

# Digestate from agricultural biogas plants as a reservoir of anti-microbials and antibiotic resistance genes – implications for the environment

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**Table S1.** Procedures used in the determination of antimicrobials in digestate samples.

Class of antimicrobials	Compound	Fraction	References
betalactams	AMO	S	[1]
		L	[2]
	AMP	S	[1]
		L	[2]
tetracyclines	TC	S	[1]
		L	[2]
	OXY	S	[1]
		L	[2]
	DOX	S	[1]
		L	[2]
sulphonamides	CHLOR	S	[1]
		L	[2]
	SMX	S	[1]
		L	[2]
fluoroquinolones	SFD	S	[3]
		L	[2]
	ENF	S	[1]
		L	[2]
macrolides	CIP	S	[1]
		L	[2]
lincosamides	CLR	S	[3]
		L	[2]
dihydrofolic acid reductase inhibitor	CLD	S	[3]
		L	[2]
	TRI	S	[3]
		L	[2]

AMO – amoxicillin ; AMP – ampicillin; TC – tetracycline; OXY – oxytetracycline; DOX – doxycycline; CHLOR – chlortetracycline; SMX – sulfamethoxazole; SFD – sulphonamide; ENF – enrofloxacin; CIP – ciprofloxacin; CLR – clarithromycin ; CLD – clindamycin; TRI – trimethoprim; S – solid fraction; L – liquid fraction

**Table S2.** Parameters of the qPCR reaction.

Gene	Primer (5'–3')	Amplicon size (bp)	Annealing temperature (°C)	Reference
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<i>cfxA</i>	CGTAGTTTTGAGTATAGCTTT GATGTTGCCTATATATGTC	802	56	[1]
<i>bla<sub>TEM</sub></i>	AGTGCTGCCATAACCATGAGTG CTGACTCCCCGTCGTGTAGATA	431	61	[2]
<i>bla<sub>OXa</sub></i>	ATTATCTACAGCAGCGCCAGTG TGCATCCACGTCTTTGGTG	296	61	
<i>tetA</i>	GCTACATCCTGCTTGCCTTC GCATAGATCGCCGTGAAGAG	211	54	[3]
<i>tetM</i>	ACAGAAAGCTTATTATATAAC TGGCGTGTCTATGATGTTTAC	171	55	
<i>aac 6'- Ib-cr</i>	TTGCAGTGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	482	57	[4]
<i>qepA</i>	GCCGGTGATGCTGCTGA CAGTAACAGCGCACCAA	204	63	[5]
<i>sul1</i>	CGCACCGGAAACATCGCTGCAC TGAAGTTCCGCCGCAAGGCTCG	163	55.9	[4]
<i>sul2</i>	TCCGGTGGAGGCCGGTATCTGG CGGGAATGCCATCTGCCTTGAG	191	57.5	
<i>intI1</i>	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	280	54	[5]
<i>intI2</i>	TTATTGCTGGGATTAGGC ACGGCTACCCTCTGTTATC	233	50	
<i>ermF</i>	TAGATATTGGGGCAGGCAAG GGAAATTGCGGAAGTCAAA	126	58	[6]

**Table S3.** Antibiotic concentrations in digestate samples.

Samples of digestate from biogas plants								
Antibiotic	Fraction	Parameters	BP1W	BP1S	BP2W	BP2S	BP3W	BP3S
SFD	LF	Av.	136.5	98.2	106.8	n.d	n.d	n.d
		SD.	21.6	14.3	8.8	-	-	-
	SF	Av.	n.d	n.d	n.d	n.d	n.d	n.d
		SD	-	-	-	-	-	-
TC	LF	Av.	368.7	275.6	n.d	n.d	n.d	n.d
		SD	27.2	32.3	-	-	-	-
	SF	Av.	1164.4	464.8	n.d	n.d	n.d	n.d
		SD	216.3	104.8	-	-	-	-
DOX	LF	Av.	<LOQ	620.4	854.6	1555.9	n.d	n.d
		SD	-	34.2	142.7	305.9	-	-
	SF	Av.	218.1	800.2	396.7	1282.5	n.d	n.d
		SD	26.4	131.1	69.5	193.8	-	-
CIP	LF	Av.	n.d	n.d	n.d	n.d	n.d	n.d
		SD	-	-	-	-	-	-
	SF	Av.	145.3	92.4	n.d	n.d	n.d	n.d
		SD	21.1	13.4	-	-	-	-
ENF	LF	Av.	n.d	n.d	n.d	n.d	n.d	n.d
		SD	-	-	-	-	-	-
	SF	Av.	147.1	387.9	n.d	n.d	n.d	n.d
		SD	21.8	56.2	-	-	-	-
CLR	LF	Av.	208.9	56.97	n.d	n.d	n.d	n.d
		SD	27.2	8.3	-	-	-	-

