SUPPORTING INFORMATION

Aerobic Bioremediation of PAH Contaminated Soil Results in Increased Genotoxicity and Developmental Toxicity

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SI contains:

10 pages

4 tables

Contents

Materials and Methods

Chemicals. Isotopically labeled standards used as surrogates for parent, methyl, and heterocyclic PAHs were d_{10}-fluorene, d_{10}-phenanthrene, d_{10}-pyrene, d_{12}-triphenylene, d_{12}-benzo[a]pyrene, d_{12}-benzo[ghi]perylene, for oxygenated PAHs were (d_6-1,4-naphthoquinone, d_8-anthraquinone), and for nitrated PAHs were (d_9-1-nitropyrene, d_7-1-nitronaphthalene, d_{11}-6-nitrochrysene, d_9-5-nitroacenaphthene, d_9-9-nitroanthracene and d_9-3-nitrofluoranthene). Isotopically labeled standards used as internal standards for parent, methyl and heterocyclic PAHs were d_{10}-acenaphthene, d_{10}-fluoranthene, d_{12}-benzo[k]fluoranthene, for oxygenated PAHs was d_8-9-fluorenone and for nitrated PAHs were d_9-nitrobiphenyl and d_9-2-nitrofluorene.

T**able S1**. Mean concentration in dry weight and standard errors (SE) of PAHs in unfractionated soil extracts pre- and post-bioremediation. Compounds with asterisks (*) showed significant changes in concentration post-bioremediation ($p < 0.05$, $n = 3$). No nitrated PAHs were detected above the limit of detection (1 pg μL^{-1}). (n.d. = not detected).

PAHs	**Abbr.**	**Pre-bioremediation $\mu g\ g^{-1}$**	**Post-bioremediation $\mu g\ g^{-1}$**	***p* value**
Naphthalene	NAP	6.26 ± 0.49	8.58 ± 3.82	0.58
2-Methylnaphthalene	2MNAP	3.90 ± 0.67	3.76 ± 0.67	0.89
1-Methylnaphthalene	1MNAP	3.35 ± 0.62	2.14 ± 0.41	0.18
2,6-Dimethylnaphthalene	2,6MNAP	3.40 ± 0.52	0.93 ± 0.22	0.01*
1,3-Dimethylnaphthalene	1,3MNAP	3.54 ± 0.50	1.08 ± 0.27	0.01*
Acenaphthylene	ACEY	8.03 ± 0.30	13.78 ± 2.94	0.12
Acenaphthene	ACE	6.13 ± 0.71	1.31 ± 0.43	<0.01*
Fluorene	FLU	4.22 ± 0.68	3.02 ± 0.58	0.25
Phenanthrene	PHE	78.32 ± 11.54	28.65 ± 7.60	0.02
Anthracene	ANT	11.17 ± 1.21	10.21 ± 1.88	0.69
2-Methylphenanthrene	2MPHE	57.48 ± 4.72	16.22 ± 4.38	<0.01*
2-Methylanthracene	2MANT	6.50 ± 0.56	3.85 ± 0.73	0.04*
1-Methylphenanthrene	1MPHE	122.95 ± 8.45	30.32 ± 7.62	<0.01*
3,6-Dimethylphenanthrene	3,6MPHE	21.57 ± 1.50	6.48 ± 1.55	<0.01*
Fluoranthene	FLA	63.32 ± 4.15	19.99 ± 3.98	<0.01*
Pyrene	PYR	78.84 ± 3.99	32.03 ± 6.62	<0.01*
Retene	RET	92.97 ± 6.15	59.57 ± 11.20	0.06
Benz[c]fluorine	BcF	9.02 ± 0.57	4.17 ± 0.89	0.01*
1-Methylpyrene	1MPYR	3.98 ± 0.38	2.35 ± 0.54	0.07
Cyclopenta[cd]pyrene	CdeP	1.65 ± 0.16	2.04 ± 0.35	0.36
Benzo(a)anthracene	BaA	38.38 ± 2.76	17.22 ± 3.30	0.01*
Chrysene + Triphenylene	CHR+TRI	27.17 ± 1.90	11.99 ± 2.40	0.01*
6-Methylchrysene	6MCHR	2.09 ± 0.14	1.25 ± 0.28	0.05
Benzo(b)fluoranthene	BbF	30.42 ± 2.24	21.76 ± 3.87	0.12
Benzo(k)fluoranthene	BkF	11.40 ± 0.95	7.87 ± 1.53	0.12
Benz[j][e]aceanthrylene	BjeA	0.42 ± 0.04	0.43 ± 0.08	0.94
Benz(e)pyrene	BeP	19.47 ± 1.98	16.58 ± 2.99	0.46
Benzo(a)pyrene	BaP	31.00 ± 2.51	21.15 ± 3.84	0.10
Dibenz(a,c)anthracene	DacP/DahP	0.31 ± 0.02	0.26 ± 0.04	0.33
Indeno(1,2,3-cd)pyrene	IcdP	15.43 ± 0.95	16.77 ± 2.82	0.67
Benzo(ghi)perylene	BghiP	15.67 ± 0.80	18.25 ± 3.08	0.46
Anthranthrene	ANTH	1.88 ± 0.16	2.15 ± 0.37	0.53
OPAHs				
9-Fluorenone	9FLO	0.13 ± 0.02	0.07 ± 0.01	0.05
1,4-Naphthoquinone	1,4NQ	n.d.	0.00 ± 0.00	n.d.
Acenaphthenequinone	ACEN	0.08 ± 0.02	0.20 ± 0.03	0.05

