

Supplemental material

Distribution and characterization of quaternary ammonium biocides resistant bacteria in different soils

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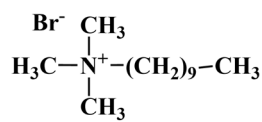
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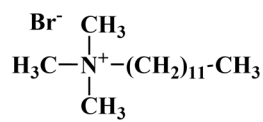
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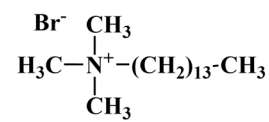
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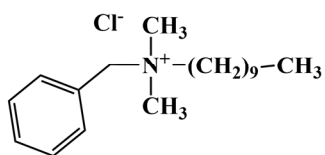
ATMAC-10



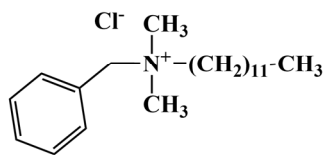
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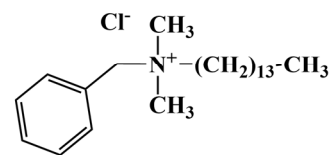
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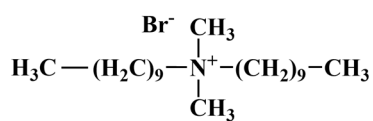
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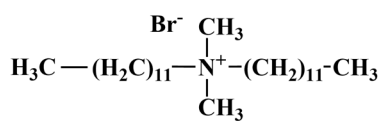
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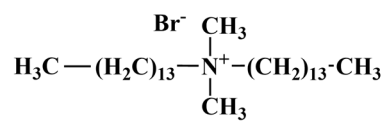
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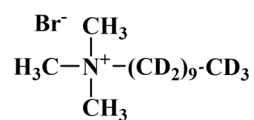
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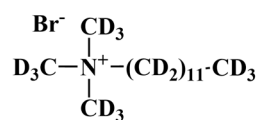
DADMAC-12



DADMAC-14



ATMAC-10D



ATMAC-12D

Figure S1. Structural formula of the target QACs.

Table S1. Physicochemical properties of produced water samples in different periods.

Sample	COD (mg/L)	TOC (mg/L)	BOD5 (mg/L)	Cl ⁻ (mg/L)	pH
1	1880	620.55	949.74	5611.8	7.8
2	1800	601.99	760.63	4888.8	7.94
3	1600	455.11	678.41	4954.5	7.31
4	1696	512.23	711.30	5134.5	7.57
5	1632	461.35	703.08	5646.5	7.65
6	1344	342.79	548.65	5789.1	7.62
7	1288	304.02	603.37	5923.5	7.36
8	1208	280.01	512.17	5350.7	7.42
9	1600	231.56	648.65	5662	7.26
10	1304	108.65	543.37	7775.6	7.43
11	1192	56.38	502.17	8413.6	7.93

COD: chemical oxygen demand,

BOD5: 5-days biochemical oxygen demand,

TOC: total organic carbon.

Table S2. The mobile phases and elution gradients procedure

	Time (min)	Percentage A (%)	Percentage B (%)	Percentage C (%)
Elution gradients procedure	0	0	80	20
	7	80	0	20
	13	80	0	20
	18	80	0	20
	25	0	80	20
Mobile phases	A	B	C	
	Methanol	Pure water	10 mM NH ₄ Ac in iso-propanol	

Table S3. The targeted analytes, retention time, and monitored multiple reaction monitoring (MRM).

