Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Supporting Information

Effects of disinfectants and particles on occurrence of different microorganisms in drinking water distribution systems

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Table S1 The primers, probes and amplification programs of opportunistic pathogens

Targeted microbe	Targeted genes	Primer sequences	Size (bp)	Program
Legionella spp	23S rRNA	F: CCCATGAAGCCCGTTGAA R:ACAATCAGCCAATTAGTACGAGTTAGC P:TCCACACCTCGCCTATCAACGTCGTAGT	92	95°C for 2 min, 40 cycles of 95°C for 5 s and 58.5°C for 10 s ^{1}
Legionella pneumophila	*mip*	F:AAAGGCATGCAAGACGCTATG R:GAAACTTGTTAAGAACGTCTTTCATTTG P:TGGCGCTCAATTGGCTTTAACCGA	78	95°C for 2 min, 40 cycles of 95°C for 5 s and 60°C for 10 s ^{1}
Mycobacteria spp.	16S rRNA	F:CCTGGGAAACTGGGTCTAAT R: CGCACGCTCACAGTTA P:TTTCACGAACAACGCGACAAACT	462	95°C for 2 min, 40 cycles of 95°C for 5 s, 55°C for 15 s and 72°C for 10 s ^{1}
Mycobacteria avium	16S rRNA	F：AGAGTTTGATCCTGGCTCAG R：ACCAGAAGACATGCGTCTTG	180	98°C for 2 min, 40 cycles of 98°C for 5 s and 68°C for 18 s ^{1}
Pseudomonas aeruginosa	*oprl*	F:GACGTACACGCGAAAGACCT R:GCCCAGAGCCATGTTGTACT	99	95°C for 5 min, 40 cycles: 95°C for 15 s, 60°C for 45 s ^{2}
Acanthamoeba spp	18S rRNA	F: TTTTCAGTTGGTTTTGGCAG R: GGTCGGCATCGTTTATGG	219	95°C for 2 min, 40 cycles of 95°C for 5 s and 60°C for 10 s ^{3}
Harmanella vermiformis	18S rRNA	F: TTACGAGGTCAGGACACTGT R: GACCATCCGGAGTTCTCG	512	98°C for 2 min, 40 cycles of 98°C for 5 s, 72°C for 18 s ^{1}
Total bacteria	16S rRNA	F: CGGTGAATACGTTCYCGG R: GGYTACCTTGTTACGACTT P: CTTGTACACACCGCCCGTC	124	95°C for 30 s, 35 cycles of 95°C for 15 s and 56°C for 60 s ^{1}

Table S2 Quantification limits and amplification efficiencies for qPCR

Target microorganism	Quantification limit (gene copies/μL DNA)	Slope	Amplification efficiency (%)	R^2
Legionella spp.	4	-3.410	96.43	0.9962
Legionella pneumophila	6	-3.411	96.43	0.9561
Mycobacteria spp.	7	-3.331	99.62	0.9918
Mycobacterium avium	4	-3.388	97.33	0.9779
Pseudomonas aeruginosa	67	-3.420	96.06	0.9921
Acanthamoeba spp.	18	-3.516	92.49	0.9924
Harmanella vermiformis	16	-3.361	98.38	0.9813
Total bacteria (16S rRNA)	8	-3.360	98.43	0.9910

Note: The amplification efficiency was calculated by the formula: Amplification efficiency = $10^{-1/\text{slope}}$-1

Table S3 Characteristics of water quality in different regions

Area	Turbidity (NTU)	Temperature (℃)	pH	TOC （mg/L)	DO （mg/L)	Total Fe （μg/L)	particles number
FC	0.30±0.28	18.87±7.12	8.05±0.33	1.88±0.61*	9.37±2.04	63.45±50.12	492±447
TC	0.26±0.15	19.78±6.82	8.06±0.32	2.31±0.45*	9.36±1.5	59.51±40.76	389±535

*, significant difference with 95% confidence ($p < 0.05$) in two areas.

Table S4 Number of particles with different sizes

Area	2~3μm	3~5μm	5~7μm	7~10μm	10~15μm	15~20μm	20~25μm	>25μm
FC	164±149	222±223	57±57	30±32	13±18	5±6	1±2	1±2
TC	136±173	168±236	44±65	25±42	10±19	4±6	1±2	2±2

The value is expressed as mean±SD. *, significant difference with 95% confidence ($p < 0.05$) in the two areas.

