Supplementary Information (SI) for Environmental Science: Water Research & Technology.

Well Exposed Supplemental Information:

Table S1: Summary table of known Aquifer information

Aquifer	
Alluvial	The Apple-Plum Alluvial aquifer, an unconsolidated sand and gravel formation in the Apple-Plum watershed primarily serving the Dubuque area in eastern Iowa[1,2], has an alluvial composition that allows for high permeability and water movement, making it a significant groundwater source. With depths ranging from approximately 50 to 250[2] feet and a cumulative confining layer thickness of less than 25 feet[3], the aquifer has minimal protection from surface contamination sources, such as agricultural runoff, chemical spills, and leaking underground storage tanks. The aquifer's vulnerability is managed through rigorous treatment to maintain water quality for municipal use.
Cambrian-Ordovician	The Cambrian-Ordovician aquifer is a main water source for municipalities in eastern half of Iowa[4] and is also commonly known as the Jordan Aquifer. Its bedrock is mainly comprised of sandstone with smaller portions of limestone and dolomite. Where the aquifer is deeper, there have been high levels of fluoride, chloride and sulfate dissolved minerals found along with excessive amounts of radium, but these are municipally removed in Iowa. This aquifer has low susceptibility to contamination due to its thick confining layers, which provide natural protection against surface pollutants, including contaminants from leaking underground storage tanks, chemical spills, and excessive fertilizer application[5]. Wells on the Cambrian-Ordovician aquifer can range from 300 to 2000 ft deep, with extremes reaching down to 3000 ft.
Silurian	The Silurian aquifer serves many public, rural, and industrial users in eastern and northern Iowa. Its bedrock consists of dolomite and limestone, both primarily composed of calcium carbonate, with dolomite distinguished by the addition of magnesium[6]. This aquifer reaches depths of up to 700 feet in some areas, with wells typically ranging from 100 to 700 feet deep[4]. However, due to its shallow depth in certain locations and minimal protective overlying materials, the Silurian aquifer is highly susceptible to contamination[7]. This vulnerability exposes it to pollutants from surface sources, including leaking underground storage tanks, chemical spills, and excessive fertilizer application.

Galena	The Galena aquifer is one of the more restricted aquifer sources that is locally used in northeastern Iowa[4]. Although its exact depth isn't conclusive, it is known to be shallower than both the Silurian and the Cambrian-Ordovician aquifers. Its bedrock is a comprised of shale with some embedded dolomite and limestone[8]. Uniquely, contamination from bacteria and nitrates are a concern for the Galena aquifer because potions of its strata are exposed in its eastern region[4].

Table S2: Summary table of known Municipal treatment information

Municipality	# of samples in municipality	
1. Asbury Municipal Water System	1	The Asbury Municipal System in Asbury, Iowa, sources its water from the Cambrian-Ordovician (Jordan) aquifer[9] and treats it using chlorination to achieve "breakpoint" chlorination, ensuring a minimum chlorine residual of 0.3 mg/L free chlorine or 1.5 mg/L total chlorine across the distribution

		system[10]. This treatment process, overseen by the Iowa Department of Natural Resources (IDNR), maintains water safety for public consumption.
2. Cascade Municipal Water Supply	1	The Cascade Municipal Water Supply in Iowa sources its water from the Cambrian-Ordovician aquifer[11], specifically the Jordan Sandstone and Silurian Limestone formations, which offer natural protection against surface contaminants. The wells have a combined daily capacity of approximately 1,440,000 gallons. The system includes three well houses, a 400,000-gallon water tower, and 12 miles of underground distribution lines, with an average daily consumption of about 230,000 gallons[12]. Cascade employs chlorination with a Maximum Residual Disinfectant Level (MRDL) of 4.0 ppm. In 2023, the Running Annual Average (RAA) chlorine residual in the system is 2.3 ppm, within a range of 1.53 to 3.1 ppm[13]. The Iowa Department of Natural Resources (IDNR) oversees the water quality, ensuring it meets all state and federal safety standards.
3. Dubuque Water Works	6	The City of Dubuque sources its water from the Apple-Plum Alluvial aquifer and the Jordan (Cambrian-Ordovician) aquifer[14,15]. To ensure water safety, Dubuque employs chlorination for disinfection, maintaining microbiological safety by destroying pathogens, with a Maximum Residual Disinfectant Level (MRDL) of 4.0 ppm and a Running Annual Average (RAA) chlorine residual of 1.1 ppm[16]. Additionally, fluoride is detected at 0.7 mg/L in the treated water – this concentration results from a combination of added fluoride to promote dental health, natural erosion of deposits. Phosphate is introduced to chemically stabilize the water, reducing lead leaching from pipes[16]. The Iowa Department of Natural Resources (IDNR) oversees the water quality, ensuring adherence to all safety standards.
4. Dyersville Municipal Water Co	3	The Dyersville water supply in Iowa draws from the Cambrian-Ordovician and Silurian aquifers, which consist of sandstone and dolomite rock layers[16]. To ensure safe drinking water, Dyersville uses chlorination for disinfection, maintaining a Maximum Residual Disinfectant Level (MRDL) of 4.0 ppm and a Running Annual Average (RAA) chlorine residual of 1.8 ppm, with levels ranging from 1.1

