

Assessment of extracellular polymeric substances production and antioxidant defences in periphytic communities exposed to effluent contaminants.

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Table S1. Summarized characterization of sampling sites (data collected from Silva et al. (2022)). General water and sediment quality parameters are provided first, then quantified concentrations of PPCPs, metallic elements, and PAHs in sediments are given for compounds found in at least one sample analyzed in the present study.

	WWTPa			WWTPb			WWTPc		
	Upa	D1a	D2a	Upb	D1b	D2b	Upc	D1c	D2c
Water									
Flow speed (m/s)	11.11	12.36	0.25	25	27.22	9.72	7.69	9.64	8.99
Mean water depth (cm)	41	60	37	50	40	50	20	20	60
Channel width (m)	1 - 5	1 - 5	3 - 5	5 - 10	3 - 5	3 - 5	3 - 5	3 - 5	3 - 5
Temperature (°C)	18.3	17.7	17.2	17.3	18.2	17.8	28.9	24.1	23.6
Conductivity (µS/cm)	398	400	360	505	458	1435	619	799	1025
Dissolved oxygen (mg/L)	8.81	8.94	7.85	9.88	9.46	9.72	12.26	5.30	5.42
Oxygen saturation (%)	93.9	93.7	81.1	102	100.3	102.6	158.7	63.4	63.8
pH	7.13	7.35	7.37	7.24	7.41	7.03	7.64	8.45	8.28
Total dissolved solids (TDS)	256	260	ND	328	297	933	402	521	ND
Total Nitrogen (N)	0.28	0.00	0.00	0.00	0.00	17.41	19.32	34.56	30.59
Total Phosphorous (P)	0.00	0.03	0.00	0.00	0.07	0.05	0.17	0.49	0.64
Sediment									
Organic matter (% w/w)	1.34	1.34	0.19	0.44	0.37	0.44	0.49	0.58	0.42
Silt >3.9-63 µm (% w/w)	4.67	4.67	0.43	1.21	3.49	6.41	2.47	2.72	3.46
Sand >63-2000 µm (% w/w)	95.12	95.12	94.86	83.06	90.79	92.40	90.01	76.02	86.62
Gravel >2000 (% w/w)	0.23	0.23	4.70	15.72	5.71	1.20	7.53	21.27	9.91
Sediment									
PPCPs (µg/kg)									
Amisulpride	bdl	bdl	bdl	bdl	bdl	bdl	bql	1.52	1.13
Amitriptyline	bdl	bdl	bdl	bdl	bdl	bdl	bdl	2.48	1.54
Atenolol	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.19	1.13
Bisoprolol	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.19	1.21
Caffeine	bql	bql	bql	bql	bql	0.20	13.1	15.9	8.64
Citalopram	bdl	bdl	bdl	bdl	bql	bql	bql	10.95	7.67
Propranolol	bdl	bdl	bdl	bdl	bdl	bdl	bdl	3.14	3.09
Sertraline	bdl	bdl	bdl	bdl	bdl	bdl	bdl	4.58	3.41
Tramadol	bdl	bdl	bdl	bdl	bdl	bdl	bql	20.2	22.5
Triclosan	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.72	1.35
Trimethoprim	bdl	bdl	bdl	0.07	48.0	bql	bql	bql	bql
Venlafaxine	bdl	bdl	bdl	bql	bql	bdl	bql	12.4	10.1
Metals and metalloids (mg/Kg)									
Li	1.91	1.38	0.62	1.17	1.51	1.32	2.98	3.38	3.33
Be	0.18	bdl	bdl	bdl	bdl	bdl	0.29	0.52	bdl

