Electronic Supplementary Information for

Small and Large-Scale Distribution of Four Classes of Antibiotics in Sediment: Association with Metals and Antibiotic Resistance Genes

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Environmental Science: Processes & Impacts

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Table S1. Global positioning system (GPS) coordinates of river surface sediment collection sites and their abbreviations.

River Sampling Site	Abbreviation	GPS Location	Date Collected
Big Stone Lake	BSL	45.303492, -96.453089	July 13, 2015
Marsh Lake	ML	45.171606, -96.094239	July 13, 2015
Lac qui Parle	LQP	45.022186, -95.868581	July 13, 2015
Granite Falls	GF	44.812499, -95.535147	July 13, 2015
St. Peter	SP	44.324499, -93.953020	June 25, 2015
Jordan	JD	44.692811, -93.641017	June 25, 2015
Grand Rapids	GR	47.231792, -93.530150	July 6, 2015
Brainerd	BRD	46.378194, -94.183337	July 6, 2015
Little Falls	LF	45.975469, -94.368498	July 7, 2015
St. Cloud	STC	45.548207, -94.147166	July 7, 2015
Coon Rapids	CR	45.144222, -93.312308	July 7, 2015
Hastings	HG	44.762600, -92.873418	June 25, 2015
Lake Pepin	LP	44.499750, -92.294170	August 5, 2014

Table S2. Global positioning system (GPS) coordinates of Lake Winona surface sediment collection sites relative to the outfall of to the Alexandria Lake Area Sanitary District wastewater treatment plant (WWTP).

Distance from WWTP (km)	GPS Location
0.15	45.87219, -95.40524
0.48	45.87501, -95.40402
0.93	45.87740, -95.39924
1.41	45.88030, -95.39468
1.9	45.88293, -95.38963
2.19	45.88477, -95.38699
2.51	45.88752, -95.38596

Table S3. Percent organic, carbonate, and inorganic of Lake Winona surface sediment relative to distance (km) from wastewater treatment plant (WWTP) effluent and percent water of sample determined by loss-on-ignition.

Lake Winona Surface Sediment Loss-On-Ignition Results

Distance from WWTP (km)	*Organic*	*Carbonate*	*Inorganic*	*Water*
0.15	26.6	46.1	27.3	85.0
0.48	18.1	58.6	23.3	86.6
0.93	19.0	55.1	26.0	86.4
1.41	19.8	49.1	31.1	88.3
1.90	20.1	46.9	33.0	90.7
2.19	20.3	46.3	33.4	87.6
2.51	21.2	41.9	36.9	86.5

Table S4. Percent organic, carbonate, and inorganic of Minnesota and Mississippi River surface sediment and percent water of sample determined by loss-on-ignition.

Minnesota and Mississippi River Loss-On-Ignition Results

Sample Site	*Organic*	*Carbonate*	*Inorganic*	*Water*
Big Stone Lake	0.6	7.0	92.3	23.6
Marsh Lake	9.4	14.4	76.2	55.5
Lac Qui Parle	3.4	12.6	84.0	33.2
Granite Falls	4.8	14.8	80.4	39.1
St. Peter	3.8	13.2	83.0	39.5
Jordan	1.9	9.8	88.3	30.4
Grand Rapids	26.8	9.2	63.9	84.2
Brainerd	8.4	8.0	83.6	63.8
Little Falls	17.4	8.5	74.2	75.1
St. Cloud	0.7	3.1	96.2	18.1
Coon Rapids	1.8	3.7	94.5	27.0
Hastings	7.8	9.4	82.8	51.6
Lake Pepin	13.4	11.5	75.1	84.5

Table S5. Particle size distribution of river sediment samples organized by sand (50 – 200 µm), silt (2 – 50 µm), and clay (less than 2 µm) content.

Textural Analysis			
Sample Site	*Sand %*	*Silt %*	*Clay %*
Minnesota River			
Big Stone Lake	99	<1	<1
Marsh Lake	22	48	30
Lac Qui Parle	68	19	13
Granite Falls	52	32	16
St. Peter	56	28	16
Jordan	68	22	10
Mississippi River			
Grand Rapids†	--	--	--
Brainerd	26	54	19
Little Falls	51	24	26
St. Cloud	99	<1	<1
Coon Rapids	90	2	8
Minnesota & Mississippi River			
Hastings	22	56	22
Lake Pepin†	--	--	--

† textural analysis was not performed due to insufficient sample volume

Table S6. Long-term median stream flow (ft^3/s) in the Minnesota and Mississippi River Basin at monitored Minnesota cities recorded by the United States Geological Survey.[2]

Station Site	Closest Sediment Collection Site	Long-Term Median Flow (ft^3/s)
Minnesota River Basin		
Ortonville	Big Stone Lake & Marsh Lake	88
Near Lac qui Parle	Lac qui Parle	909
Montevideo	–	970
Granite Falls	Granite Falls	3,150
Morton	–	3,550
Mankato	St. Peter	4,680
Near Jordan	Jordan	6,770
Fort Snelling State Park	–	9,920
Mississippi River Basin (above Minnesota River)		
Grand Rapids	Grand Rapids	1,019
Aitkin	–	3,750
Brainerd	Brainerd	4,470
Royalton	Little Falls	5,900
St. Cloud	St. Cloud	8,580
Brooklyn Park	Coon Rapids	10,500
Mississippi River Basin (below Minnesota River)		
St. Paul	–	18,400
Hastings	Hastings	28,100

Table S7. Municipal wastewater treatment plants with design flows greater than 1 million gallons per day (MGD) that discharge into either the Minnesota and Mississippi river.

Wastewater Treatment Plant	ID	Closest Downstream Sample Location	Approx. Distance Between WWTP & Sampling Location (km)	Design Flow (MGD)	Latitude	Longitude
Met Council - Metropolitan	1	HG	29	314	44.92579687	-93.04530563
Met Council - Blue Lake	2	HG	71	42	44.78882217	-93.40595245
Met Council - Seneca	3	HG	53	38	44.82766073	-93.20736532
Saint Cloud	4	CR	94	17.9	45.55372238	-94.15486145
Grand Rapids	5	BRD	274	15.2	47.21850962	-93.49687607
Met Council - Eagles Point	6	HG	4	11.9	44.78831863	-92.9203186
Mankato	7	SP	22	11.25	44.18256875	-94.00033578
Winona	8	–	–	9.6	44.03337973	-91.60286644
Brainerd	9	LF	54	6	46.33846086	-94.22622637
Saint Peter	10	JD	79	4	44.33962873	-93.95740632
Red Wing	11	LP	23	4	44.57117916	-92.52793498
Met Council - Hastings	12	LP	59	2.69	44.74224518	-92.84880245
Bemidji	13	GR	159	2.5	47.46446757	-94.87602514
Little Falls	14	STC	61	2.4	45.96585397	-94.36857616
Monticello	15	CR	46	2.36	45.29854791	-93.77659364
Elk River	16	CR	28	2.2	45.29961395	-93.5594101
Minnesota River Valley Public Utilities Commission	17	JD	54	1.842	44.47514862	-93.90383704
Lake City	18	–	–	1.52	44.44788742	-92.26652527
Camp Ripley	19	LF	18	1.44	46.10596085	-94.42694855
Redwood Fall	20	SP	177	1.321	44.57040689	-95.10811858

Table S8. List of antibiotics included in the study and their acronyms and general uses. Also noted is whether the antibiotic is naturally produced.

Antibiotic	Acronym	Natural Product	General Uses[1]
		Sulfonamides	
sulfachlorpyridazine	SCP	no	swine, calves, dogs
sulfadiazine	SDZ	no	horses, humans
sulfadimethoxine	SDM	no	fish, poultry
sulfamethazine	SMZ	no	swine, cattle
sulfamethoxazole	SMX	no	human
sulfapyridine	SPD	no	human
		Macrolides	
erythromycin[a]	EMC	yes	humans, poultry, swine
tylosin	TYL	yes	chicken, swine, cattle
		Tetracyclines	
chlortetracycline[b]	CTC	yes	swine, poultry, cattle, sheep, ducks
doxycycline	DXC	no	human, dogs
oxytetracycline	OTC	yes	poultry, fish, swine, cattle, sheep
tetracycline	TCC	yes	human, dogs, cattle
		Fluoroquinolones	
ciprofloxacin	CFC	no	human, swine, chickens
enrofloxacin	EFC	no	cattle, swine, poultry, dogs, cats
norfloxacin	NFC	no	human, poultry
ofloxacin	OFC	no	poultry, human
		Non-Categorized	
carbadox	CBX	no	swine
trimethoprim	TMP	no	human, dogs, horses
lincomycin	LMC	yes	poultry, swine

[a] includes the presence of erythromycin-H_2O
[b] includes the presence of epi-chlortetracycline, iso-tetracycline, and epi-iso-tetracycline

Table S9. Limits of detection (LODs) and quantification (LOQs) in ng/g for antibiotics in Lake Winona surface sediment extractions. Also displayed are absolute recoveries of internal standards and relative recoveries of surrogates and target antibiotics.

Limits of Detection and Recovery in Lake Winona

Analytes	LOD [ng/g]	LOQ [ng/g]	Recovery (%)
Sulfonamides			
Sulfapyridine	0.85	2.54	110 ± 16
Sulfadiazine	0.09	0.26	120 ± 24
Sulfamethoxazole	0.12	0.36	94 ± 0
Sulfamethazine	0.18	0.55	91 ± 9
Sulfachloropyridazine	0.01	0.04	112 ± 5
Sulfadimethoxine	0.15	0.44	83 ± 4
$^{13}C_6$-Sulfamethazine[a]	-	-	56 ± 5
$^{13}C_6$-Sulfamethoxazole[b]	-	-	52 ± 14
Tetracyclines			
Tetracyclines	1.43	4.29	19 ± 5
Doxycycline	1.11	3.32	18 ± 4
Oxytetracycline	4.07	12.20	5 ± 1
Chlortetracycline	1.92	5.76	71 ± 11
Demeclocycline[a]	-	-	10 ± 11
Fluoroquinolones			
Norfloxacin	1.46	4.37	23 ± 3
Ciprofloxacin	2.06	6.18	26 ± 5
Enrofloxacin	0.10	0.30	38 ± 12
Ofloxacin	0.47	0.80	33 ± 5
Nalidixic Acid[a]	-	-	54 ± 6
Clinafloxacin[b]	-	-	18 ± 5
Macrolides			
Erythromycin	0.45	1.35	99 ± 20
Tylosin	0.05	0.15	218 ± 24
$^{13}C_2$-Erythromycin[b]	-	-	27 ± 20
Non-categorized			
Carbadox	0.42	0.76	13 ± 2
Trimethoprim	0.04	0.06	24 ± 5
Lincomycin	0.09	0.15	6 ± 6
Simeton[b]	-	-	67 ± 3

[a] surrogate
[b] internal standard

Table S10. Limit of detection (LOD) and quantification (LOQ) in ng/g for antibiotics in Minnesota and Mississippi River sediment extracts.

