
D15,2.7ºC_1	19.1	19465000	26252	90	13.8
D15,2.7ºC_2	14.1	13640000	11084	61	11.8
D15,2.7ºC_3	23.1	42070000	35302	111	17.3
D15,12ºC_1	5.2	4987500	40904	232	53.6
D15,12ºC_2	7.3	3855500	67038	355	57.9
D15,12ºC_3	4.4	3412000	29446	220	39.9

D15,20ºC_1	3.7	1780000	29607	262	48.4
D15,20ºC_2	6.8	3527000	44546	435	69.1
D15,20ºC_3	< 2	609350	41431	370	65.2
D15,20ºC wo chem_1	< 2	420850	35918	434	79.9
D15,20ºC wo chem_2	2.6	854350	34426	332	63.9
D15,20ºC wo chem_3	2.1	673150	26885	346	63.4
Blank 1	< 2	Below detection limit	7709	76	18.4
Blank 2	< 2	Below detection limit	7407	67	14.2

Table ESI14: qPCR data for Gudenaa Summer inoculum, and during incubation (D15 = day 15)

Sample ID	Extracted DNA Conc. [ng/uL]	Prokaryotic concentration estimated from 16S rRNA qPCR [copies/uL]	Number of Reads after sequencing, QC	Obs. number of ASVs	Faith's Phylogenetic Diversity
Inoculum_1	< 2	32295	43955	406	64.5
Inoculum_2	< 2	3197.5	46720	441	66.1
Inoculum_3	< 2	Below detection limit	47520	270	36.5
D15,2.7ºC_1	< 2	3015.5	38813	141	31.5
D15,2.7ºC_2	< 2	199100	64302	162	45.9
D15,2.7ºC_3	< 2	43215	54292	172	36.9
D15,12ºC_1	< 2	68200	57385	246	50.6
D15,12ºC_2	< 2	12625	44979	228	52.4
D15,12ºC_3	2	1153000	66232	270	52.6
D15,20ºC_1	< 2	4906.5	42672	374	61.9
D15,20ºC_2	< 2	Below detection limit	36185	231	42.5
D15,20ºC_3	< 2	16085	65164	448	63.5

D15,20ºC wo chem_1	< 2	2769.5	41130	479	73.4
D15,20ºC wo chem_2	< 2	Below detection limit	48662	222	40.8
D15,20ºC wo chem_3	< 2	Below detection limit	44950	205	40.0
Blank 1	< 2	Below detection limit	15487	30	7.2
Blank 2	< 2	Below detection limit	11102	26	5.3
Blank 3	< 2	Below detection limit	44951	131	21.2



ESI15:

ESI30

Figure ESI15: The 15 most abundant sequences at 2.7 and 12ºC across all samples.

ESI16:

