



Supplementary Materials

Ecotoxicological estimation of 4-cumylphenol, 4-*t*-octylphenol, nonylphenol and volatile leachate phenol degradation by the microscopic fungus *Umbelopsis isabellina* using a battery of biotests

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Figure S1. Location of leachate collection from post-industrial landfills. Study area location in Zgierz city (Poland, Central Europe). A. The municipal and industrial wastewater treatment plant, B. The former "Boruta" Dye Industry Plant area, C. The closed landfill for hazardous waste of the former "Boruta" Dye Industry Plant, D. The closed energy ash and gypsum landfill.

Table S1. Basic analysis of the landfill leachate collected from the hazardous waste landfill of the former “Boruta” dye production plants in Zgierz.

Parameter	Method	Unit	Value	Standard
pH	PN-EN ISO 10523:12	pH	7.2 ± 0.4	6.5 – 9.0
Colour	PN-EN ISO 7887:2012	Pt-Co	2920 ± 230	-
Unit weight	ASTM D854	g cm ⁻³	1.018 ± 0.003	-
Conductivity	PN-EN 27888:1999	µS	11805 ± 673	-
COD _{Mn}	PN-ISO 15705:2005	mg L ⁻¹ O ₂	348.8 ± 92.4	125
TOC	PN-EN 1484:1999	mg L ⁻¹ C	1040.5 ± 190.4	30
BOD ₅	PN-EN 1899-2:2002	mg L ⁻¹ O ₂	300 ± 15.2	-

Table S2. Chemical contaminants of the landfill leachate.

Parameter	Method	Unit	Value	Standard
Nitrites	PN-EN ISO 13395:2001	mg L ⁻¹ NO ₂	0.026 ± 006	1
Nitrates	PN-EN ISO 10304-1:2009	mg L ⁻¹ NO ₃	< 1.7	30
Sulphates	PN-EN ISO 10304-1:2009	mg L ⁻¹ SO ₄	15 ± 3	500
Chlorides	PN-EN ISO 10304-1:2009	mg L ⁻¹ Cl	2.1 ± 0.4	1000
Cyanides (free)	PN-EN ISO 14403-2:2012	mg L ⁻¹	< 0.008	-
Cyanides (bound)	PN-EN ISO 14403-2:2012	mg L ⁻¹	0.045 ± 0.008	-
Antimony	PN-EN ISO 11885:2009	mg L ⁻¹ Sb	<0.020	0.3
Arsenic	PN-EN ISO 11885:2009	mg L ⁻¹ As	<0.020	0.1
Barium	PN-EN ISO 11885:2009	mg L ⁻¹ Ba	0.37 ± 0.08	2
Beryllium	PN-EN ISO 11885:2009	mg L ⁻¹ Be	<0.004	1
Boron	PN-EN ISO 11885:2009	mg L ⁻¹ B	20.35 ± 4.68	1
Chromium (total)	PN-EN ISO 11885:2009	mg L ⁻¹ Cr _{total}	0.055 ± 0.011	0.1
Zinc	PN-EN ISO 11885:2009	mg L ⁻¹ Zn	0.067 ± 0.015	2
Aluminum	PN-EN ISO 11885:2009	mg L ⁻¹ Al	0.071 ± 0.016	3
Cadmium	PN-EN ISO 11885:2009	mg L ⁻¹ Cd	0.0015 ± 0.0003	0.4

