

Treatment of micropollutants in municipal wastewater: Ozone or powdered activated carbon?

Supplementary data

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Materials and methods – complementary information

Analyses of micropollutants – Synthesis of the analytical method

Upon arrival in the laboratory, samples were immediately acidified to pH 2.5 with 5 N HCl and filtered at 0.7 μm through glass fibre filters (type GF/F, Whatman). Analysis of 58 hydrophilic micropollutants (36 pharmaceuticals, 13 biocides and pesticides, 2 corrosion inhibitors and 7 endocrine compounds, Table S1), were conducted on the filtrate as described by Morasch et al. (2010). The target compounds were extracted less than 1 h after acidification by an automated solid phase extraction (SPE) system (GX-274 ASPEC, Gilson, USA) on hand-assembled two-layered cartridges (Oasis HLB and mixture of Strata X-CW, Strata X-AW and Isolute ENV+ phases). The eluent was then analysed by ultra-performance liquid chromatography (UPLC) (Acquity UPLC system, with HSS T3 or BEH C18 column depending of the compounds, from Waters, USA) coupled to a tandem quadrupole mass spectrometer (MS/MS) (Acquity TQ Detector, Waters). To account for losses during SPE and the matrix effect, samples were spiked with deuterated surrogates, as described by Morasch et al. (2010). UPLC-MS/MS conditions, extraction efficiency of the associated deuterated standards and repeatability of the method are detailed by Morasch et al. (2010).

Yeast Estrogen Screen (YES) – Synthesis of the method

The yeast estrogen screen with the recombinant yeast *Saccharomyces cerevisiae* was performed according to Routledge and Sumpter (1996) in 96-well microtitre plates using yeast cells provided by J. Sumpter (Brunel University, Uxbridge, UK). In brief, yeast cells were cultured in minimal medium on an orbital shaker at 30°C for 24 h before the onset of the test. At the beginning of the test, 1:2 dilution series of the reference substance, the enriched wastewater samples and the solvent control were pipetted onto the plates. The solvent was evaporated completely on a sterile bench. In the meantime the cell density of the yeast cells was determined, and an assay medium prepared (seeded with 4×10^7 yeast cells). Subsequently, the yeast-cell suspension was pipetted on the test plate (200 μl /well). The plate was incubated at 30°C. After 72 h, cell density ($\text{OD}_{620 \text{ nm}}$) and colour change ($\text{OD}_{540 \text{ nm}}$) were measured using a plate reader (Synergy 4, Biotek, Winooski, USA).

Combined Algae Assay– Synthesis of the method

The combined algae assay on the green algae *Pseudokirchneriella subcapitata* was conducted as described by Escher et al. (2008). The herbicide diuron served as the reference substance and ethanol as the solvent control (50 μl /well, 8 wells/plate). After a complete ablation of the solvent, the samples were re-suspended in 100- μl algae medium. Finally, 100 μl of algae suspension with an OD_{685} of 0.1 were added to each well. Photosynthesis inhibition by means of effective quantum yield was measured after 2 and 24 h using a Maxi-Imaging PAM (pulse amplitude modulation, IPAM) device (Walz, Effeltrich, Germany) as described by Schreiber et al. (2007). Algae growth was measured by means of absorbance at 685 nm in a microtitre plate photometer (Synergy 4, Biotek, Winooski, USA) at the test start and end as well as on two occasions in between. The toxicity of the wastewater samples was expressed as diuron-equivalent concentrations (DEQs) for the endpoint “inhibition of Photosystem II” and toxic equivalent concentrations (TEQs, virtual baseline toxicant) for growth inhibition (Escher et al., 2008).

Fish early life stage test with rainbow trout – Synthesis of the method

This test was performed according to OECD guideline 210 (OECD, 1992b). Details of the methodology are described by Stalter et al. (2010). In brief, freshly fertilized eggs (< 1 h) of rainbow trout (*Oncorhynchus mykiss*) were exposed to the test waters in 8-l stainless steel vessels in a flow-through system. Reconstituted water (OECD guideline 203, OECD, 1992a) served as the control medium. At the start of the test, 70 eggs/replicate were randomly distributed to the test vessels and gradually reduced to 40 eggs the next day. The fish embryos were exposed at $10 \pm 2^\circ\text{C}$ and in darkness. Flow of test media into each test vessel was adjusted to 11 ml min^{-1} , corresponding to two test vessel volume exchanges per day. For the post hatch period the temperature was raised to $12 \pm 2^\circ\text{C}$ and a 12/12 h photoperiod was set. Flow-through rates in the test vessels were adjusted weekly depending on the fish developmental stage to reach 44 ml min^{-1} seven days before the test end, achieving a eight-fold medium exchange in the test vessels per day (OECD, 1992b). From the beginning of swim-up onwards, the fish were fed four times per day (trout starter, 4% body weight per day). In total four control and three replicate treatments for all wastewaters were assessed. During the test period several endpoints were determined daily, namely: hatching, mortality, swim up, malformations and abnormal behaviour. After the end of the test fish were humanely killed with an overdose of MS222 (tricaine methanesulfonate, Sigma–Aldrich, St. Louis, USA). Afterwards individual fish were blotted dry and fresh weight and length were measured. The plasma vitellogenin concentration was determined in whole body homogenates of 20 fish per control and wastewater as described by Holbech et al. (2006) using a vitellogenin ELISA test kit for rainbow trout (Biosense, Bergen, Norway) in a 1:20 dilution.

Table S1. Physico-chemical properties of the 58 micropollutants routinely analysed.

