

SUPPLEMENTARY MATERIAL

Helichrysum microphyllum subsp. *tyrrhenicum*, its root associated microorganisms, and wood chips represent an integrated green technology for remediation of petroleum hydrocarbon-contaminated soils

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Figure S1. Sampling of contaminated substrate at the former SIPSA Srl plant (Brabau - Torregrande, OR, Italy).



Figure S2. Samplings of substrates and plants at the end of the phytoremediation test with *Helichrysum microphyllum* subsp. *tyrrhenicum* in refinery substrate with aged contamination (July 2021).

Table S1. Main characteristics of refinery substrate and organic amendment.

Parameter	Substrate	Wood chips
Substrate moisture (%)	12%	49%
Substrate pH (H ₂ O)	8.4 ± 0.2	7.2 ± 0.1
Total petroleum hydrocarbons (g kg ⁻¹ dry w)	3.3 ± 0.8	nd
Dehydrogenase activity (INT-F g ⁻¹ wet w)	6.0 ± 5.4	15.8 ± 0.3
Viable titer of fungi (log MPN g ⁻¹ wet w)	3.2 ± 0.2	7.6 ± 0.4
Viable titer of bacteria (log MPN g ⁻¹ wet w)	5.1 ± 0.4	7.7 ± 0.2
Viable titer of diesel degraders (log MPN g ⁻¹ wet w)	5.4 ± 0.3	8.5 ± 0.4
Viable titer of paraffin degraders (log MPN g ⁻¹ wet w)	4.9 ± 0.1	8.0 ± 0.5

Table S2. Statistical analysis of growth parameters (plant height, crown diameter, stem base diameter) of *H. tyrrhenicum* measured at the beginning and at the end of the greenhouse test in three different substrates.

				Kruskal-Wallis	
				Chi-sqr	p
Height (cm)		mean	dev.st		
GARD	initial (0 d)	20.5	2.8	33.6	6.7E-09
	final (199 d)	26.9	3.7		
UNAM	initial (0 d)	20.5	3.5	2.6	0.11
	final (199 d)	22.3	5.1		
WCAM	initial (0 d)	20.8	3.4	10.5	1.2E-03
	final (199 d)	24.3	4.2		
Crown diameter (cm)					
GARD	initial (0 d)	9.3	2.5	43.8	3.7E-11
	final (199 d)	20.6	4.9		
UNAM	initial (0 d)	11.0	4.0	9.3	2.2E-03
	final (199 d)	8.0	2.7		
WCAM	initial (0 d)	9.5	2.3	10.5	1.2E-03
	final (199 d)	11.9	2.8		
Stem base diameter (cm)					
GARD	initial (0 d)	0.3	0.1	39.2	3.1E-10
	final (199 d)	0.5	0.1		
UNAM	initial (0 d)	0.3	0.1	11.8	5.4E-04
	final (199 d)	0.4	0.1		
WCAM	initial (0 d)	0.2	0.1	27.9	1.0E-07
	final (199 d)	0.4	0.1		

GARD: commercial garden substrate, UNAM: contaminated substrate without amendment, WCAM: contaminated substrate amended with wood chips.

Table S3. Statistical analysis of growth parameters (plant height, crown diameter, stem base diameter) of *H. tyrrhenicum* measured at each kinetic time during the greenhouse test in three different substrates.

