Supporting Materials for

Reversible transformations of sulfamethoxazole and its submoieties by manganese-oxidizing bacteria and biogenic manganese oxides in the presence of humic substances

Adam Sochacki[1*], Hana Šubrtová Salmonová[2], Sylwia Bajkacz[3], Ewa Felis[4], Lucie Schneider[2], Zdeněk Chval[5], Zuzana Vaňková[6], Martin Lexa[7], Michael Pohořelý[8,9], Philippe François-Xavier Corvini[10], Markéta Marečková[2]

[1]Czech University of Life Sciences Prague, Faculty of Environmental Sciences, Department of Applied Ecology, Kamýcká 129, 165 00 Prague 6, Czech Republic

[2]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetics, Kamýcká 129, Prague 6, 16500, Czech Republic

[3]Silesian University of Technology, Faculty of Chemistry, Department of Inorganic Chemistry, Analytical Chemistry and Electrochemistry, ul. M. Strzody 7, 44-100 Gliwice, Poland

[4]Silesian University of Technology, Faculty of Energy and Environmental Engineering, Environmental Biotechnology Department, ul. Akademicka 2, 44-100 Gliwice, Poland

[5]Institute of Laboratory Diagnostics and Public Health, Faculty of Health and Social Studies, University of South Bohemia, J. Boreckého 27, 37011, České Budějovice, Czech Republic

[6] Czech University of Life Sciences Prague, Faculty of Environmental Sciences, Department of Environmental Geosciences, Kamýcká 129, 165 00 Prague 6, Czech Republic

[7] Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Department of Department of Wood Processing and Biomaterials, Kamýcká 129, 165 00 Prague 6, Czech Republic

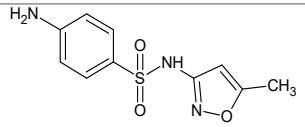
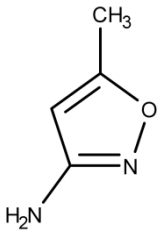
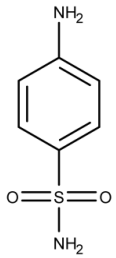
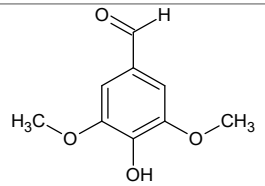
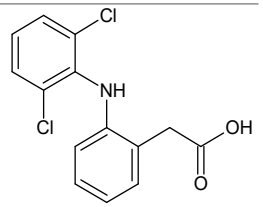
[8] University of Chemistry and Technology, Prague, Faculty of Environmental Technology, Department of Power Engineering, Technická 5, Prague 16628, Czech Republic

[9] Czech Academy of Sciences, Institute of Chemical Process Fundamentals, Research Group of Waste Management and Sustainable Technologies, Rozvojová 135, Prague 16500, Czech Republic

[10] University of Applied Sciences and Arts Northwestern Switzerland. School of Life Sciences, , 4132 Muttenz, Switzerland

*Corresponding author. Tel. +420 224 38 2993; E-mail address: sochacki@fzp.czu.cz

ESI1 Substances used in the experiment as target contaminants and electron mediator (syringaldehyde) (Source: Kim et al., 2023; except electrophilicity index and ionisation potential calculated as described in Section 2.7 of the main text)

Property		**Shortened name and abbreviation used in this study**				
		Sulfamethoxazole (SMX)	**Isoxazole (ISX)**	**Sulfanilamide (SNM)**	**Syringaldehyde (SAH)**	**Diclofenac (DCF)**
IUPAC name		4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide	5-methyl-1,2-oxazol-3-amine	4-aminobenzenesulfonamide	4-hydroxy-3,5-dimethoxybenzaldehyde	2-[2-(2,6-dichloroanilino)phenyl]acetic acid
Molecular Formula		$C_{10}H_{11}N_3O_3S$	$C_4H_6N_2O$	$C_6H_8N_2O_2S$	$C_9H_{10}O_4$	$C_{14}H_{11}Cl_2NO_2$
Molecular Structure						
CAS no.		723-46-6	1072-67-9	63-74-1	134-96-3	15307-86-5
p*K*a		pKa1 = 1.6; pKa2 = 5.7	2.5	10.6	7.30	4.15
log *Kow*		0.89	0.10	-0.62	1.07	4.51
Molecular weight		253.28	98.10	172.21	182.17	296.1
Electrophilicty index (eV)	pH4.5	2.813	2.195	2.565	4.361	2.659
	pH 7.5	2.238	2.184	2.561	3.857	2.603
Ionisation potential (eV)	pH4.5	6.172	6.711	6.184	6.394	5.560
	pH 7.5	5.673	6.700	6.178	5.878	5.460

ESI2 Substances used in the experiment as micropollutants or electron mediator (syringaldehyde). All substances were purchased from Sigma Aldrich (Czech Republic)

Substance		Purity	Concentration, mg/L
Redox mediator	Syringaldehyde	98%	Added in a molar 2:1 or 20:1, syringaldehyde:target substance
Positive control	Diclofenac, DCF	Sodium salt, ≥98%	10
Sulfonamides	Isoxazole, ISX (3-Amino-5-methylisoxazole)	≥97%	10
	Sulfadiazine, SDZ	99.0-101.0%	10
	Sulfadimethoxine, SDM	98.0-102.0%	2
	Sulfamerazine, SMR	≥99%	10
	Sulfamethazine, SMZ	99.0-101.0%	10
	Sulfamethizole, SMI	≥99%	10
	Sulfamethoxazole, SMX	≥98%	10
	Sulfanilamide, SFN	≥98%	10
	Sulfapyridine, SPD	≥99%	10
	Sulfathiazole, STZ	99.0-101.0%	10