Table S5 Pearson correlation analysis between the microorganisms in all samples (n = 98).

	16S rRNA	Myc	Ma	Leg	Lp	Ps	Ac	Hv
16S rRNA	1	**0.323**	0.149	**0.477**	0.109	**0.382**	**0.408**	**0.228**
		(p=0.001)	(p=0.142)	**(p=0)**	(p=0.285)	**(p=0)**	**(p=0)**	**(p=0.024)**
Myc		1	**0.335**	**0.343**	-0.059	0.094	**0.563**	-0.001
			(p=0.001)	**(p=0.001)**	(p=0.562)	(p=0.356)	**(p=0)**	(p=0.99)
Ma			1	0.106	-0.023	0.19	**0.286**	0.049
				(p=0.301)	(p=0.822)	(p=0.062)	**(p=0.004)**	(p=0.63)
Leg				1	**0.208**	0.009	**0.415**	0.107
					(p=0.04)	(p=0.932)	**(p=0)**	(p=0.294)
Lp					1	0.106	0.062	0.171
						(p=0.3)	(p=0.545)	(p=0.093)
Ps						1	0.103	0.06
							(p=0.315)	(p=0.55)7
Ac							1	0.159
								(p=0.119)
Hv	□	□	□	□	□	□	□	1

The bold numbers represented significance under $p < 0.05$. Myc: *Mycobacterium* spp., Ma: *M. avium*, Leg: *Legionella* spp., Lp: *L. pneumophila*, Ps: *P. aeruginosa*, Ac: *Acanthamoeba* spp., Hv: *H.vermiformis*.

Table S6 Pearson correlation analysis between the microorganisms in FC areas (n=60).

	16S rRNA	Myc	Ma	Leg	Lp	Ps	Ac	Hv
16S rRNA	1	**0.300** **(p=0.020)**	0.157 (p=0.232)	**0.489** **(p=0)**	-0.029 (p=0.825)	**0.382** **(p=0.003)**	**0.460** **(p=0)**	0.213 (p=0.102)
Myc		1	**0.417** **(p=0.001)**	**0.272** **(p=0.035)**	-0.085 (p=0.517)	0.030 (p=0.818)	**0.558** **(p=0)**	-0.030 (p=0.819)
Ma			1	0.122 (p=0.355)	-0.185 (p=0.157)	0.225 (p=0.085)	**0.311** **(p=0.015)**	-0.037 (p=0.777)
Leg				1	0.245 (p=0.059)	0.012 (p=0.930)	**0.287** **(p=0.026)**	0.166 (p=0.204)
Lp					1	0.064 (p=0.627)	0.036 (p=0.787)	0.150 (p=0.254)
Ps						1	0.143 (p=0.277)	0.216 (p=0.098)
Ac							1	0.078 (p=0.553)
Hv								1

The bold numbers represented significance under $p < 0.05$. Myc: *Mycobacterium* spp., Ma: M. avium, Leg: *Legionella* spp., Lp: *L. pneumophila*, Ps: *P. aeruginosa*, Ac: *Acanthamoeba* spp., Hv: *H.vermiformis*.

Table S7 Pearson correlation analysis between the microorganisms in TC areas (n=38).

	16S rRNA	Myc	Ma	Leg	Lp	Ps	Ac	Hv
16S rRNA	1	0.236	0.312	0.280	**0.358**	**0.397**	0.123	0.152
		(p=0.154)	(p=0.056)	(p=0.089)	**(p=0.027)**	**(p=0.014)**	(p=0.463)	(p=0.363)
Myc		1	0.314	**0.348**	-0.070	0.186	**0.498**	-0.064
			(p=0.055)	**(p=0.032)**	(p=0.675)	(p=0.263)	**(p=0.001)**	(p=0.705)
Ma			1	0.228	0.259	0.161	**0.392**	0.226
				(p=0.168)	(p=0.116)	(p=0.336)	**(p=0.015)**	(p=0.173)
Leg				1	0.099	-0.047	**0.500**	-0.108
					(p=0.555)	(p=0.779)	**(p=0.001)**	(p=0.518)
Lp					1	0.164	0.048	0.170
						(p=0.324)	(p=0.774)	(p=0.307)
Ps						1	-0.001	-0.173
							(p=0.993)	(p=0.300)
Ac							1	0.165
								(p=0.323)
Hv								1

The bold numbers represented significance under $p < 0.05$. Myc: *Mycobacterium* spp., Ma: *M. avium*, Leg: *Legionella* spp., Lp: *L. pneumophila*, Ps: *P. aeruginosa*, Ac: *Acanthamoeba* spp., Hv: *H.vermiformis*.