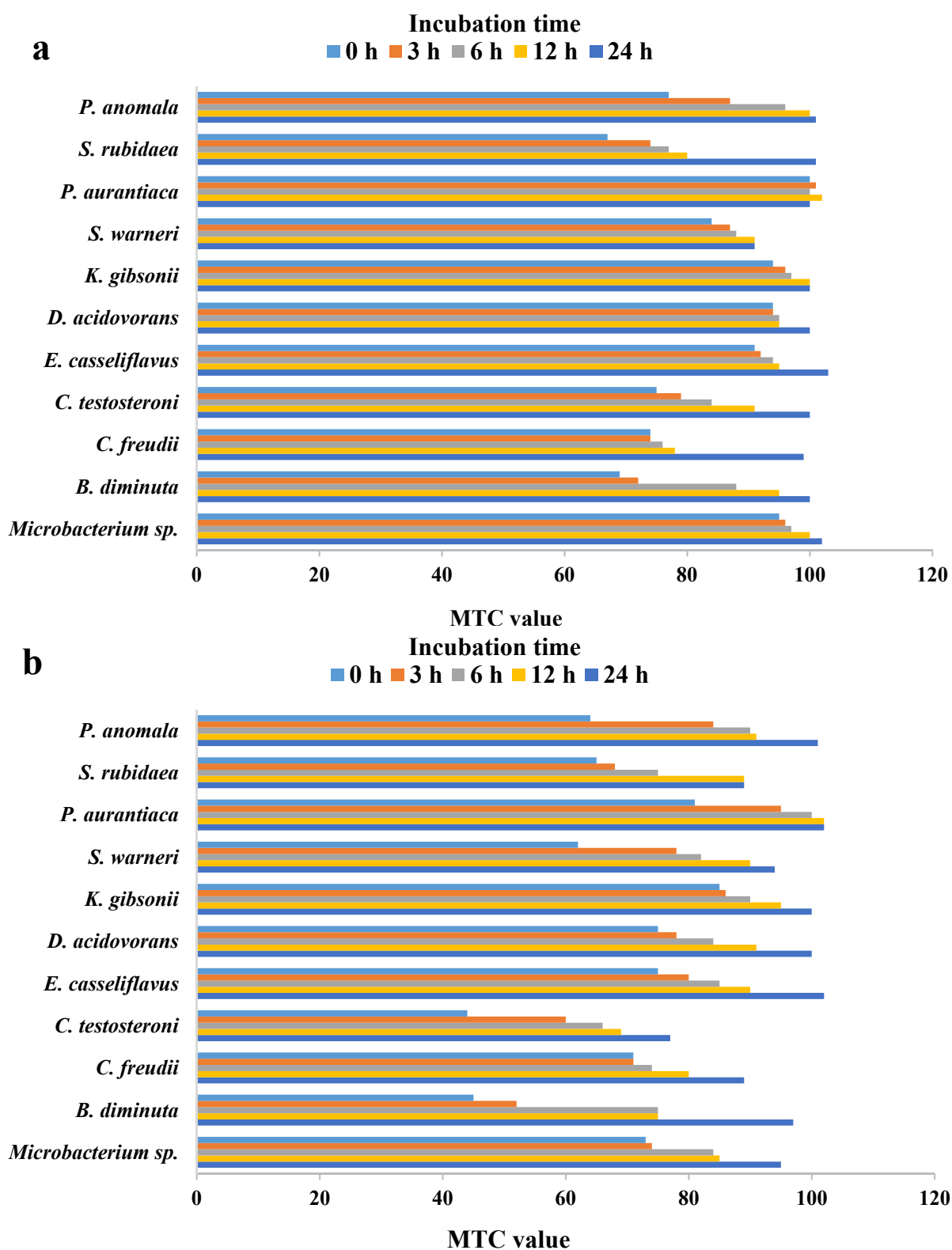
Cobalt	PN-EN ISO 11885:2009	mg L ⁻¹ Co	<0.002	1
Manganese	PN-EN ISO 11885:2009	mg L ⁻¹ Mn	0.17 ± 0.04	-
Copper	PN-EN ISO 11885:2009	mg L ⁻¹ Cu	0.014 ± 0.003	0.5
Molybdenum	PN-EN ISO 11885:2009	mg L ⁻¹ Mo	0.057 ± 0.009	1
Nickel	PN-EN ISO 11885:2009	mg L ⁻¹ Ni	0.23 ± 0.04	0.5
Lead	PN-EN ISO 11885:2009	mg L ⁻¹ Pb	0.023 ± 0.004	0.5
Selenium	PN-EN ISO 11885:2009	mg L ⁻¹ Se	<0.050	-
Silver	PN-EN ISO 11885:2009	mg/L Ag	<0.010	0.1
Thallium	PN-EN ISO 11885:2009	mg L ⁻¹ Tl	<0.020	1
Titanium	PN-EN ISO 11885:2009	mg L ⁻¹ Ti	0.024 ± 0.005	1
Vanadium	PN-EN ISO 11885:2009	mg L ⁻¹ V	<0.006	2
Iron	PN-EN ISO 11885:2009	mg L ⁻¹ Fe	30.88 ± 5.87	10
Mercury	EPA 7473 02.2007	mg L ⁻¹ Hg	0.0014 ± 0.0002	0.06
Volatile phenols	PN-EN ISO 14402 : 2004	mg L ⁻¹	1.68 ± 0.48	0.1
Petroleum hydrocarbons	PN-EN ISO 9377-2:2003	mg L ⁻¹	7.2 ± 2.3	15