ESI3 Components of the growth medium for *Pseudomonas putida* MnB6

All reagents were used as received without further purification. Yeast extract was purchased from Oxoid; casamino acids from Difco™; HEPES and $CoCl_2 \cdot 6\ H_20$ from VWR Chemicals; D-glucose, $CaCl_2$, $MgSO_4$, $FeCl_3$ and $CoCl_2 \cdot 6\ H_20$ from Lach-Ner; $CuSO_4 \cdot 5\ H_20$ from Penta; and $ZnSO_4 \cdot 7\ H_20$ and $MnCl_2$ from Sigma-Aldrich.

Compound	**Supplier**	**CAS Number**	**Dosage per 1 L**
Yeast extract	Oxoid		0.5 g
Casamino acids	Difco™		0.5 g
HEPES	VWR Chemicals	7365-45-9	5mM
D-Glucose	Lach-Ner	50-99-7	10 mM
$CaCl_2$	Lach-Ner	10043-52-4	0,48 mM
$MgSO_4$	Lach-Ner	7487-88-9	0,83 mM
$FeCl_3$	Lach-Ner	7705-08-0	3.7 µM
$CuSO_4 \cdot 5\ H_20$	Penta	7758-99-8	10 mg
$ZnSO4 \cdot 7\ H_20$	Sigma-Aldrich	7446-20-0	44 mg
$CoCl_2 \cdot 6\ H_20$	Lach-Ner	7791-13-1	20 mg
$Na_2MoO_4 \cdot 2\ H_20$	VWR Chemicals	10102-40-6	13 mg
$MnCl_2$	Sigma-Aldrich	7773-01-5	29 mg

ESI4 Additional information on the analysis of BioMnOx

The BioMnOx obtained after 24-h cultivation was pretreated by 0.17% sodium hypochlorite (12% Cl, stabilized, technical, Carl Roth, Germany) in several iterative steps to eliminate the adsorbed organic fraction originating from the cultivation media. The cleaning procedure was based on the protocol of Villalobos et al. (2003). Scanning electron microscopy (SEM) of the samples was performed using a MIRA 3 electron microscope (Tescan Orsay Holding, Brno, Czech Republic) with a secondary electron detector operated at 12 kV acceleration voltage. The energy-dispersive X-ray spectroscopy (EDX) of the samples was conducted using an energy-dispersive spectroscopy system (Bruker XFlash X-ray detector, Karlsruhe, Germany, and ESPRIT 2 software). The photoelectron spectrum was measured by X-ray photoelectron spectroscopy (XPS) method (Kratos ESCA 3400, X-ray source MgKa 120 W). A wide scan was performed using 0-1100eV, 0.2eV/step, 5x acquisitions, and then the identified elements were measured in high resolution (0.05eV/step, 15x-30x acquisitions). Additionally, the Mn3s region was separately measured to determine the oxidation state of manganese in the supplied sample (as received) and after Ar^+ ion sputtering. The C 1s 284.8 eV lines were used as calibration for the evaluation. The specific surface area was determined from the adsorption isotherm of N2 at T = 77 K using the method of BET surface analysis (Coutler SA 3100 automatic analyzer, Beckman Coutler, Inc., Brea, CA, USA).

The X-ray diffraction (XRD) analysis was performed using a desktop diffractometer Bruker D2 Phaser with an LYNXEYE XE detector (CuKα radiation, 30 kV, 10 mA, and measuring increment step of 0.022° 2Θ, time step 2.5 s, in the range from 5° to 80° 2Θ). The identification of all phases was performed using Diffrac. Suite EVA software (version 4.3) and the ICDD PDF-2 database (2018).

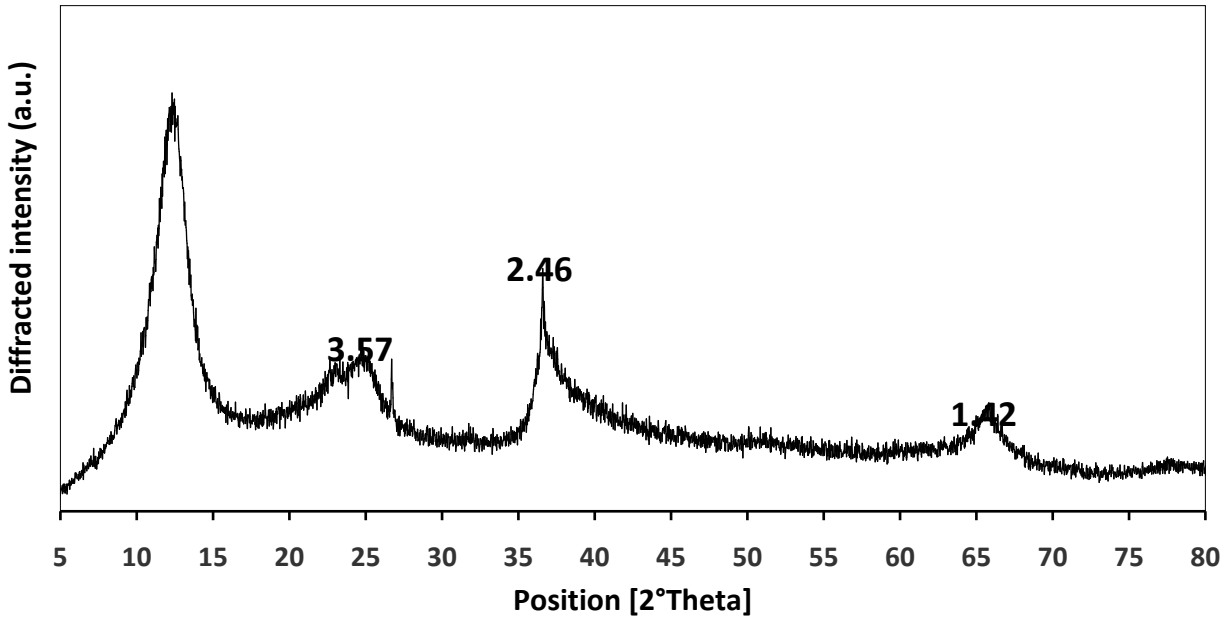
ESI5 HPLC analysis details: all the analyses were performed with a mobile phase: A – acetonitrile, B- formic acid/0.01M ammonium formate, pH 3.3.