Table S8 Pearson correlation between HPC and other indicators (FC areas: n=60, TC areas: n=38).

Area	Tur	Temp	pH	TOC	DO	Total Fe	PN
FC	**0.279** **(p=0.031)**	0.056 (p=0.671)	-0.091 (p=0.490)	**0.355** **(p=0.005)**	0.013 (p=0.921)	**0.417** **(p=0.001)**	0.156 (p=0.232)
TC	**0.334** **(p=0.040)**	**0.348** **(p=0.032)**	**-0.336** **(p=0.039)**	-0.117 (p=0.485)	**0.364** **(p=0.025)**	**0.482** **(p=0.002)**	0.125 (p=0.455)

Tur: Turbidity, Temp: temperature, PN: particles number.

Table S9 The Pearson correlation between microorganisms and water quality indicators (n=98).

	Turbidity	Total iron	Particles number	2~3μm	3~5μm	5~7μm	7~10μm	10~15μm	15~20μm	20~25μm	>25μm
16S rRNA	0.196	**0.349**	0.026	0.004	0.024	0.04	0.08	0.078	0.052	-0.08	-0.178
	(p=0.054)	**(p=0)**	(p=0.801)	(p=0.972)	(p=0.812)	(p=0.693)	(p=0.434)	(p=0.445)	(p=0.612)	(p=0.435)	(p=0.079)
Myc	**0.203**	0.025	0.176	0.195	0.137	0.161	**0.216**	**0.204**	**0.219**	**0.201**	0.136
	(p=0.045)	(p=0.806)	(p=0.084)	(p=0.055)	(p=0.179)	(p=0.114)	**(p=0.033)**	**(p=0.044)**	**(p=0.031)**	**(p=0.047)**	(p=0.182)
Ma	**0.206**	0.058	**0.2420**	**0.238**	**0.217**	**0.227**	**0.248**	**0.260**	**0.298**	**0.248**	0.103
	(p=0.042)	(p=0.569)	**(p=0.017)**	**(p=0.018)**	**(p=0.032)**	**(p=0.025)**	**(p=0.014)**	**(p=0.01)**	**(p=0.003)**	**(p=0.014)**	(p=0.315)
Leg	**0.296**	**0.230**	0.144	0.171	0.106	0.105	**0.200**	0.194	0.179	0.142	0.091
	(p=0.003)	**(p=0.022)**	(p=0.158)	(p=0.093)	(p=0.299)	(p=0.302)	**(p=0.048)**	(p=0.055)	(p=0.079)	(p=0.162)	(p=0.373)
Lp	-0.032	0.056	**-0.206**	-0.178	-0.162	**-0.220**	**-0.348**	**-0.353**	**-0.316**	**-0.352**	**-0.336**
	(p=0.757)	(p=0.586)	**(p=0.042)**	(p=0.079)	(p=0.111)	**(p=0.03)**	**(p=0)**	**(p=0)**	**(p=0.002)**	**(p=0)**	**(p=0.001)**
Ps	**0.214**	**0.228**	0.043	0.036	0.112	0.053	-0.198	**-0.273**	-0.185	-0.135	-0.182
	(p=0.034)	**(p=0.024)**	(p=0.671)	(p=0.723)	(p=0.274)	(p=0.601)	(p=0.051)	**(p=0.007)**	(p=0.068)	(p=0.186)	(p=0.072)
Ac	**0.314**	0.116	**0.300**	**0.301**	**0.285**	**0.289**	**0.260**	**0.203**	0.186	0.174	0.145
	(p=0.002)	(p=0.257)	**(p=0.003)**	**(p=0.003)**	**(p=0.005)**	**(p=0.004)**	**(p=0.01)**	**(p=0.045)**	(p=0.067)	(p=0.086)	(p=0.155)
Hv	-0.006	0.102	-0.036	-0.02	0.02	-0.052	**-0.230**	**-0.280**	**-0.245**	**-0.215**	**-0.273**
	(p=0.953)	(p=0.318)	(p=0.724)	(p=0.843)	(p=0.842)	(p=0.614)	**(p=0.023)**	**(p=0.005)**	**(p=0.015)**	**(p=0.033)**	**(p=0.006)**
HPC	**0.529**	**0.390**	0.034	0.064	0.044	0.013	-0.062	-0.094	-0.069	-0.086	-0.116
	(p=0)	**(p=0)**	(p=0.742)	(p=0.533)	(p=0.665)	(p=0.902)	(p=0.546)	(p=0.356)	(p=0.498)	(p=0.397)	(p=0.254)

The bold numbers represented significance under $p < 0.05$. Myc: *Mycobacterium* spp., Ma: *M. avium*, Leg: *Legionella* spp., Lp: *L. pneumophila*, Ps: *P. aeruginosa*, Ac: *Acanthamoeba* spp., Hv: *H.vermiformis*.

