

**Effective bioremediation of clarithromycin and diclofenac in wastewater by microbes and *Arundo donax* L.**

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**Table S1** Selection Reaction monitoring (SRM) transitions and retention times of the studied analytes.

SRM Transition <sup>a</sup>	Prec. Ion <sup>b</sup>	Prod. Ion	DP (V)	FP (V)	CE (eV)	CXP (V)	RT (min)
DCF Qual <sup>c</sup>	296	250.2	0	117	16	4.6	7.05
DCF Quant <sup>d</sup>	296	214.2	0	117	46.5	5.9	7.05
4-OH-DCF Quant <sup>d</sup>	312.1	230	3	146	44	1.8	6.32
4-OH-DCF Qual <sup>c</sup>	312.1	265.9	3	146	19	6	6.32
CLA Quant <sup>d</sup>	748.6	158.2	16	180	40	3.8	5.73
CLA Qual <sup>c</sup>	748.6	590.5	16	180	25	10.8	5.73

<sup>a</sup> Source parameters: Nebulizer gas (NEB, air), 12 (arbitrary units); curtain gas (CUR, nitrogen), 7 (arbitrary units); temperature (TEM), 400°C; ionspray voltage (IS), 5500 V; collision gas (CAD, nitrogen), 6 (arbitrary units).

<sup>b</sup> Prec. Ion: precursor Ion; Prod. ion: Product Ion; DP: Declustering Potential; FP: Focusing Potential; CE: Collision Energy; CXP: Collision cell eXit Potential; RT: Retention Time, Selected Reaction Monitoring;

<sup>c</sup> SRM transition used for qualitative confirmation of the analyte identity;

<sup>d</sup> SRM transition used for quantitation.

**Table S2** *P*-values of two-way ANOVAs evaluating the effect of PhC concentration (Dose) and incubation time ( $T_{inc}$ ) on the removal rate (%) of diclofenac (DCF) and clarithromycin (CLA) in the nutrient medium.

Microorganism/factor	<i>Streptomyces rochei</i>		<i>Phanaerochete chrysosporium</i>		<i>Trametes versicolor</i>	
	CLA	DCF	CLA	DCF	CLA	DCF
Dose <sup>a</sup>	<b>0.001<sup>c</sup></b>	<b>&lt;0.001</b>	<b>0.045</b>	<b>0.034</b>	0.181	<b>&lt;0.001</b>
$T_{inc}$ <sup>b</sup>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Dose x $T_{inc}$	0.145	<b>0.018</b>	0.324	<b>&lt;0.001</b>	<b>0.006</b>	<b>0.002</b>

<sup>a</sup> CLA: 10 and 100  $\mu\text{g L}^{-1}$ ; DCF: 1 and 10  $\text{mg L}^{-1}$ ;

<sup>b</sup> 0, 20, 48, 72, 144 h;

<sup>c</sup> In bold statistically significant values ( $P \leq 0.05$ ). At least three replicates per treatment.

**Table S3** Total plant fresh weight (FW), root FW, shoot FW, root length, leaf area, leaf and stem number and chlorophyll meter (SPAD) data of *Arundo donax* L. treated with 0 (control), 10  $\mu\text{g L}^{-1}$  and 100  $\mu\text{g L}^{-1}$  of clarithromycin (CLA) and sampled at 18 and 30 days of growth after the beginning of the experiment (T18 and T30, respectively). *P*-values of the two-way ANOVA testing the effect of CLA dose and time are also reported.