	Limit of Detection [ng/g]				Limit of Quantification [ng/g]			
Analyte	**Mean**	**Median**	**Max**	**Min**	**Mean**	**Median**	**Max**	**Min**
Sulfonamides								
Sulfapyridine	0.007	0.008	0.012	0.002	0.035	0.036	0.037	0.029
Sulfadiazine	0.020	0.023	0.035	0.005	0.103	0.105	0.107	0.087
Sulfamethoxazole	0.126	0.138	0.145	0.049	0.431	0.440	0.449	0.362
Sulfamethazine	0.009	0.010	0.014	0.002	0.028	0.028	0.029	0.023
Sulfachloropyridazine	0.138	0.146	0.155	0.048	0.432	0.442	0.451	0.363
Sulfadimethoxine	0.006	0.007	0.009	0.001	0.028	0.029	0.029	0.024
Fluoroquinolones								
Norfloxacin	3.31	1.20	18.94	0.27	8.97	3.25	51.39	0.73
Ciprofloxacin	11.62	5.34	39.73	0.83	35.74	16.44	122.20	2.54
Enrofloxacin	0.39	0.19	1.60	0.04	0.48	0.24	1.97	0.05
Ofloxacin	0.08	0.05	0.20	0.02	0.26	0.18	0.67	0.07
Tetracyclines								
Tetracyclines	9.17	5.94	29.14	1.88	27.82	18.00	88.37	5.70
Doxycycline	9.42	6.68	26.51	2.74	29.07	20.63	81.85	8.46
Oxytetracycline	179.6	113.6	676.7	16.19	558.9	353.7	2106	50.38
Chlortetracycline	4.25	3.20	9.82	1.21	13.73	10.36	31.76	3.91
Macrolides								
Erythromycin	0.45	0.49	0.59	0.23	1.36	1.46	1.76	0.68
Tylosin	0.95	1.00	1.82	0.39	3.07	3.23	5.84	1.27
Non-Categorized								
Carbadox	1.25	0.65	5.66	0.36	6.42	3.36	29.08	1.86
Trimethoprim	0.20	0.19	0.27	0.12	0.43	0.42	0.58	0.26
Lincomycin	0.08	0.04	0.49	0.02	0.26	0.11	1.53	0.06

Table S11. Absolute recoveries of internal standards and relative recoveries of surrogates and antibiotics in Minnesota and Mississippi River sediment extracts.

	Absolute and Relative Recovery (%)			
Analyte	**Mean**	**Median**	**Max**	**Min**
Sulfonamides				
Sulfapyridine	178%	107%	497%	73%
Sulfadiazine	170%	102%	471%	70%
Sulfamethoxazole	97%	82%	234%	77%
Sulfamethazine	212%	131%	753%	93%
Sulfachloropyridazine	107%	93%	281%	76%
Sulfadimethoxine	191%	110%	743%	96%
$^{13}C_6$-Sulfamethazine[a]	141%	109%	603%	68%
$^{13}C_6$-Sulfamethoxazole[b]	37%	30%	82%	4%
Fluoroquinolones				
Norfloxacin	31%	26%	114%	2%
Ciprofloxacin	12%	9%	54%	1%
Enrofloxacin	25%	20%	95%	2%
Ofloxacin	35%	34%	91%	9%
Nalidixic Acid[a]	215%	206%	305%	146%
Clinafloxacin[b]	48%	45%	120%	2%
Tetracyclines				
Tetracyclines	10%	9%	29%	2%
Doxycycline	10%	8%	22%	2%
Oxytetracycline	4%	2%	16%	0%
Chlortetracycline	115%	89%	253%	31%
Demeclocycline[a]	22%	18%	60%	0%
Macrolides				
Erythromycin	73%	64%	138%	54%
Tylosin	155%	131%	305%	73%
$^{13}C_2$-Erythromycin[b]	27%	24%	48%	12%
Non-categorized				
Carbadox	47%	47%	84%	6%
Trimethoprim	90%	89%	130%	66%
Lincomycin	110%	89%	212%	9%
Simeton[b]	32%	33%	24%	41%

[a] denotes surrogate
[b] denotes internal standard

Table S12. List of genes corresponding to their resistance function. "Other" category includes biomass surrogate (16S rRNA), kanamycin, rifampicin, esterase, and streptomycin resistance.

Resistance/Function	Genes
aminoglycoside	*aacD, aadA5*
β-lactamase	*ampC*, *bla*$_{KPC}$, *bla*$_{NDM-1}$, *bla*$_{NPS}$, *bla*$_{OXA}$,
chloramphenicol	*catB8, cmlB, floR*
erythromycin	*ermB, ermF*
integrons	*intI1, intI2, intI3*
macrolides	*mefE, mphBM*
metal	*cadA, chrA, copA, merA, nikA, rcnA*
multidrug efflux	*acrD, mexB*
quaternary ammonium	*qacF, qacG*
quinolones	*qnrA, qnrB*
sulfonamide	*sul1, sul2, sul3*
tetracycline	*tet*(A), *tet*(L), *tet*(M), *tet*(S), *tet*(W), *tet*(X)
trimethoprim	*dfr13*
vancomycin	*vanA, vanB*
other	16S rRNA, *aadD, arr2, ereB, strB*

Table S13. Detected antibiotics in Lake Winona surface sediment [ng/g] corresponding to Figure 3.

	Antibiotic Sediment Concentration [ng/g] in Lake Winona							
	Distance from WWTP (m)							
Antibiotic	*0.15*	*0.48*	*0.93*	*0.93*	*1.41*	*1.9*	*2.19*	*2.51*
Sulfapyridine	19.6	15.8	3.8	3.9	3.8	5.0	4.1	2.9
Sulfadiazine	n.d.[a]	n.d.	n.d.	n.d.	0.3	0.4	0.3	n.d.
Sulfamethoxazole	1.0	2.0	1.6	2.2	2.6	8.9	3.8	2.8
Sulfamethazine	0.7	n.d.	n.d.	0.5	0.5	0.5	0.5	0.5
Sulfachlorpyridazine	n.d.	n.d.	0.4	0.6	0.6	2.4	1.0	0.8
Sulfadimethoxine	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.4	n.d.
Ciprofloxacin	298.3	58.9	30.0	30.5	19.7	12.1	24.1	7.3
Enrofloxacin	1.0	n.d.	0.4	0.6	1.4	1.6	1.1	0.4
Ofloxacin	711.0	117.9	92.2	93.8	73.6	48.0	74.2	24.3
Erythromycin	3.1	1.7	1.3	3.2	2.0	n.d.	2.0	1.7
Trimethoprim	24.7	5.2	4.5	5.1	n.d.	3.7	4.2	n.d.
Tetracycline	2.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chlortetracycline	n.d.	n.d.	n.d.	n.d.	n.d.	5.9	n.d.	n.d.

[a] non-detect

Table S14. Detected antibiotics in Mississippi and Minnesota rivers surface sediment [ng/g] corresponding to Figure 4.

	Antibiotic Concentration in River Sediment [ng/g]								
Sampling Location[a]	SPD	SDZ	SMX	SMZ	SDM	OFC	EMC	TMP	OTC
BSL	n.d.[b]	n.d.	n.d.	< 0.03	n.d.	n.d.	0.48	n.d.	< 50.38
ML	0.08	n.d.	n.d.	0.08	0.06	n.d.	n.d.	< 0.47	n.d.
LQP	0.03	n.d.	n.d.	0.03	n.d.	n.d.	n.d.	< 0.40	n.d.
GF	0.06	n.d.	< 0.43[c]	0.05	n.d.	n.d.	0.14	n.d.	n.d.
SP	0.03	n.d.	n.d.	n.d.	n.d.	0.94	0.01	0.61	n.d.
JD	0.08	n.d.	< 0.43	1.11	0.01	n.d.	n.d.	n.d.	n.d.
GR	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 0.58	n.d.
BRD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.44	n.d.	n.d.
LF	n.d.	n.d.	n.d.	n.d.	n.d.	3.42	0.80	< 0.44	n.d.
STC	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	0.10	n.d.	n.d.
CR	n.d.	n.d.	n.d.	n.d.	n.d.	0.67	n.d.	< 0.30	n.d.
HG	0.14	n.d.	n.d.	0.03	n.d.	0.75	n.d.	0.89	n.d.
LP	1.91	0.44	n.d.	0.68	n.d.	6.20	0.71	< 1.43	n.d.

[a] See Table S1 for corresponding sampling location

[b] non-detect

[c] Sediment concentration less than LOQ, but greater than LOD. Sediment concentration reported as < LOQ.

SPD = sulfapyridine; SDZ = sulfadiazine; SMX = sulfamethoxazole; SMZ = sulfamethazine; SDM = sulfadimethoxine; OFC = ofloxacin; EMC = erythromycin; TMP = trimethoprim; OTC = oxytetracycline

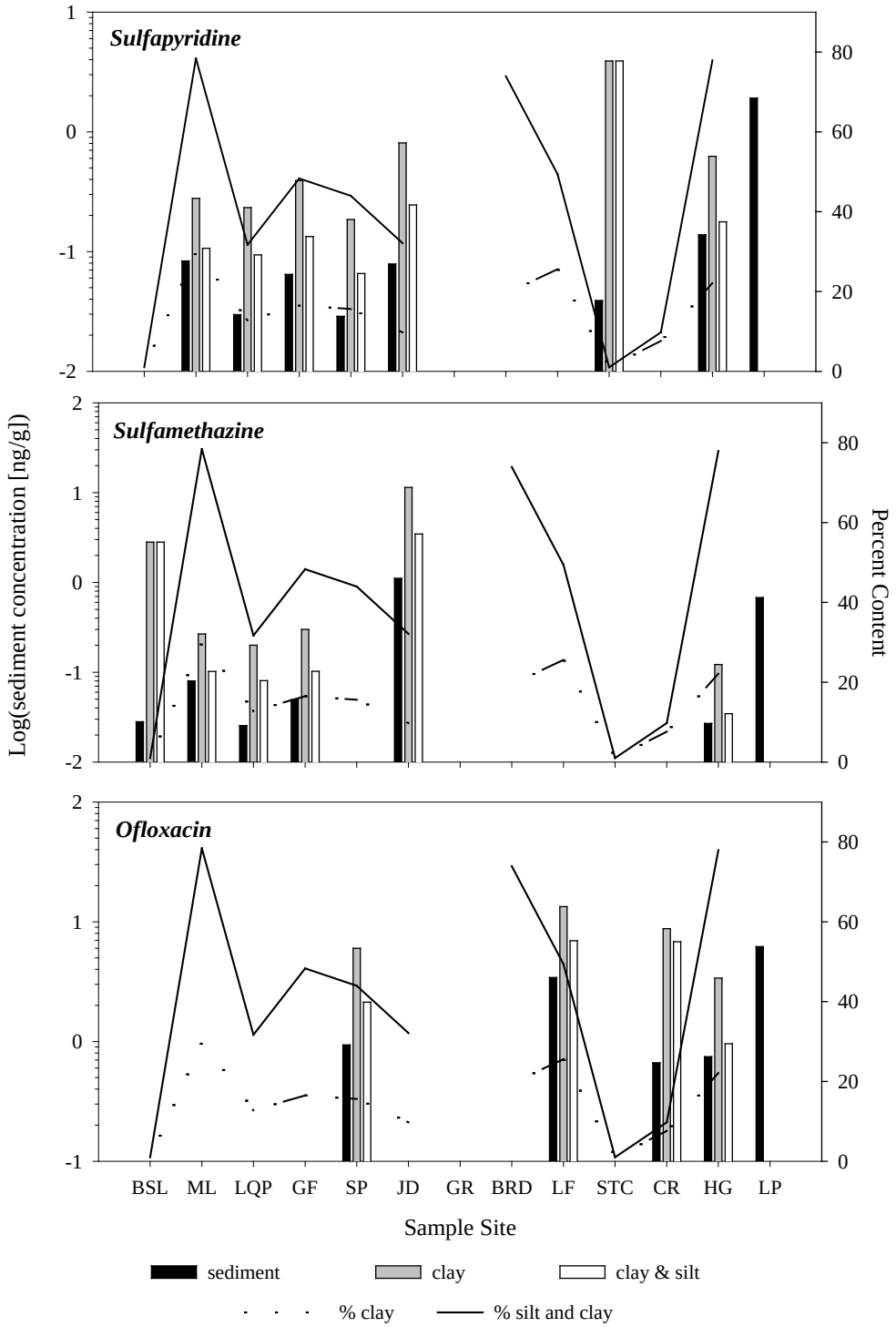

Figure S1. Log_{10} transformed antibiotic sediment concentration expressed as per gram of sediment (black bars), per gram clay (gray bars), and per gram clay and silt (white bars) with percent clay (dashed line) and percent silt and clay (solid line) of sediment samples.