Na	bdl	bdl	bdl	bdl	bdl	294	321	220
Mg	174	105	35.0	292	365	4389	5012	4804
Al	1341	790	303	662	1005	984	5107	6221
P	172	253	94.7	101	116	118	651	685
K	304	212	bdl	bdl	207	238	585	745
Ca	1122	796	185	36393	35934	22553	40874	40602
V	1.54	0.85	0.33	1.85	2.29	2.35	23.0	26.6
Cr	1.71	1.07	bdl	1.92	2.94	2.65	17.7	23.7
Mn	21.8	14.4	4.71	52.3	33.7	49.5	210	192
Fe	2762	1452	660	1631	2092	2225	11237	12232
Co	0.64	bdl	bdl	1.93	1.48	1.66	8.53	10.6
Ni	1.51	1.02	bdl	1.74	2.23	2.28	21.6	26.0
Cu	3.92	4.62	0.73	3.72	4.25	5.00	11.06	19.3
Zn	11.9	9.63	2.54	9.05	15.4	15.9	50.2	84.8
As	0.88	bdl	bdl	bdl	bdl	bdl	0.78	0.71
Se	bdl	bdl	bdl	bdl	bdl	bdl	0.63	0.70
Rb	3.30	1.97	0.95	1.77	2.85	2.86	3.98	5.04
Sr	3.89	3.53	2.04	19.9	43.4	14.7	72.1	76.9
Y	1.20	0.62	0.32	1.20	1.31	1.38	3.43	3.77
Mo	bdl	bdl	bdl	bdl	bdl	bdl	0.22	1.34
Ag	0.05	bdl	bdl	bdl	bdl	0.34	0.23	0.23
Cd	bdl	bdl	bdl	bdl	bdl	bdl	0.09	0.09
Sn	0.22	0.25	0.53	0.23	0.22	0.22	0.13	0.50
Ba	13.7	10.7	30.3	11.6	834	15.0	66.1	61.4
La	3.06	1.69	1.15	2.67	3.53	4.73	9.85	13.6
Ce	6.47	3.54	2.39	5.54	7.32	10.3	19.3	21.4
Pr	0.80	0.43	0.29	0.68	0.88	1.20	2.38	2.61
Nd	3.12	1.70	1.08	2.63	3.43	4.53	9.37	10.4
Sm	0.58	0.31	0.21	0.53	0.77	0.83	1.69	1.86
Eu	0.12	0.08	0.12	0.11	2.97	0.16	0.64	0.66
Gd	1.12	0.61	0.41	0.96	1.44	1.72	3.38	3.67
Tb	0.09	0.05	0.03	0.08	0.10	0.13	0.28	0.31
Dy	0.31	0.16	0.10	0.28	0.33	0.40	0.98	1.07
Ho	0.05	0.03	0.01	0.05	0.05	0.06	0.18	0.19
Er	0.13	0.07	0.03	0.11	0.13	0.14	0.41	0.44
Tm	0.02	0.01	0.01	0.02	0.02	0.02	0.07	0.07
Yb	0.09	0.04	0.01	0.08	0.08	0.10	0.28	0.30
Lu	bdl	bdl	bdl	bdl	bdl	bdl	0.05	0.04
Pb	2.08	1.32	1.09	2.67	56.38	4.98	10.28	6.32
U	0.17	0.08	0.09	0.23	0.27	0.31	0.34	0.35

PAHs ($\mu\text{g}/\text{Kg}$)

Naphthalene (NP)	bdl	bdl	bdl	2.49	2.49	3.73	1.71	2.05	2.91
Acenaphthylene (ACY)	bdl	bdl	bdl	bdl	bdl	bdl	1.26	1.26	1.69
Acenaphthene (ACE)	bdl	bdl	bdl	bdl	1.77	bdl	3.86	bdl	bdl
Fluorene (FLU)	bdl	bdl	bdl	bdl	bdl	bdl	6.66	6.13	bdl
Phenanthrene (PHE)	1.85	2.01	1.35	1.61	1.01	1.22	32.70	24.30	11.90
Anthracene (ANT)	bdl	bdl	bdl	bdl	bdl	bdl	5.90	bdl	bdl
Fluoranthene (FLA)	1.76	2.21	1.26	1.99	1.15	1.59	44.10	28.60	17.70
Pyrene (PYR)	3.25	3.87	2.12	2.25	1.17	1.64	48.40	52.10	25.40
Benzo[a]anthracene (BAA)	bdl	bdl	bdl	1.34	bdl	bdl	16.30	1.71	4.14
Chrysene (CRY)	bdl	bdl	bdl	2.15	1.52	1.73	16.30	3.42	5.50
Benzo[b]fluoranthene (BBF)	bdl	bdl	bdl	1.60	1.00	1.33	16.30	2.87	7.04
Benzo[k]fluoranthene (BKF)	bdl	bdl	bdl	bdl	bdl	bdl	8.19	1.64	3.50
Benzo[a]pyrene (BAP)	bdl	bdl	bdl	1.17	bdl	1.22	23.80	2.81	8.62
Indeno[1,2,3-c,d]pyrene (IND)	bdl	bdl	bdl	bdl	bdl	bdl	15.80	2.49	7.16
Dibenzo[a,h]anthracene (DBAH)	bdl	bdl	bdl	bdl	bdl	bdl	3.53	bdl	bdl
Benzo[g,h,i]perylene (BGHI)	bdl	bdl	bdl	1.46	1.26	1.78	18.90	3.41	9.63
PAHs sum	6.86	8.10	4.73	16.06	11.37	14.24	262.45	132.79	105.19