QACs	Retention time (min)	MRM Transitions	MRM transitions for IS
ATMAC-12	5.24	228.30>60.10	228.30>57.20
ATMAC-14	7.09	256.30>60.10	256.30>57.15
BAC-10	4.58	276.30>91.00	276.30>58.15
BAC-12	5.75	304.30>91.10	304.30>58.05
ATMAC-D10	4.12	221.40>61.10	221.40>50.15
ATMAC-D12	6.21	262.55>105.00	262.55>70.20
ATMAC-10	4.19	200.25>60.20	200.25>89.20
BAC-14	8.15	332.35>91.10	332.35>240.25
DADMAC-10	9.04	326.40>186.20	326.40>57.15
DADMAC-12	13.70	382.45>214.25	382.45>57.10
DADMAC-14	23.73	438.50>242.25	438.50>57.15

Table S4. Calibration curves for QACs standards

Compounds	Calibration curves	Linear regression coefficients	LOD (ng/g)	LOQ (ng/g)
ATMAC-10	$y = 0.5311x + 0.009$	$R^2 = 0.9958$	0.016	0.053
ATMAC-12	$y = 0.442x + 0.0166$	$R^2 = 0.9963$	0.036	0.122
ATMAC-14	$y = 0.3645x + 0.0097$	$R^2 = 0.9971$	0.095	0.317
BAC-10	$y = 0.2707x + 0.0157$	$R^2 = 0.9963$	0.024	0.081
BAC-12	$y = 0.2158x + 0.0203$	$R^2 = 0.9938$	0.072	0.239
BAC-14	$y = 0.1654x - 0.0198$	$R^2 = 0.9967$	0.012	0.039
DADMAC-10:10	$y = 0.3374x + 0.013$	$R^2 = 0.9957$	0.007	0.022
DADMAC-12:12	$y = 0.2189x + 0.008$	$R^2 = 0.9959$	0.010	0.033
DADMAC-14:14	$y = 0.2506x + 0.0267$	$R^2 = 0.9934$	0.011	0.038

LOD: limit of detection,

LOQ: limit of quantitation.

Table S5. Longitude, latitude, and land use types of soil sampling points.

Sampling Point	Longitude	Latitude	Land Use (Crop)
S1	107°36'6.85"	29°44'13.01"	Wasteland
S2	107°36'9.46"	29°44'10.44"	Wasteland
S3	107°36'7.80"	29°44'14.04"	Wasteland
S4	107°36'6.28"	29°44'9.69"	Wasteland
S5	107°36'3.77"	29°44'24.84"	Farmland (Corn)
S6	107°36'5.40"	29°44'24.79"	Farmland (Corn)
S7	107°36'2.80"	29°44'28.56"	Farmland (Corn)
S8	107°35'50.36"	29°44'40.22"	Wasteland (Sichuan Pepper)
S9	107°35'50.86"	29°44'42.53"	Wasteland
S10	107°35'49.63"	29°44'47.01"	Wasteland
S11	107°35'19.22"	29°45'22.21"	Farmland (Corn)
S12	107°35'17.82"	29°45'22.08"	Wasteland
S13	107°35'16.55"	29°45'23.26"	Farmland (Corn)
S14	107°34'40.01"	29°44'41.02"	Farmland (Corn)
S15	107°34'39.21"	29°44'41.80"	Farmland (Corn)
S16	107°34'38.86"	29°44'41.97"	Farmland (Corn)
S17	107°34'45.09"	29°44'39.12"	Wasteland
S18	107°34'46.07"	29°44'39.97"	Wasteland
S19	107°34'46.47"	29°44'39.92"	Wasteland
S20	107°34'51.25"	29°43'58.92"	Wasteland
S21	107°34'54.20"	29°43'58.26"	Farmland (Corn)
S22	107°34'55.28"	29°43'58.29"	Farmland (Corn)
S23	107°37'6.56"	29°47'20.07"	Wasteland
S24	107°35'23.13"	29°45'57.81"	Farmland (Rice)
S25	107°36'11.00"	29°45'33.17"	Wasteland

Table S6. Physicochemical properties of soil samples.