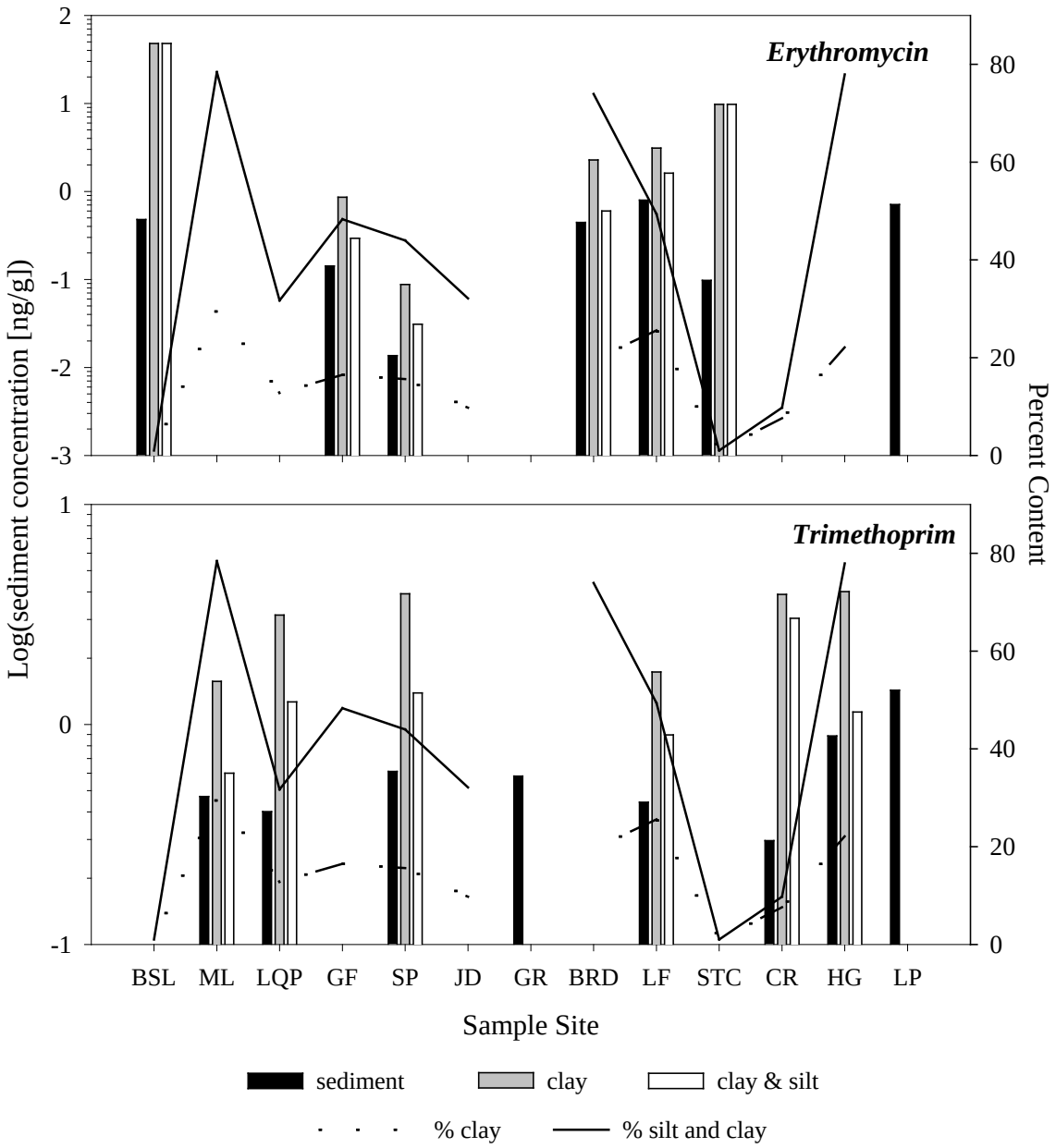

Figure S1. Continued

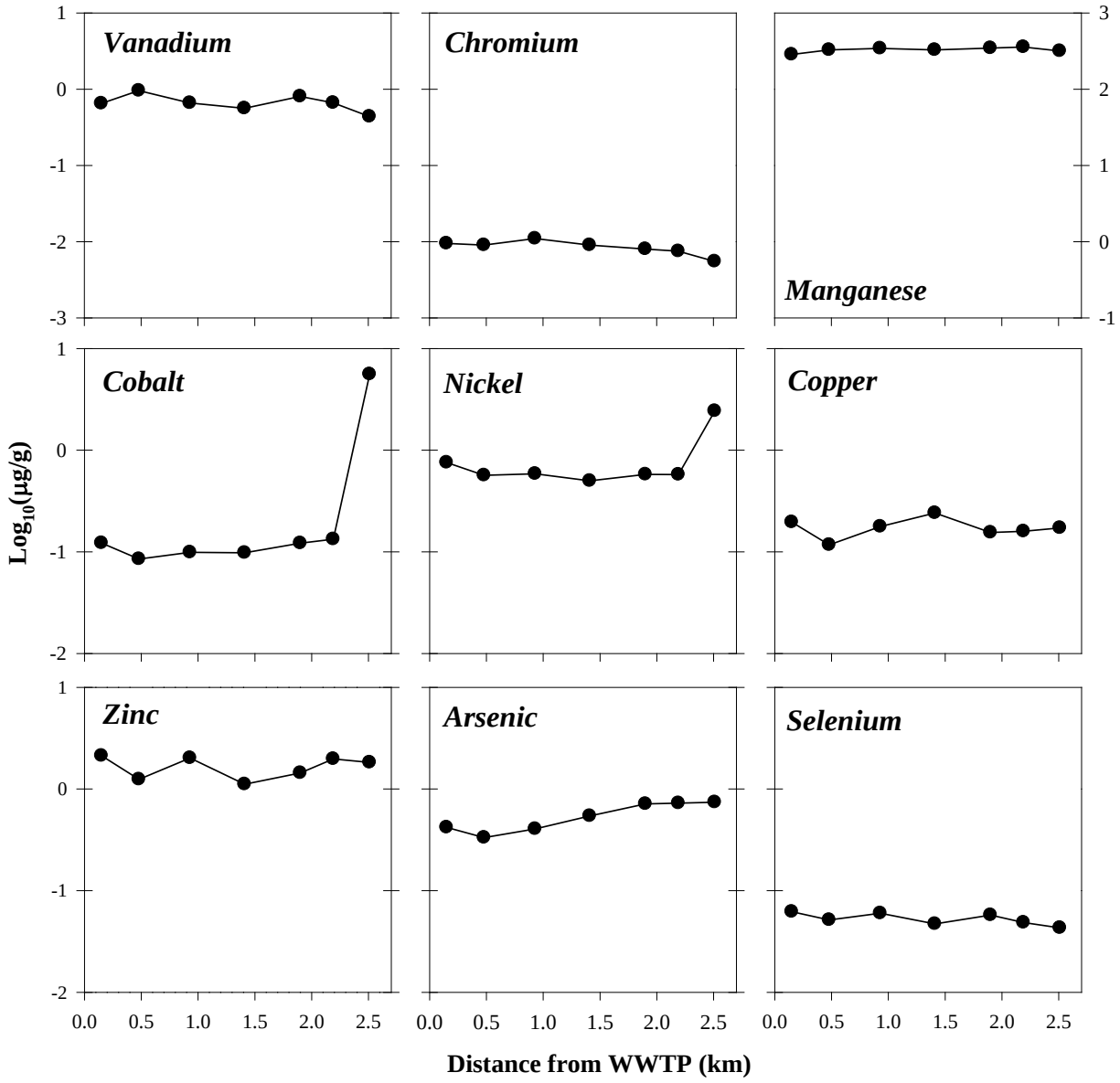

Figure S2. Concentration of metals in Lake Winona surface sediment as a function of distance from the discharge of wastewater treatment plant (km). Data are also provided in Table S15.

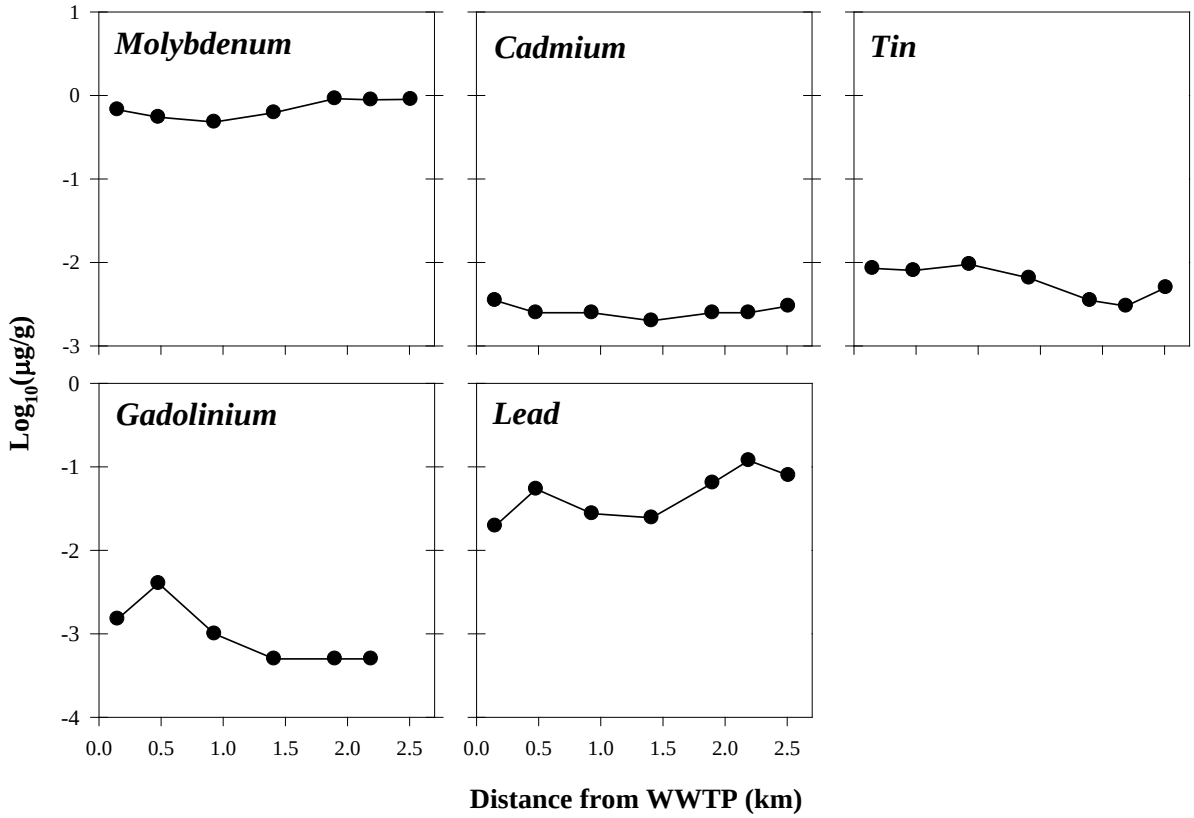

Figure S2. Continued.

Table S15. Concentration of metals in Lake Winona surface sediment as a function of distance from the discharge of wastewater treatment plant corresponding to Figure S2.