Effluent

WWTPa WWTPb WWTPc

PPCPs (ng/L)

Abacavir	14.1	7.42	bql	Lincomycin	Bdl	80.3	bdl
Acetaminophen	3714.0	19.5	21.9	Losartan	611	57.6	345

Amisulpride	16.04	16.42	69.25	Metformin	297	3.18	11.9
Amitriptyline	bdl	1.70	bdl	Metoprolol	bdl	1.70	6.95
Amoxicillin	bdl	28.527	29.268	Omeprazole	bdl	74.0	Bdl
Atenolol	49.4	59.0	90.4	Pantoprazole	bdl	10.8	1.62
Atorvastatin	132	bdl	bdl	Prilocaine	13.8	3.09	4.84
Azithromycin	Bdl	bdl	33.9	Propranolol	bdl	10.81	9.67
Bezafibrate	34.1	8.62	50.6	Quetiapine	7.89	bdl	Bdl
Bisoprolol	55.3	7.21	104	Rosurvastatin	711	bdl	20.4
Bupropion	Bdl	bdl	8.07	Sertraline	bdl	4.96	7.07
Caffeine	3681	74.0	76.5	Sulfamethoxazole	17.1	41.4	63.3
Carbamazepine	154	274	317	Telmisartan	671	575	629
Cetirizine	96.8	159	420	Timolol	bdl	bdl	12.7
Citalopram	10.2	12.2	bdl	Tramadol	219	542	679.
Clarithromycin	Bdl	18.7	bdl	Trimethoprim	3.70	12.9	6.56
Clindamycin	Bdl	8.40	bdl	Valsartan	2251	198	1423
DEET	113	32.9	116	Venlafaxine	54.6	109	204
Diclofenac	558	824	742	BP1	3.36	bdl	4.20
Enalapril	9.22	bdl	bdl	Caffeic acid	800	2.94	6.50
Fluconazole	6.24	28.6	44.5	Diclofenac	284	1128	782
Gabapentin	197	84.3	174	Furosemide	241	181	311
Gliclazide	3.22	25.5	34.3	Ibuprofen	35.6	18.3	47.3
Indomethacin	Bdl	24.7	26.8	Naproxen	2.52	2.25	2.32
Irbesartan	231	836	901	Triclosan	bdl	0.45	6.12
Ketoprofen	21.7	30.7	24.4				
Lamotrigine	Bdl	57.9	194				
Levofloxacin	Bdl	2.43	bdl				
Lidocaine	3.22	47.0	45.5				

Elements ($\mu\text{g L}^{-1}$)

Li	5.59	6.45	13.4	Ni	5.47	10.4	9.47
Na	528	115	133	Cu	45.5	7.91	2.41
Mg	19.2	13.0	19.5	Zn	144	49.8	22.6
Al	510	35.1	15.5	As	1.32	1.46	0.60
P	10760	6423	1581	Rb	37.9	22.1	18.77
K	52.0	28.7	30.7	Sr	297.08	186.16	226.5
Ca	115	72.0	65.5	Ag	0.72	0.07	0.06
V	1.81	0.54	1.40	Cd	0.20	bql	bql
Cr	3.92	5.87	0.63	Sn	2.63	0.47	0.39
Mn	193	31.3	67.7	Sb	0.60	bql	0.37
Fe	1008	106	331	Pb	10.3	0.22	0.28
Co	1.63	1.49	1.07	Ba	52.4	40.6	7.27