Compound	CAS-No	M[g/mol] ^a	Log K _{ow} ^a	pK _a ^a	Charge at pH 7 ^b	Log D _{ow} (pH 7) ^c	Type ^d
Pharmaceuticals							
Acipimox	[51037-30-0]	154.1	-0.52	3.3	-1	-2.1	A
Atenolol	[29122-68-7]	266.3	0.16	9.6	1	-1.3	B
Azithromycin	[83905-01-5]	749	4.02	8.7; 9.5	2	2.8	B
Bezafibrate	[41859-67-0]	361.8	4.25	3.7; 13.6	-1	2.7	A
Carbamazepine	[298-46-4]	236.3	2.45	13.9	0	2.5	N
Ciprofloxacin	[85721-33-1]	331.4	0.28	6.1; 8.8	1; Z; 0; -1	0.3	Z
Clarithromycin	[81103-11-9]	748	3.16	9.0	1	1.8	B
Clindamycin	[18323-44-9]	425	2.16	7.5	1; 0	1.4	B
Clofibric acid	[882-09-7]	214.7	2.57	3.5	-1	1.0	A
Diatrizoic acid	[117-96-4]	613.9	1.37	1.2; 7.9; 11.7	-1	-0.4	A
Diclofenac	[15307-86-5]	296.2	4.51	4.1	-1	3.0	A
Fenofibrate	[49562-28-9]	360.8	5.19	NA	0	5.2	N
Gabapentin	[60142-96-3]	171.2	-1.1	3.7; 10.0	Z	-1.1	Z
Gemfibrozil	[25812-30-0]	250.3	4.77	4.7	-1	3.4	A
Ibuprofen	[15687-27-1]	206.3	3.97	4.9	-1	2.6	A
Iohexol	[66108-95-0]	821.1	-3.05	NA	0	-3.1	N
Iomeprol	[78649-41-9]	777.1	-2.79	11.7; 12.6; 13.6	0	-2.8	N
Iopamidol	[60166-93-0]	777.1	-2.42	11.1; 12.9	0	-2.4	N
Iopromide	[73334-07-3]	791.1	-2.05	11.4	0	-2.1	N
Iothalamic acid	[2276-90-6]	613.9	0.5	2.1; 11.2; 12.6	-1	-1.2	A
Ketoprofen	[22071-15-4]	254.3	3.12	4.5	-1	1.7	A
Mefenamic acid	[61-68-7]	241.3	5.12	4.2	-1	3.7	A
Metoprolol	[37350-58-6]	267.4	1.88	9.7	1	0.4	B
Metronidazole	[443-48-1]	171.2	-0.02	2.5	0	0.0	N
Nadolol	[42200-33-9]	309.4	0.81	9.7	1	-0.6	B
Naproxen	[22204-53-1]	230.3	3.18	4.2	-1	1.7	A
Norfloxacin	[70458-96-7]	319.3	-1.03	6.4; 8.7	Z; 0; -1	-1.0	Z
Ofloxacin	[82419-36-1]	361.4	-0.39	5.7; 7.1	Z; 0; -1	-0.4	Z
Paracetamol	[103-90-2]	151.2	0.46	9.4	0	0.5	N
Pravastatin	[81093-37-0]	424.5	3.1	4.5	-1	1.7	A
Primidone	[125-33-7]	218.3	0.91	NA	0	0.9	N
Propranolol	[525-66-6]	259.3	3.48	9.4	1	2.1	B
Simvastatin	[79902-63-9]	418.6	4.68	13.5	0	4.7	N
Sotalol	[3930-20-9]	272.4	0.24	8.2; 9.1	1	-0.9	B
Sulfadimethoxine	[122-11-2]	310.3	1.63	2.0; 6.7	-1	1.0	A
Sulfamethoxazole	[723-46-6]	253.3	0.89	1.8; 5.8	-1	-0.2	A
Trimethoprim	[738-70-5]	290.3	0.91	1.3; 7.2	1; 0	0.4	B
Endocrine disrupting compounds							
17 α -Ethinylestradiol	[57-63-6]	296.4	3.67	10.4	0	3.7	N
Bisphenol A	[80-05-7]	228.3	3.32	10.1	0	3.3	N
Estriol	[50-27-1]	288.4	2.45	10.4	0	2.5	N
Estrone	[53-16-7]	270.4	3.13	10.3	0	3.1	N
Nonylphenol	[84852-15-3]	220.4	5.92	11.1	0	5.9	N
β -Estradiol	[50-28-2]	272.4	4.01	10.5	0	4.0	N
Pesticides and other common chemicals							
Atrazine	[1912-24-9]	215.7	2.61	1.7	0	2.6	N
Benzotriazole	[95-14-7]	119.1	1.44	8.4	0	1.4	N
Carbendazim	[10605-21-7]	191.2	1.52	4.2	0	1.5	N
Chloridazon	[1698-60-8]	221.6	1.14	3.4	0	1.1	N
Diazinon	[333-41-5]	304.4	3.81	2.4	0	3.8	N
Diuron	[330-54-1]	233.1	2.68	13.6	0	2.7	N
IPBC	[55406-53-6]	281.1	2.54	NA	0	2.5	N
Irgarol	[28159-98-0]	253.4	4.07	NA	0	4.1	N
Isoproturon	[34123-59-6]	206.3	2.87	NA	0	2.9	N
Mecoprop	[93-65-2]	214.7	3.13	3.1	-1	1.5	A
Methylbenzotriazole	[29385-43-1]	133.2	1.71	8.8	0	1.7	N
Propiconazole	[60207-90-1]	342.2	3.72	1.1	0	3.7	N
Tebufenozide	[112410-23-8]	352.5	4.25	NA	0	4.3	N
Terbutryn	[886-50-0]	241.4	3.74	4.3	0	3.7	N
Triclosan	[3380-34-5]	289.5	4.76	7.8	0; -1	4.8	N

^a Source: Morasch et al. (2010), completed with Escher et al. (2011) and Reungoat et al. (2012). ^b Source:

www.chemicalize.org (last accessed 25.10.2012) ^c $\log D_{ow} = \log K_{ow} - \log(1+10^{(pH-pK_a)})$ for acids and $\log D_{ow} = \log K_{ow} -$

$\log(1+10^{(pK_a-pH)})$ for bases (Schwarzenbach et al. 2003). ^d A: acidic, B: basic, N: neutral, Z: zwitterion

Table S2. Sample preparation for estrogens analyses and enrichment for the bioassays (YES, algae assay).

	Solid phase extraction for estrogens	Solid phase extraction for bioassays
General Information		
Sample type	Water samples	
Sample volumes	250 ml wastewater influent 500 ml wastewater effluent	200 ml wastewater influent 500 ml wastewater effluent
Blank	500 ml ultrapure water	
Sample preparation		
Filtration	Yes, with glass fibre filter type APFD 09050 (1 µm) (Millipore)	
Acidification	Yes, with HCl to pH 3	
Addition of isotope-labelled internal mixed standard solution (IS)	30 ng EE2-D4, E2-13C2, E1-D4, BPA-D16 and NP-13C6 to each sample	No
Sample enrichment	Solid phase extraction (SPE)	
SPE cartridges	LiChrolut EN RP-18 (bottom: 100 mg LiChrolut EN, top: 200 mg LiChrolut RP 18)	
Conditioning	6 ml Hexane 2 ml Acetone 6 ml Methanol 10 ml Water (pH 3.0)	2 ml Hexane 2 ml Acetone 6 ml Methanol 6 ml Water (pH 3.0)
Washing	8 ml Methanol/Water (70:30, v/v) 6 ml Acetonitrile/Water (30:70, v/v)	No, only filling of the cartridge with water (pH 3.0)
Elution	4 ml Acetone	4 ml Acetone 1 ml Methanol
Evaporation	With N ₂ to ca. 100 µl	With N ₂ to ca. 500 µl, then completing to 1000 µl with ethanol
Enrichment factor	1250 × wastewater influent 2500 × wastewater effluent	200 × wastewater influent 500 × wastewater effluent
Purification and storage of sample extract		
Sorbent	Mini silica gel columns (1.00 ± 0.01 g)	No
Application of sample	100 µl sample + 2 × 0.2 ml Hexane/Acetone (60:40, v/v)	
Elution	7.1 ml Hexane/Acetone (60:40, v/v)	
Evaporation	To dryness, fill-up with 200 µl Ethanol	
Storage	In the dark, at -20°C	

Table S3. Specification for LC-MS/MS analytics of estrogenic active substances.

LC-MS/MS analysis	
LC-MS/MS instrument	API 4000 LC-MS/MS (Applied Biosystems, Warrington, UK)
HPLC separation	Gradient elution Eluent A = water/acetonitrile (90:10, v/v) Eluent B = acetonitrile/water (90:10, v/v)
HPLC column	MS C18 HPLC column (2.1 mm x 100 mm, particle size 3.5 μ m)
Ionisation	Negative electrospray ionisation (ESI)
Calibration	0 - 200 ng/ml E1, E2 and EE2 mixed standards 0 - 2500 ng/ml NP+BPA standards
Replicates	2
Limit of quantification	E1 0.6 ng/l; E2 1.1 ng/l; EE2 3.0 ng/l; BPA 4.9 ng/l; NP 22.9 ng/l

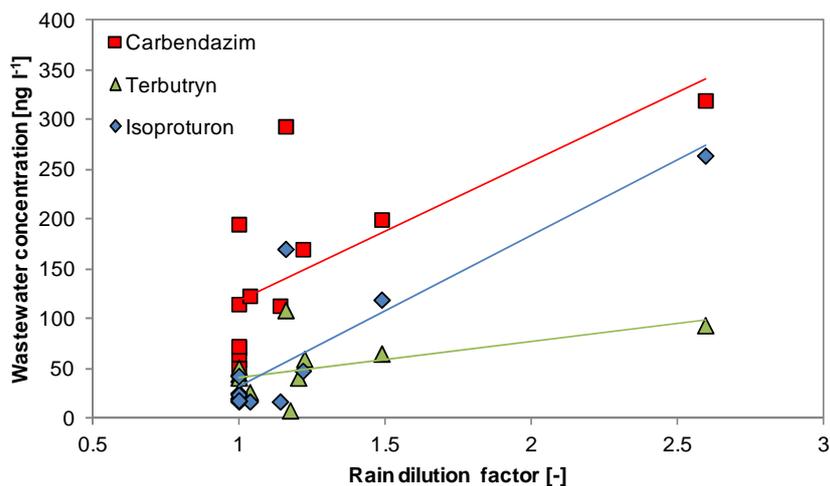


Figure S1. Concentration of selected pesticides in raw wastewater as a function of wastewater dilution by runoff water. Correlations with the dilution factor (wet weather flow/dry weather flow): Isoproturon ($r = 0.875$, $p < 0.001$), carbendazim ($r = 0.712$, $p < 0.01$), terbutryn ($r = 0.612$, $p < 0.05$).