	Log_{10} Transformed Metal Concentration in Sediment [µg/g]						
	Distance from WWTP (km)						
Metal	*0.15*	*0.48*	*0.93*	*1.41*	*1.9*	*2.19*	*2.51*
V	-0.19	-0.02	-0.18	-0.25	-0.09	-0.18	-0.36
Cr	-2.02	-2.05	-1.96	-2.05	-2.10	-2.12	-2.26
Mn	2.45	2.52	2.54	2.52	2.54	2.55	2.50
Co	-0.91	-1.07	-1.00	-1.01	-0.92	-0.88	0.75
Ni	-0.12	-0.25	-0.23	-0.30	-0.24	-0.24	0.39
Cu	-0.71	-0.93	-0.75	-0.62	-0.81	-0.80	-0.76
Zn	0.33	0.09	0.30	0.05	0.16	0.30	0.26
As	-0.38	-0.48	-0.39	-0.26	-0.15	-0.14	-0.13
Se	-1.21	-1.29	-1.22	-1.33	-1.24	-1.31	-1.37
Mo	-0.17	-0.26	-0.32	-0.21	-0.04	-0.05	-0.05
Cd	-2.46	-2.60	-2.60	-2.70	-2.60	-2.60	-2.52
Sn	-2.07	-2.10	-2.02	-2.19	-2.46	-2.52	-2.30
Gd	-2.82	-2.40	-3.00	-3.30	-3.30	-3.30	n.d.[a]
Pb	-1.71	-1.27	-1.56	-1.61	-1.19	-0.92	-1.10

[a] non-detect

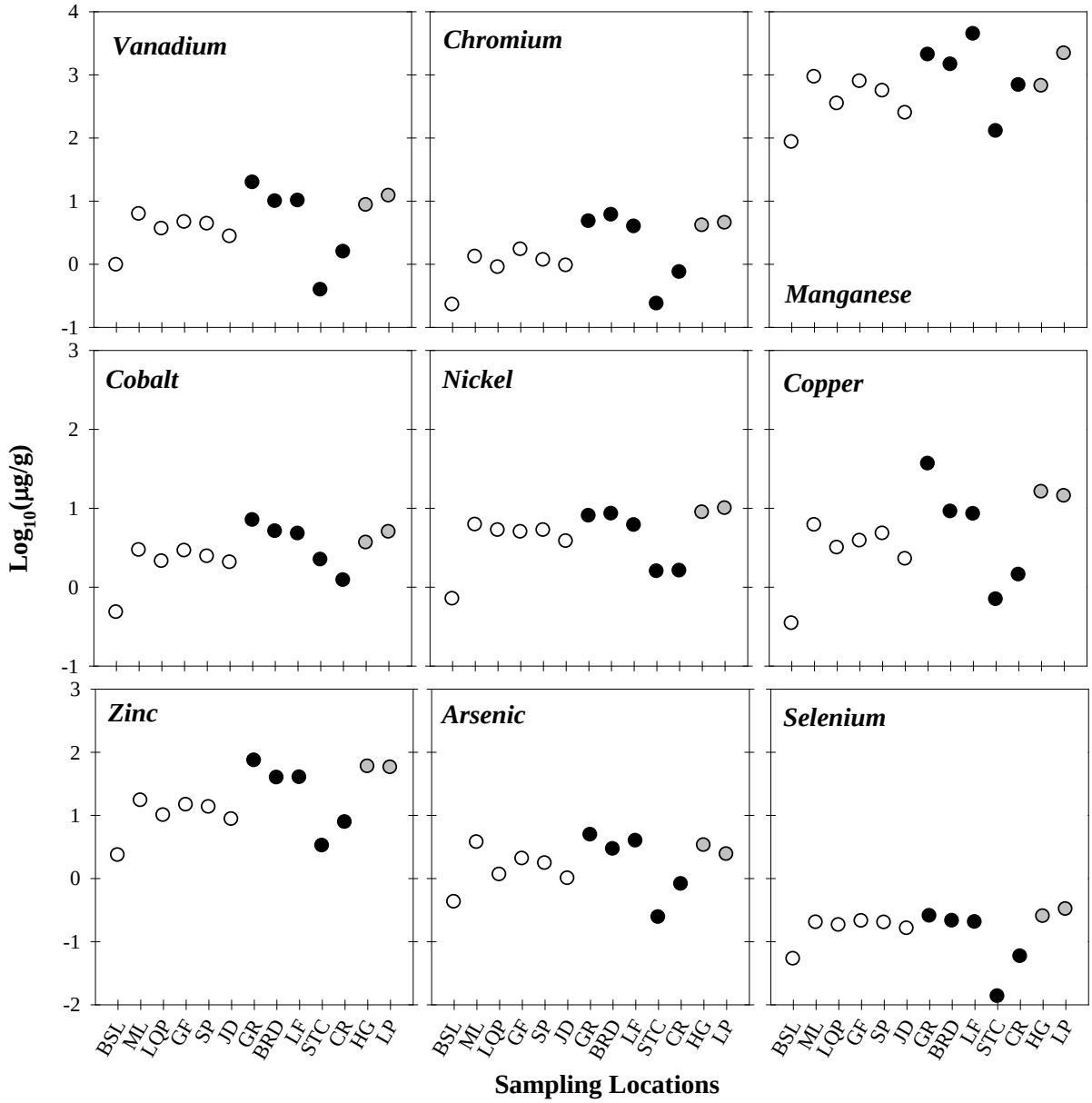

Figure S3. Metal concentration in river sediment from the Minnesota River (white circle), Mississippi River (black circle), and after they converge (grey circle). Data also provided in Table S16.

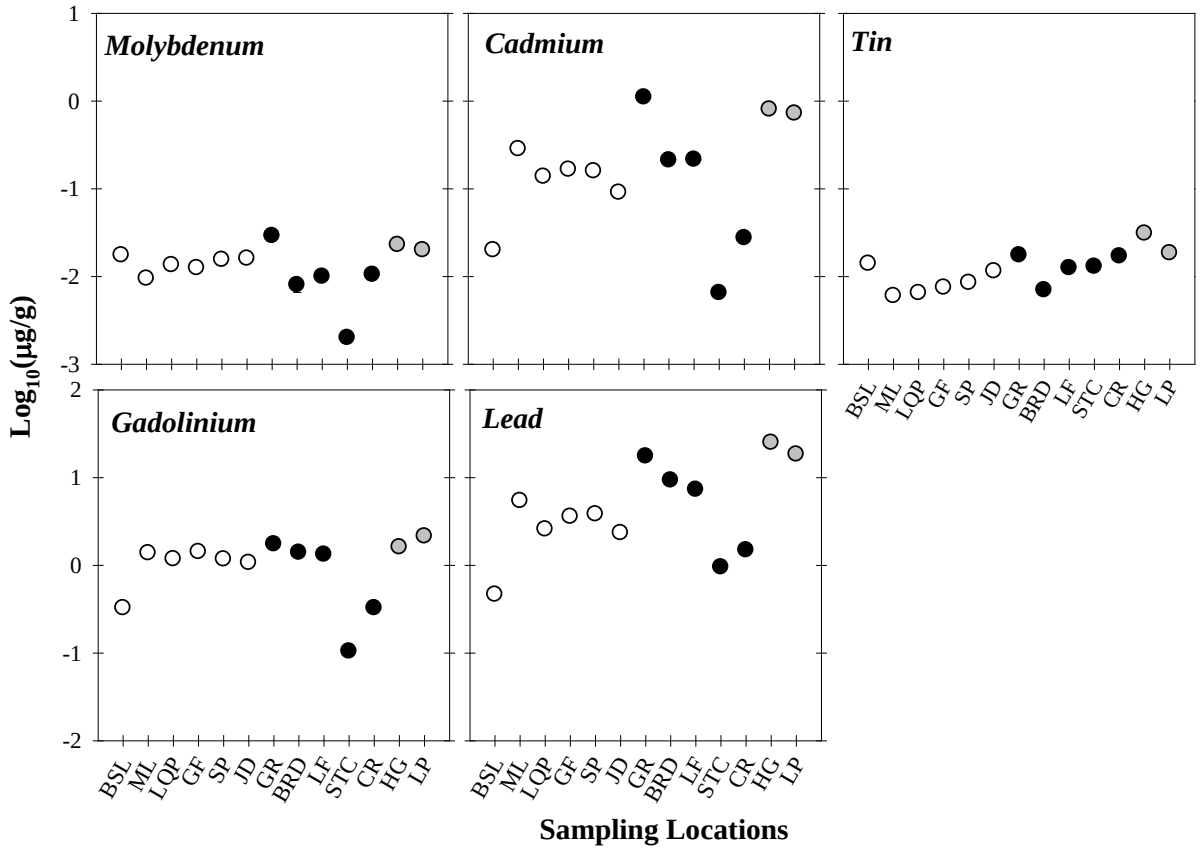

Figure S3. Continued.

Table S16. Concentration of metals in river surface sediment corresponding to Figure S3.

Sampling Location	**Log_{10} Transformed Metal Concentration in River Sediment [µg/g]**													
	V	*Cr*	*Mn*	*Co*	*Ni*	*Cu*	*Zn*	*As*	*Se*	*Mo*	*Cd*	*Sn*	*Gd*	*Pb*
BSL	-0.012	-0.640	1.933	-0.320	-0.150	-0.460	0.367	-0.374	-1.276	-1.757	-1.699	-1.854	-0.487	-0.333
ML	0.793	0.118	2.967	0.468	0.792	0.788	1.233	0.572	-0.699	-2.022	-0.547	-2.222	0.139	0.734
LQP	0.562	-0.049	2.543	0.327	0.720	0.499	0.999	0.059	-0.741	-1.870	-0.860	-2.187	0.070	0.411
GF	0.667	0.233	2.896	0.461	0.699	0.586	1.164	0.311	-0.677	-1.903	-0.781	-2.125	0.153	0.555
SP	0.639	0.067	2.747	0.388	0.721	0.680	1.130	0.239	-0.700	-1.810	-0.800	-2.071	0.068	0.582
JD	0.439	-0.021	2.397	0.312	0.581	0.357	0.937	0.000	-0.795	-1.796	-1.043	-1.939	0.029	0.368
GR	1.293	0.680	3.318	0.849	0.902	1.563	1.866	0.688	-0.596	-1.538	0.043	-1.757	0.241	1.243
BRD	0.997	0.781	3.164	0.707	0.930	0.957	1.593	0.464	-0.673	-2.097	-0.676	-2.155	0.146	0.969
LF	1.007	0.597	3.646	0.678	0.786	0.926	1.600	0.593	-0.693	-2.000	-0.668	-1.903	0.124	0.862
STC	-0.406	-0.625	2.108	0.347	0.199	-0.156	0.517	-0.616	-1.870	-2.699	-2.187	-1.886	-0.981	-0.020
CR	0.197	-0.126	2.836	0.088	0.206	0.158	0.888	-0.091	-1.237	-1.979	-1.561	-1.770	-0.487	0.173
HG	0.937	0.613	2.825	0.564	0.948	1.207	1.771	0.525	-0.600	-1.638	-0.096	-1.509	0.206	1.399
LP	1.083	0.653	3.340	0.699	0.999	1.154	1.755	0.381	-0.489	-1.699	-0.141	-1.733	0.332	1.266

Table S17. P-values for Tukey tests comparing metal concentration in river sediments.