CLA dose ( $\mu\text{g L}^{-1}$ )	Time	Total FW (g)	Root FW (g)	Shoot FW (g)	Root length (cm)	Leaf area ( $\text{cm}^2$ )	Leaves (n)	Stems (n)	SPAD (unit)
0	18 d	3.01 $\pm$ 0.43 <sup>a</sup>	0.35 $\pm$ 0.11	2.66 $\pm$ 0.37	529.0 $\pm$ 50.3	122.7 $\pm$ 21.8	11.90 $\pm$ 0.81	1.25 $\pm$ 0.16	40.5 $\pm$ 0.5 a
10	18 d	4.71 $\pm$ 1.34	0.46 $\pm$ 0.11	4.25 $\pm$ 1.24	696.8 $\pm$ 85.2	150.5 $\pm$ 38.3	14.68 $\pm$ 1.02	1.50 $\pm$ 0.09	42.5 $\pm$ 0.8 ab
100	18 d	3.67 $\pm$ 0.19	0.62 $\pm$ 0.09	3.05 $\pm$ 0.13	373.3 $\pm$ 60.2	117.7 $\pm$ 6.9	12.83 $\pm$ 0.83	1.33 $\pm$ 0.14	42.6 $\pm$ 0.6 ab
0	30 d	5.89 $\pm$ 0.48	1.33 $\pm$ 0.17	4.56 $\pm$ 0.31	793.2 $\pm$ 26.1	155.5 $\pm$ 11.4	17.33 $\pm$ 1.81	1.83 $\pm$ 0.40	44.8 $\pm$ 1.1 b
10	30 d	5.78 $\pm$ 0.36	1.34 $\pm$ 0.07	4.44 $\pm$ 0.33	787.6 $\pm$ 15.2	151.7 $\pm$ 12.8	16.25 $\pm$ 2.48	1.25 $\pm$ 0.16	44.1 $\pm$ 1.2 b
100	30 d	5.59 $\pm$ 0.48	1.86 $\pm$ 0.17	3.73 $\pm$ 0.32	597.2 $\pm$ 50.0	136.5 $\pm$ 10.3	13.84 $\pm$ 0.55	1.42 $\pm$ 0.16	42.2 $\pm$ 0.4 ab
<i>P</i> -value CLA dose		0.463 <sup>b</sup>	<b>0.010</b>	0.246	<b>0.001</b>	0.497	0.341	0.663	0.557
<i>P</i> -value Time		<b>0.002</b>	<b>&lt;0.001</b>	0.064	<b>&lt;0.001</b>	0.294	<b>0.033</b>	0.426	<b>0.015</b>
<i>P</i> -value CLA dose x Time		0.399	0.327	0.329	0.300	0.732	0.264	0.164	<b>0.036</b>

<sup>a</sup> Values are means  $\pm$  SE of six replicates;

<sup>b</sup> *P*-values in bold indicate a statistical significant difference according to two-way ANOVA, testing the treatments CLA dose and time, used as fixed factors, and following the post-hoc Tukey-B test ( $P \leq 0.05$ ). For SPAD, numbers followed by different letters are significantly different, according to the significance of the interaction between CLA dose and time. Results of the significant main effects of CLA dose and time and of interaction dose x time are reported in Fig. 3. In Fig. 3, for shoot FW, the main effects of CLA dose and time are also reported.

**Table S4** Root, shoot and total clarithromycin (CLA) concentration and content of *Arundo donax* L. treated with 0 (control), 10  $\mu\text{g L}^{-1}$  and 100  $\mu\text{g L}^{-1}$  of CLA at 18 and 30 days of growth after the beginning of the experiment (T18 and T30, respectively). Concentration data are expressed for fresh dry weight. *P*-values of the two-way ANOVA testing the effect of CLA dose and time are also reported.

CLA dose ( $\mu\text{g L}^{-1}$ )	Time	Root CLA conc. ( $\mu\text{g g}^{-1}$ )	Shoot CLA conc. ( $\mu\text{g g}^{-1}$ )	Root CLA content ( $\mu\text{g plant}^{-1}$ )	Shoot CLA content ( $\mu\text{g plant}^{-1}$ )	Total CLA content ( $\mu\text{g plant}^{-1}$ )
0	18 d	0.00 $\pm$ 0.00 <sup>a</sup> a	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
10	18 d	1.16 $\pm$ 0.09 c	1.06 $\pm$ 0.05	0.53 $\pm$ 0.16 b	4.51 $\pm$ 2.18	5.04 $\pm$ 1.16
100	18 d	1.82 $\pm$ 0.08 d	1.80 $\pm$ 0.43	1.13 $\pm$ 0.21 c	5.49 $\pm$ 2.81	6.62 $\pm$ 1.66
0	30 d	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
10	30 d	0.89 $\pm$ 0.05 b	0.77 $\pm$ 0.04	1.19 $\pm$ 0.07 c	3.42 $\pm$ 1.70	4.61 $\pm$ 0.26
100	30 d	0.98 $\pm$ 0.08 bc	1.21 $\pm$ 0.10	1.82 $\pm$ 0.13 d	4.51 $\pm$ 2.22	6.34 $\pm$ 0.49
<i>P</i> -value CLA dose		<b>&lt;0.001</b> <sup>b</sup>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<i>P</i> -value Time		<b>&lt;0.001</b>	0.063	<b>&lt;0.001</b>	0.267	0.705
<i>P</i> -value CLA dose x Time		<b>&lt;0.001</b>	0.289	<b>0.016</b>	0.718	0.956