Sample	pH	TN (g/kg)	TP (g/kg)	CEC (cmol/kg)	Organic Matter (g/kg)	Soil Types ^a
S1	7.5	1.1423	0.5769	4.654402736	21	Loam
S3	4.62	1.5113	0.6388	6.090064387	24	Clay
S5	4.58	1.3531	1.1098	6.574698193	22.4	Clay
S6	7.75	1.3044	0.5797	8.004635376	16.6	Clay
S7	5.97	1.6894	0.9318	5.643528991	24.9	Clay
S8	6.24	1.7351	0.7286	3.38506065	25.2	Clay
S9	7.42	0.8085	0.2668	7.252036447	9.6	Clay
S10	5.08	1.9063	0.6416	6.601178647	26.7	Clay
S11	4.95	1.381	0.6162	9.559883644	15.7	Clay
S12	7.79	0.665	0.3558	7.271012979	6.5	Clay
S13	4.48	1.1483	1.0182	9.045844836	14.2	Clay
S14	6.94	1.9472	0.4542	4.422590566	13.1	Clay
S15	6.98	1.8677	0.4289	11.16358276	24.3	Clay
S16	5.21	2.9326	0.6912	8.661684197	27.2	Loam
S18	6.71	1.7516	0.4494	6.383603441	10.8	Loam
S20	8.26	3.2523	0.3027	9.621506807	18.1	Clay
S21	7.86	1.8832	0.6665	6.020594392	24.2	Clay
S22	6.34	2.2457	0.5506	4.5987901	24.6	Clay
S23	7.87	3.3473	0.7216	9.536874008	39.6	Sand
S24	8.13	2.135	0.9114	7.137998978	22.1	Sand
S25	7.95	1.9493	0.8751	12.2948131	16.9	Sand

TN: total nitrogen,

TP: total phosphorus,

CEC: cation exchange capacity,

^a Classification of soil samples according to “Chinese soil” prepared by Institute of Soil Science, Chinese Academy of Sciences.

Table S7. The difference of QACs concentration between exploitation area and non-exploitation area.

Compound	ATMAC-10	ATMAC-12	ATMAC-14	Σ ATMAC	BAC-10	BAC-12
<i>P-value</i>	0.923	0.807	0.633	0.606	0.166	0.403
Compound	Σ BAC	DADMAC-10	DADMAC-12	DADMAC-14	Σ DADMAC	Σ QACs
<i>P-value</i>	0.391	0.435	0.484	0.131	0.487	0.881

Table S8. Content of heavy metals in soil samples.

Samp	As	Ba	Cd	Cr	Cu	Ni	Pb	Sr	Zn
le	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
S1	8.79	626.22	0.27	73.98	61.69	39.32	36.33	130.18	126.53
S3	8.43	221.17	0.28	74.67	47.44	35.26	37.87	49.65	109.44
S5	6.32	421.48	0.24	74.94	28.89	30.75	31.13	39.30	82.11
S6	11.13	352.80	0.34	91.77	35.41	36.89	34.27	130.08	98.45
S7	4.46	201.54	0.35	68.35	18.69	17.78	26.81	66.48	66.94
S8	6.67	318.45	0.32	90.59	31.51	36.75	37.43	87.48	119.39
S9	8.22	972.78	0.39	111.01	40.54	50.97	38.18	224.21	134.29
S10	6.55	292.13	0.58	85.85	32.36	26.06	39.14	77.71	88.78
S11	10.35	1025.44	0.30	86.14	26.83	29.69	40.00	104.26	95.25
S12	2.00	325.64	0.20	52.46	23.65	14.68	31.57	79.83	70.21
S13	7.46	488.74	0.24	69.04	27.20	25.70	42.51	59.88	94.58
S14	5.48	320.10	0.14	58.38	21.50	16.11	30.89	54.17	86.45
S15	5.34	452.08	0.19	73.24	22.13	24.45	33.22	82.76	81.02
S16	7.22	264.87	0.19	68.00	27.53	23.86	40.22	71.84	85.91
S18	5.75	269.83	0.12	72.43	27.15	25.65	37.60	48.37	86.59
S20	11.65	1553.24	0.22	90.60	33.64	47.77	29.32	220.13	138.11
S21	3.51	47.19	0.20	81.95	41.17	37.39	23.09	47.84	86.95
S22	4.63	111.65	0.26	81.46	36.47	44.68	26.25	36.90	82.53
S23	38.94	305.92	0.38	84.97	34.51	37.23	41.69	53.15	111.40
S24	7.12	260.69	0.32	64.78	28.72	27.39	38.57	87.71	92.38
S25	18.41	2200.63	0.39	133.55	48.23	54.60	48.00	131.09	138.78