Table S3. Toxicity bioassays selected for the ecotoxicological analysis of filtrates from *U. isabellina* cultures supplemented with xenobiotics and landfill leachate.

Trophic level	Species	Measure of toxic effect (toxicological endpoints)	4NP, 4-CP, 4- <i>t</i> -OP samples analyzed	VPs samples analyzed
Decomposer	<i>Aliivibrio fischeri</i>	Bioluminescence activity	X	X
	<i>Microbacterium</i> sp.	Growth rate inhibition	X	
	<i>Brevundimonas diminuta</i>	Growth rate inhibition	X	
	<i>Citrobacter freundii</i>	Growth rate inhibition	X	
	<i>Comamonas testosteroni</i>	Growth rate inhibition	X	
	<i>Enterococcus casseliflavus</i>	Growth rate inhibition	X	

	<i>Delftia acidovorans</i>	Growth rate inhibition	X	
	<i>Kurthia gibsonii</i>	Growth rate inhibition	X	
	<i>Staphylococcus warneri</i>	Growth rate inhibition	X	
	<i>Pseudomonas aurantiaca</i>	Growth rate inhibition	X	
	<i>Serratia rubidaea</i>	Growth rate inhibition	X	
	<i>Pichia anomala</i>	Growth rate inhibition	X	
	<i>Saccharomyces cerevisiae</i>	Estrogenic/anti-estrogenic activity	X	
		Androgenic/anti-androgenic activity		
Producer	<i>Sorghum saccharatum</i>	Seed germination rate, root length reduction	X	X
	<i>Lepidium sativum</i>	Seed germination rate, root length reduction	X	
	<i>Sinapis alba</i>	Seed germination rate, root length reduction	X	
	<i>Rhapidocelis subcapitata</i>	Growth rate inhibition	X	
	<i>Phaeodactylum tricornutum</i>	Growth rate inhibition	X	
Consumer	<i>Thamnocephalus platyurus</i>	Reduction or complete cessation of food intake	X	
	<i>Daphnia magna</i>	Mortality	X ^a	X
	<i>Artemia franciscana</i>	Mortality	X ^a	X

^a – tests were carried out in previous studies [19]



