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

Effect of microbially available phosphorous removal on *Legionella* spp. in multi-storey residential dwelling in Latvia

Marta Zemīte[a,b,d], Daina Pūle[b,c], Olga Kiriļina-Gūtmane[c], Laima Ķimse[c], Mārtiņš Strods[a], Jurģis Zemītis[d], Linda Mežule[a], Olga Valciņa[c], Tālis Juhna[a]

[a] Water Research and Environmental Biotechnology Laboratory, Riga Technical University, Riga, Latvia,
[b] Department of Water Engineering and Technology, Riga Technical University, Riga, Latvia,
[c] The Institute of Food Safety, Animal Health, and Environment "BIOR", Riga, Latvia,
[d] Department of Heat Engineering and Technology, Riga Technical University, Riga, Latvia

Table S1 shows electrical conductivity values for groundwater pumping stations "Baltezers", "Remberģi" and "Zaķumuiža". According to hydraulic modelling, performed by municipal water provider, the water from these plants reaches the pilot study area.

Table S1. Electrical conductivity values (µS cm^{-1}) from groundwater pumping plants that are related to pilot site buildings` water supply.

"Baltezers"			"Remberģi"			"Zaķumuiža"		
average	min	max	average	min	max	average	min	max
695	646	779	337	302	356	305	250	338

Fig. S1 shows cumulative water consumption in both buildings, indicating greater water consumption around initial six weeks and more similar water usage during the remaining time. **Fig. S2** shows water consumption pattern that was similar in both buildings during the study time.

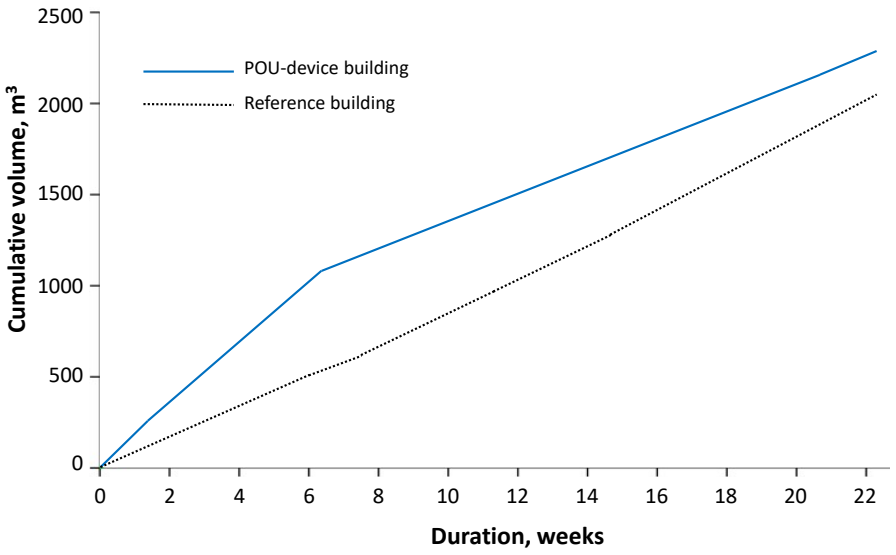

Fig. S1 Cumulative water consumption throughout the sampling time.