Substance	Program (isocratic or gradient) ACN/buffer	Retention time [min]	Wavelength [nm]	LOD/LOQ [mg/L]	R^2
Diclofenac	Iso., 70/30	5.55	283	0.06/0.17	0.999
Isoxazole	Iso., 30/70	3.95	220	0.4/0.12	0.999
Sulfanilamide	Iso., 30/70	3.92	260	0.02/0.05	0.999
Sulfamethoxazole	Iso., 40/60	5.64	269	0.03/0.08	0.997
Syringaldehyde and Sulfamethoxazole	Iso., 30/70	5.71 8.06	307 269	0.22/0.66 0.03/0.08	0.998 0.998
Isoxazole and Sulfanilamide and Syringaldehyde	Gradient mode, 15/85 0-7 min 30/70 7-14 min 80/20 14-22 min 15/85 22-30 min	5.06 4.66 12.11	260 220 307	0.02/0.07 0.07/0.11 0.20/0.66	0.995 0.998 0.993
Sulfadiazine	Iso., 30/70	5.92	269	0.04/0.0.13	0.995
Sulfadimethoxine	Iso., 50/50	4.79	271	0.03/0.09	0.999
Sulfamerazine	Iso., 30/70	4.92	269	0.02/0.07	0.999
Sulfapyridine	Iso., 30/70	4.66	266	0.02/0.07	0.999
Sulfathiazole	Iso., 30/70	4.39	287	0.02/0.07	0.999
Sulfamethizole	Iso., 30/70	5.28	282	0.02/0.07	0.999
Sulfamethazine	Iso., 30/70	5.48	268	0.03/0.08	0.996

ESI6 Identification of transformation products

The information-dependent acquisition (IDA) mode, combining EMS with EPI, was used to maximize the information obtained in one scan. Data recorded by the EMS-IDA-EPI method were collected in positive electrospray ionization (ESI+) and negative (ESI-) ionization modes, while the p-MRM method data were collected only in positive ionization mode. The EMS and EPI mass ranges were from m/z 50 to m/z 700, and the scan rates were 1000 Da/s. The IDA criteria were as follows: the trigger for EPI was the 1–2 most intense ions that exceeded 100 cps; the mass tolerance was 250 mDa; former target ions were excluded for 30 s after two occurrences; the maximum rolling collision energy allowed was 80 eV in ESI+ and -80 eV in ESI-; and the dynamic background subtraction was turned on. The presence of TPs in the samples identified by the p-MRM mode was confirmed by analyzing the mass spectra recorded in the EMS-IDA-EPI mode. Non-targeted analysis was performed using a retrospective approach to mass spectral analysis.