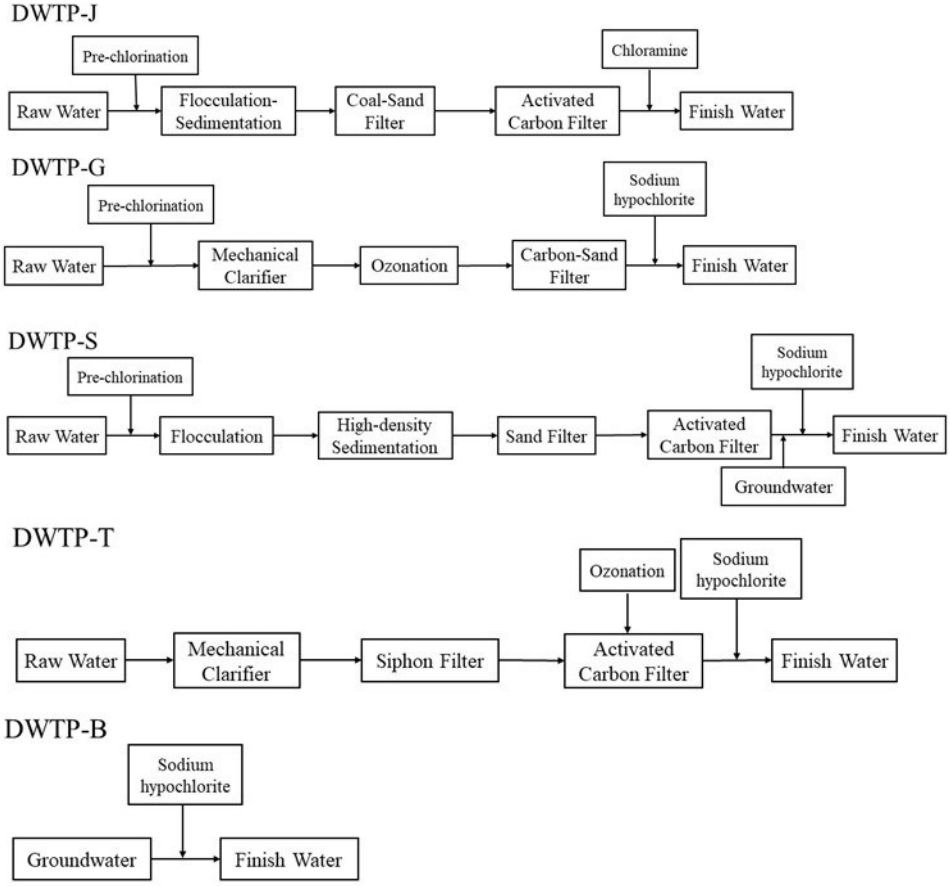

Fig. S1 The main water treatment processes for the five drinking water treatment plants.

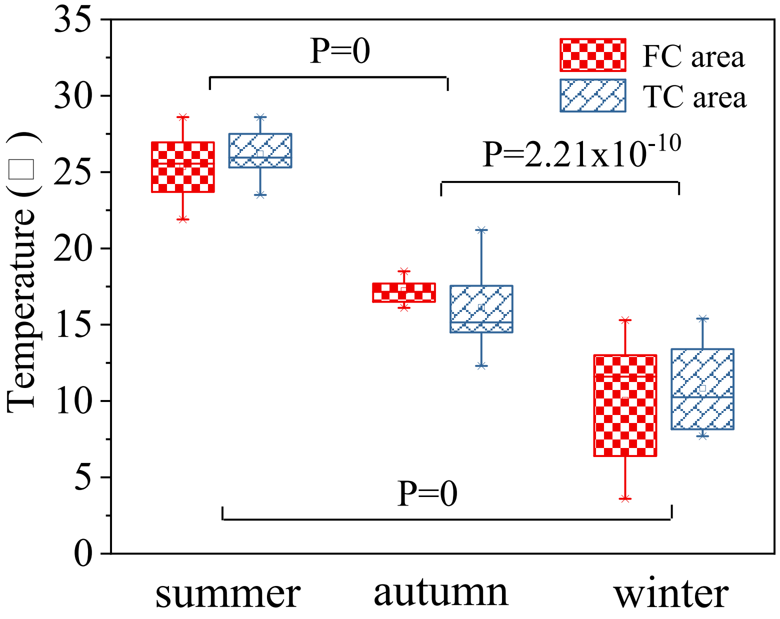

Fig. S2 Temperature profiles in different seasons of the two areas.