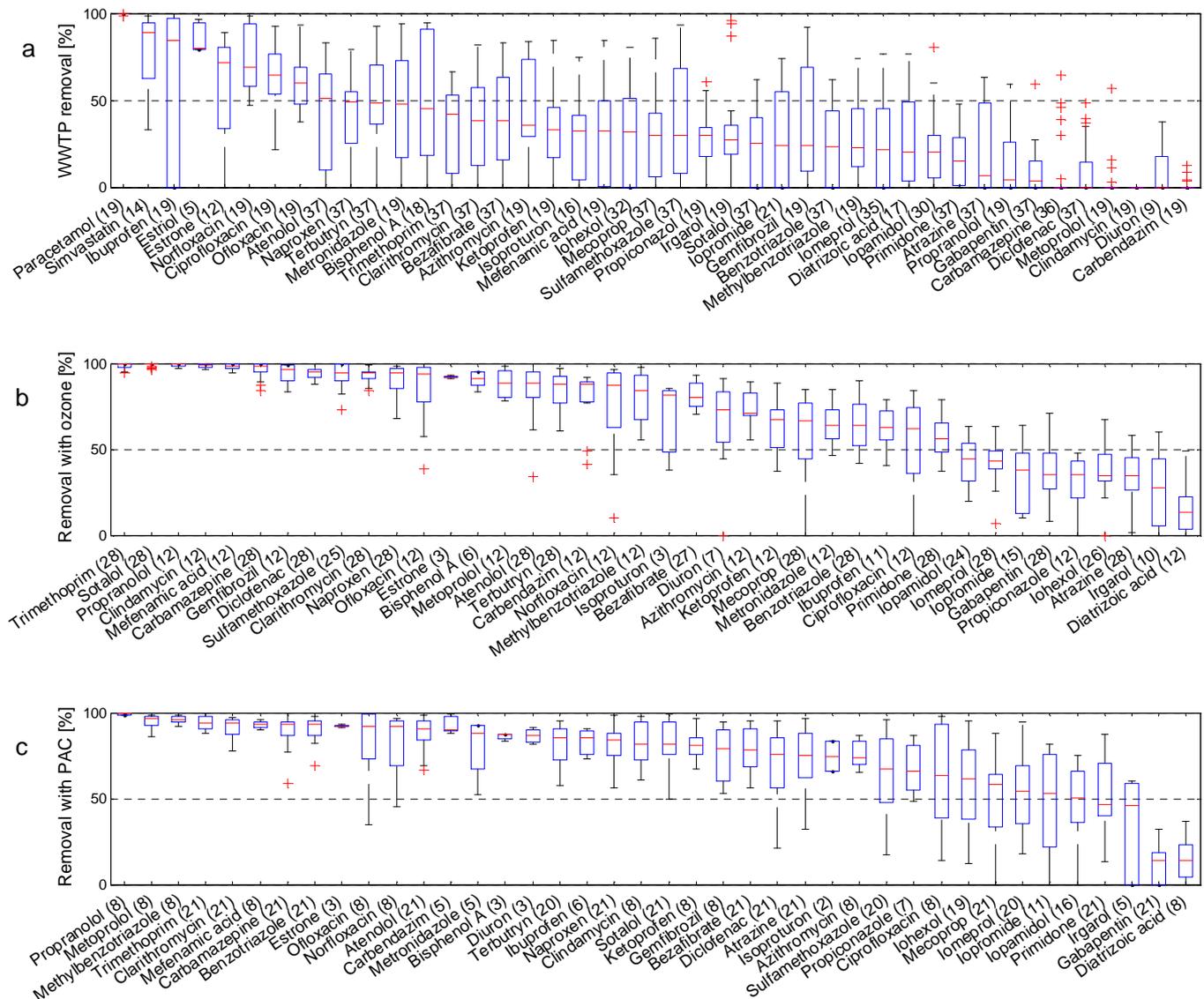
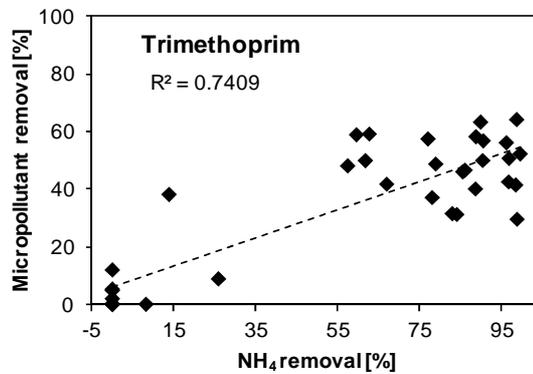
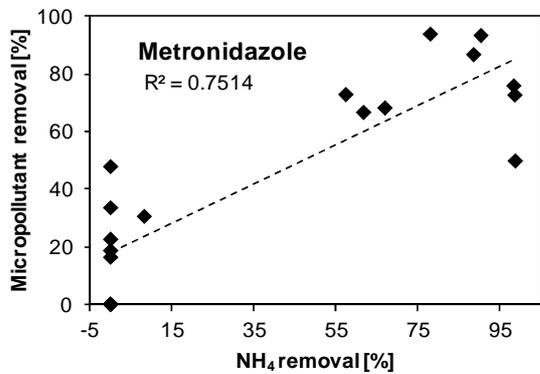
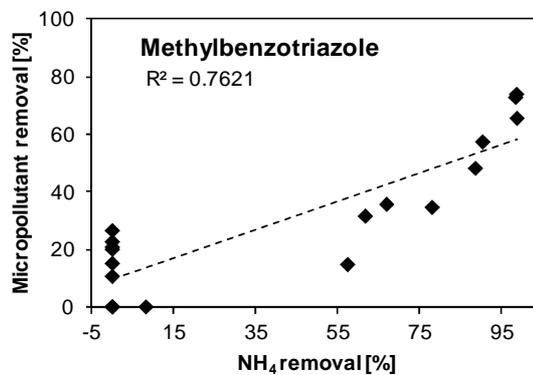
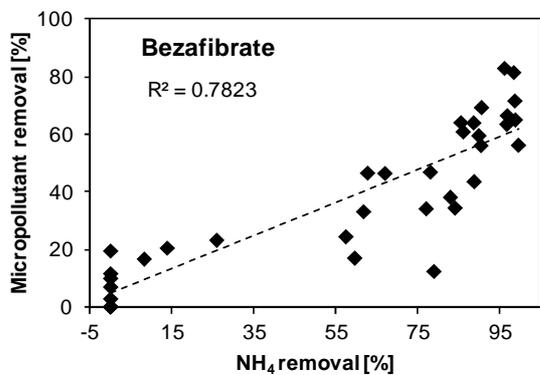
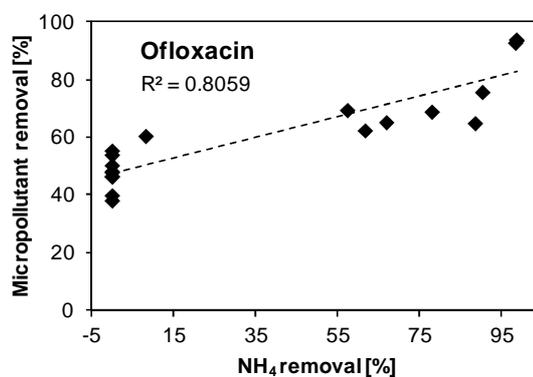
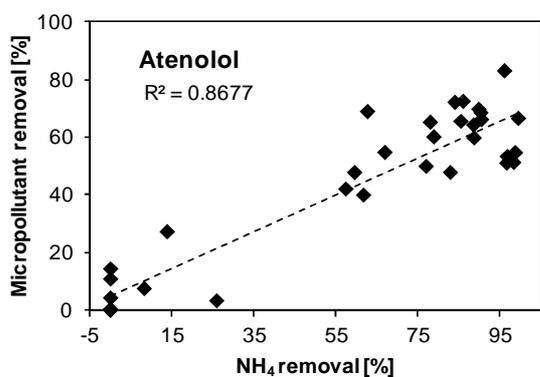
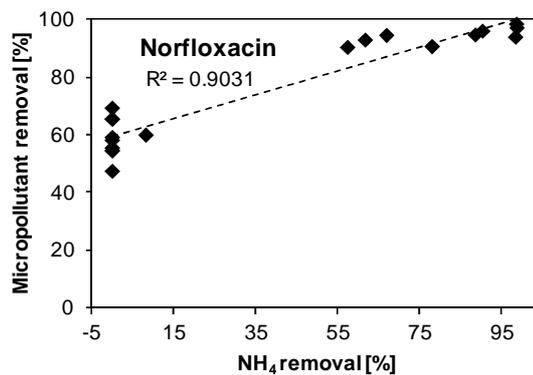
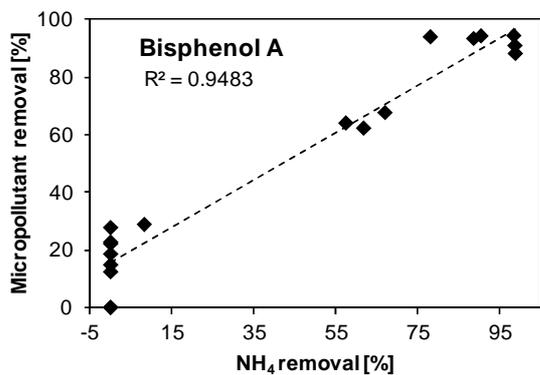