ESI7 X-ray diffraction spectrum for MnOx used in the experiment



ESI8 SEM-EDS analysis

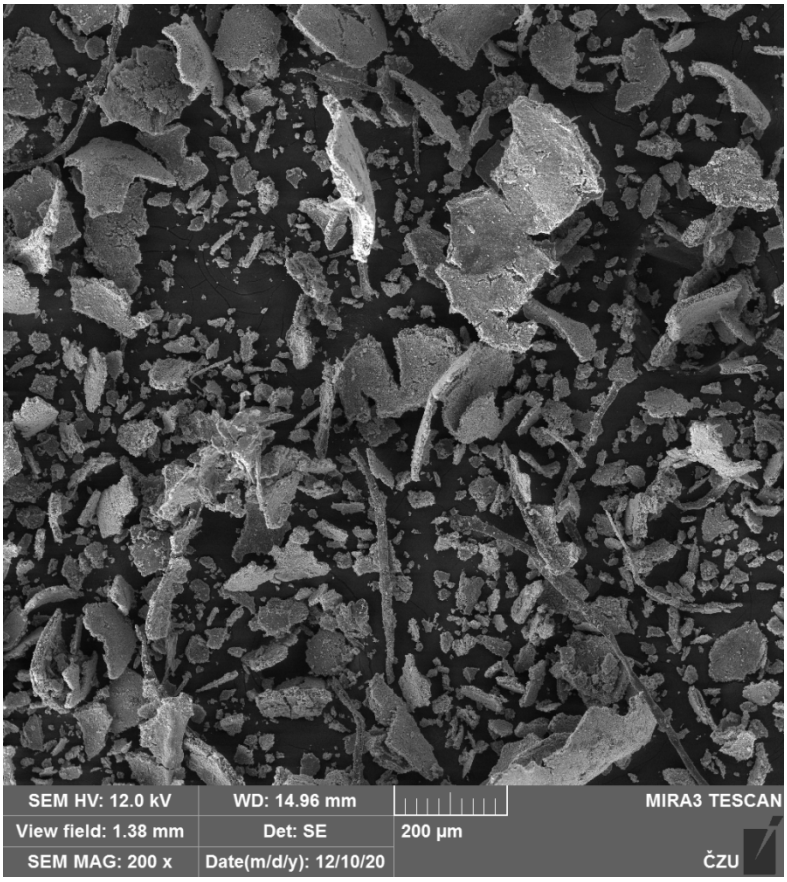


Figure SEM morphology

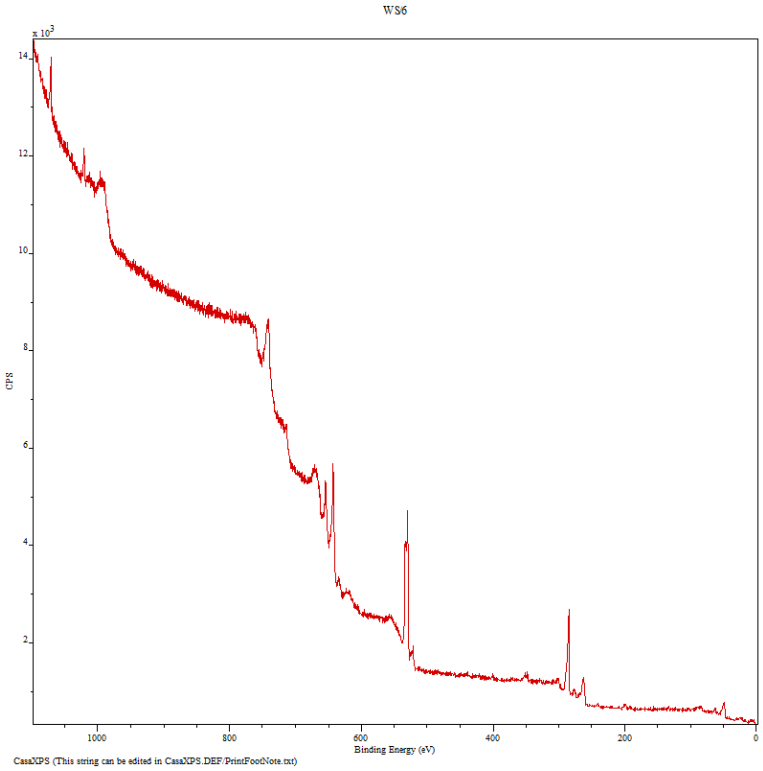
Table SEM-EDS analysis of the elemental composition of the filtration materials and amendments (wt%), n=10

	C	O	Na	Al	Si	P	Cl	Ca	Mn
Average	12.28%	26.02%	3.20%	0.94%	0.84%	0.46%	2.27%	2.36%	51.62%
SD	2.83%	3.61%	0.30%	0.01%	0.01%	0.01%	0.38%	0.20%	4.52%

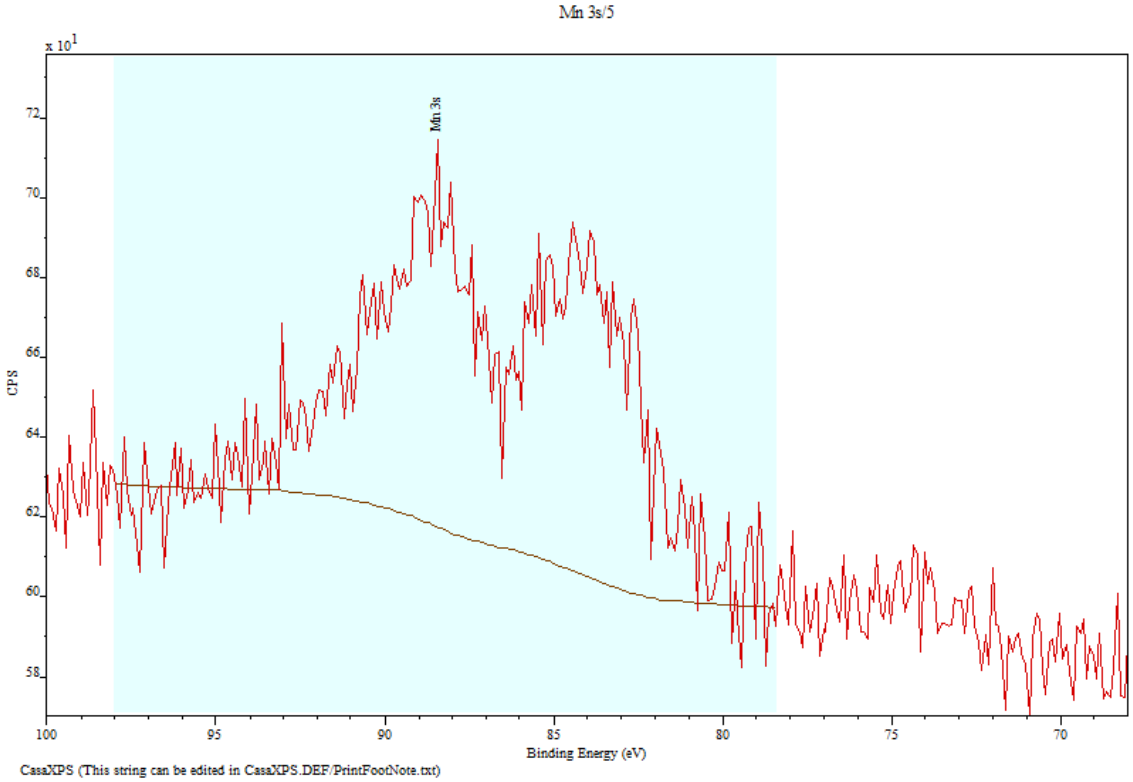
ESI9 Details on the XPS analysis of BioMnOx produced by *Pseudomonas putida* MnB6