Phenanthrene-1,4-dione	1,4PD	0.16 ± 0.02	0.05 ± 0.01	<0.01*
9,10-Anthraquinone	9,10AQ	8.28 ± 0.40	2.18 ± 0.30	<0.001*
1,4-Anthraquinone	1,4AQ	n.d.	n.d.	
2-methyl-9,10-anthraquinone	2M9,10AQ	3.26 ± 0.57	1.16 ± 0.16	0.02*
2-Ethyl-9,10-Anthraquinone	2E9,10AQ	1.69 ± 0.06	0.77 ± 0.10	<0.01*
9,10-Phenanthrenequinone	9,10PQ	n.d.	n.d.	
Benzo[a]fluorenone	BaF	2.05 ± 0.13	0.89 ± 0.10	<0.01*
Benzanthrone	BZ	1.00 ± 0.12	0.74 ± 0.06	0.13*
Aceanthrenequinone	ACEAN	n.d.	n.d.	
Benzo[c]phenanthrene-[1,4]quinone	Bc1,4Q	0.73 ± 0.06	0.40 ± 0.06	0.01*
7,12-Benzo[a]anthracene dione	7,12BaAD	1.75 ± 0.07	1.03 ± 0.08	<0.01*
Benzo[cd]pyrenone	BcdP	0.50 ± 0.05	0.67 ± 0.07	0.11
5,12-Napthacenequinone	5,12NQ	n.d.	n.d.	
1,6-Benzo[a]pyrene quinone	1,6BaPQ	n.d.	n.d.	
HPAHs				
2-Methylbenzofuran	2MBZ	n.d.	n.d.	
Thianapthene	THN	0.07 ± 0.00	0.05 ± 0.01	0.09
Quinoline	QUI	0.03 ± 0.00	0.03 ± 0.01	0.92
Indole	IND	0.12 ± 0.02	0.03 ± 0.01	0.01*
8-Methylquinoline	8MQ	n.d.	n.d.	
Dibenzofuran	DBZ	0.53 ± 0.06	0.47 ± 0.08	0.57
Xanthene	XAN	n.d.	n.d.	
5,6-Benzoquinoline	5,6BQ	0.86 ± 0.07	0.19 ± 0.06	<0.01*
Acridine	ACR	0.76 ± 0.06	0.15 ± 0.04	<0.001*
Carbazole	CAR	0.24 ± 0.02	0.22 ± 0.03	0.70
Dibenzothiophene	DBZ	0.41 ± 0.04	0.24 ± 0.04	0.05

Table S2. Maximum allowable concentrations (MACs) of the 16 priority PAHs in industrial soils regulated in by the U.S. E.P.A. and Canadian Council of Ministers versus observed mean PAH concentrations and standard errors (SE) in unfractionated soil extracts in study.