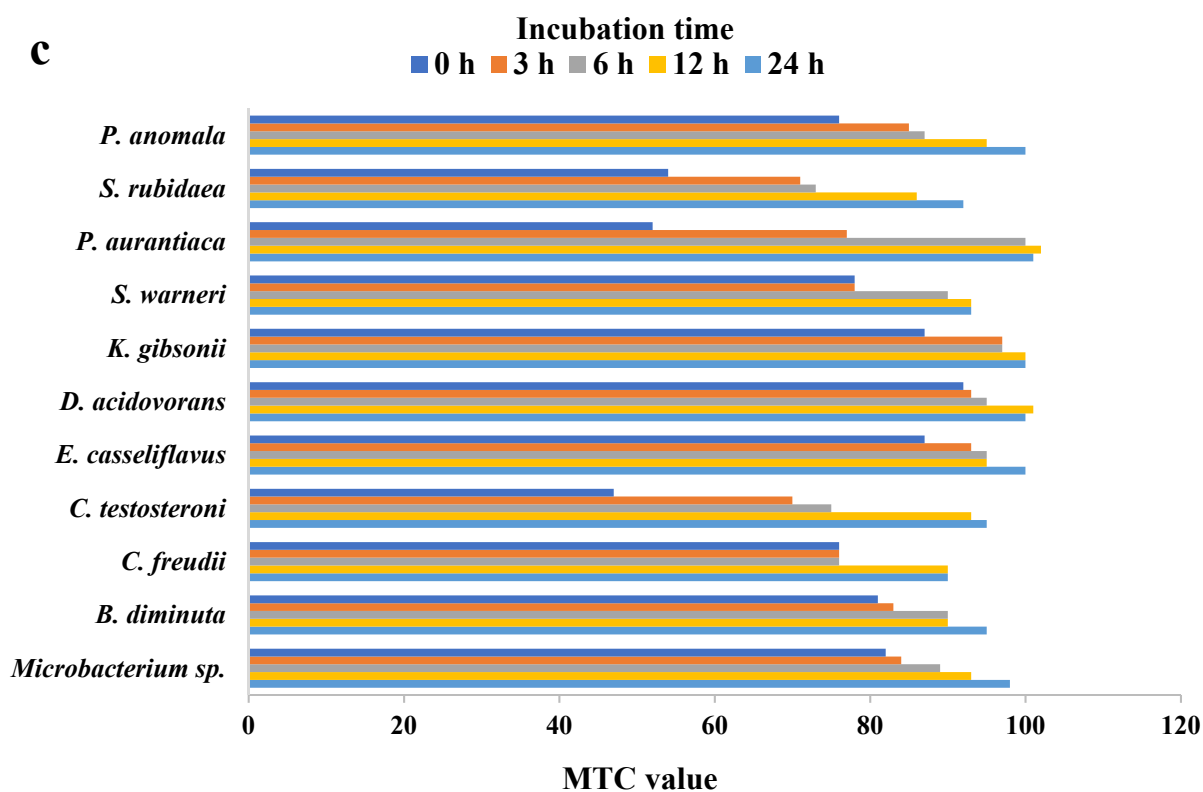


Figure S2. MARA species responses (MTC value) to *U. isabellina* cultures treated with test xenobiotics: a – 4-CP, b – NP, c – 4-*t*-OP.