	atom.%
C 1s	48.27
Mn 2p	7.38
O 1s	38.28
Cl 2p	1.34
Zn 2p	0.39
P 2p	0.70
Na 1s	2.63
Ca 2p	1.00

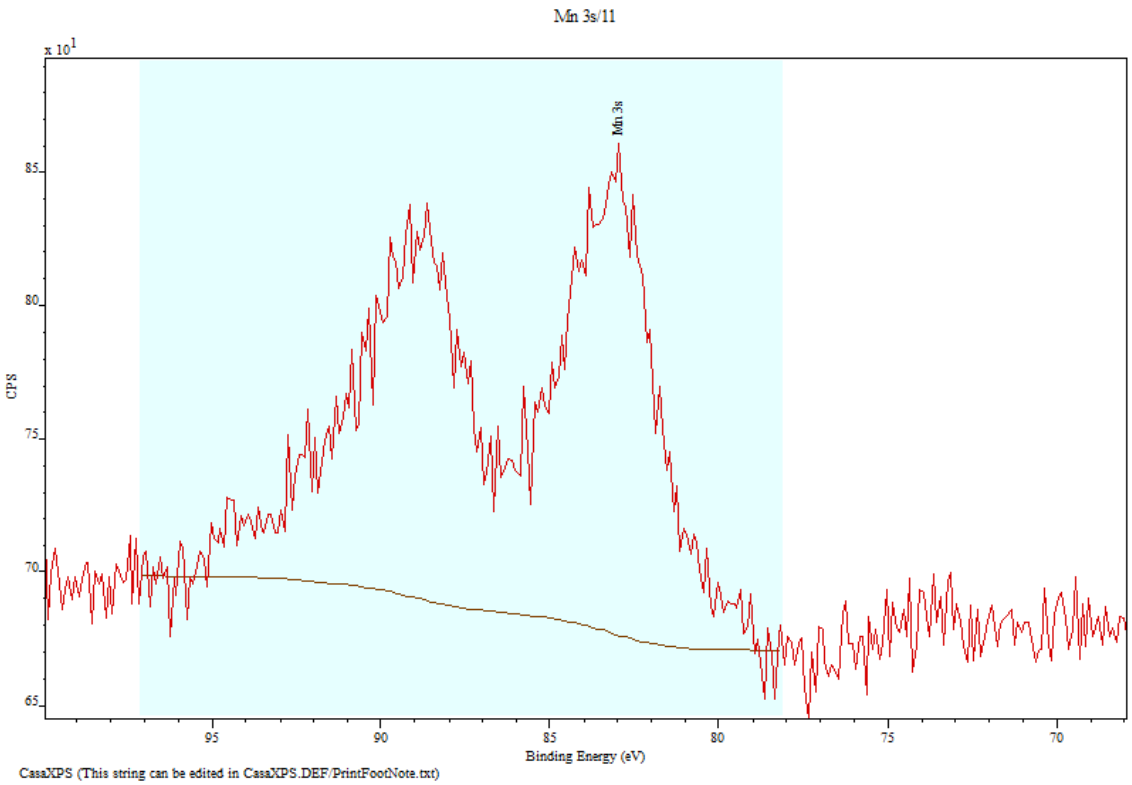
Full scan reveals that biogenic material contains mostly Mn, O and C.