	MS-MN	Both[a] -MN	Both-MS
V	0.86	0.17	0.33
Cr	0.21	0.023*	0.30
Mn	0.076	0.14	0.96
Co	0.082	0.075	0.82
Ni	0.95	0.087	0.15
Cu	0.41	0.036*	0.25
Zn	0.16	5.4×10^{-3}*	0.13
As	0.90	0.35	0.55
Se	0.41	0.40	0.088
Mo	0.17	0.41	0.040*
Cd	0.97	0.049*	0.040*
Sn	0.025*	5.0×10^{-5}*	9.9×10^{-3}*
Gd	0.44	0.38	0.088
Pb	0.31	1.4×10^{-3}*	0.023*

[a] Both = Lake Pepin and Hastings sites
*significant correlation

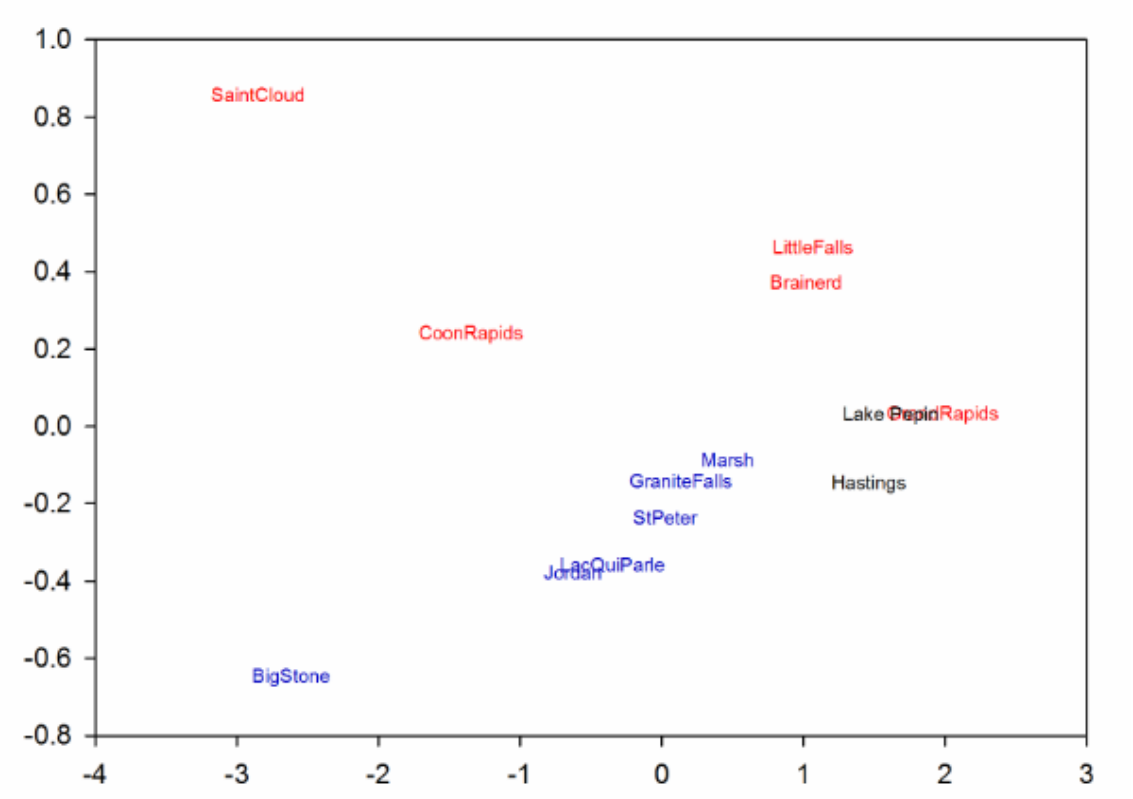

Figure S4. PCoA visualization of differences between samples based on heavy metal concentrations. Samples in red are those collected from the Mississippi River, blue are those collected from the Minnesota River, and black are those collected after the confluence of the two rivers.

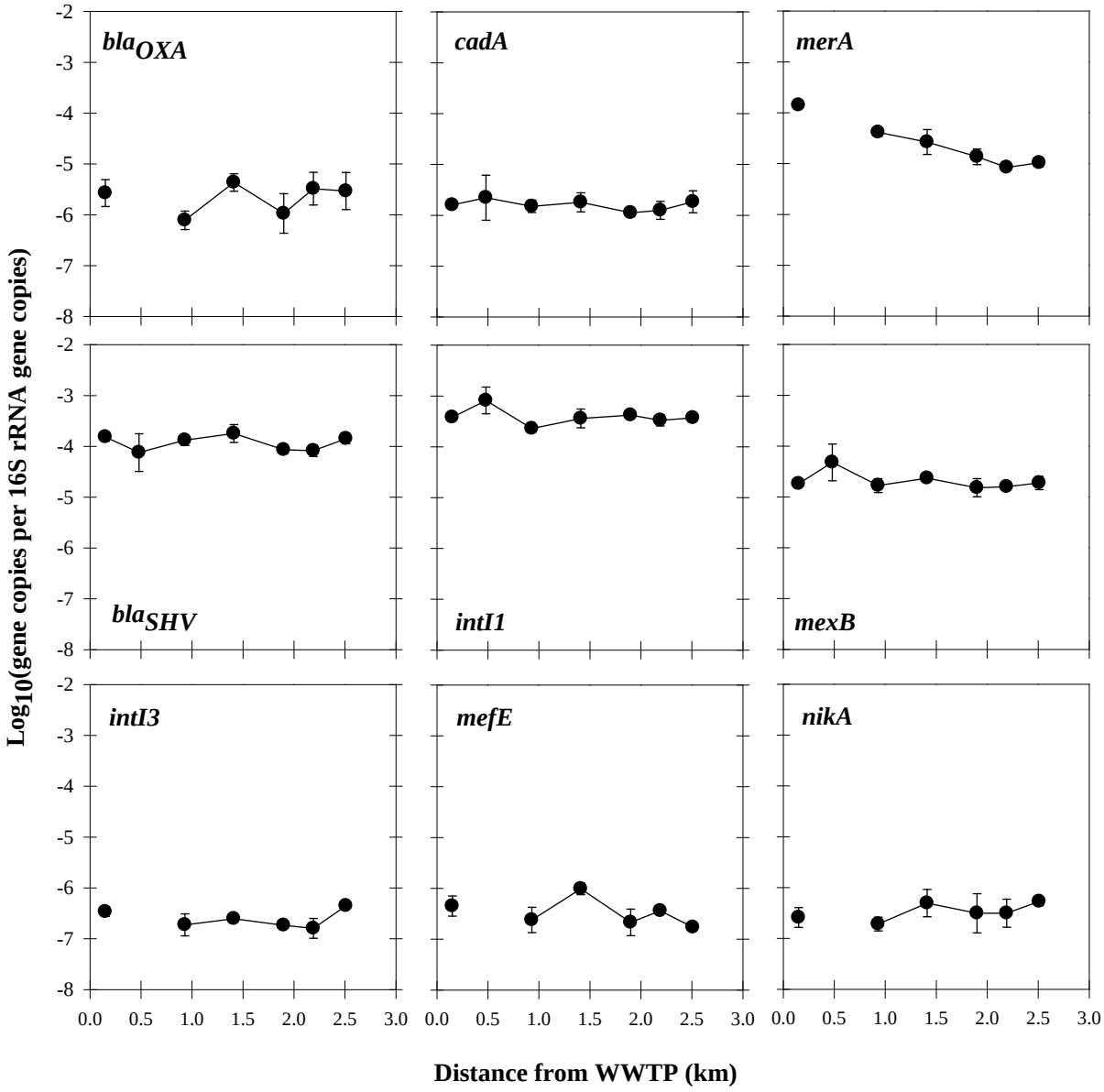

Figure S5. Concentrations of resistance genes that were quantified in more than half of the Lake Winona surface sediment samples as a function of distance from the wastewater treatment plant (WWTP) outfall. Data also provided in Table S18.

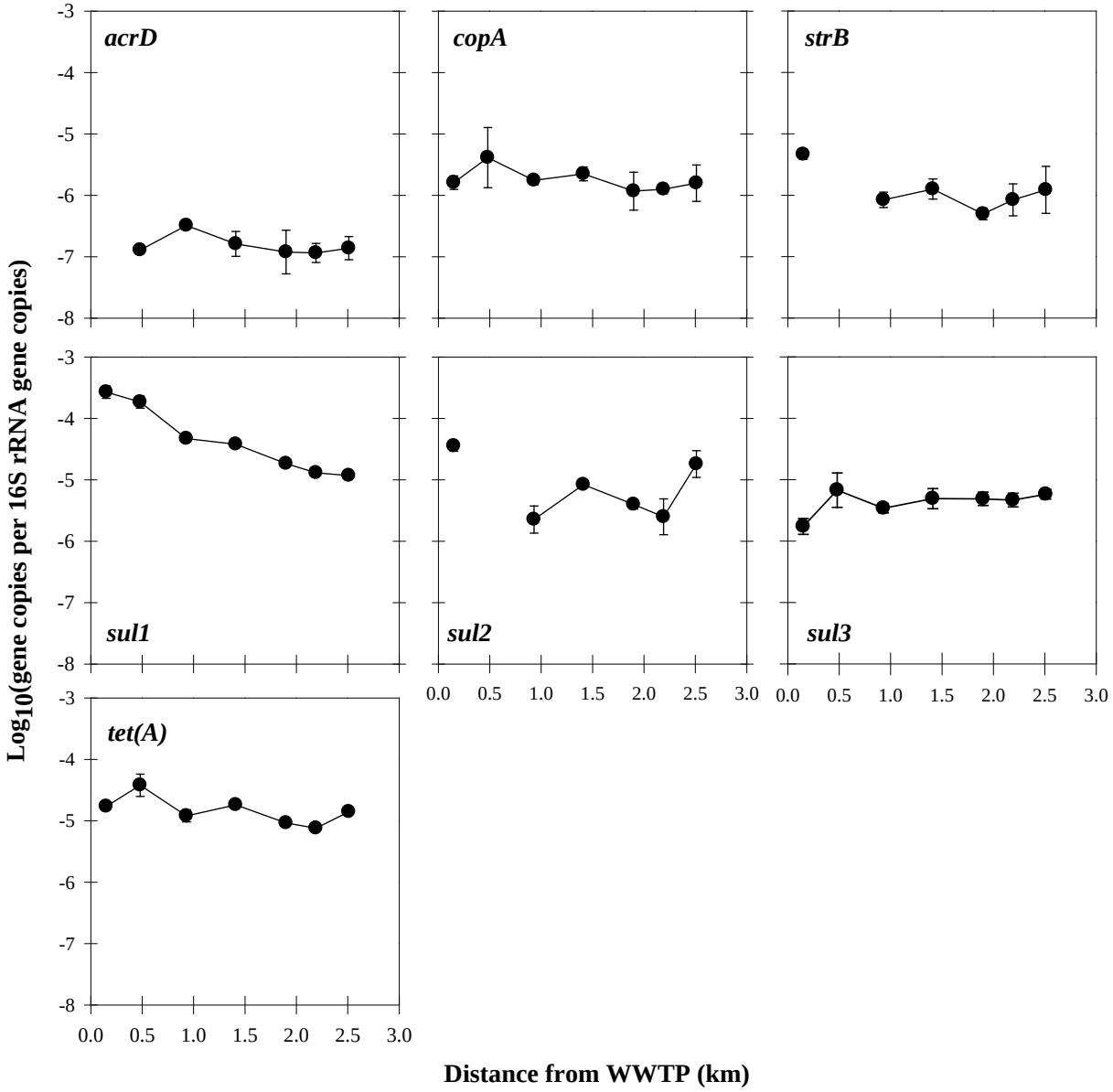

Figure S5. Continued.

Table S18. Concentrations of genes that were quantified in more than half of the Lake Winona surface sediment samples as a function of distance from the wastewater treatment plant (WWTP) outfall. Data also provided in Figure 5 and S5.