Table S9. Antibiotic resistance profiles of 48 strains of soil QACs-resistant bacteria.

Antibiotic	Strains	Proportion (%)
AMO	S1-2, S1-3, S2-1, S2-2, S3-1, S3-2, S3-3, S5-1, S6-2, S7-1, S8-1, S8-2, S9-1, S10-1, S10-2, S11-1, S11-2, S11-3, S13-1, S13-2, S14-1, S14-2, S14-3, S15-1, S15-2, S17-1, S21-1, S23-1, S23-2, S23-3, S24-1, S24-2, S24-3, S25-1, S25-2	72.92
T/S	S1-2, S1-3, S2-1, S3-1, S3-2, S3-3, S5-1, S6-1, S6-2, S7-1, S8-1, S8-2, S9-1, S10-2, S11-1, S11-2, S11-3, S13-1, S13-2, S14-1, S14-3, S15-2, S17-1, S21-1, S23-1, S23-2, S23-3, S24-1, S24-2, S24-3, S25-1, S25-2	66.67
CHA	S1-2, S1-3, S2-2, S3-1, S3-2, S3-3, S5-1, S7-1, S8-1, S8-2, S9-1, S10-1, S10-2, S11-1, S11-2, S11-3, S13-1, S13-2, S14-1, S14-3 S15-1, S15-2, S17-1, S21-1, S23-2, S23-3, S24-1, S24-2, S24-3, S25-1, S25-2	64.58
TET	S1-2, S5-1, S6-1, S6-2, S7-1, S8-2, S10-1, S11-1, S11-2, S11-3, S13-1, S13-2, S14-1, S14-3, S15-1, S21-1, S23-1, S23-2, S23-3, S24-1, S24-2, S25-1, S25-2	47.92
VAN	S1-2, S2-1, S2-2, S3-1, S3-2, S3-3, S4-1, S4-2, S4-3, S4-4, S7-1, S8-1, S10-1, S14-2, S14-3, S15-1, S15-2, S17-1	37.50
OFX	nd	0.00
None	S1-1, S2-3, S6-3, S16-1, S18-1, S20-1, S20-2, S21-2	16.67
nd: not detect		

Table S10. Antibiotic resistance profiles of 21 strains of produced water QACs-resistant bacteria.

Antibiotic	Strains	Proportion (%)
VAN	PW2-1, PW3-1, PW3-2, PW4-1, PW5-1, PW5-2, PW5-3, PW6-1, PW7-1, PW7-2, PW8-1, PW8-2, PW9-1, PW9-2, PW10-3, PW10-4	76.19
CHA	PW2-1, PW3-1, PW3-2, PW4-1, PW5-1, PW5-2, PW5-3, PW6-1, PW7-1, PW8-1, PW8-2, PW9-1, PW9-2, PW10-3, PW10-4, PW11- 1,	76.19
AMO	PW2-1, PW3-1, PW3-2, PW4-1, PW5-1, PW5-3, PW7-1, PW9-1, PW9-2, PW10-2, PW10-3, PW10-4, PW11-1,	61.90
TET	PW2-1, PW3-1, PW3-2, PW5-3, PW6-1, PW7-1, PW7-2, PW9-1, PW9-2, PW10-3, PW10-4	52.38
T/S	PW3-1, PW7-1, PW10-2, PW10-4, PW11-1	23.80
OFX	nd	0.00
None	PW1-1, PW1-2, PW10-1	14.29

nd: not detect