		to 1.95 ppm[17]. Fluoride was detected at a level of 0.9 ppm in the water, a concentration that results from a combination of added fluoride to promote dental health, natural erosion of deposits, and possible discharge from fertilizer and aluminum factories[17]. Additionally, sodium was measured at 26.1 ppm, originating both from the natural erosion of deposits and from additives introduced during the treatment process[17]. The Iowa Department of Natural Resources (IDNR) oversees the water quality, ensuring compliance with all required safety standards.
5. Epworth Water Supply	2	The Epworth Water Supply in Iowa sources its water from the Silurian aquifer[18], specifically from dolomite formations[7]. Epworth employs chlorination for disinfection to maintain microbiological safety, with a Maximum Residual Disinfectant Level (MRDL) of 4.0 ppm and a Running Annual Average (RAA) chlorine residual reported to ensure consistent disinfection compliance[7]. The Epworth water system is overseen by the IDNR, which ensures adherence to water quality standards.
6. Farley Water Supply	1	The Farley Water Supply in Iowa sources its water from the sandstone and dolomite of the Cambrian-Ordovician aquifer[19,20]. Farley uses chlorination as its primary disinfection method to control microbes in the water, maintaining a Maximum Residual Disinfectant Level (MRDL) of 4.0 ppm. In 2020, the system reported a Running Annual Average (RAA) chlorine residual of 1.8 ppm, with recorded values ranging from 1.33 to 2.16 ppm[19]. The Iowa Department of Natural Resources (IDNR) oversees the water quality, ensuring adherence to safety and compliance standards.
7. Holy Cross Water Supply	1	The Holy Cross Water Supply in Iowa sources its water from the Cambrian-Ordovician aquifer[21].
8. Luxemburg Water Supply	1	The City of Luxemburg water supply in Iowa sources its water from the Cambrian Jordan Sandstone aquifer[22]. This aquifer is not susceptible to contamination due to its depth and protective overlying materials, which prevent contaminants from easily accessing the water source. The Luxemburg water

		supply employs chlorination as its primary disinfection method to control microbial growth, maintaining a Maximum Residual Disinfectant Level (MRDL) of 4.0 ppm and a Running Annual Average (RAA) chlorine residual of 1.66 ppm, with levels ranging between 1.00 and 2.24 ppm[23]. The Iowa Department of Natural Resources (IDNR) oversees the water quality, ensuring compliance with all safety standards.
9. New Vienna Water Supply	1	The New Vienna Water Supply in Iowa sources its water from the Silurian and Cambrian-Ordovician aquifers[24].
10. Peosta Water Supply	2	The Peosta water supply in Iowa sources its water from the Cambrian-Ordovician aquifer, specifically from sandstone and dolomite formations[25]. Peosta uses chlorination as a primary disinfection method, maintaining a Maximum Residual Disinfectant Level (MRDL) of 4.0 ppm and a Running Annual Average (RAA) chlorine residual of 0.8 ppm, with recorded levels ranging from 0.33 to 1.6 ppm[26]. Fluoride was detected at 0.5 ppm, reflecting a combination of added fluoride to support dental health, natural erosion of deposits, and possible discharge from fertilizer and aluminum factories[26]. Additionally, sodium levels measured at 85.8 ppm stem from both natural deposits and additives used during the treatment process[26]. The Iowa Department of Natural Resources (IDNR) oversees the water quality to ensure compliance with all safety standards.
11. Worthington Water Supply	1	The Worthington water supply in Iowa sources its water from the Cambrian-Ordovician aquifer, specifically from sandstone and dolomite formations[27]. Chlorination is used as the primary disinfection method, with a Maximum Residual Disinfectant Level (MRDL) of 4.0 ppm and a Running Annual Average (RAA) chlorine residual of 1.0 ppm, ranging from 0.4 to 2.15 ppm, to control microbial growth[28]. Fluoride was detected at 1.6 ppm, sourced from a combination of added fluoride to promote dental health, natural erosion of deposits, and potential discharge from fertilizer and aluminum factories[28]. Additionally, sodium levels were measured at 50.6 ppm, stemming from both the natural erosion of deposits and additives introduced during the treatment process . The Iowa

		Department of Natural Resources (IDNR) oversees the water quality to ensure compliance with all safety standards.