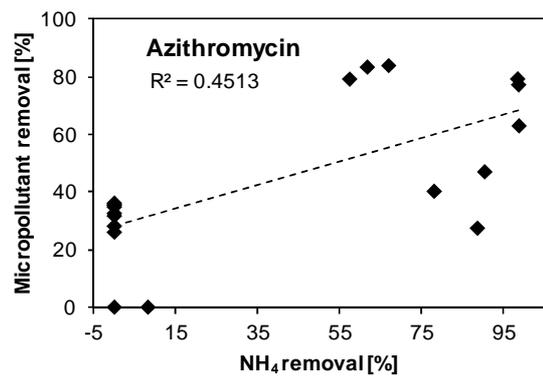
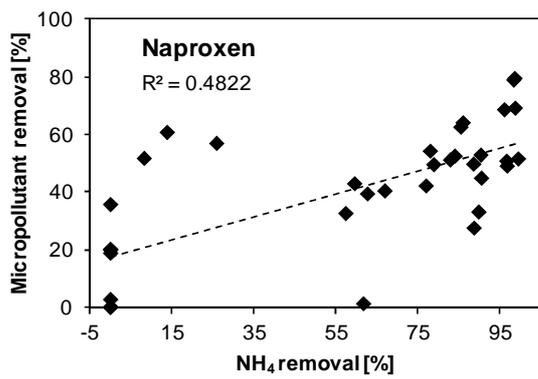
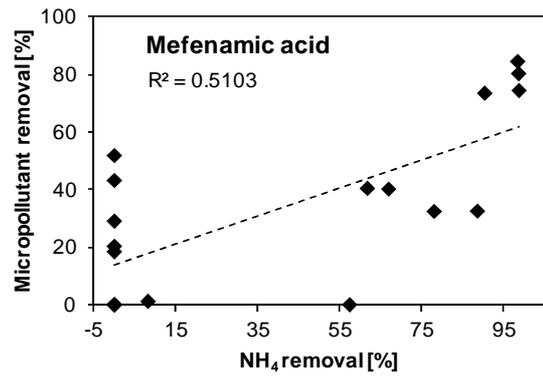
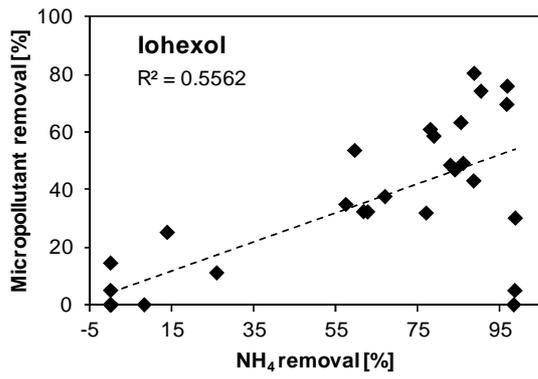
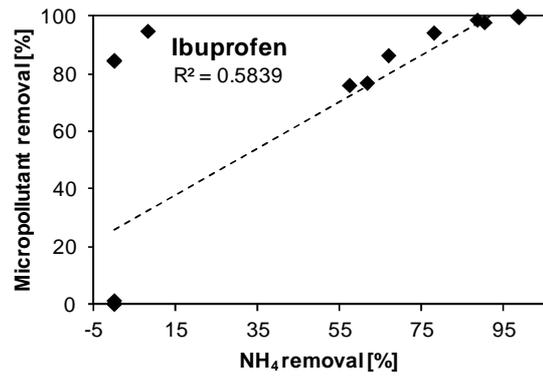
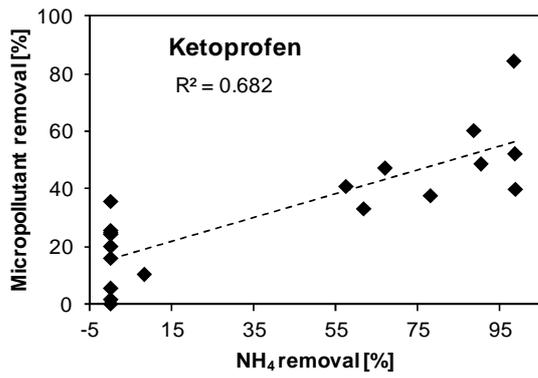
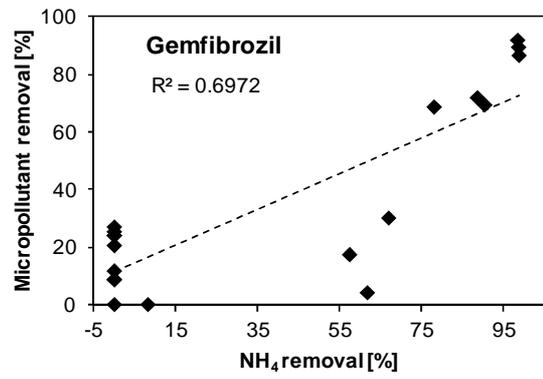
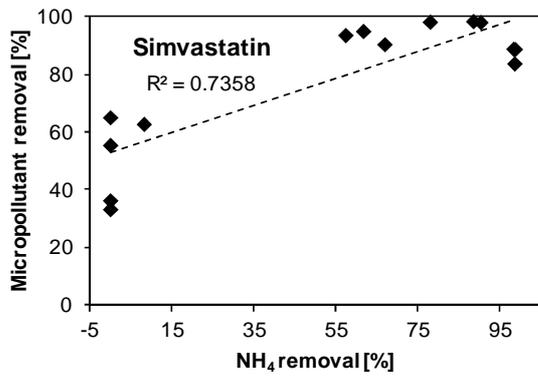