	Gene Concentrations in Lake Winona Surface Sediment [gene copies per 16S rRNA copies]													
	Distance from WWTP													
	0.15 km		*0.48 km*		*0.93 km*		*1.41 km*		*1.9 km*		*2.19 km*		*2.51 km*	
Gene	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
16S rRNA[a]	10.76	0.10	10.12	0.53	10.84	0.03	10.85	0.07	10.93	0.08	10.87	0.06	10.79	0.05
acrD	-6.89	–[b]	n.d.[c]		-6.49	–	-6.79	0.20	-6.92	0.36	-6.94	0.16	-6.86	0.19
bla$_{OXA}$	-5.57	0.26	n.d.		-6.11	0.18	-5.36	0.17	-5.97	0.39	-5.48	0.32	-5.53	0.36
bla$_{SHV}$	-3.81	0.08	-4.12	0.37	-3.88	0.11	-3.75	0.18	-4.07	0.07	-4.08	0.11	-3.85	0.10
cadA	-5.80	0.04	-5.65	0.44	-5.82	0.12	-5.74	0.19	-5.95	0.08	-5.90	0.18	-5.73	0.22
copA	-5.79	0.11	-5.39	0.49	-5.76	0.08	-5.65	0.11	-5.93	0.31	-5.90	0.07	-5.80	0.30
intI1	-3.42	0.03	-3.09	0.26	-3.64	0.07	-3.44	0.18	-3.38	0.05	-3.48	0.11	-3.43	0.02
intI3	-6.47	0.09	-6.49	–	-6.77	0.15	-6.70	0.04	-6.69	0.47	n.d.		n.d.	
mefE	-6.35	0.20	n.d.		-6.62	0.25	-6.01	0.11	-6.67	0.26	-6.44	0.03	-6.77	–
merA	-3.85	0.06	n.d.		-4.38	0.08	-4.57	0.24	-4.86	0.16	-5.07	0.04	-4.98	0.08
mexB	-4.74	0.02	-4.32	0.36	-4.77	0.14	-4.63	0.05	-4.82	0.18	-4.80	0.04	-4.72	0.13
nikA	-6.59	0.20	n.d.		-6.72	0.13	-6.31	0.27	-6.51	0.38	-6.50	0.28	-6.27	0.08
strB	-5.34	0.08	n.d.		-6.08	0.13	-5.90	0.16	-6.31	0.10	-6.08	0.26	-5.91	0.38
sul1	-3.57	0.10	-3.73	0.10	-4.33	0.04	-4.42	0.04	-4.74	0.02	-4.89	0.04	-4.93	0.05
sul2	-4.45	0.09	n.d.		-5.65	0.22	-5.08	0.05	-5.40	0.08	-5.60	0.29	-4.74	0.22
sul3	-5.76	0.13	-5.17	0.28	-5.47	0.08	-5.31	0.16	-5.31	0.11	-5.33	0.11	-5.24	0.08
tet(A)	-4.77	0.05	-4.42	0.18	-4.92	0.10	-4.74	–	-5.04	–	-5.12	0.02	-4.85	0.04

[a] units in gene copies per gram sediment
[b] only one replicate was detected above LOQ
[c] non-detect

Table S19. Concentrations of genes that were quantified in more than half of the river sediment samples. Data also provided in Figure 6 and 7.

		Gene Concentration in River Surface Sediment [gene copies per 16S rRNA copies]									
		16S rRNA[a]	*blaSHV*	*cadA*	*floR*	*intI1*	*mexB*	*nikA*	*sul1*	*sul3*	*tet(A)*
BSL	*AVG*	2.06E+09	-2.63	-4.26	-4.28	-2.48	-3.44	-5.64	n.d.	-4.40	-4.00
	SD	2.75E+08	0.07	0.10	0.14	0.08	0.21	0.20		0.01	0.05
ML	*AVG*	1.07E+10	-3.04	-4.85	-5.03	-2.91	-3.97	-5.85	-5.06	-5.24	-4.36
	SD	6.71E+08	0.08	0.10	0.44	0.15	0.13	0.12	0.16	0.06	0.10
LQP	*AVG*	5.10E+09	-2.79	-4.65	-4.85	-2.92	-3.78	-5.75	n.d.	-5.11	-4.41
	SD	4.21E+08	0.05	0.09	0.11	0.08	0.15	0.25		0.19	0.18
GF	*AVG*	7.29E+09	-3.01	-4.90	-4.77	-2.97	-3.90	-6.17	-5.20	-5.28	-4.48
	SD	3.66E+08	0.02	0.06	0.18	0.02	0.15	0.02	0.25	0.15	0.13
SP	*AVG*	5.77E+09	-3.04	-5.01	-4.77	-3.07	-3.93	-5.96	-4.94	-5.49	-4.48
	SD	1.42E+09	0.17	0.11	0.30	0.09	0.27	–	0.37	0.34	0.06
JD	*AVG*	1.35E+09	-2.58	-4.53	-4.16	-2.60	-3.49	-5.27	-4.78	-4.73	-4.18
	SD	2.50E+08	0.11	0.01	0.06	0.10	0.30	0.43	–	0.18	0.10
GR	*AVG*	3.92E+09	-2.84	-4.86	-4.59	-2.76	-3.82	-5.73	n.d.	-5.28	-4.00
	SD	6.87E+08	0.11	0.03	0.53	0.08	0.41	–		0.41	0.10
BRD	*AVG*	7.26E+09	-2.98	-4.98	-4.80	-3.01	-4.00	-5.48	-5.09	-5.64	-4.48
	SD	9.57E+08	0.11	0.22	0.13	0.10	0.05	0.28	0.12	0.18	0.28
LF	*AVG*	1.52E+10	-3.78	-5.32	-5.05	-3.39	-4.13	n.d.	-5.47	-5.51	-5.18
	SD	2.64E+09	0.43	0.21	0.11	0.24	0.30		0.20	–	0.10
STC	*AVG*	7.22E+08	-2.26	-4.14	-3.97	-2.54	-3.67	-5.37	n.d.	-4.97	-4.00
	SD	1.67E+08	0.11	0.10	–	0.13	0.42	0.15		0.11	–
CR	*AVG*	6.18E+09	-2.96	-4.68	-4.98	-2.84	-3.96	-5.96	-4.84	-5.15	-4.76
	SD	3.26E+08	0.04	0.05	0.41	0.05	0.03	0.42	0.14	0.28	0.04
HG	*AVG*	2.67E+09	-2.51	-4.58	n.d.[c]	-2.65	-3.55	-5.69	-4.39	-5.54	-3.99
	SD	4.89E+08	0.05	0.08		0.13	0.26	0.09	0.08	0.30	0.12
LP	*AVG*	3.77E+10	-5.20	-5.90	n.d.	-3.33	n.d.	n.d.	-5.81	n.d.	n.d.
	SD	2.13E+10	–[b]	–		0.12			0.11		

[a] units in gene copies per gram sediment; [b] only one replicate was detected above LOQ; [c] non-detect

Table S20. P-values for Tukey tests comparing resistance gene concentrations in river sediments. Single asterisks signify statistically significant differences where the first sample listed in the pair is significantly greater. Double asterisks signify statistically significant differences where the second sample listed is significantly greater.

Target Gene	MN-MS	MN-Both	MS-Both
bla$_{SHV}$	0.93	1.7×10^{-3}*	4.6×10^{-3}*
cadA	0.70	6.2×10^{-3}*	0.033*
floR	0.59	0.020*	0.12
intI1	0.69	0.46	0.83
mexB	0.65	2.8×10^{-3}*	0.019*
nikA	0.92	9.1×10^{-4}**	1.6×10^{-3}**
sul1	0.88	0.96	1
sul3	0.059	6.3×10^{-4}*	0.069
tet(A)	0.42	6.1×10^{-3}*	0.070

Table S21. P-values generated from Pearson correlations between metals and antibiotics [log_{10}(ng/g)] with target genes [log_{10}(gene copies per 16S rRNA gene copies)] in Lake Winona. Shaded regions indicate p-values less than 0.05.

	acrD	***bla$_{OXA}$***	***bla$_{SHV}$***	***cadA***	***copA***	***intI1***	***intI3***	***mefE***	***merA***	***mexB***	***nikA***	***strB***	***sul1***	***sul2***	***sul3***	***tetA***
V	0.767	0.263	0.061	0.869	0.399	0.184	0.075	0.974	0.748	0.308	0.139	0.581	0.276	0.329	0.842	0.509
Cr	0.183	0.406	0.860	0.988	0.509	0.787	0.350	0.410	0.127	0.751	0.093	0.761	0.116	0.615	0.299	0.620
Mn	0.977	0.535	0.203	0.314	0.695	0.703	0.055	0.716	0.100	0.742	0.860	***0.010***	0.124	***0.004***	0.155	0.286
Co	0.752	0.644	0.579	0.663	0.624	0.833	0.098	0.272	0.424	0.698	0.208	0.947	0.260	0.409	0.586	0.807
Ni	0.820	0.704	0.538	0.582	0.688	0.887	0.051	0.252	0.659	0.750	0.328	0.717	0.471	0.277	0.818	0.945
Cu	0.503	0.308	***0.008***	0.819	0.464	0.121	0.547	***0.036***	0.321	0.282	0.545	0.307	0.813	0.420	0.304	0.575
Zn	0.558	0.627	0.852	0.412	0.186	0.181	0.846	0.295	0.642	0.146	0.224	0.519	0.901	0.891	0.140	0.246
As	0.334	0.586	0.952	0.174	***0.047***	0.470	0.945	0.537	***0.013***	0.116	0.180	0.266	***0.006***	0.763	0.576	0.051
Se	0.332	0.158	0.899	0.425	0.780	0.727	0.545	0.910	0.120	0.654	***0.022***	0.698	0.187	0.893	0.079	0.904
Mo	0.095	0.546	0.636	0.240	0.094	0.991	0.821	0.505	0.167	0.265	0.304	0.658	0.112	0.803	0.722	0.171
Cd	0.811	0.949	0.840	0.990	0.597	0.997	0.231	0.388	0.407	0.657	0.659	0.178	0.493	0.173	0.159	0.997
Sn	0.097	0.717	0.295	0.121	0.136	0.938	0.493	0.660	***0.040***	0.323	0.489	0.234	***0.041***	0.500	0.404	0.094
Gd	0.913	0.730	0.667	0.085	0.066	0.164	0.222	0.847	***0.029***	0.067	0.220	0.139	***0.033***	0.312	0.997	***0.040***
Pb	0.104	0.868	0.087	0.551	0.567	0.676	0.672	0.299	***0.014***	0.865	0.568	0.220	0.094	0.452	0.122	0.397
SPD	0.604	0.925	0.785	0.604	0.430	0.235	0.698	0.646	0.054	0.378	0.414	0.076	***0.006***	0.240	0.219	0.222
SMX	0.421	0.618	0.465	0.075	0.148	0.714	0.345	0.515	0.076	0.253	0.653	***0.025***	***0.033***	0.272	0.342	0.099
SMZ	***0.014***	0.127	0.994	0.945	0.703	***0.012***	0.424	0.563	0.964	0.708	0.296	0.356	0.796	0.152	0.791	0.732
SCP	0.179	0.765	0.171	0.159	0.105	0.161	0.716	0.578	0.312	0.323	0.915	0.129	0.351	0.925	0.503	0.325
TMP	0.136	0.557	0.113	0.823	0.859	0.820	***0.026***	0.232	0.115	0.788	0.836	***0.014***	0.244	***0.046***	***0.025***	0.812
EMC	0.155	0.720	0.389	0.377	0.436	0.415	0.973	0.648	***0.026***	0.339	0.227	0.130	0.306	0.579	< ***0.001***	0.686
CFC	0.588	0.895	0.760	0.763	0.660	0.819	0.855	0.457	***0.021***	0.744	0.265	0.053	***0.012***	0.400	***0.034***	0.468
EFC	0.340	0.677	0.582	0.321	0.695	0.217	0.390	0.258	0.994	0.982	0.869	0.868	0.842	0.889	0.944	0.792
OFC	0.468	0.909	0.625	0.956	0.863	0.985	0.906	0.405	***0.018***	0.970	0.269	0.066	***0.030***	0.419	***0.012***	0.639