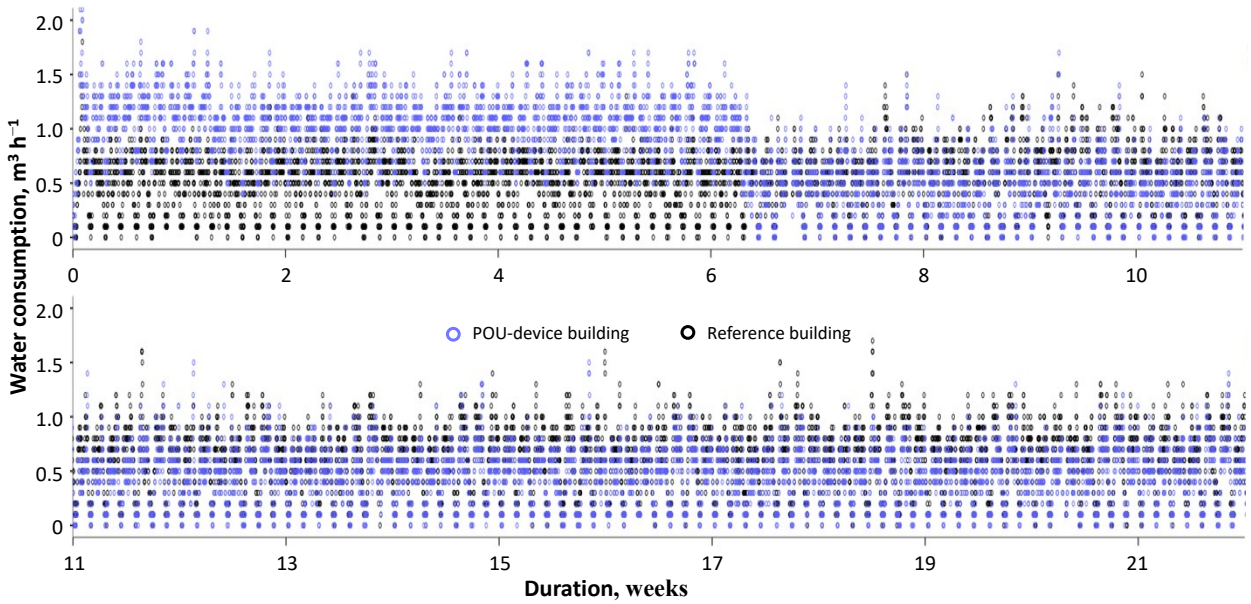

Fig. S2 Water consumption pattern through weeks 0-11 (top) and 11-22 (bottom).

Table S2 summarises microbially available phosphorus (MAP) concentrations in samples collected from inlet water, domestic cold water (DCW) system and domestic hot water (DHW) system, and analysed by three different time intervals, representing the entire sampling period (samples from weeks 0-22), regular (samples from weeks 0-12) or dynamic (samples from weeks 16-22) heat exchanger setpoint. All samples were collected as grab-samples.

Table S2. Microbially available phosphorus (MAP) concentration in samples analysed for the entire sampling duration (weeks 0-22), Regular temperature setting (weeks 0-12), and Changed temperature setting (weeks 16-22).

Sampling time	Microbially available phosphorus (MAP), µg l^{-1}		p-value [a]
	Reference building	POU-device building	
Water inlet (from water main (Ref. building) or after sorption filter (POU-dev. building)			
weeks 0-22, n=12 for each building	10.29* (4.23)	3.56* (1.49)	**<0.001**
weeks 0-12, n=6 for each building	11.81* (3.32)	2.93* (1.63)	**<0.001**
weeks 16-22, n=6 for each building	8.76* (4.77)	4.19* (1.12)	0.066
DCW, kitchen taps			
weeks 0-22, n=33 (RB) and 39 (POU)	1.06 (0-14.14)	0 (0-4.28)	**<0.001**
weeks 0-12, n=17 (RB) and 22 (POU)	2.69 (0-12.60)	0.41 (0-4.28)	**0.001**
weeks 16-22, n=16 (RB) and 17 (POU)	0.67 (0-14.14)	0 (0-0.18)	**0.001**
DHW, showerheads			
weeks 0-22, n=33 (RB) and 39 (POU)	0.33 (0-9.01)	0 (0-9.31)	**0.012**
weeks 0-12, n=17 (RB) and 22 (POU)	2.01 (0-9.01)	0.36 (0-9.31)	**0.018**
weeks 16-22, n=16 (RB) and 17 (POU)	0 (0-3.66)	0 (0-0.08)	**0.014**
DHW, circulation return			
weeks 0-22, n=12 for each building	1.45* (1.64)	0.20 (0-4.95)	0.060
weeks 0-12, n=6 for each building	2.41* (1.79)	0.57 (0-4.95)	0.132
weeks 16-22, n=6 for each building	0.49* (0.72)	0 (0-0.26)	**0.001**

[a] 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[b] Total Cell Count = Intact Cell Count + Damaged Cell Count.