Day	Test	Height (cm)				Crown diameter (cm)								Stem base diameter (cm)						
				Kruskal-Wallis		Mann-Whitney				Kruskal-Wallis		Mann-Whitney				Kruskal-Wallis		Mann-Whitney		
		mean	dev.st	Chi-sqr	p	Comparison	p	mean	dev.st	Chi-sqr	p	Comparison	p	mean	dev.st	Chi-sqr	p	Comparison	p	
0	GARD	20.5	2.8	0.459	0.795	ND	9.3	2.5	3.17	0.2045	ND	0.258	0.075	4.60	0.100	ND				
	UNAM	20.5	3.5				11.0	4.0				0.281	0.121							
	WCAM	20.8	3.4				9.5	2.3				0.232	0.087							
14	GARD	20.8	2.9	0.489	0.783	ND	9.9	2.5	1.32	0.5171	ND	0.277	0.096	0.81	0.666	ND				
	UNAM	20.8	3.4				10.8	3.3				0.284	0.129							
	WCAM	21.1	3.3				9.8	2.3				0.256	0.082							
28	GARD	21.7	3.0	0.955	0.620	ND	10.5	2.3	1.48	0.4765	ND	0.273	0.070	2.30	0.315	ND				
	UNAM	21.3	3.5				10.8	3.1				0.282	0.133							
	WCAM	21.6	3.4				9.9	2.3				0.253	0.090							
43	GARD	22.1	2.9	0.499	0.779	ND	11.0	2.5	1.32	0.5164	ND	0.284	0.069	3.40	0.182	ND				
	UNAM	21.9	3.7				11.0	3.0				0.280	0.132							
	WCAM	21.9	3.5				10.2	2.4				0.266	0.092							
56	GARD	22.6	3.0	1.097	0.578	ND	11.3	2.3	1.61	0.4472	ND	0.300	0.072	4.10	0.128	ND				
	UNAM	22.2	3.8				10.9	2.9				0.289	0.135							
	WCAM	22.2	3.6				10.5	2.4				0.280	0.093							
70	GARD	22.8	2.7	2.127	0.345	ND	11.6	2.2	2.85	0.2404	ND	0.318	0.075	4.71	0.094	ND				
	UNAM	22.2	3.9				10.9	2.9				0.305	0.143							
	WCAM	22.2	3.6				10.6	2.5				0.286	0.095							
84	GARD	23.0	2.8	1.966	0.374	ND	12.0	2.2	4.15	0.1256	ND	0.344	0.084	7.65	0.022	GARD	UNAM	0.091		
	UNAM	22.4	4.1				11.1	2.9				0.308	0.146			GARD	WCAM	0.031		
	WCAM	22.3	3.6				10.7	2.5				0.298	0.095			UNAM	WCAM	1.000		
102	GARD	23.3	2.7	3.443	0.179	ND	12.2	2.3	2.95	0.2285	ND	0.359	0.088	8.36	0.015	GARD	UNAM	0.097		
	UNAM	22.4	4.2				11.1	3.0				0.315	0.140			GARD	WCAM	0.015		
	WCAM	22.5	3.7				11.1	2.6				0.306	0.094			UNAM	WCAM	1.000		
112	GARD	23.4	2.7	3.250	0.197	ND	12.5	2.7	3.97	0.137	ND	0.525	0.849	8.45	0.015	GARD	UNAM	0.076		
	UNAM	22.5	4.2				11.0	3.1				0.327	0.127			GARD	WCAM	0.018		
	WCAM	22.7	3.6				11.1	2.5				0.321	0.089			UNAM	WCAM	1.000		
130	GARD	23.7	2.8	4.506	0.105	ND	14.3	3.3	20.60	3.2E-05	GARD	UNAM	2.4E-04	0.405	0.081	GARD	UNAM	0.008		
	UNAM	22.4	4.1				10.7	3.1			GARD	WCAM	5.3E-04	0.335	0.122	GARD	WCAM	0.002		
	WCAM	22.9	3.7				11.4	2.7			UNAM	WCAM	0.791	0.334	0.100	UNAM	WCAM	1.000		
144	GARD	23.9	2.7	5.670	0.059	ND	15.2	3.2	34.25	3.5E-08	GARD	UNAM	2.5E-07	0.437	0.075	GARD	UNAM	0.002		
	UNAM	22.3	4.0				9.9	3.4			GARD	WCAM	5.1E-05	0.360	0.120	GARD	WCAM	2.9E-05		
	WCAM	23.2	3.7				11.7	2.8			UNAM	WCAM	0.129	0.343	0.106	UNAM	WCAM	1.000		
158	GARD	24.4	2.7	8.864	0.012	GARD	UNAM	0.010	16.2	3.3	44.91	1.7E-10	GARD	UNAM	2.2E-09	0.465	0.075	GARD	UNAM	9.7E-04
	UNAM	22.2	4.0			GARD	WCAM	0.169	9.5	2.8			GARD	WCAM	1.5E-05	0.375	0.124	GARD	WCAM	1.2E-04
	WCAM	23.2	3.8			UNAM	WCAM	0.944	12.0	2.8			UNAM	WCAM	0.009	0.366	0.112	UNAM	WCAM	1.000
175	GARD	25.0	3.0	12.890	0.002	GARD	UNAM	0.002	17.3	3.4	56.86	4.4E-13	GARD	UNAM	2.0E-10	0.491	0.081	GARD	UNAM	3.7E-05
	UNAM	22.1	4.1			GARD	WCAM	0.089	8.9	2.6			GARD	WCAM	7.3E-08	0.385	0.112	GARD	WCAM	9.5E-05
	WCAM	23.4	3.8			UNAM	WCAM	0.370	11.8	2.8			UNAM	WCAM	9.8E-04	0.384	0.117	UNAM	WCAM	1.000
184	GARD	25.7	3.1	18.290	1.1E-04	GARD	UNAM	8.4E-05	18.6	4.0	59.91	9.6E-14	GARD	UNAM	1.9E-10	0.521	0.109	GARD	UNAM	2.7E-06
	UNAM	21.8	4.3			GARD	WCAM	0.053	8.3	2.9			GARD	WCAM	2.1E-08	0.357	0.129	GARD	WCAM	7.3E-05
	WCAM	23.8	4.0			UNAM	WCAM	0.126	11.8	2.8			UNAM	WCAM	1.5E-04	0.390	0.127	UNAM	WCAM	0.777
199	GARD	26.9	3.7	20.600	3.3E-05	GARD	UNAM	4.3E-05	20.6	4.9	63.39	1.7E-14	GARD	UNAM	2.0E-10	0.532	0.119	GARD	UNAM	1.5E-05
	UNAM	22.3	5.1			GARD	WCAM	0.018	8.0	2.7			GARD	WCAM	5.2E-09	0.380	0.109	GARD	WCAM	4.4E-05
	WCAM	24.3	4.2			UNAM	WCAM	0.104	11.9	2.8			UNAM	WCAM	1.7E-05	0.390	0.127	UNAM	WCAM	1.000