SPD = sulfapyridine; SMX = sulfamethoxazole; SMZ = sulfamethazine; SCP = sulfachloropyridazine; TMP = trimethoprim; EMC = erythromycin; CFC = ciprofloxacin; EFC = enrofloxacin; OFC = ofloxacin

Table S22. Pearson coefficients generated from Pearson correlations between metals and antibiotics [$\log_{10}$(ng/g) with target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in Lake Winona. Shaded regions indicate p-values less than 0.05.

	acrD	***bla*$_{OXA}$**	***bla*$_{SHV}$**	***cadA***	***copA***	***intI1***	***intI3***	***mefE***	***merA***	***mexB***	***nikA***	***strB***	***sul1***	***sul2***	***sul3***	***tetA***
V	-0.157	-0.546	-0.733	-0.078	0.381	0.567	-0.767	0.017	0.169	0.453	-0.678	-0.287	0.480	-0.486	0.093	0.303
Cr	0.626	-0.421	0.083	-0.007	0.303	-0.126	-0.467	0.418	0.693	0.148	-0.739	0.161	0.647	-0.263	-0.460	0.230
Mn	0.015	-0.321	-0.548	-0.447	-0.183	-0.177	-0.802	-0.192	-0.729	-0.154	-0.094	***-0.919***	-0.637	***-0.950***	0.600	-0.471
Co	-0.167	0.242	0.256	0.202	-0.227	-0.099	0.732	-0.537	-0.406	-0.181	0.600	0.035	-0.494	0.418	0.252	-0.114
Ni	-0.121	0.200	0.283	0.255	-0.187	-0.067	0.810	-0.556	-0.232	-0.149	0.486	0.191	-0.329	0.532	0.108	-0.032
Cu	0.345	0.504	***0.887***	-0.107	-0.334	-0.640	0.312	***0.841***	0.493	-0.474	0.314	0.505	-0.111	0.410	-0.456	-0.259
Zn	0.304	-0.254	0.087	-0.371	-0.566	-0.571	0.103	-0.516	0.243	-0.609	-0.583	0.333	-0.058	0.073	-0.617	-0.507
As	-0.481	0.284	-0.028	-0.578	***-0.761***	-0.330	-0.036	-0.320	***-0.906***	-0.647	0.630	-0.543	***-0.897***	-0.159	0.258	-0.753
Se	0.483	-0.655	-0.060	-0.362	-0.131	-0.163	-0.314	-0.060	0.702	-0.208	***-0.875***	0.204	0.564	-0.071	-0.702	-0.057
Mo	-0.737	0.313	-0.220	-0.512	-0.678	-0.006	0.120	-0.344	-0.644	-0.489	0.508	-0.232	-0.653	0.132	0.166	-0.581
Cd	-0.127	-0.034	0.095	0.006	-0.245	0.002	0.576	-0.436	0.420	-0.207	-0.231	0.632	0.314	0.638	-0.594	-0.002
Sn	0.734	-0.191	0.463	0.641	0.622	0.036	0.353	0.231	***0.832***	0.440	-0.356	0.573	***0.774***	0.347	-0.378	0.679
Gd	0.068	-0.214	-0.226	0.751	0.782	0.649	0.664	-0.120	***0.915***	0.781	-0.666	0.757	***0.848***	0.573	0.002	***0.832***
Pb	-0.723	0.088	-0.689	-0.275	-0.264	0.195	-0.222	-0.512	***-0.904***	-0.080	0.297	-0.588	-0.678	-0.384	0.639	-0.383
SPD	-0.271	0.050	-0.128	0.240	0.358	0.517	0.204	0.240	0.803	0.397	-0.414	0.766	***0.895***	0.568	-0.532	0.529
SMX	-0.409	-0.260	-0.333	-0.707	-0.607	-0.171	-0.472	-0.336	-0.765	-0.500	0.236	***-0.867***	***-0.793***	-0.537	0.425	-0.671
SMZ	***-0.785***	0.693	-0.357	0.443	0.497	***0.916***	0.406	0.300	0.024	0.673	0.514	0.462	0.433	0.662	0.205	0.613
SCP	-0.708	-0.169	-0.584	-0.501	-0.338	0.426	-0.172	-0.310	-0.255	-0.133	0.038	-0.269	-0.123	0.017	0.102	-0.220
TMP	0.146	-0.274	-0.151	-0.251	-0.122	-0.002	***-0.125***	-0.014	0.690	-0.130	-0.766	***0.517***	0.648	***0.180***	***-0.792***	-0.007
EMC	0.568	0.314	0.546	0.312	0.143	-0.318	0.279	0.432	***0.700***	-0.002	-0.297	0.811	0.527	0.386	***-0.694***	0.188
CFC	0.282	0.070	0.143	0.141	0.205	0.107	0.097	0.380	***0.879***	0.152	-0.544	0.806	***0.863***	0.426	***-0.792***	0.331
EFC	-0.555	0.219	-0.290	-0.319	-0.023	0.378	-0.434	0.551	0.004	0.091	0.088	-0.088	0.138	-0.074	0.009	-0.008
OFC	0.372	0.061	0.227	0.026	0.081	-0.009	0.063	0.422	***0.887***	0.017	-0.540	0.783	***0.803***	0.410	***-0.864***	0.218

SPD = sulfapyridine; SMX = sulfamethoxazole; SMZ = sulfamethazine; SCP = sulfachloropyridazine; TMP = trimethoprim; EMC = erythromycin; CFC = ciprofloxacin; EFC = enrofloxacin; OFC = ofloxacin

Table S23. P-values generated from Pearson correlations among target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in Lake Winona. Shaded regions indicate p-values less than 0.05.

	acrD	***blaOXA***	***blaSHV***	***cadA***	***copA***	***intI1***	***intI3***	***mefE***	***merA***	***mexB***	***nikA***	***strB***	***sul1***	***sul2***	***sul3***
blaOXA	0.349	--	--	--	--	--	--	--	--	--	--	--	--	--	--
blaSHV	0.297	0.517	--	--	--	--	--	--	--	--	--	--	--	--	--
cadA	0.859	0.324	0.587	--	--	--	--	--	--	--	--	--	--	--	--
copA	0.872	0.545	0.871	***0.010***	--	--	--	--	--	--	--	--	--	--	--
intI1	0.194	0.351	0.241	0.242	0.102	--	--	--	--	--	--	--	--	--	--
intI3	0.900	0.424	0.158	0.074	0.495	0.508	--	--	--	--	--	--	--	--	--
mefE	0.976	0.208	0.348	0.557	0.192	0.823	0.868	--	--	--	--	--	--	--	--
merA	***0.044***	0.840	0.231	0.572	0.386	0.774	0.623	0.493	--	--	--	--	--	--	--
mexB	0.763	0.169	0.593	***0.022***	***<0.001***	***0.022***	0.321	0.119	0.651	--	--	--	--	--	--
nikA	0.295	0.113	0.601	0.341	0.657	0.219	0.308	0.703	0.324	0.208	--	--	--	--	--
strB	0.787	0.366	0.185	0.255	0.437	0.768	0.180	0.451	0.075	0.407	0.957	--	--	--	--
sul1	0.638	0.964	0.843	0.265	0.144	0.348	0.660	0.417	***<0.001***	0.197	0.348	0.054	--	--	--
sul2	0.620	0.317	0.196	0.199	0.579	0.274	***0.016***	0.755	0.324	0.341	0.457	***0.042***	0.299	--	--
sul3	0.096	0.849	0.309	0.588	0.458	0.331	0.774	0.687	***0.010***	0.324	0.188	0.069	0.269	0.387	--
tetA	0.991	0.498	0.907	***0.006***	***0.001***	0.068	0.117	0.337	0.169	***0.003***	0.575	0.126	0.065	0.094	0.720

Table S24. Pearson coefficients generated from Pearson correlations among target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in Lake Winona. Shaded regions indicate p-values less than 0.05.

	acrD	***bla$_{OXA}$***	***bla$_{SHV}$***	***cadA***	***copA***	***intI1***	***intI3***	***mefE***	***merA***	***mexB***	***nikA***	***strB***	***sul1***	***sul2***	***sul3***
bla$_{OXA}$	-0.539	--	--	--	--	--	--	--	--	--	--	--	--	--	--
bla$_{SHV}$	0.514	0.335	--	--	--	--	--	--	--	--	--	--	--	--	--
cadA	0.095	0.490	0.251	--	--	--	--	--	--	--	--	--	--	--	--
copA	0.086	0.313	-0.076	***0.873***	--	--	--	--	--	--	--	--	--	--	--
intI1	-0.615	0.467	-0.511	0.510	0.667	--	--	--	--	--	--	--	--	--	--
intI3	-0.079	0.407	0.655	0.769	0.351	0.341	--	--	--	--	--	--	--	--	--
mefE	-0.019	0.600	0.469	0.305	0.617	0.119	-0.088	--	--	--	--	--	--	--	--
merA	***0.889***	-0.107	0.576	0.294	0.437	-0.152	0.257	0.353	--	--	--	--	--	--	--
mexB	-0.160	0.642	-0.247	***0.826***	***0.968***	***0.825***	0.493	0.704	0.237	--	--	--	--	--	--
nikA	-0.590	0.712	0.273	0.475	0.233	0.589	0.504	0.201	-0.490	0.600	--	--	--	--	--
strB	0.168	0.454	0.624	0.553	0.396	0.156	0.631	0.385	0.768	0.420	-0.029	--	--	--	--
sul1	0.247	-0.024	0.093	0.489	0.613	0.420	0.231	0.412	***0.990***	0.554	-0.469	0.803	--	--	--
sul2	-0.303	0.496	0.613	0.610	0.289	0.535	***0.896***	0.165	0.490	0.475	0.381	***0.828***	0.512	--	--
sul3	-0.734	0.101	-0.452	0.251	0.339	0.434	-0.152	-0.212	***-0.918***	0.439	0.621	-0.777	-0.486	-0.437	--
tetA	0.006	0.349	0.055	***0.901***	***0.943***	0.719	0.706	0.479	0.642	***0.926***	0.292	0.694	0.726	0.738	0.167

Table S25. P-values generated from Pearson correlations among target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in river sediments. Shaded regions indicate p-values less than 0.05.

	bla$_{SHV}$	*cadA*	*floR*	*intI1*	*mexB*	*nikA*	*sul1*	*sul3*
cadA	***< 0.001***	--	--	--	--	--	--	--
floR	***0.002***	***0.003***	--	--	--	--	--	--
intI1	***0.001***	***< 0.001***	***0.002***	--	--	--	--	--
mexB	***0.001***	***0.001***	***0.001***	***< 0.001***	--	--	--	--
nikA	***0.016***	0.079	***0.018***	***0.049***	0.074	--	--	--
sul1	***0.001***	***0.000***	0.189	***0.002***	***0.016***	0.442	--	--
sul3	0.101	***0.006***	***0.022***	***0.011***	***0.010***	0.302	0.669	--
tetA	***< 0.001***	***0.006***	***0.007***	***< 0.001***	***0.002***	0.081	***0.018***	0.154

Table S26. Pearson coefficients generated from Pearson correlations among target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in river sediments. Shaded regions indicate p-values less than 0.05.