Mean* (standard deviation) values are presented for normally distributed data or otherwise, **Median** (min-max) is used.

Tables S3 and **S4** represent characteristics of domestic hot water (DHW) grab samples, collected from showerheads (first draw) and circulation return. The analysis summary corresponds to the entire sampling time (weeks 0-22). **Table S3** summarises data for Reference building, while **Table S4** is for POU-device building.

Table S3. Characteristics of domestic hot water samples of the Reference building.

Parameter	DHW, showerhead (n=33)	DHW, circulation return (n=12)	p-value [a]
Physical and chemical parameters			
Temperature, °C	21.94* (2.29)	44.35* (2.62)	**<0.001**
pH	8.00* (0.12)	8.23* (0.11)	**<0.001**
Electrical conductivity, µS cm^{-1}	303 (270-602)	315.42* (40.12)	0.409
Ca, mg l^{-1}	36.93* (4.27)	35.47* (3.04)	0.282
Mg, mg l^{-1}	10.8 (8.5-16.3)	10.0 (8.5-11.3)	0.424
Mn, mg l^{-1}	0.014 (0.008-0.178)	0.014 (0.011-0.081)	1.000
Cu, µg l^{-1}	142 (73-289)	99 (30)	**0.002**
Zn, mg l^{-1}	0.052 (0.027-0.142)	0.042 (0.033-0.076)	0.052
Fe, mg l^{-1}	0.032 (0-0.122)	0.061* (0.047)	0.115
Pb, µg l^{-1}	2.20 (1.45-9.87)	2.84 (1.70-6.36)	0.109
Main microbial nutrients			
Total organic carbon (TOC), mg l^{-1}	1.57 (1.04-8.14)	1.42 (1.03-4.18)	0.409
Microbially available phosphorus (MAP), µg l^{-1}	0.33 (0-9.01)	1.45* (1.64)	0.367
Microbiological parameters			
Total Cell Count, cells ml^{-1} [b]	$3.31x10^5$ ($1.11x10^5$ - $8.71x10^5$)	$2.25x10^5$* ($9.47x10^4$)	**0.007**
Damaged Cell Count, cells ml^{-1}	$9.61x10^4$ ($6.06x10^4$ - $3.31x10^5$)	$8.43x10^4$* ($2.36x10^4$)	0.142
Intact Cell Count (ICC), cells ml^{-1}	$2.73x10^5$* ($1.59x10^5$)	$1.41x10^5$* ($7.97x10^4$)	**0.001**
Low Nucleic Acid content cells, % ICC	24.65 (6.32-68.13)	35.76* (10.99)	**0.009**
High Nucleic Acid content cells, % ICC	75.35 (31.87-93.81)	64.32* (11.01)	**0.009**
L. pneumophila, CFU l^{-1}	400 (0-3100)	100 (0-2600)	0.409

[a] 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[b] Total Cell Count = Intact Cell Count + Damaged Cell Count.

Mean* (standard deviation) values are presented for normally distributed data or otherwise, **Median** (min-max) is used.

Table S4. Characteristics of domestic hot water samples of the POU-device building.

Parameter	DHW, showerhead (n=39)	DHW, circulation return (n=12)	p-value [a]
Physical and chemical parameters			
Temperature, °C	30.40 (15.9-43.9)	45.85* (2.06)	**<0.001**
pH	7.98* (0.15)	8.09* (0.11)	**0.025**
Electrical conductivity, µS cm^{-1}	384 (293-524)	373.5* (51.3)	0.222
Ca, mg l^{-1}	38.80 (31.4-42.3)	37.47* (3.03)	0.339
Mg, mg l^{-1}	11.75* (1.12)	10.98* (0.97)	**0.036**
Mn, mg l^{-1}	0.008 (0-0.397)	0.11 (0.005-0.099)	0.131
Cu, µg l^{-1}	232 (38.8-2421)	157.8* (103.5)	**0.079**
Zn, mg l^{-1}	0.097 (0.046-0.627)	0.078* (0.042)	**0.012**