Pesticides ($\mu\text{g L}^{-1}$)

2,4-D	0.09	bql	bql	Metribuzin	bdl	bdl	0.12
Triclopyr	0.07	bdl	bdl				

PAHs (ng/L)

Naphthalene (NP)	bql	bql	80.1	Pyrene (PYR)	bql	bql	40.7
Phenanthrene (PHE)	bql	bql	69.0	Indeno(1,2,3 cd)pyrene (IND)	bql	bql	41.0
Fluoranthene (FLA)	bql	bql	52.2				

Table S2. Average, with standard deviation within parenthesis (n = 3), activity of antioxidant enzymes and malondialdehyde concentration for LPO assessment, as found in periphytic communities sampled in three sites (Up, D1 and D2) in WWTPa, WWTPb and WWTPc.

	WWTPa			WWTPb			WWTPc		
	Upa	D1a	D2a	Upb	D1b	D2b	Upc	D1c	D2c
LPO	0.06 (0.02)	0.07 (0.02)	0.06 (0.02)	0.11 (0.04)	0.13 (0.03)	0.07 (0.05)	0.17 (0.01)	0.12 (0.03)	0.10 (0.06)
CAT	325.74 (42.73)	422.87 (41.39)	601.98 (190.52)	131.38 (28.14)	247.79 (93.19)	279.88 (66.56)	188.31 (28.86)	134.97 (18.80)	391.38 (131.55)
SOD	1.00 (0.05)	0.80 (0.08)	0.76 (0.13)	0.95 (0.08)	0.96 (0.09)	0.96 (0.17)	1.02 (0.08)	1.12 (0.08)	0.96 (0.14)
GPx	0.25 (0.13)	0.50 (0.17)	0.74 (0.07)	0.14 (0.03)	0.26 (0.04)	0.30 (0.03)	0.29 (0.02)	0.14 (0.03)	0.36 (0.10)
GST	0.08 (0.05)	0.04 (0.05)	0.09 (0.08)	0.03 (0.01)	0.10 (0.04)	0.34 (0.12)	0.18 (0.12)	0.08 (0.01)	0.16 (0.11)

Section S3. Brief description of the ecological quality status determination based on diatom communities in the three sites within each WWTP studied.

Each site within each case-study WWTP was sampled for benthic biofilms by scrubbing and washing five pebble-to-cobble (5-15 cm) sized stones selected to ensure coverage of the different microhabitats and shading conditions. A sub-sample was preserved with Lugol's iodine solution and then carefully transported in an ice cooler box to the laboratory. Upon arrival, samples were stored in a refrigerator shielded from light until they were ready for further processing. Samples were cleaned using a combination of nitric acid and potassium dichromate to eliminate organic matter. Subsequently, the samples underwent thorough washing with distilled water. A small droplet of the cleaned sample was placed on a cover slip and left to air-dry at room temperature. This prepared cover slip was then affixed to a glass slide using Naphrax® to create permanent slides. Taxonomic identification of diatoms to the species level (Table S2) was carried out using these permanent slides, under a light microscope (Leica model DM6 B) with Differential Interference Contrast (DIC) imaging. In each slide at least 400 valves were counted and identified using international floras (Krammer & Lange-Bertalot, 1986, 1988, 1991b, 1991a; Lange-Bertalot et al., 2017). For the determination of the ecological status by diatom communities in compliance with the WFD, the biotic index IPS (Indice de Polluosensibilité Spécifique; Cemagref, 1982) was calculated using the OMNIDIA software (version 5.3; Lecointe et al., 1993). Finally, the Ecological Quality Ratio (EQR) was calculated taking into account the reference benchmarks for the river typology of each sampled site according to A.P.A. (2021) and the summarized results are presented in the table below.

	WWTPa			WWTPb			WWTPc		
	Up	D1	D2	Up	D1	D2	Up	D1	D2
EQR	0.99	1.02	0.93	0.86	0.78	0.86	0.15	0.12	0.35
Ecological status	High	High	Good	Good	Good	Good	Bad	Bad	Poor

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