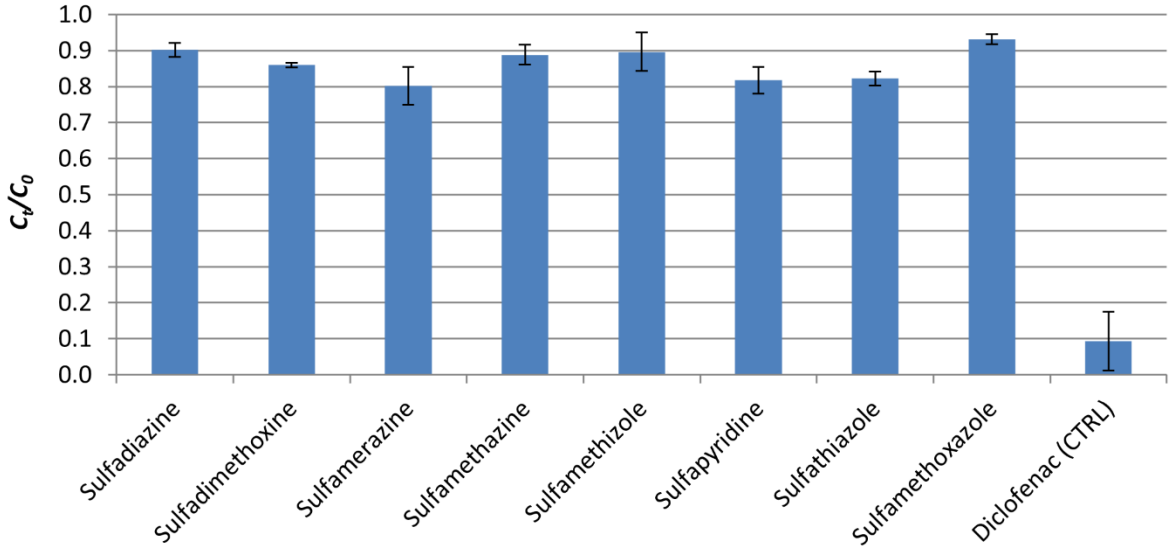
Mn 3s/5 before sputtering



Mn 3s/5 after sputtering


Mn 3s/11
x 10^1
Mn 3s
85
80
75
70
65
CPS
95
90
85
80
75
70
Binding Energy (eV)
CasaXPS (This string can be edited in CasaXPS.DEF/PrintFootNote.txt)

ESI10 Removal (C_t/C_0) of selected sulfonamides and diclofenac (positive control) in active cultures of *P. putida* MnB6 with peroxidized BioMnOx (Bio-Mn experiment) after time (t) 48 h.



ESI11 Improvements of the degradation of SMX, ISX and SNM in the presence of SAH

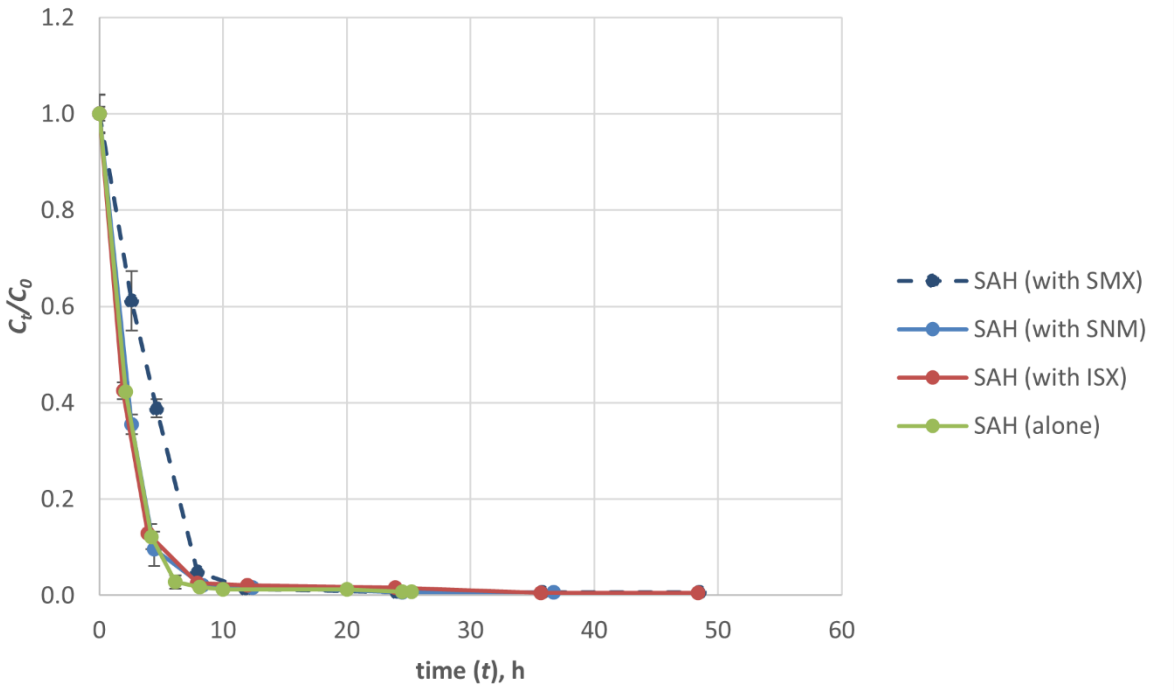
	SMX	SNM	ISX
Bio-Mn	+26%	+58%	+27%
Bio-noMn	+6%	+5%	+13%
Abio-Mn	+14%	+34%	+6%

ESI12 First-order kinetics parameters in the Bio-Mn and Bio-Mn-SAH tests (for the SAH experiments without the decoupling period)

Substance	*k, 1/h*	*SE*	$t_{50\%}$, *h*	$t_{99\%}$, *d*	*R*
SMX, w/o SAH	0.001	0.0002	693	192	0.842
SMX, w. SAH	0.029	0.0042	23.9	6.62	0.893
ISX, w/o SAH	0.001	0.0001	693	192	0.983
ISX, w. SAH	0.065	0.0128	10.6	2.95	0.906
SNM, w/o SAH	no fit				
SNM, w. SAH	0.174	0.0351	3.98	1.10	0.907
SAH (alone)	0.452	0.0194	1.53	0.42	0.998