	bla$_{SHV}$	*cadA*	*floR*	*intI1*	*mexB*	*nikA*	*sul1*	*sul3*
cadA	***0.934***	--	--	--	--	--	--	--
floR	***0.820***	***0.802***	--	--	--	--	--	--
intI1	***0.814***	***0.912***	***0.818***	--	--	--	--	--
mexB	***0.814***	***0.827***	***0.849***	***0.890***	--	--	--	--
nikA	***0.700***	0.551	***0.723***	***0.605***	0.559	--	--	--
sul1	***0.898***	***0.918***	0.562	***0.884***	***0.807***	0.350	--	--
sul3	0.496	***0.742***	***0.678***	***0.701***	***0.705***	0.343	0.181	--
tetA	***0.884***	***0.742***	***0.761***	***0.876***	***0.801***	0.548	***0.797***	0.438

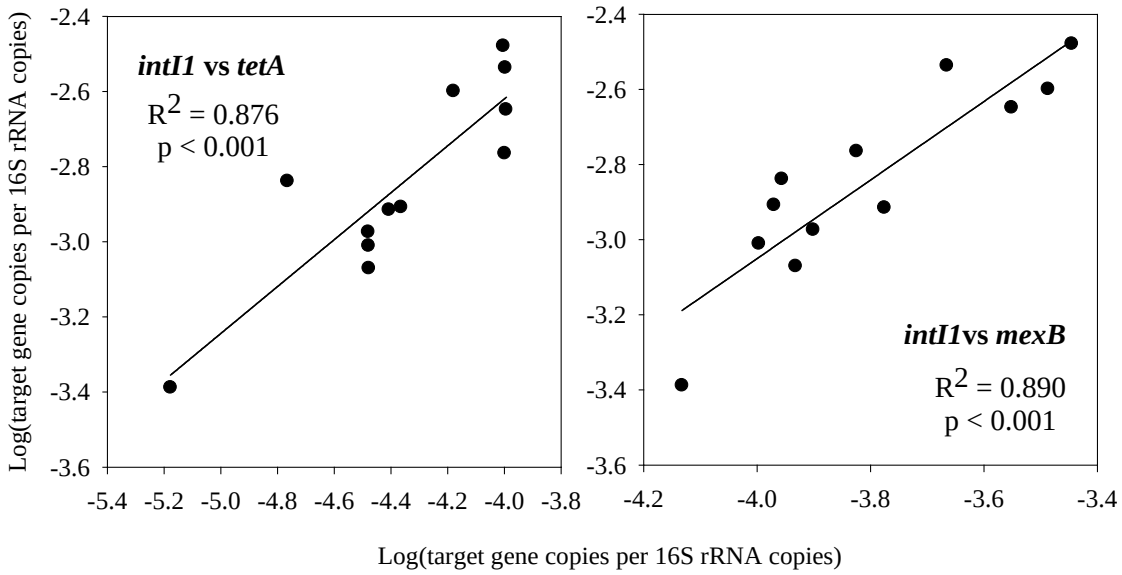

Figure S6. Representative Pearson correlations between target genes (log_{10}(gene copies per 16S rRNA gene copies)) that were significant (p-value < 0.05) in river surface sediments. Linear trendline, R^2 value, and p-value are displayed.

Appendix A: Methods

Texture Analysis

Approximately 45-50 g of oven dried sample (when available) were dispersed in 2.5% sodium hexametaphosphate (100 mL of 5% SHMP and 100 mL of distilled water) by shaking for 16 hours on a rotary benchtop shaker at 30 rpm. The resulting dispersed slurry was transferred completely into a 1000 mL settling column and filled to volume with 800 mL of distilled water. A weighted brass plunger was used to completely mix and distribute the particles throughout the column, at which point the beginning of settling time was recorded. A hydrometer reading (corrected by a factor of 0.36 for every °C above 20) was taken at 40 seconds, 4 hours, and 8 hours. For these samples, which contained appreciable organic matter that remained undigested (no pretreatment with H_2O_2 to remove organic matter), the 4 hour hydrometer reading was used to determine the clay fraction,[3] while the 40 second reading gave the sand fraction. The silt fraction was determined by difference.

Antibiotic Extraction Method

Antibiotics were extracted from the sediment using accelerated solvent extraction, and the analytes were detected and quantified by liquid chromatography tandem mass spectrometry with a Phenomenex Kinetex F5 column. A detailed description is given in Kerrigan et al 2018.[4] Extraction efficiencies of antibiotics were determined by spiking 100 ng of each antibiotic in a methanolic solution onto the sediment and measuring the recovered mass from the extraction process. In Lake Winona, triplicate spike and recovery analyses were performed on sediment that was deposited in Lake Winona pre-1900s. This sediment was collected via piston coring for the study described Kerrigan et al 2018.[4] Due the high variability in sediment composition amongst the river sediments, relative recovery of antibiotics was assessed at each sample site.

Method blanks were run every eight samples to monitor for carry over contamination during the extraction process. Method blanks consisted of Ottawa sand spiked with surrogates and internal standards and were processed in an identical manner to the river sediments. Limits of detection (LODs) for each antibiotic were 3× the peak area near the analyte retention time in method blank. Limits of quantification (LOQs) were 10× the peak area in method blank near the analyte retention time minus the mass determined in the method blank. Sediment concentrations, LODs, and LOQs were determined by internal standard dilution methodology and were recovery corrected.

Metal Quantification

Fourteen metals were quantified in the sediment samples: vanadium, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, molybdenum, cadmium, tin, gadolinium, and lead. Samples were freeze-dried and crushed into a fine powder (diameter < 0.15 mm) using a clean mortar and pestle. Samples were partially digested to limit quantification of metals that are loosely bound to the sediment and bioavailable to bacteria. This digestion used 0.5 g of dried and powdered sediment which was leached into 20 mL of 0.5 N HCl in Teflon vials at 80 °C for 30 minutes. Metals were quantified using a Thermo Scientific XSeries2 ICP-MS fitted with a hexapole collision/reaction cell. Unknowns were quantified by comparing intensities of the unknowns to a curve prepared by 4 multi-analyzed standards from SPEX industries that were diluted accordingly. Elements of mass less than 39 were analyzed at standard mass resolution with no reactive or collision gasses. Elements of mass 39 or greater were analyzed at standard mass resolution using Helium/Hydrogen collision reaction mode (CCT) with kinetic energy discrimination (KED). All elements had a dwell time of 15 ms with 30 sweeps; 5 replicates were used to determine means and standard deviations. An ESI PC3 FAST system with sample loops

was used for sample introduction and to reduce oxide formation and carryover between samples. ^{155}In was used as an internal standard to compensate for matrix effects and signal drift.

DNA Extraction and Purification

Prior to DNA extraction, samples were mixed with 500 μL of CLS-TS buffer (MP Biomedicals LLC; Solon, OH) and placed in Lysis Matrix E bead beating tubes (MP Biomedicals). Bacterial cells were lysed by placing each tube in a BIO 101 Thermo Savant Fast-Prep FP120 Cell Disruptor (Qbiogene, Inc., Carlsbad, CA) for 30 seconds. DNA was extracted and purified using a FastDNA Spin Kit for Soil (MP Biomedicals) following the manufacturer's instructions. Extracted DNA was stored at -20 °C.

Microfluidic qPCR

Microfluidic quantitative polymerase chain reaction (MF-qPCR) was used to quantify the 16S rRNA gene as well as 45 antibiotic resistance, metal resistance, and antibiotic resistance-associated genes (Table S4). Fluidigm Biomark Gene Expression 48.48 IFC or 192.24 gene expression chips (Fluidigm; South San Francisco, CA) were run according to the protocols developed by Fluidigm. An MX IFC controller (Fluidigm; South San Francisco, CA) was used to load the samples and reagents onto the chip and a Biomark HD was used to analyze the chip. The chip was run following the following thermal protocol: 95 °C for 60 seconds, 40 cycles of 96 °C for 5 seconds and 60 °C for 20 seconds, followed by 3 seconds at 60 °C and slow heating to 95 °C at a rate of 1 °C per 3 seconds. Following MF-qPCR, melt curves were analyzed to ensure that non-specific amplification was not present.

Due to the small volumes of template DNA used for MF-qPCR, a preamplification step was needed in order to amplify the DNA into a quantifiable range. This preamplification used the same primers that were used for the MF-qPCR and a low number of PCR cycles. A standard curve, which also underwent the preamplification step, was prepared using serial 10-fold dilutions of a mixture of DNA standards for all genes of interest. Reaction volumes were 25 μL and consisted of: 12.5 μL EvaGreen, 6.25 μL mixture of 50 nM of each primer, and 0.625 μL of DNA template. The thermal protocol used was as follows: initial denaturation at 95 °C for 10 minutes followed by 17 cycles of a 15 second denaturation at 95 °C and anneal and extension for 4 minutes at 60 °C. Preamplification was performed on a Bio-Rad (Hercules, CA) CFX Connect Real-Time System. Preamplification products were diluted 10-fold with DNase and RNase free water and stored at -20 °C.

The 16S rRNA gene was quantified using conventional qPCR as the concentrations in the samples were too high to quantify using MF-qPCR. In addition, *intI1* for the Lake Pepin samples were run using conventional qPCR as the standard curve for the 192.24 MF-qPCR chip did not amplify well. For conventional qPCR, a Bio-Rad (Bio-Rad; Hercules, CA) CFX Connect Real-Time System was used. Reaction volumes were 25 μL and consisted of: 12.5 μL of EvaGreen MasterMix (Bio-Rad; Hercules, CA), 25 μg of bovine serum albumin, optimized quantities of forward and reverse primers, and approximately 1 ng of template DNA. The thermal protocol used was: 2 minutes initial denaturation at 95 °C followed by 40 cycles of denaturation at 95 °C for 15 seconds and a one-minute annealing/extending step at 60 °C. Standard curves were prepared by performing a serial 10-fold dilution of a DNA solution with known concentration, the slopes of which were used to calculate amplification efficiency (Table A1). Amplification curves were

inspected to ensure that no inhibition had taken place and melt curves were inspected to ensure that non-specific amplification did not occur.

Table A1. Amplification details for all target genes in this study. These values correspond to conditions during quantification of all samples.

Gene	*Amplification Efficiency*	*Quantification Limit (lower), log(copies/µL of DNA extract)*
16S rRNA	0.973	5.04
aacA	1.150	3.59
aacA5	1.003	3.63
aadD	1.056	1.63
acrD	1.095	2.54
ampC	0.985	3.65
arr2	1.090	3.49
blaKPC	0.933	2.54
blaNDM-1	0.830	2.78
blaNPS	1.005	2.54
blaOXA	0.966	2.60
blaSHV	0.843	3.20
blaVIM	1.035	2.65
cadA	1.077	2.54
catB8	0.946	1.56
chrA	0.780	2.57
cmlB	1.063	2.66
copA	1.002	3.56
ctxm32	1.079	1.65
dfr13	1.081	2.62
ereB	0.873	2.59
floR	0.757	2.62
imp13	1.071	1.62
intI1	0.940	3.55
intI2	0.959	2.58
intI3	1.023	2.54
mefE	0.905	2.59
merA	0.806	4.56
mexB	0.794	2.56
nikA	1.083	1.56
qacF	0.985	3.70
qnrA	1.051	3.70
qnrB	1.026	2.62
rcnA	1.116	1.62
strB	1.076	1.55
sul1	1.000	2.56
sul2	0.867	3.65
sul3	1.039	1.70
tet(A)	0.983	2.58
tet(L)	1.090	3.37
tet(M)	1.023	2.37
tet(S)	0.996	2.62
tet(W)	1.227	3.70
tet(X)	1.039	3.60
vanA	1.056	3.59
vanB	0.910	2.49

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