Figure S2. Removal efficiency of 40 to 43 micropollutants during **(a)** the conventional biological wastewater treatment with either activated sludge without nitrification or moving bed bioreactor with partial to complete nitrification (average removal of 35%), **(b)** the ozonation (ozone dose between 2.3 to 9.1 mg O₃ l⁻¹, median 5.9 mg O₃ l⁻¹ or 0.83 g O₃ g⁻¹ DOC, average removal of 71%) and **(c)** the PAC-UF treatment (PAC dose between 10 to 20 mg PAC l⁻¹, median 12 mg l⁻¹, average removal of 73%). Results of (n) analyses (24 h to 72 h composite samples) conducted between June 2009 and October 2010. Representation of the median removal, the quartiles 25-75 %, the minimum and maximum values and the outliers.

Effect of the nitrification level on the removal of 18 micropollutants





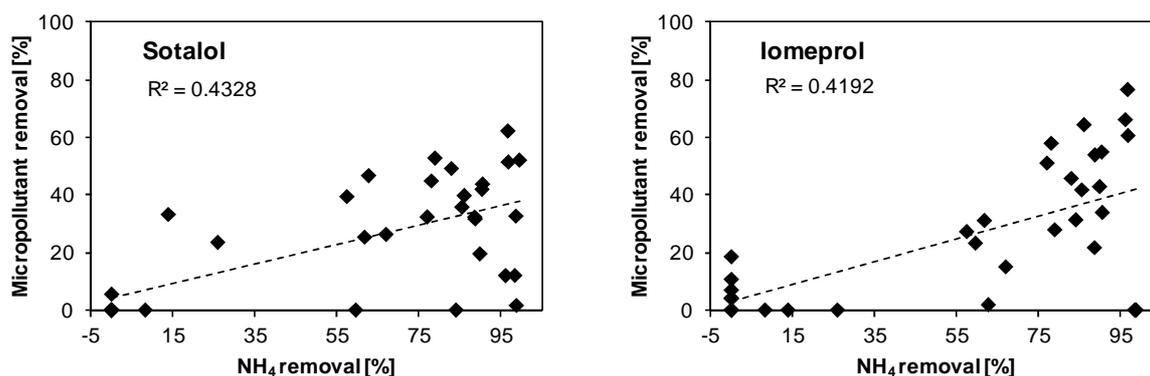


Figure S3. Removal of 18 micropollutants in the biological treatment as a function of the level of nitrification (ammonium removal). Results of 19 to 36 campaigns on 24 to 72-h composite samples at the entrance of the WWTP and at the outlet of the biological treatment. Diverse levels of nitrification were obtained by varying the hydraulic residence time or the aeration either in an activated sludge tank with a sludge age of 2 d (0 to 26% of nitrification, 9 to 21 mg N-NH₄ l⁻¹ in the effluent) or in a moving bed bioreactor (57 to 99% of nitrification, 0.1 to 10 mg N-NH₄ l⁻¹ in the effluent). Of the 42 compounds regularly detected, 24 had a significant ($p < 0.05$) positive correlation of their removal with the level of nitrification, among which 11 had a strong correlation ($r > 0.8$) and seven a medium correlation ($0.6 < r < 0.8$) (Table S4). Compounds with $r > 0.6$ are presented here. There were 18 compounds that were not significantly influenced by the nitrifying efficiency of the biological treatment, including the very common pollutants carbamazepine, diclofenac, gabapentin, sulfamethoxazole, benzotriazole and mecoprop.

Table S4. Correlation coefficients between the removal of 42 micropollutants and the level of nitrification (% of ammonium removal) in the biological treatment. Pearson correlation on 19 to 36 analyses. Correlations were considered significant for p values < 0.05 .

Substance	Correlation	Substance	Correlation
Bisphenol A	0.97 ***	Irgarol	0.48 *
Norfloxacin	0.95 ***	Clarithromycin	0.43 **
Atenolol	0.93 ***	Terbutryn	0.36 *
Ofloxacin	0.90 ***	Paracetamol	0.29 ns
Bezafibrate	0.88 ***	Isoproturon	0.27 ns
Methylbenzotriazole	0.87 ***	Benzotriazole	0.26 ns
Metronidazole	0.87 ***	Carbendazim	0.24 ns
Trimethoprim	0.86 ***	Estrone	0.20 ns
Simvastatin	0.86 ***	Propiconazol	0.20 ns
Gemfibrozil	0.83 ***	Mecoprop	0.19 ns
Ketoprofen	0.83 ***	Iopamidol	0.16 ns
Ibuprofen	0.76 ***	Diclofenac	0.14 ns
Iohexol	0.75 ***	Carbamazepine	0.12 ns
Mefenamic acid	0.71 ***	Ciprofloxacin	0.12 ns
Naproxen	0.69 ***	Gabapentin	0.05 ns
Azithromycin	0.67 **	Clindamycin	0.00 ns
Sotalol	0.66 ***	Sulfamethoxazole	-0.08 ns
Iomeprol	0.65 ***	Diatrizoic + iothalamic acid	-0.13 ns
Propranolol	0.57 *	Metoprolol	-0.22 ns
Primidone	0.53 ***	Atrazine	-0.41 *
Iopromide	0.50 *	Diuron	-0.42 ns

ns: no significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

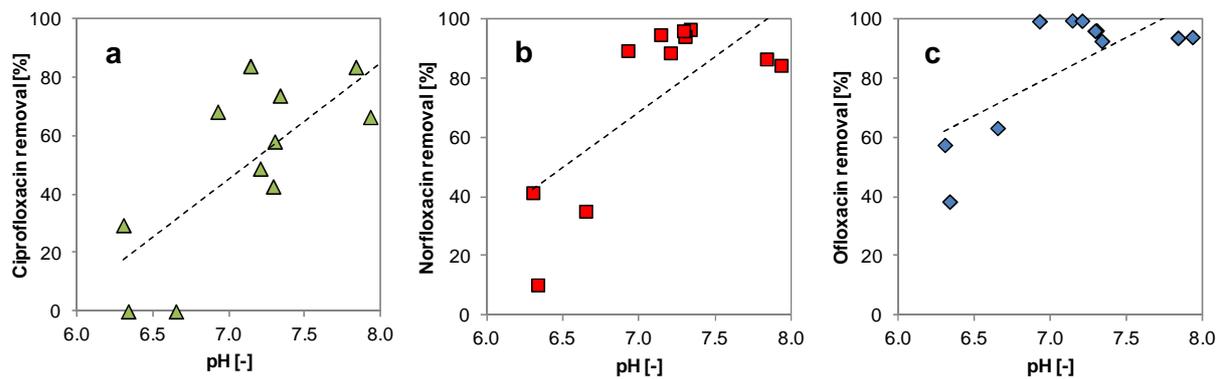


Figure S4. Removal of fluoroquinolone antibiotics by ozonation (in the pilot plant) as a function of the feed water pH. (a) Ciprofloxacin. (b) Norfloxacin. (c) Ofloxacin. Ozone doses varied between 3 and 7 mg O₃ l⁻¹ to maintain the same residual dissolved ozone concentration in the third chamber of the reactor. No clear link between the ozone dose and the removal of these three compounds was evident, suggesting that the pH was the most influential factor. Correlations of the removal rate with the pH: Ciprofloxacin ($r = 0.76$, $p = 0.004$), norfloxacin ($r = 0.73$, $p = 0.007$), ofloxacin ($r = 0.74$, $p = 0.006$).