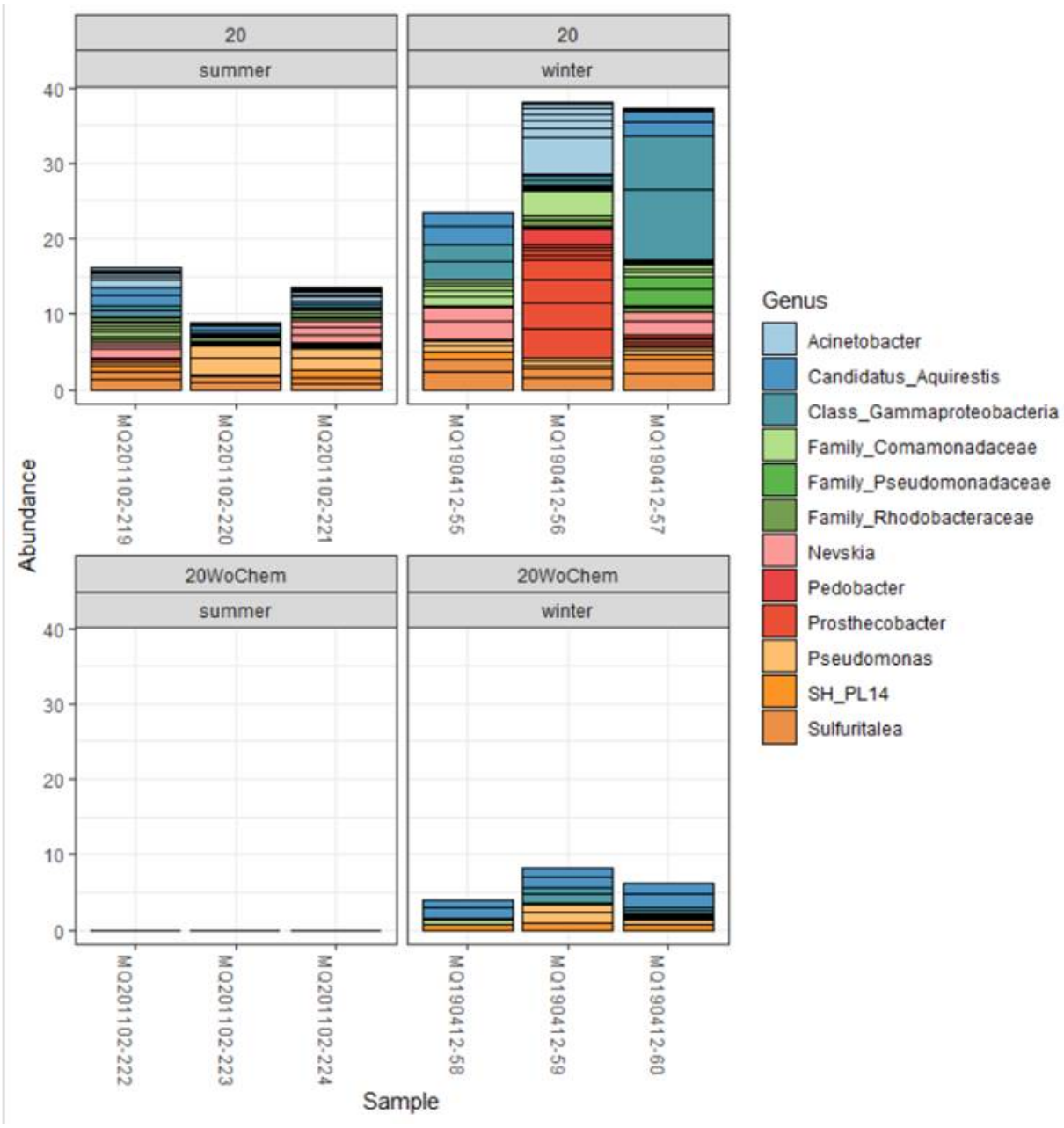
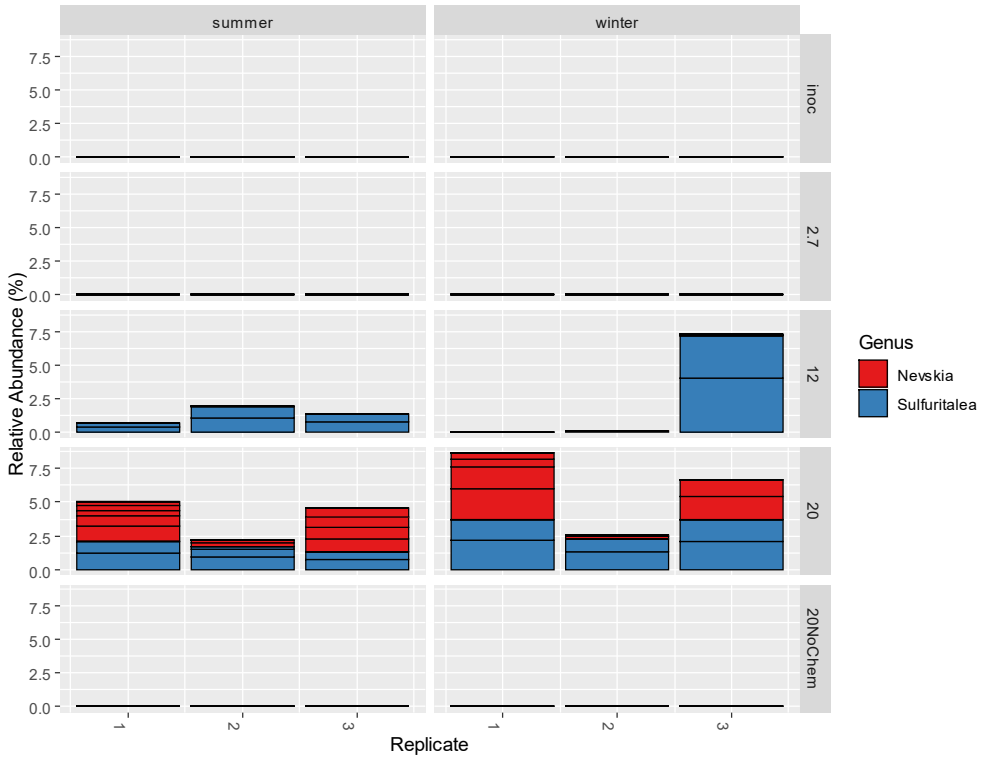


Figure ESI16: Sequences more abundant in the presence of chemicals compared to without chemicals.



ESI17: Abundance of two genera only observed in the presence of chemicals.

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