Priority PAH	**U.S. E.P.A[1] μg g^{-1}**	**Canada[2] μg g^{-1}**	**Germany [3] μg g^{-1}**	**Pre-bioremediation μg g^{-1}**	**Post-bioremediation μg g^{-1}**
NAP	17			6.26 ± 0.49	8.58 ± 3.82
ACE	45000			6.13 ± 0.71	1.31 ± 0.43
ACEY				8.03 ± 0.30	13.78 ± 2.94
FLU	30000			4.22 ± 0.68	3.02 ±0.58
PHE		50		78.32 ± 11.54	28.65 ±11.54
ANT	230000			11.17 ±1.21	10.21 ± 1.88
FLA	30000			63.32 ± 4.15	19.99 ± 3.98
PYR	23000	100		78.84 ± 3.99	32.03 ± 6.62
CHR	290				
BaA	2.9	10		38.38 ± 2.76	17.22 ± 3.30
BkF	29	10		11.40 ± 0.95	7.87 ± 1.53
BbF	2.9	10		30.42 ± 2.24	21.76 ± 3.87
BaP	0.29	0.7	12	31.00 ± 2.51	21.51 ± 3.84
IcdP	2.9	10		15.43 ± 0.95	16.77 ± 2.82
DahA	0.29	10			
BghiP					

Table S3. Mean lethal concentration (LC_{50}) with standard errors bars of unfractionated soil extract and soil extract fractions (A – F) pre- and post-bioremediation for *DT40*, *Rad54*$^{-/-}$, and *Rev1*$^{-/-}$ cells in mg soil residue per mL DMSO. The LC_{50} values with asterisks (*) showed a significant decrease post-bioremediation (increased toxicity), while (‡) showed a significant increase post-bioremediation (decreased toxicity) ($p < 0.05$ $n = 4$). The LC_{50} for soil extract fraction B post-bioremediation could not be determined because the full dose-response curve could not be captured from the exposure concentrations (N.D. = not determined).

Cell line	Soil/fraction	Pre-bioremediation mg mL^{-1}	Post-bioremediation mg mL^{-1}	*p* value
DT40	Unfractionated	0.90 ± 0.06	0.48 ± 0.03	<0.001*
	A	1.94 ± 0.28	12.6 ± 0.65	<0.0001
	B	32.2 ± 2.81	N.D.	-
	C	5.48 ± 0.51	116 ± 3.59	<0.0001ǂ
	D	3.55 ± 0.44	156 ± 10.5	<0.0001ǂ
	E	0.59 ± 0.09	0.35 ± 0.04	<0.05*
	F	5.39 ± 0.78	3.80 ± 0.18	0.09
Rad54$^{-/-}$	Unfractionated	0.71 ± 0.05	0.31 ± 0.02	<0.001*
	A	1.62 ± 0.23	9.20 ± 1.13	<0.001ǂ
	B	19.8 ± 1.22	N.D.	-
	C	3.80 ± 0.57	62.7 ± 8.77	<0.001ǂ
	D	2.64 ± 0.18	73.1 ± 9.45	<0.001ǂ
	E	0.49 ± 0.03	0.25 ± 0.03	<0.01*
	F	3.93 ± 0.45	3.08 ± 0.21	0.14
Rev1$^{-/-}$	Unfractionated	1.12 ± 0.09	0.50 ± 0.06	<0.01*
	A	1.29 ± 0.01	6.75 ± 0.40	<0.0001ǂ
	B	17.4 ± 1.74	N.D.	-
	C	5.39 ± 0.75	45.8 ± 4.67	<0.001ǂ
	D	3.39 ± 0.25	36.3 ± 6.84	<0.01ǂ
	E	0.51 ± 0.05	0.09 ± 0.02	<0.001*
	F	4.60 ± 0.59	1.18 ± 0.26	<0.01*

Table S4. Median effective concentrations (EC_{50}) and standard errors of fractionated soil extracts (A-F) pre- and post-bioremediation in embryonic zebrafish. Median EC_{50} values with asterisks (*) showed a significant decrease post-bioremediation (increased developmental toxicity), while (‡) showed a significant increase post-bioremediation (decreased developmental toxicity) ($p < 0.05$ $n = 32$). The median EC_{50}s of fractions E and F post-bioremediation were unable to be calculated because the concentrations tested were too low to capture the full dose-response curve (N.D. = not determined).

Soil fraction	**Pre-bioremediation** **μg mL^{-1}**	**Post-bioremediation** **μg mL^{-1}**	***p* value**
A	0.89 ± 0.04	0.94 ± 0.03	0.36
B	1.29 ± 0.04	1.34 ± 0.05	0.43
C	2.11 ± 0.06	1.01 ± 0.04	<0.001*
D	3.30 ± 0.07	4.74 ± 0.07	<0.001ǂ
E	5.39 ± 0.09	N.D.	-
F	8.90 ± 0.12	N.D.	-

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