Fe, mg l^{-1}	0 (0-1240)	0.155 (0.041-0.553)	**<0.001**
Pb, µg l^{-1}	2.77 (0-133)	4.29* (2.64)	0.689
Main microbial nutrients			
Total organic carbon (TOC), mg l^{-1}	2.21 (1.46-8.57)	1.97 (1.37-4.29)	0.152
Microbially available phosphorus (MAP), µg l^{-1}	0 (0-9.31)	0.2 (0-4.95)	0.307
Microbiological parameters			
Total Cell Count, cells ml^{-1} [b]	$4.12x10^5$ ($1.28x10^5$ - $9.57x10^5$)	$3.12x10^5$* ($7.39x10^4$)	**0.008**
Damaged Cell Count, cells ml^{-1}	$1.13x10^5$ ($7.50x10^4$ - $5.09x10^5$)	$1.17x10^5$* ($3.25x10^4$)	0.318
Intact Cell Count (ICC), cells ml^{-1}	$3.13x10^5$* ($1.67x10^5$)	$1.95x10^5$* ($6.65x10^4$)	**0.001**
Low Nucleic Acid content cells, % ICC	25.29 (12.78-74.03)	24.47 (18.11-58.15)	0.756
High Nucleic Acid content cells, % ICC	74.64 (26.15-87.28)	75.41 (42.15-81.53)	0.657
L. pneumophila, CFU l^{-1}	400 (0-9500)	1925 (0-10 000)	0.304

[a] 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.
[b] Total Cell Count = Intact Cell Count + Damaged Cell Count.
Mean* (standard deviation) values are presented for normally distributed data or otherwise, **Median** (min-max) is used.

Tables S5 and **S6** represent microbiological characteristics of domestic hot water (DHW) grab samples, collected from showerheads (first draw) and circulation return within two different temperature-setting periods. Mean (standard deviation) values are presented for normally distributed data or otherwise, Median (min-max) is used, and the analysis summary corresponds to the entire sampling time (weeks 0-22). **Table S5** summarises data for the showerhead samples, while **Table S6** is for circulation return samples.

Table S5. Microbiological characteristics of domestic hot water showerhead first-draw samples.

Parameter	Reference building	POU-device building	p-value [a]
Regular temperature setting (samples from weeks 0-12), n=17 (RB) and 22 (POU)			
Total Cell Count, cells ml^{-1} [b]	$4.75x10^5$* ($2.36x10^5$)	$5.09x10^5$* ($2.56x10^5$)	0.675
Damaged Cell Count, cells ml^{-1}	$1.03x10^5$ ($6.06x10^4$ - $3.31x10^5$)	$1.11x10^5$ ($7.50x10^4$ - $5.09x10^5$)	0.347
Intact Cell Count (ICC), cells ml^{-1}	$3.38x10^5$* ($1.73x10^5$)	$3.50x10^5$* ($1.99x10^5$)	0.837
Low Nucleic Acid content cells, % ICC	21.99 (6.32-68.13)	25.28 (12.78-74.03)	0.210
High Nucleic Acid content cells, % ICC	77.67 (31.87-93.81)	74.48 (26.15-87.28)	0.232
L. pneumophila, CFU l^{-1}	150 (0-3100)	150 (0-700)	0.124
Changed temperature setting (samples from weeks 16-22), n=16 (RB) and 17 (POU)			
Total Cell Count, cells ml^{-1} [b]	$2.51x10^5$ ($1.62x10^5$ - $6.53x10^5$)	$4.02x10^5$* ($1.34x10^5$)	**0.028**
Damaged Cell Count, cells ml^{-1}	$8.47x10^4$ ($6.09x10^4$ - $2.21x10^5$)	$1.18x10^5$ ($7.61x10^4$ - $2.53x10^5$)	**0.025**
Intact Cell Count (ICC), cells ml^{-1}	$1.68x10^5$ ($9.06x10^4$ - $4.51x10^5$)	$2.64x10^5$* ($9.93x10^4$)	0.081
Low Nucleic Acid content cells, % ICC	27.63* (10.32)	26.12* (2.25)	0.605
High Nucleic Acid content cells, % ICC	72.41* (10.30)	73.92* (5.25)	0.606
L. pneumophila, CFU l^{-1}	550 (0-3000)	4335* (3202)	**<0.001**

[a] 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.
[b] Total Cell Count = Intact Cell Count + Damaged Cell Count.
Mean* (standard deviation) values are presented for normally distributed data or otherwise, **Median** (min-max) is used.