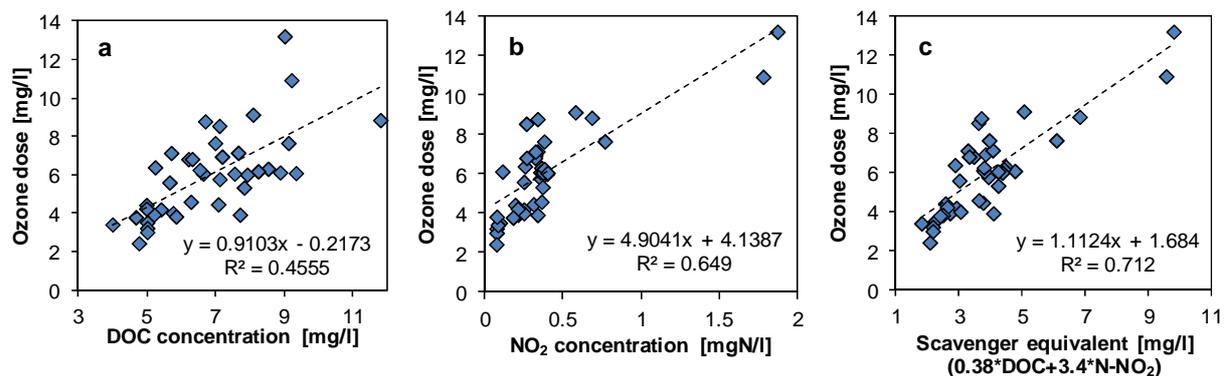
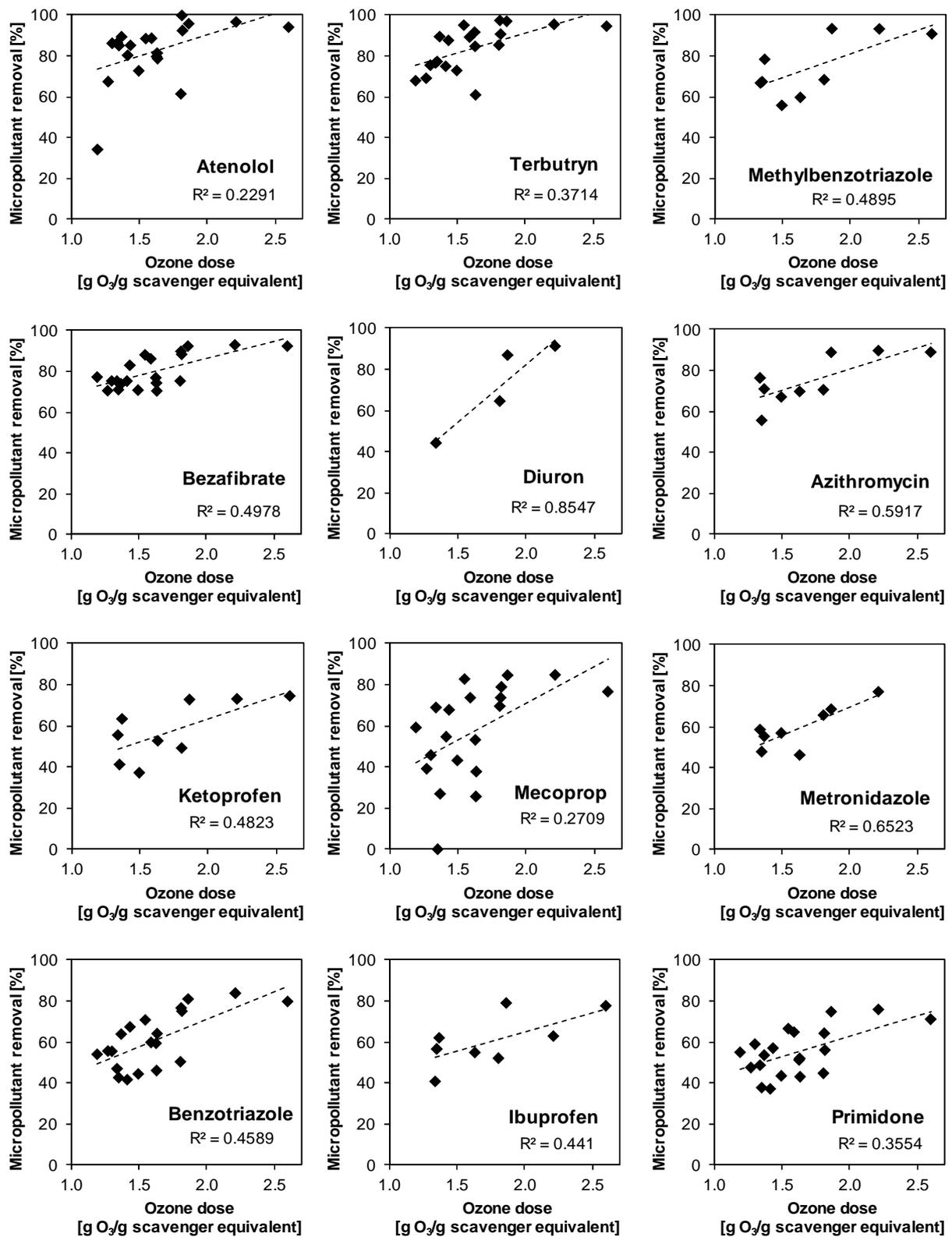


Figure S5. Influence of the daily average ozone dosage in the reactor as a function of daily average concentrations of (a) dissolved organic carbon (DOC), (b) nitrite, and (c) scavenger equivalent, calculated by the optimal (maximizing R^2) weighted sum of DOC and NO₂ concentrations (in mg l⁻¹): $0.38 \text{ DOC} + 3.4 \text{ N-NO}_2$. The ozone dose was regulated to maintain the same residual dissolved ozone concentration ($\sim 0.1 \text{ mg l}^{-1}$) in the third chamber of the reactor and thus varied depending of the oxidative demand of the water, mainly due to DOC and nitrite concentration.

Influence of the ozone dose on the removal of 15 micropollutants by ozonation

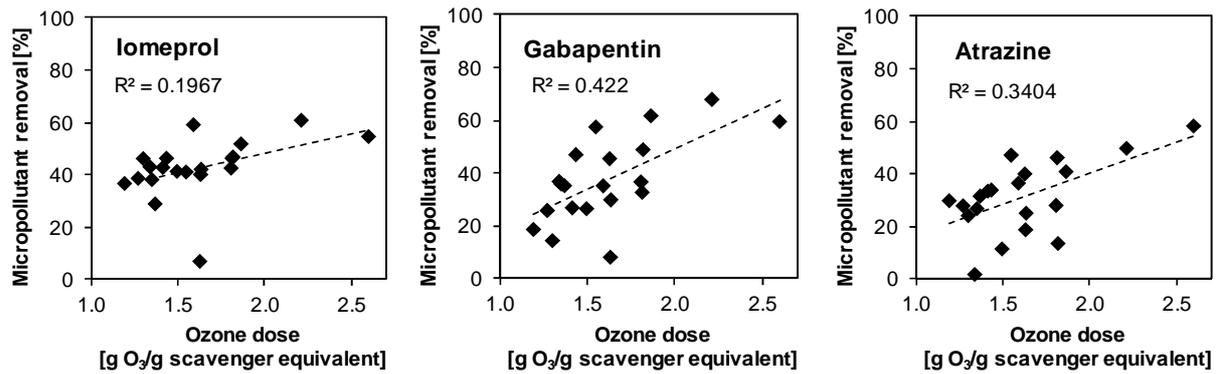


Figure S6. Influence of the daily average ozone dose on the removal of 15 micropollutants by ozonation. Results of 20 campaigns conducted on the effluent of a moving bed bioreactor with partial nitrification. The ozone dose is normalized by the scavenger equivalent concentration, calculated by the weighted sum of DOC and NO_2 concentrations (in mg l^{-1}): $0.38 \text{ DOC} + 3.4 \text{ N-NO}_2$.