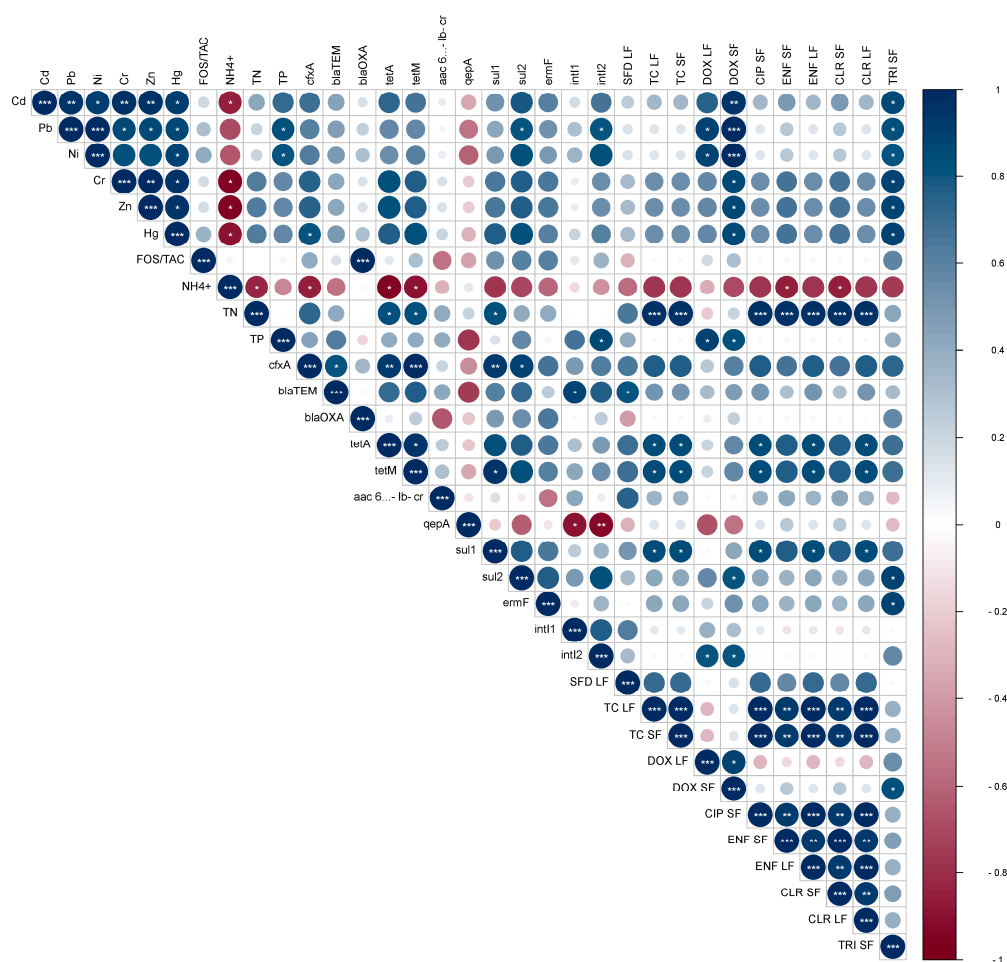
CLD	SF	Av.	62.8	70.7	n.d	n.d	n.d	n.d
		SD	9.8	9.4	-	-	-	-
	LF	Av.	268.4	88.6	n.d	n.d	n.d	n.d
		SD	29.1	8.7	-	-	-	-
	SF	Av.	n.d	n.d	n.d	n.d	n.d	n.d
		SD	-	-	-	-	-	-
TRI	LF	Av.	n.d	n.d	n.d	n.d	n.d	n.d
		SD	-	-	-	-	-	-
	SF	Av.	66.8	226.1	<LOQ	261.9	n.d	n.d
		SD	7.6	42.5	-	53.2	-	-

BP – biogas plant; 1,2,3 – Number of BP; W – winter; S – spring; SFD - sulfadiazine; TC - tetracycline; DOX - doxycycline; CIP - ciprofloxacin; ENF - enrofloxacin; CLR - clarithromycin; CLD - clindamycin; TRI - trimethoprim; Av. – Average (ng/g in solid fraction or ng/L In liquid fraction); SD – standard deviation; n.d – not detected;

**Table S4.** The presence and absolute abundance of antibiotic resistance genes (ARGs) in digestate samples (abbreviations are explained in Table S3) (number of copies/g of digestate).

Targeted genes and class of antibiotics												
Sample	betalactams			tetracyclines		fluoroquinolones		sulfonamides		MLS group	Integrase genes	
	<i>cfxA</i>	<i>bla<sub>TEM</sub></i>	<i>bla<sub>OXA</sub></i>	<i>tetA</i>	<i>tetM</i>	<i>aac 6'-Ib-cr</i>	<i>qepA</i>	<i>sul1</i>	<i>sul2</i>	<i>ermF</i>	<i>intI1</i>	<i>intI2</i>
BP1S	4.98E3	1.07E4	2.60E3	2.47E5	1.26E6	9.50E3	2.34E5	5.19E10	1.63E5	4.13E7	4.37E5	4.37E5
BP1W	3.33E5	7.78E4	3.05E3	2.22E6	2.25E6	5.02E3	5.10E4	8.89E10	7.2E5	7.08E7	7.24E6	8.14E6
BP2S	4.98E3	3.11E4	4.25E4	7.87E4	9.65E5	2.60E3	1.60E4	2.11E10	3.14E6	1.00E8	6.94E6	5.84E7
BP2W	2.79E3	3.21E4	1.16E3	1.28E4	2.89E5	4.25E4	1.60E4	2.9E9	1.42E5	1.06E7	8.76E6	9.39E6
BP3S	1.50E3	3.46E3	3.70E3	1.10E4	2.29E5	3.70E3	6.15E4	2.98E9	1.19E5	2.30E7	5.19E5	1.39E5
BP3W	5.73E2	2.83E3	1.67E3	1.21E4	1.64E5	3.05E3	3.31E5	2.45E9	6.25E4	2.47E7	2.78E5	1.11E5

S – spring; W – winter



**Figure S1.** Spearman's non-parametric correlation analysis between antibiotic concentrations and concentrations of ARGs, integrase genes, physicochemical parameters (TN – total nitrogen; TP – total phosphorus), and concentration of HMs (Cd - cadmium, Pb – lead, Ni – nickel, Cr – chromium, Zn – zinc, and Hg – mercury) (other abbreviations are explained in Table S1 and Table S3). Significant statistical results are marked with an asterisk: \* for  $p < 0.05$ ; \*\* for  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

## References

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