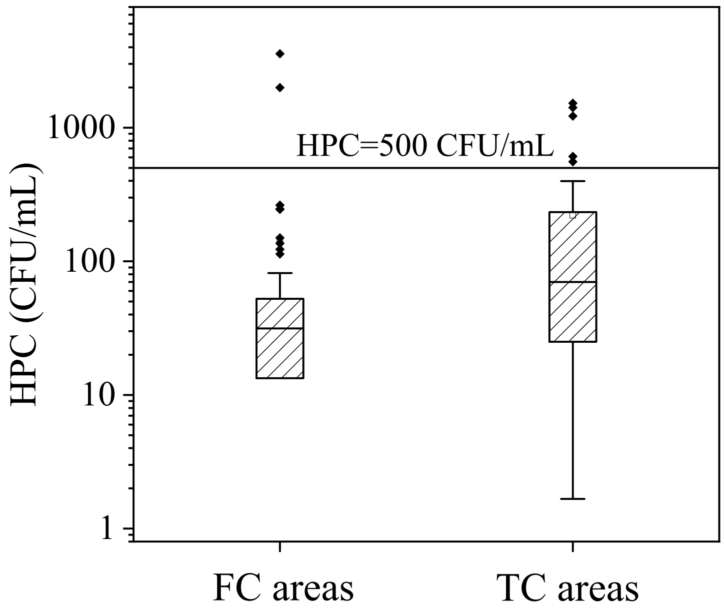

Fig. S3 The distribution of HPC in free chlorine (FC) areas and total chlorine (TC) areas.

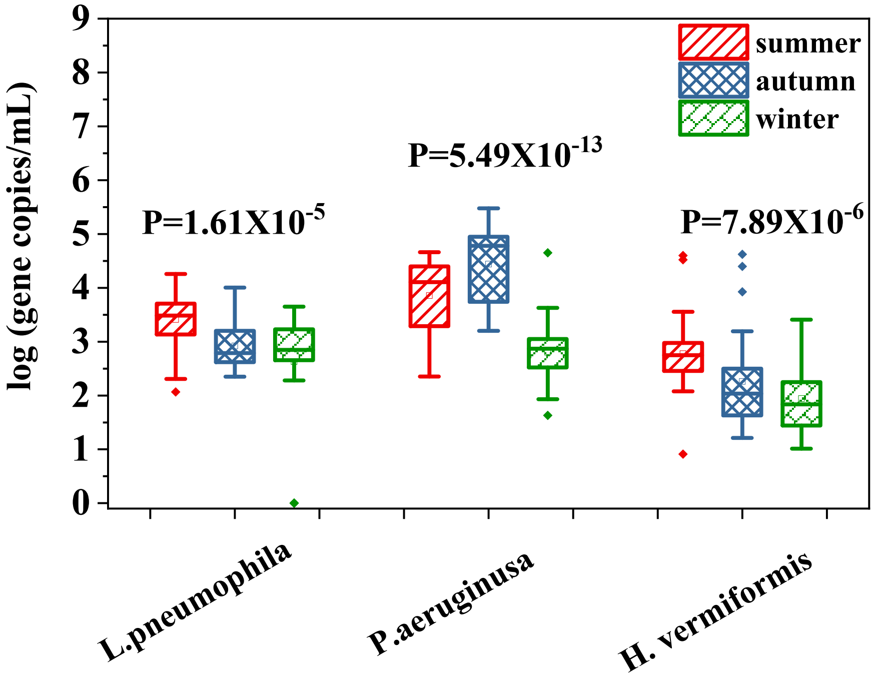

Fig. S4 The seasonal differences of *L. pneumophila*, *P. aeruginosa*, and *H. vermiformis* for all the samples.