<sup>a</sup> Values are means  $\pm$  SE of six replicates;

<sup>b</sup> *P*-values in bold indicate a statistical significant difference according to two-way ANOVA testing the treatments CLA dose and time, used fixed factors and following the post-hoc Tukey-B test ( $P \leq 0.05$ ). For root CLA concentration and content, numbers followed by different letters are significantly different, according to the significance of the interaction between CLA dose and time. Results of the significant main effect CLA dose and of the interaction dose x time are reported in Fig. 4.

**Table S5** Bioaccumulation factor (BF) in roots and shoot and translocation factor (TF) of clarithromycin (CLA) of *Arundo donax* L. treated with 10  $\mu\text{g L}^{-1}$  and 100  $\mu\text{g L}^{-1}$  of CLA at 18 and 30 days of growth after the beginning of the experiment (T18 and T30, respectively). *P*-values of the two-way ANOVA testing the effect of CLA dose and time are also reported.

CLA dose ( $\mu\text{g L}^{-1}$ )	Time	Root BAF (n)	Shoot BAF (n)	TF (n)
10	18 d	574.3 $\pm$ 61.6 <sup>a</sup> b	698.1 $\pm$ 33.3 c	0.93 <sup>a</sup> $\pm$ 0.07
100	18 d	121.6 $\pm$ 40.7 a	114.8 $\pm$ 28.9 a	0.97 $\pm$ 0.20
10	30 d	841.2 $\pm$ 90.4 c	418.3 $\pm$ 36.0 b	0.88 $\pm$ 0.09
100	30 d	9.8 $\pm$ 0.8 a	67.4 $\pm$ 9.4 a	1.23 $\pm$ 0.01
<i>P</i> -value CLA dose		< <b>0.001</b> <sup>b</sup>	< <b>0.001</b>	0.110
<i>P</i> -value Time		0.209	< <b>0.001</b>	0.361
<i>P</i> -value CLA dose x Time		<b>0.007</b>	<b>0.002</b>	0.203

<sup>a</sup> Values are means  $\pm$  SE of six replicates;

<sup>b</sup> *P*-values in bold indicate a statistical significant difference according to two-way ANOVA testing the treatments CLA dose and time, used fixed factors and following the post-hoc Tukey-B test ( $P \leq 0.05$ ). For root and shoot BAF, numbers followed by different letters are significantly different, according to the significance of the interaction between CLA dose and time. Results of the significant interaction CLA dose x time are reported in Fig. 5.

**Table S6** Total plant fresh weight (FW), root FW, shoot FW, root length, leaf area, leaf and stem number, chlorophyll meter (SPAD) data of *Arundo donax* L. treated with 0 (control), 1 and 10  $\mu\text{g L}^{-1}$  of diclofenac (DCF) at 18 days of growth after the beginning of the experiment. *P*-values of the the one-way ANOVA testing the effect of DCF dose are also reported.

DCF dose ( $\text{mg L}^{-1}$ )	Total FW (g)	Root FW (g)	Shoot FW (g)	Root length (cm)	Leaf area ( $\text{cm}^2$ )	Leaves (n)	Stems (n)	SPAD (unit)
0	3.96 <sup>a</sup> ± 0.76 <sup>a</sup> b	0.28 ± 0.05	3.68 ± 0.71 b	360.8 ± 41.6 b	129.6 ± 24.4 b	13.0 ± 1.6	2.5 ± 0.4	28.3 ± 0.9
1	4.02 ± 0.63 b	0.46 ± 0.07	3.56 ± 0.57 b	334.9 ± 32.2 b	136.9 ± 15.6 b	12.3 ± 0.6	2.0 ± 0.1	26.8 ± 0.8
10	1.62 ± 0.26 a	0.29 ± 0.04	1.36 ± 0.23 a	120.6 ± 22.4 a	58.0 ± 7.4 a	10.4 ± 1.1	2.4 ± 0.3	26.8 ± 1.3
<i>P</i> -value <sup>b</sup>	<b>0.017</b> <sup>b</sup>	0.054	<b>0.013</b>	<b>&lt; 0.001</b>	<b>0.010</b>	0.310	0.503	0.488