GARD: commercial garden substrate, UNAM: contaminated substrate without amendment, WCAM: contaminated substrate amended with wood chips.

Table S4. Statistical analysis of dry biomasses of *H. tyrrhenicum* at the end of the greenhouse test in three different substrates.

			Kruskal-Wallis		Mann-Whitney		
	mean	dev.st	Chi-sqr	p	Comparison		p
Dry biomass (g dry w) of epigeal tissues							
GARD	40.9	12.2	9.38	0.009	GARD	UNAM	0.037
UNAM	15.6	4.3			GARD	WCAM	0.037
WCAM	14.4	8.8			UNAM	WCAM	
Dry biomass (g dry w) of hypogeal tissues							
GARD	8.0	4.2	5.58	0.061	ND		
UNAM	5.6	4.8					
WCAM	3.1	2.7					

GARD: commercial garden substrate, UNAM: contaminated substrate without amendment, WCAM: contaminated substrate amended with wood chips.

Table S5. Statistical analysis of properties measured in bulk, rhizosphere, and deep substrates at the end of the phytoremediation test with *H. tyrrhenicum* in refinery substrate with aged contamination unamended (UNAM) and amended (WCAM) with wood chips.

			Kruskal-Wallis	
mean		dev.st	Chi-sqr	p
Bulk substrate				
Substrate moisture (%)				
UNAM	10%	6%	6.82	0.009
WCAM	33%	8%		
Substrate pH (H2O)				
UNAM	8.1	0.2	5.28	0.021
WCAM	7.8	0.1		
Dehydrogenase activity (INT-F g-1 wet w)				
UNAM	42.0	6.7	3.94	0.047
WCAM	50.5	5.9		
Viable titer of fungi (log MPN g-1 wet w)				
UNAM	4.0	0.5	6.82	0.009
WCAM	5.7	0.3		
Viable titer of bacteria (log MPN g-1 wet w)				
UNAM	6.1	0.1	6.82	0.009
WCAM	6.5	0.2		
Viable titer of diesel degraders (log MPN g-1 wet w)				
UNAM	6.4	0.2	6.82	0.007
WCAM	7.2	0.2		
Viable titer of paraffin degraders (log MPN g-1 wet w)				
UNAM	6.7	0.3	4.36	0.036
WCAM	7.1	0.2		
Total petroleum hydrocarbons (g kg-1 dry w)				
UNAM	1.8	1.0	3.15	0.076
WCAM	0.6	0.3		
Rhizosphere substrate				
Dehydrogenase activity (INT-F g-1 wet w)				
UNAM	203.2	35.4	0.10	0.754
WCAM	244.6	105.7		
Viable titer of fungi (log MPN g-1 wet w)				
UNAM	4.8	0.3	6.82	0.009
WCAM	5.6	0.1		
Viable titer of bacteria (log MPN g-1 wet w)				
UNAM	7.0	0.3	0.01	0.917
WCAM	7.2	0.7		
Viable titer of diesel degraders (log MPN g-1 wet w)				
UNAM	7.5	0.3	0.27	0.602
WCAM	7.4	0.6		
Viable titer of paraffin degraders (log MPN g-1 wet w)				
UNAM	7.6	0.3	1.84	0.173
WCAM	7.5	0.3		
Total petroleum hydrocarbons (g kg-1 dry w)				
UNAM	0.03	0.02	5.77	0.016
WCAM	0.16	0.13		
Deep substrate				
Total petroleum hydrocarbons (g kg-1 dry w)				
UNAM	3.2	1.7	3.94	0.047
WCAM	1.3	0.7		