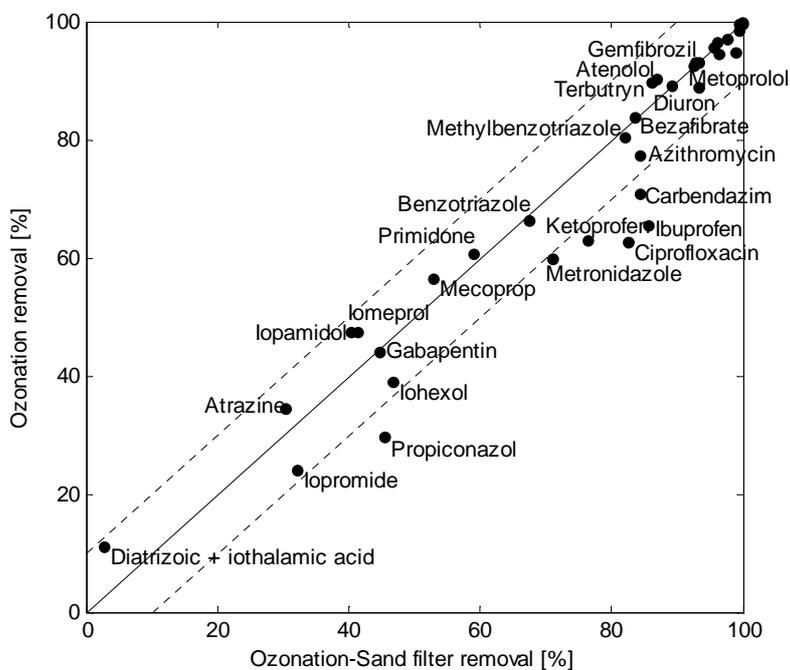


Figure S7. Comparison of the removal of 36 micropollutants with ozone alone or with ozone followed by a sand filter (SF). Black line: similar removal by ozone alone or by ozone + SF. Dashed line: 10% difference between the removal by ozone alone or by ozone + SF. Average of 8 sampling campaigns (24 to 72-h composite samples). Average removal of the 36 compounds was 73.2% for ozone and 75.8% for ozone + SF.

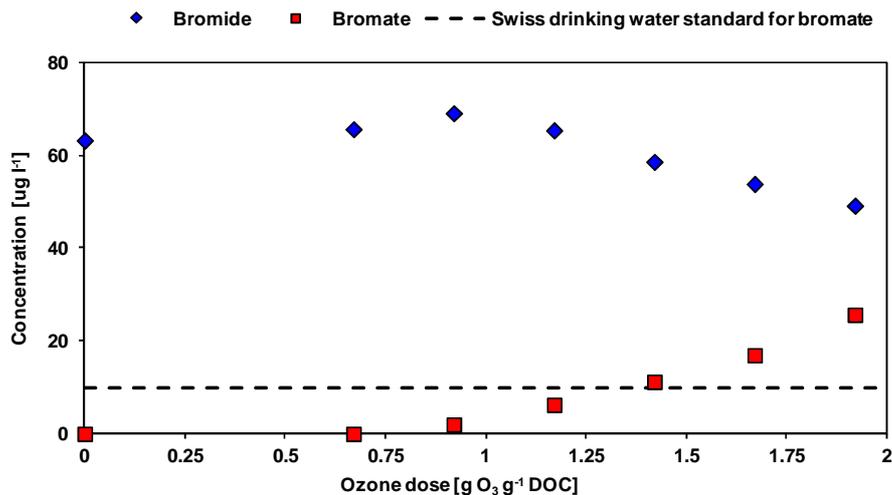


Figure S8. Influence of the ozone dose on bromate formation. Laboratory-scale oxidation experiments were conducted on 24-h composite wastewater samples collected at the Lausanne WWTP after biological treatment with full nitrification (5 mg DOC l⁻¹, 0.6 mg N-NO₂ l⁻¹). Different amounts of a stock solution of dissolved ozone (in water) were added to the samples to reach the desired ozone concentration (from 0 to 9.6 mg O₃ l⁻¹). At low doses (< 1 g O₃ g⁻¹ DOC), only negligible oxidation of bromide to bromate occurred due to fast ozone consumption by nitrite and reactive DOC. Above 0.9 g O₃ g⁻¹ DOC, a linear relation between the ozone dose and bromate formation was observed. At 1.4 g O₃ g⁻¹ DOC (7 mg O₃ l⁻¹), the Swiss drinking water standard for bromate (10 µg l⁻¹) was satisfied.