<sup>a</sup> Values are means ± SE of four replicates;

<sup>b</sup> *P*-values in bold indicate a statistical significant difference according to one-way ANOVA, testing the DCF dose as fixed factor, and following the post-hoc Tukey-B test ( $P \leq 0.05$ ). For total, shoot and root FW and leaf area, numbers followed by different letters are significantly different. Results of the significant effect of DCF dose are reported in Fig. 6.

**Table S7** Roots, shoots, and total diclofenac (DCF) and DCF metabolite (MET: 4'-hydroxy DCF, 4'-OH DCF) concentrations and content of *Arundo donax* L. treated with 0 (control), 1 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup> of DCF at 18 days of growth after the beginning of the experiment. Concentration data are expressed for fresh dry weight. *P*-values of the one-way ANOVA testing the effect of DCF dose are also reported.

DCF concentration (mg L <sup>-1</sup> )	Root conc. (µg g <sup>-1</sup> )	Shoot conc. (µg g <sup>-1</sup> )	Root content (µg plant <sup>-1</sup> )	Shoot content (µg plant <sup>-1</sup> )	Total content (µg plant <sup>-1</sup> )
DCF					
0 mg L <sup>-1</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
1 mg L <sup>-1</sup>	5.98 ± 1.09 a	0.31 ± 0.08 a	2.75 ± 0.20 a	1.10 ± 0.06 a	3.85 ± 0.23 a
10 mg L <sup>-1</sup>	59.73 ± 5.47 b	3.64 ± 0.47 b	17.32 ± 3.82 b	4.95 ± 1.36 b	22.07 ± 5.13 b
<i>P</i> -value <sup>b</sup>	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>	<b>0.001</b>	< <b>0.001</b>
MET					
0 mg L <sup>-1</sup>	0.00 ± 0.00 a	0.00 ± 0.00	0.00 ± 0.00 a	0.00 ± 0.00	0.00 ± 0.00 a
1 mg L <sup>-1</sup>	50.44 ± 6.46 b	0.00 ± 0.00	23.20 ± 4.96 b	0.00 ± 0.00	23.20 ± 4.96 b
10 mg L <sup>-1</sup>	47.27 ± 10.03 b	0.03 ± 0.01	13.71 ± 5.03 b	0.04 ± 0.01	13.75 ± 5.01 b
<i>P</i> -value	< <b>0.001</b>	0.391	<b>0.003</b>	0.391	<b>0.003</b>

<sup>a</sup> Values are means ± SE of four replicates;

<sup>b</sup> *P*-values in bold indicate a statistical significant difference according to one-way ANOVA, testing the DCF dose as fixed factor, and following the post-hoc Tukey-B test (*P* ≤ 0.05). For all parameters with the exception of MET shoot conc. and content, numbers followed by different letter are significantly different. Results of the significant effect of DCF dose are reported in Fig. 7.



**Table S8** Bioaccumulation factor (BF) in roots and shoot and translocation factor (TF) of diclofenac (DCF) of *Arundo donax* L. treated with 1 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup> of DCF at 18 days of growth after the beginning of the experiment. *P*-values of the one-way ANOVA testing the effect of DCF dose are also reported.

DCF concentration (mg L <sup>-1</sup> )	Root BAF (n)	Shoot BAF (n)	TF (n)
1	65.33 ± 11.41 <sup>a</sup>	2.94 ± 0.79	0.05 <sup>a</sup> ± 0.00
10	53.32 ± 9.80	3.17 ± 0.70	0.06 ± 0.00
<i>P</i> -value	0.443 <sup>b</sup>	0.838	0.069

<sup>a</sup> Values are means ± SE of four replicates;

<sup>b</sup> *P*>0.05 according to one-way ANOVA and the post-hoc Tuckey-B test.