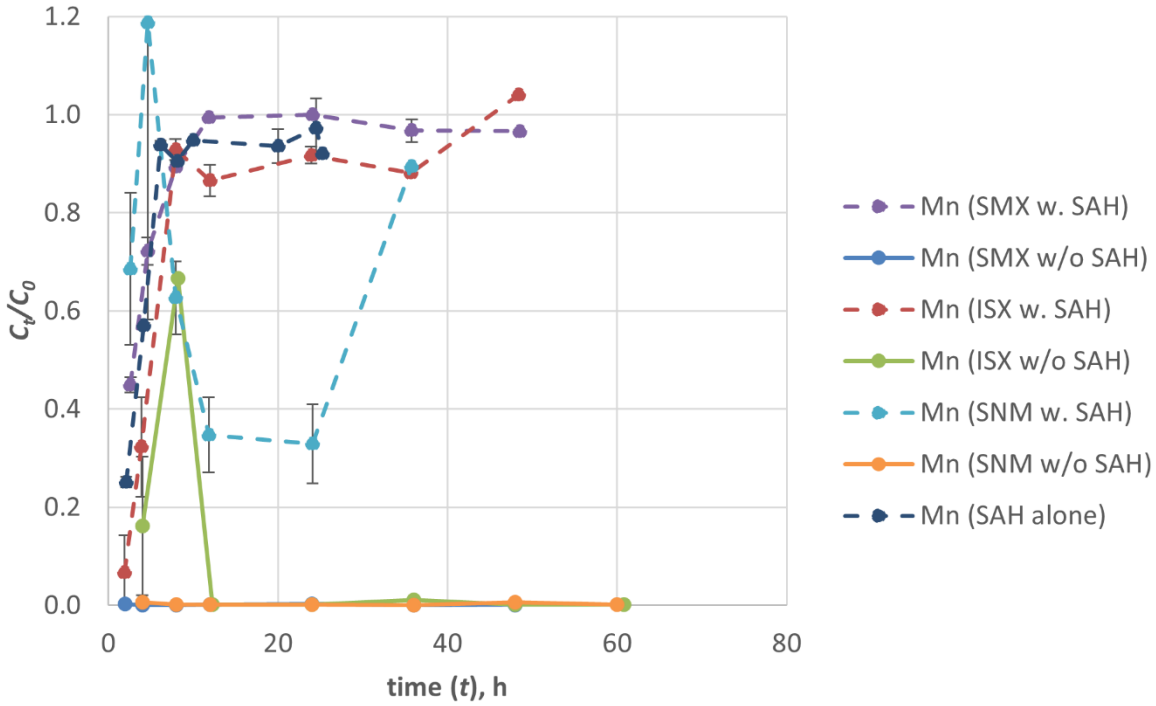
The parameters were determined using least squares non-linear regression (Levenberg-Marquardt estimation method in Software Statistica 14 (from Tibco Software Inc.).

ESI13 The removal (C_t / C_0) time profile of SAH in the Bio-Mn experiments with specific compounds SMX, SNM or ISX.

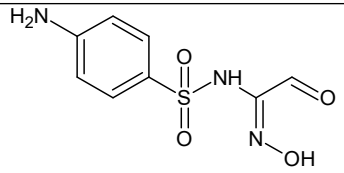
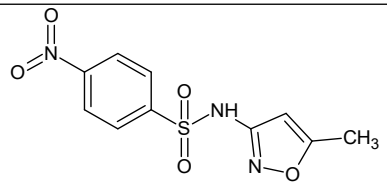
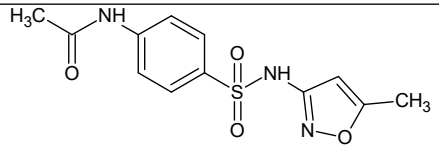
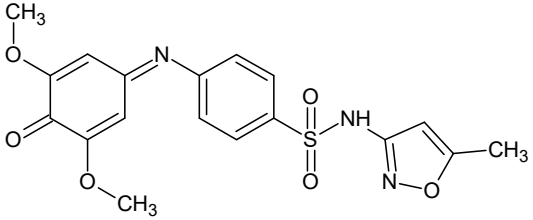


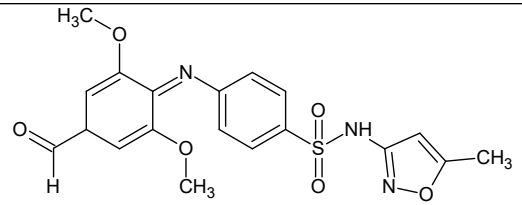
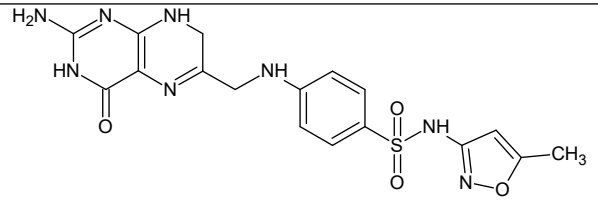
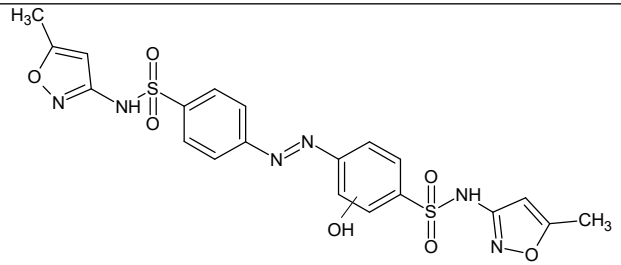
The removal (C_t/C_0) of SAH in the Abio-Mn experiment was 0.75±0.04, and in the Bio-noMn was 0.009±0.001.