Table S3: List of 33 water quality indicators/ Chemical parameters, Minimum Detection level (MDL), SWDA regulatory level (if applicable)

Water Quality Indicator/ Parameter	Minimum Detection Level	Water Quality Indicator/ Parameter	Minimum Detection Level
Aluminum	2.5144 ppb	Nickel	0.0508 ppb
Antimony	0.0145 ppb	Nitrate (as N)	0.10 ppm
Arsenic	0.0067 ppb	Phosphorus	3.7141 ppb
Barium	0.0145 ppb	Potassium	0.0045 ppm
Beryllium	0.0123 ppb	Selenium	0.2816 ppb
Boron	0.7030 ppb	Sodium	0.0176 ppm
Cadmium	0.0103 ppb	Strontium	0.0777 ppb
Calcium	0.0079 ppm	Thallium	0.0036 ppb
Chromium	0.0173 ppb	Tin	0.0145 ppb
Cobalt	0.0021 ppb	Uranium	0.0006 ppb
Copper	0.2251 ppb	Vanadium	0.0039 ppb
Iron	0.2259 ppb	Zinc	0.4222 ppb

Lead	0.0106 ppb		
Lithium	0.0608 ppb	E-coli	Presence/ Absence
Magnesium	0.0016 ppm	Total Coliform	Presence/ Absence
Manganese	0.0166 ppb	Temperature	-5 °C
Molybdenum	0.0017 ppb	pH	0

Table S4: Descriptive statistics and detection frequencies of chemicals listed on the EPA's Primary Drinking Water Regulations list in well and municipal drinking water samples

Well Samples	Municipal Samples

Parameter	Mean	Median	Range	Detection Frequency	Mean	Median	Range	Detection Frequency	Higher Source	p_value
Antimony (ppb)	0.203	0.01	0.0145 - 0.0477	100%	0.02	0.01	0.0145 - 0.0463	100%	Municipal	0.4488
Arsenic (ppb)	0.073	0.06	0.0048 - 0.1626	100%	0.20	0.10	0.0121 - 0.8262	100%	Municipal	0.1404
Barium (ppb)	82.135	63.77	0.0463 - 233.6834	100%	27.92	15.87	0.2379 - 161.9626	100%	Well	0.1636
Beryllium (ppb)	0.017	0.02	0.0123 - 0.044	100%	0.02	0.01	0.0123 - 0.0262	100%	Well	0.7455
Cadmium (ppb)	0.015	0.01	0.0103 - 0.0814	95%	0.01	0.01	0.0103 - 0.0211	90%	Well	0.3539
Copper (ppb)	8.911	5.59	0.2 - 35.833	100%	29.52	15.19	2.002 - 117.488	100%	Municipal	0.0337*
Lead (ppb)	0.203	0.12	0.0315 - 0.7772	100%	0.18	0.08	0.013 - 1.3651	100%	Well	0.1264
Nitrate (ppm)	4.054	1.80	0.24 - 11	55%	1.46	0.64	0.54 - 4.8	50%	Well	0.3911
Selenium (ppb)	0.497	0.28	0.2816 - 1.3121	70%	0.28	0.41	0.2816 - 1.154	60%	Well	0.3674
Thallium (ppb)	0.022	0.02	0.0053 - 0.095	100%	0.01	0.01	0.0049 - 0.0251	100%	Well	0.0019***
Uranium (ppb)	0.285	0.09	0.0098 - 1.1119	100%	0.28	0.23	0.009 - 0.832	100%	Well	0.9461

Table S5: Descriptive statistics and detection frequencies of chemicals listed on the EPA's Secondary Drinking Water Standards in well and municipal drinking water samples.