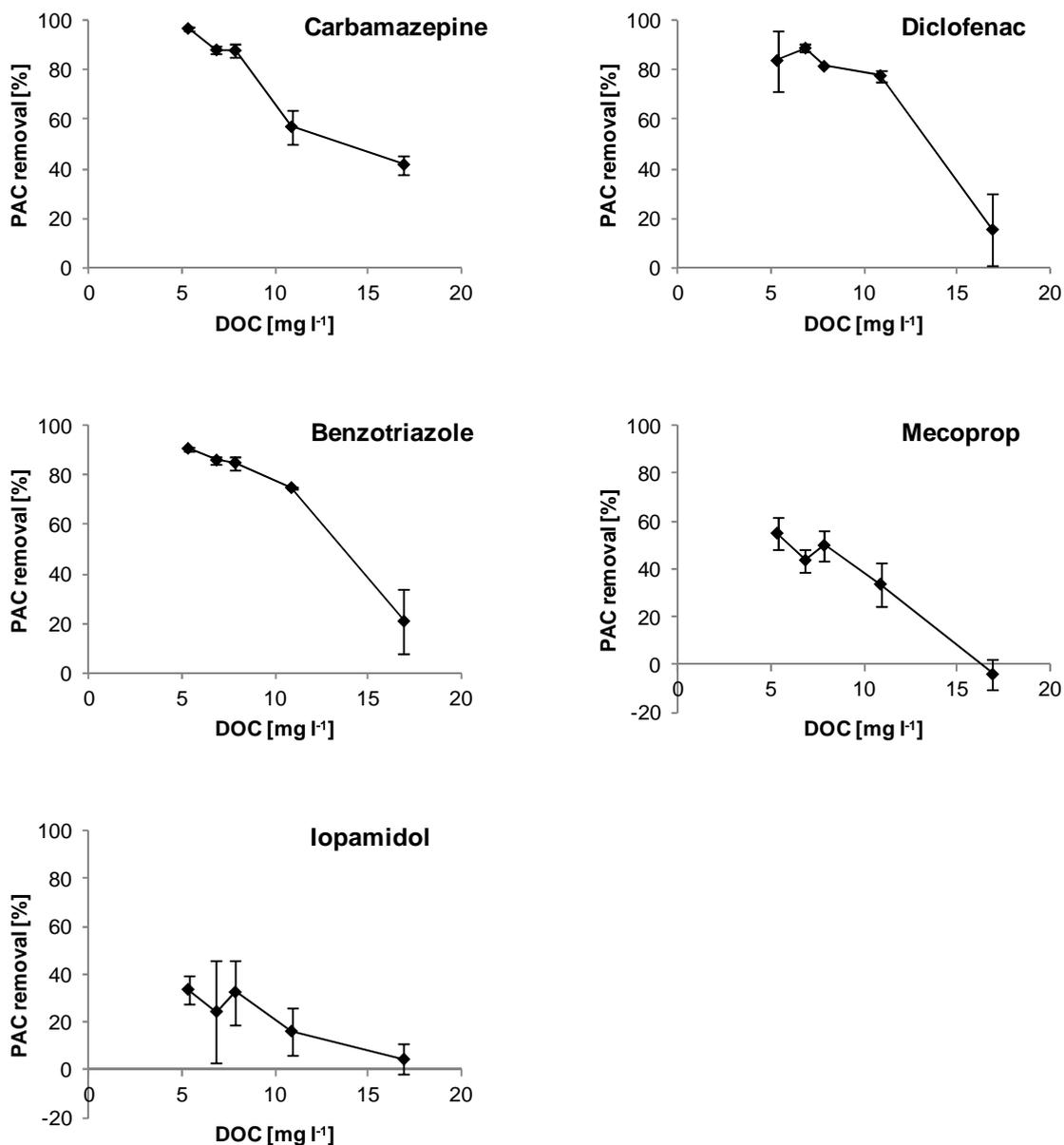


Figure S9. Influence of dissolved organic carbon (DOC) wastewater concentration on powdered activated carbon (PAC) removal efficiency of five micropollutants in wastewater. Average (diamonds) and standard deviation (vertical bars) of triplicates. Laboratory-scale batch adsorption experiments were conducted on 24-h composite wastewater samples collected during the same period at the Lausanne WWTP after either simple coagulation-precipitation treatment (DOC of 17 mg l⁻¹), activated sludge treatment without nitrification (DOC of 11 mg l⁻¹), or moving-bed bioreactor treatment with full nitrification (DOC of 5, 7 and 8 mg l⁻¹). PAC (10 mg l⁻¹, triplicates, SORBOPORTM MV-125, Envir Link SA, Switzerland) was added to the different types of wastewater and agitated at 140 rpm for 24 h in the dark at 20°C.

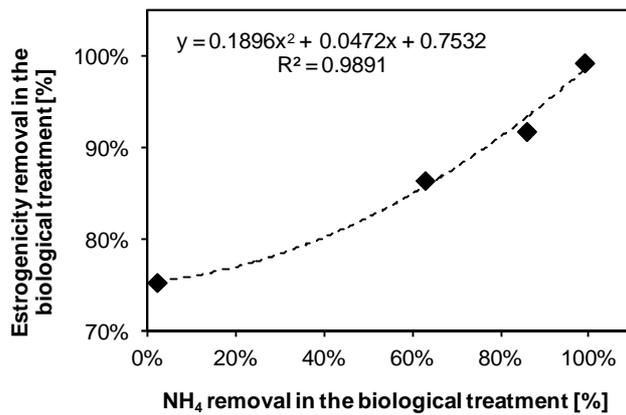


Figure S10. Estrogenic activity removal in the biological treatment (activated sludge or moving bed bioreactor) as a function of the level of nitrification (NH_4 removal). Estrogenic activity was measured with the YES on four 7-d composite samples in the influent and effluent of the biological treatment with various levels of nitrification. Dashed line: fitted quadratic trend line.

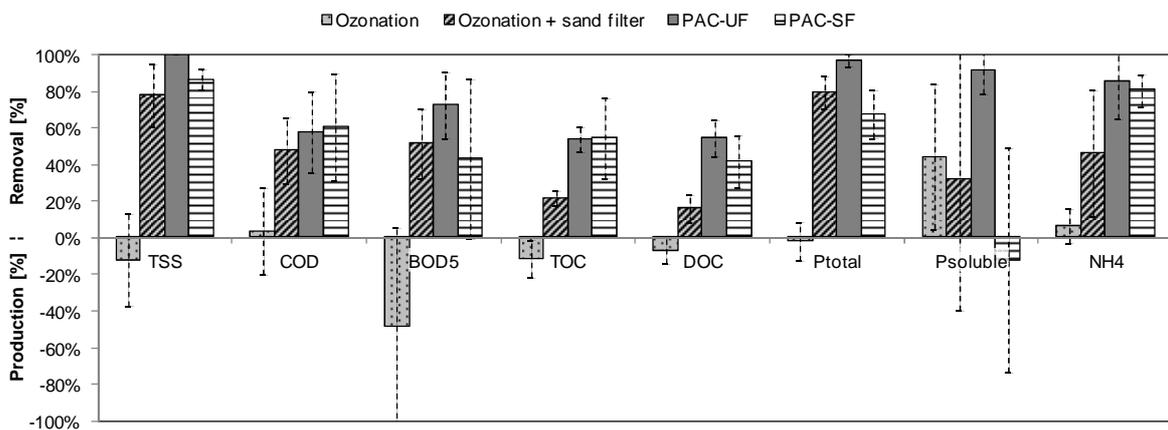


Figure S11. Removal of macropollutants with ozone, ozone/sand filter, PAC-UF and PAC-SF. Average and standard deviation of 14 (9 for PAC-SF) 24-h composite samples. Ozone dose of 3.8-7.0 $\text{mg O}_3 \text{ l}^{-1}$, PAC dose of 10-20 mg l^{-1} , coagulant (for PAC-UF only): 5-15 $\text{mg FeCl}_3 \text{ l}^{-1}$. TSS: total suspended solid, COD: chemical oxygen demand, BOD_5 : 5-d biochemical oxygen demand, TOC: total organic carbon, DOC: dissolved organic carbon, P_{total} : total phosphorus, P_{soluble} : dissolved phosphorus, NH_4 : ammonium.

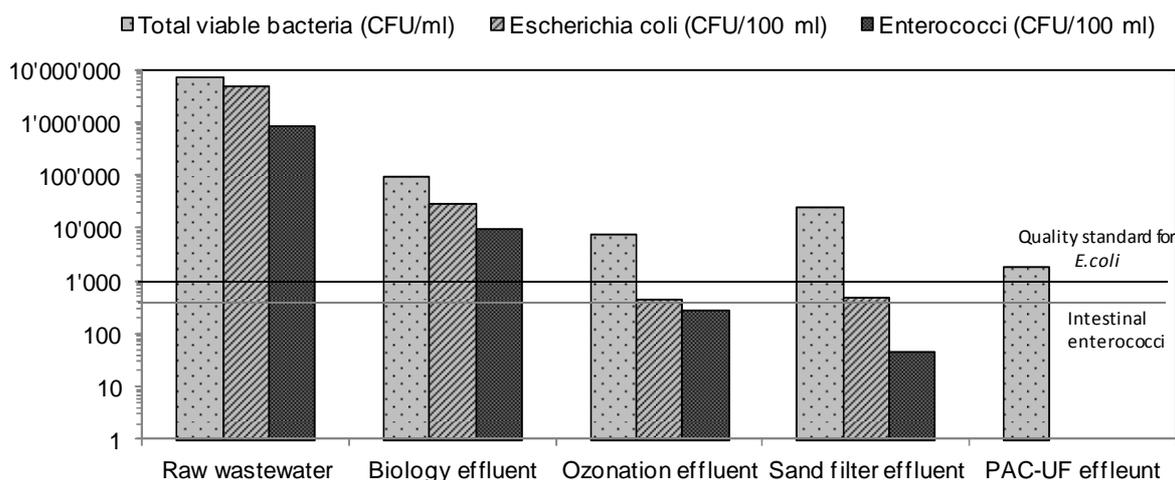


Figure S12. Influence of the treatments on the concentration of indicator bacteria in the effluent. Average of two campaigns (grab samples) with $6.9 \text{ mg O}_3 \text{ l}^{-1}$ or 20 mg PAC l^{-1} . European standards for good bathing water quality (Directive 2006/7/EC) are given for *E. coli* (1000 CFU/100 ml) and intestinal enterococci (400 CFU/100 ml) as comparative values.

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