ESI14 The removal (C_t/C_0) time profile of dissolved Mn in the experiments with specific compounds SMX, SNM or ISX (in the presence or absence of SAH) and SAH alone

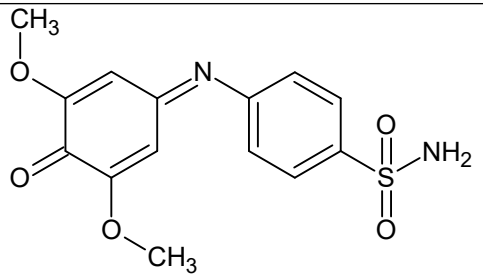
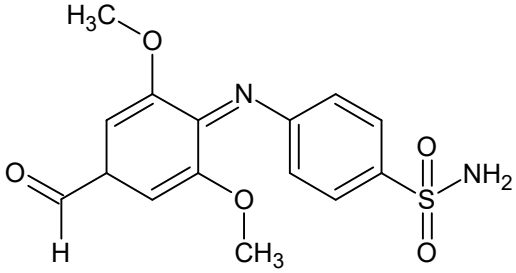


ESI15 Transformation products of sulfamethoxazole (SMX)

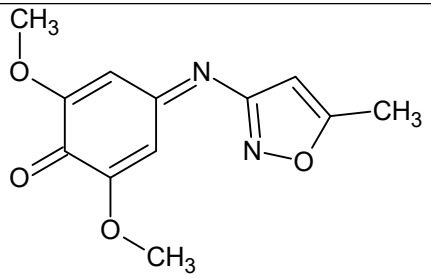
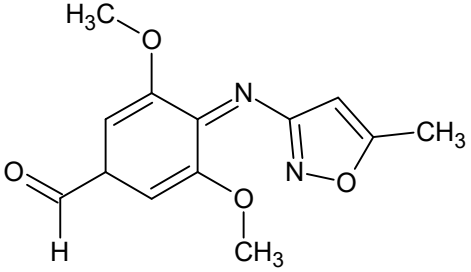
Symbol	Name	Transition ions/ product mass, *m/z*	Ionisation mode	Proposed chemical structure	Reference
TP244	4-amino-N-(1-hydroxyimino-2-oxaethyl)benzenesulfonamide	245.0 217.0	ESI+		Sochacki et al., 2021
TP283	4-nitro-SMX	284.2 267.0	ESI+		Sun et al., 2019
TP295	N4-acetyl-SMX	295.9 215.0; 249.9; 277,9	ESI+		Sochacki et al., 2021
TP403	SMX-DMBQ	403.9 243.0; 375.9	ESI+		Song et al., 2019

TP417	SMX-SAH	417.9 362.0; 389.9	ESI+		Margot et al., 2015
TP430	7,8-Dihydropterin-SMX	431.1 311.1	ESI+		Xiong et al., 2020
TP518	Hydroxyazosulfamethoxazole	519.1 419.0; 373.1	ESI+		Yang et al., 2017

ESI16 Transformation products of sulfanilamide (SNM)

Symbol	Name	Transition ions/ product mass, *m/z*	Ionisation mode	Proposed chemical structure	Reference
TP322(SNM)	**SNM-DMBQ**	322.9 263.0; 295.0	ESI+		This work
TP337(SNM)	**SNM-SAH**	338.2 303.2; 321.1	ESI+		This work

ESI17 Transformation products of isoxazole (ISX)

Symbol	Name	Transition ions/ product mass, *m/z*	Ionisation mode	Proposed chemical structure	Reference
TP248(ISX)	ISX-DMBQ	248.9 178.1; 221.1	ESI+		This work
TP262(ISX)	ISX-SAH	263.0 207.0; 234.9	ESI+		This work

References

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2023). PubChem 2023 update. Nucleic acids research, 51(D1), D1373-D1380.

Margot, J., Copin, P. J., von Gunten, U., Barry, D. A., & Holliger, C. (2015). Sulfamethoxazole and isoproturon degradation and detoxification by a laccase-mediator system: influence of treatment conditions and mechanistic aspects. Biochemical Engineering Journal, 103, 47-59.

Sochacki, A., Kowalska, K., Felis, E., Bajkacz, S., Kalka, J., Brzeszkiewicz, A., Vaňková, Z., & Jakóbik-Kolon, A. (2021). Removal and transformation of sulfamethoxazole in acclimated biofilters with various operation modes–Implications for full-scale application. Chemosphere, 280, 130638.

Song, Y., Jiang, J., Ma, J., Zhou, Y., & von Gunten, U. (2019). Enhanced transformation of sulfonamide antibiotics by manganese (IV) oxide in the presence of model humic constituents. Water research, 153, 200-207.

Sun, F., Wu, D., Chua, F. D., Zhu, W., & Zhou, Y. (2019). Free nitrous acid (FNA) induced transformation of sulfamethoxazole in the enriched nitrifying culture. Water research, 149, 432-439.

Villalobos, M., Toner, B., Bargar, J., & Sposito, G. (2003). Characterization of the manganese oxide produced by Pseudomonas putida strain MnB1. *Geochimica et Cosmochimica Acta*, *67*(14), 2649-2662.

Xiong, Q., Liu, Y. S., Hu, L. X., Shi, Z. Q., Cai, W. W., He, L. Y., & Ying, G. G. (2020). Co-metabolism of sulfamethoxazole by a freshwater microalga Chlorella pyrenoidosa. Water Research, 175, 115656.

Yang, Y., Lu, X., Jiang, J., Ma, J., Liu, G., Cao, Y., ... & Luo, C. (2017). Degradation of sulfamethoxazole by UV, UV/H2O2 and UV/persulfate (PDS): formation of oxidation products and effect of bicarbonate. Water research, 118, 196-207.