	Well Samples	Municipal Samples	

Parameter	Mean	Median	Range	Detection Frequency	Mean	Median	Range	Detection Frequency	Higher Source	p_value
Aluminum (ppb)	3.370	2.51	2.514 - 14.152	90%	9.88	2.64	2.514 - 82.337	95%	Municipal	0.0140*
Iron (ppb)	159.489	68.53	6.125 - 1035.82	100%	36.32	31.04	4.1273 - 89.170	100%	Well	0.0360**
Manganese (ppb)	10.252	2.74	0.0166 - 90.005	100%	1.00	0.68	0.187 - 4.4723	100%	Well	0.0060***
Zinc (ppb)	13.694	8.65	0.4222 - 51.847	100%	7.28	3.37	0.4222 - 49.902	100%	Well	0.0337**

Table S6: Descriptive statistics and detection frequencies of non-EPA regulated chemicals tested in well and municipal drinking water samples.

	Well Samples				Municipal Samples					
Parameter	Mean	Median	Range	Detection Frequency	Mean	Median	Range	Detection Frequency	Higher Source	p_value
Boron (ppb)	23.935	18.25	6.624 - 73.532	100%	227.12	131.56	12.177 - 911.05	100%	Municipal	0.0009***
Calcium (ppm)	47.611	58.53	0.044 - 124.695	100%	31.26	12.74	0.182 - 112.195	100%	Well	0.2503
Chromium (ppb)	0.477	0.33	0.0405 - 1.686	100%	0.39	0.35	0.0798 - 0.703	100%	Well	0.8181
Cobalt (ppb)	0.114	0.12	0.0109 - 0.2925	100%	0.08	0.06	0.0105 - 0.2574	100%	Well	0.2616
Lithium (ppb)	3.205	2.79	0.487 - 7.451	100%	30.63	18.25	0.288 - 129.604	100%	Municipal	0.0003***
Magnesium (ppm)	22.216	26.92	0.0201 - 57.669	95%	17.50	20.45	0.077 - 42.315	90%	Well	0.3104
Molybdenum (ppb)	0.425	0.28	0.0296 - 1.2182	95%	0.27	0.25	0.0155 - 0.661	100%	‘ Well	0.1559
Nickel (ppb)	0.871	0.87	0.1007 - 3.6901	100%	0.51	0.35	0.0768 - 1.6686	100%	Well	0.0617
Phosphorus (ppb)	11.819	6.41	3.714 - 55.762	70%	439.28	494.37	3.714 - 1555.63	60%	Municipal	0.0000***
Potassium (ppm)	1.142	1.06	0.1259- 4.877	100%	5.38	5.37	0.257 - 13.595	100%	Municipal	0.0000***
Sodium (ppm)	51.578	9.94	1.807 - 179.140	100%	67.19	43.29	12.57 - 197.249	100%	Municipal	0.0411*
Strontium (ppb)	65.763	62.42	0.2604 - 180.442	100%	383.53	54.38	0.2345 - 1371.643	100%	Municipal	0.1478
Tin (ppb)	0.024	0.01	0.0145 - 0.1	45%	0.01	0.01	0.0145	40%	Well	0.4094

Vanadium (ppb)	0.040	0.01	0.0044 - 0.3002	100%	0.10	0.03	0.0039 - 0.3282	100%	Municipal	0.0043***

Table S7: Module list of functional traits relating to phosphate or nitrogen metabolism.

BugBase Module ID	**Module Name**
M00145	NADPH Quinone Oxidoreductase in Chloroplasts and Cyanobacteria
M00175	Nitrogen Fixation: Nitrogen-Ammonia
M00222	Phosphate Transport System
M00434	PhoRB Phospate Starvation Response
M00438	Nitrate-Nitrite Transport System
M00443	SenX3-RegX3 Phosphate Starvation Response
M00449	CreBC Phosphate Regulation
M00473	UhpBA Hexose Phosphate Uptake
M00497	GlnLG Nitrogen Regulation
M00498	NtrYX Nitrogen Regulation
M00524	FixLJ Nitrogen Fixation
M00528	Ammonia-Nitrite Nitrification
M00529	Nitrate-Nitrogen Denitrification
M00530	Dissimilatory Nitrate Reduction: Nitrate-Ammonia
M00531	Assimilatory Nitrate Reduction: Nitrate-Ammonia
M00176	Sulfur-Sulfate Reduction

Table S8: **ddPCR assay thresholds, LOD, and LOQ**

Target taxa (gene)	**ddPCR Threshold**	**Limit of Detection**	**Limit of Quantification**
Total Bacteria	12900	5.3 gene copies / 20 µL	53 gene copies / 20 µL
L. pneumophila	8800	6.08 gene copies / 20 µL	6.08 gene copies / 20 µL

NTM	10600	5.6 gene copies / 20 µL	5.6 gene copies / 20 µL

Thresholds were determined experimentally by spiking target taxa's gblock at different concentrations into water DNA matrix and adjusting threshold until expected concentration was read out.

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