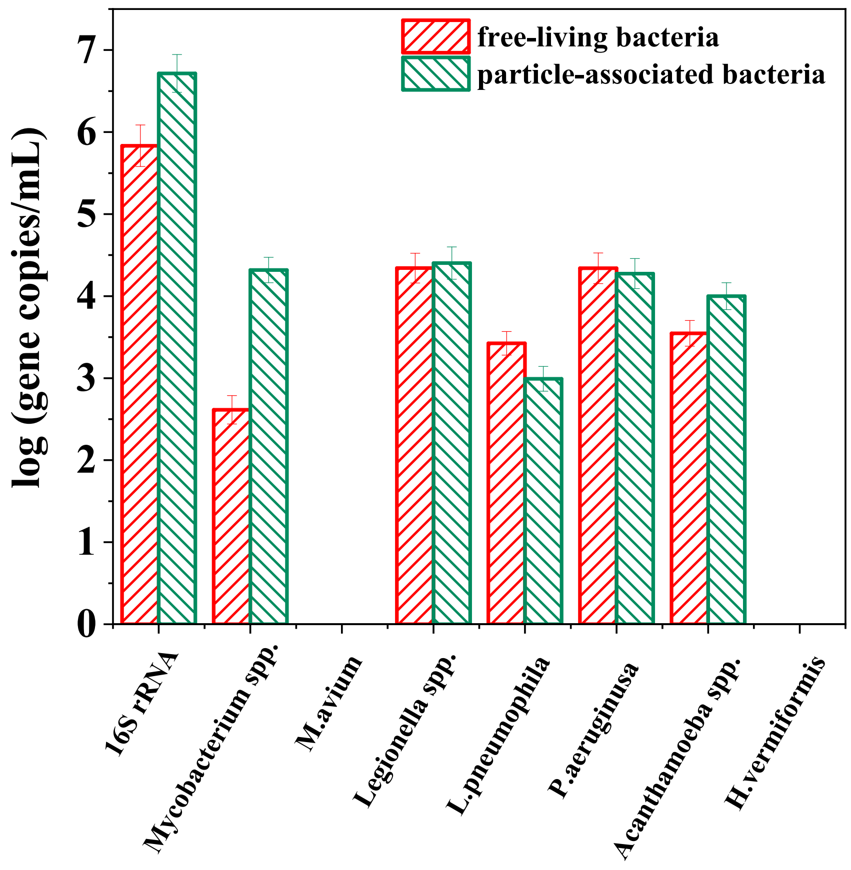

Fig. S5 The free-living and particle-associated bacteria in tap water of drinking water distribution systems.

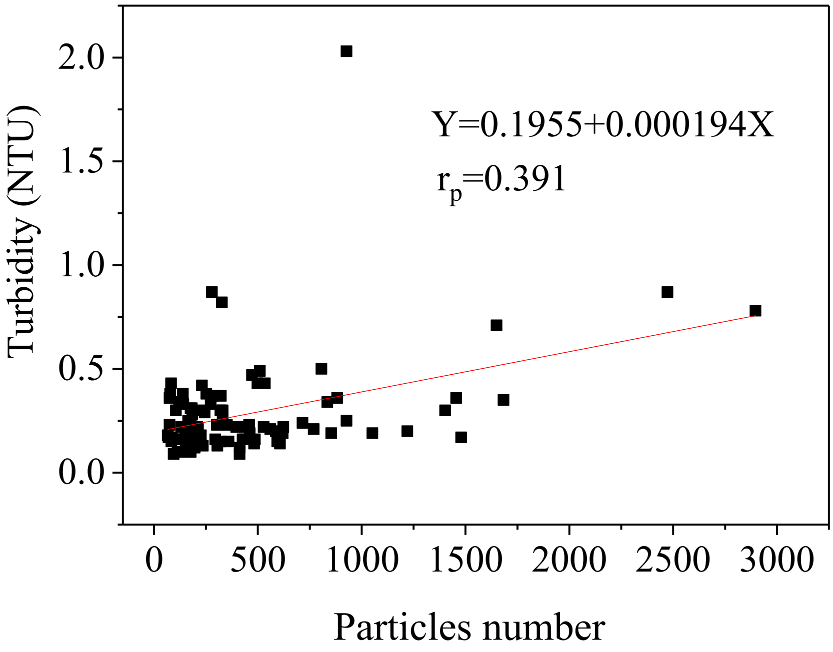

Fig. S6 The Pearson's Rank correlation of particles number and turbidity. (r_p)

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