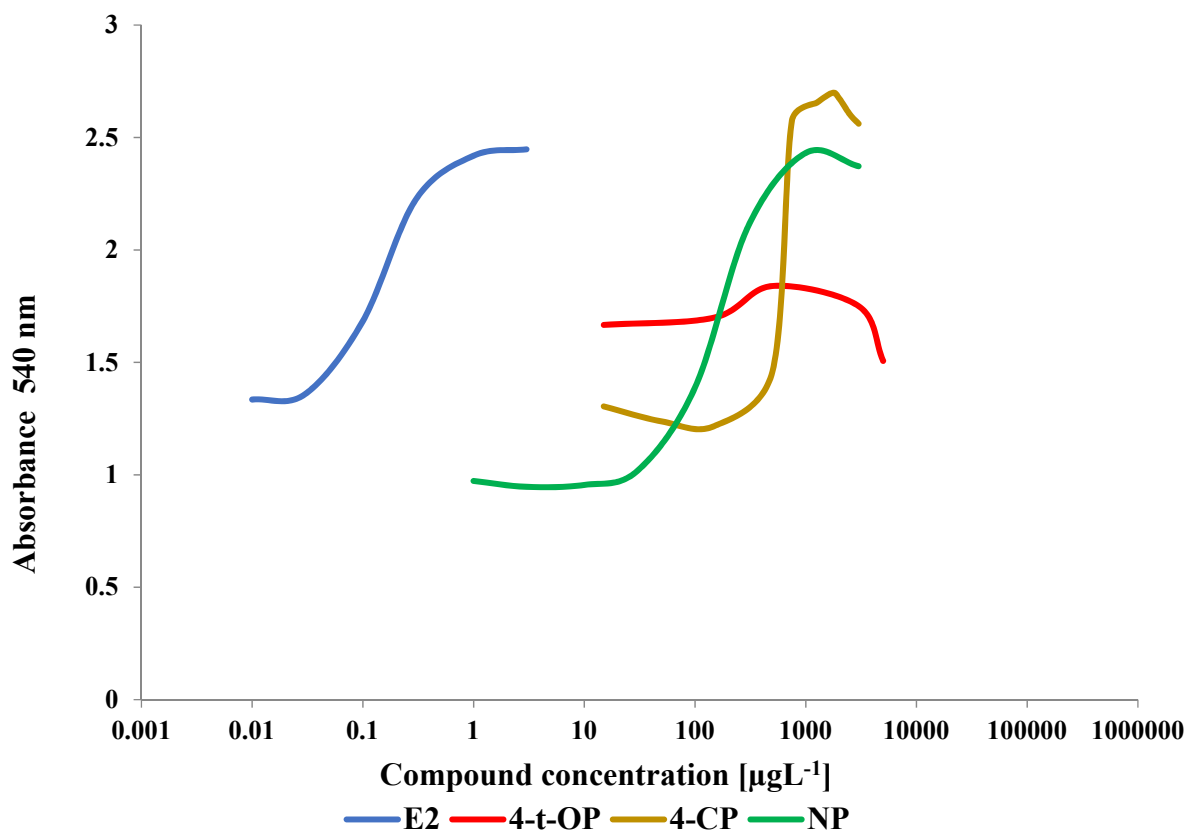


Figure S3. Oestrogenic activity of 4-CP, 4-*t*-OP and NP.

Table S4. Anti-androgenic activity of post-culture filtrates obtained after incubation of *U. isabellina* with NP or 4-CP (post-culture liquid concentration – 4%).

Time (h)	Absorbance of 4-CP (% of control, i.e. DHT)	Absorbance of 4- <i>t</i> -OP (% of control, i.e. DHT)
0	65.50 ± 1.52	75.04 ± 1.12
3	75.01 ± 0.90	83.20 ± 1.42
6	82.28 ± 1.23	85.18 ± 0.64
12	81.98 ± 0.56	87.90 ± 0.49
24	86.73 ± 0.70	93.72 ± 0.80

Table S5. Plant biological endpoints for testing the phytotoxicity of fungal post-culture filtrates supplemented with landfill leachate.

Landfill leachate (%)	Time (h)	Germination inhibition (PE%)	Root growth inhibition (PE%)	Germination index (%)
20	0	0 ± 0	32.5 ± 2.8	59.5 ± 2.0
	24	0 ± 0	39.7 ± 2.2	52.4 ± 3.0
	48	0 ± 0	49.6 ± 3.0	43.0 ± 2.9
	72	0 ± 0	29.3 ± 2.7	62.9 ± 3.6
	96	0 ± 0	20.6 ± 3.1	78.3 ± 2.8
40	0	10 ± 2	59.1 ± 3.3	40.6 ± 1.7
	24	0 ± 0	64.7 ± 2.8	35.2 ± 2.6
	48	0 ± 0	69.2 ± 3.4	30.8 ± 2.0
	72	0 ± 0	53.4 ± 3.8	44.6 ± 2.7
	96	0 ± 0	40.1 ± 2.5	59.7 ± 2.1