Table S6. Microbiological characteristics of domestic hot water circulation return samples.

Parameter	Reference building	POU-device building	p-value [a]
Regular temperature setting (samples from weeks 0-12), n=6			
Total Cell Count, cells ml^{-1} [b]	$2.82x10^5$* ($1.03x10^5$)	$2.94x10^5$* ($8.48x10^4$)	0.825
Damaged Cell Count, cells ml^{-1}	$9.44x10^4$* ($2.35x10^4$)	$1.03x10^5$* ($2.77x10^4$)	0.555
Intact Cell Count (ICC), cells ml^{-1}	$1.87x10^5$* ($8.95x10^4$)	$1.90x10^5$* ($8.28x10^4$)	0.948
Low Nucleic Acid content cells, % ICC	36.02* (15.41)	30.89* (14.29)	0.564
High Nucleic Acid content cells, % ICC	64.04* (15.41)	69.05* (14.08)	0.569
L. pneumophila, CFU l^{-1}	75 (0-1200)	125 (0-3100)	0.394
Changed temperature setting (samples from weeks 16-22), n=6			
Total Cell Count, cells ml^{-1} [b]	$1.69x10^5$* ($4.05x10^4$)	$3.31x10^5$* ($6.34x10^4$)	**<0.001**
Damaged Cell Count, cells ml^{-1}	$7.43x10^4$* ($2.06x10^4$)	$1.31x10^5$* ($3.30x10^4$)	**0.006**
Intact Cell Count (ICC), cells ml^{-1}	$9.51x10^4$* ($2.96x10^4$)	$2.00x10^5$* ($5.32x10^4$)	**0.002**
Low Nucleic Acid content cells, % ICC	35.50* (5.31)	23.68 (21.87-33.38)	0.065
High Nucleic Acid content cells, % ICC	64.59* (5.41)	76.27 (66.86-78.26)	**0.026**
L. pneumophila, CFU l^{-1}	900* (1008)	6042* (3790)	**0.020**

[a] 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.
[b] Total Cell Count = Intact Cell Count + Damaged Cell Count.
Mean* (standard deviation) values are presented for normally distributed data or otherwise, **Median** (min-max) is used.

Table S7 summarises the characteristics of water entering the internal water supply of both buildings. Only the values that are significantly different are shown. The values for the Reference building represent the main water inlet, while the values for the POU-device building represent samples collected after passing the sorption filter. Mean (standard deviation) values are presented for normally distributed data or otherwise, Median (min-max) is used, and the analysis summary corresponds to the entire sampling time (weeks 0-22). All samples were collected as grab-samples.

Table S7. Characteristics of water entering the internal water supply network.

Parameter	DHW, showerhead (n=39)	DHW, circulation return (n=12)	p-value [a]
Changed temperature setting (samples from weeks 16-22), n=12			
Electrical conductivity, µS cm^{-1}	282.67* (11.98)	367.17* (25.77)	**<0.001**
Ca, mg l^{-1}	36.08* (1.93)	39.28* (1.49)	**0.009**
Mg, mg l^{-1}	9.52* (1.18)	11.10* (1.15)	**0.040**
Mn, mg l^{-1}	0.032 (0.018-0.102)	0.014* (0.010)	**0.026**
Cu, µg l^{-1}	6.2 (3.1-31.2)	0 (0-6.1)	**0.015**
Fe, mg l^{-1}	0.153 (0.096-0.652)	0.045* (0.046)	**0.015**
Total organic carbon (TOC), mg l^{-1}	1.39* (0.37)	2.15* (0.32)	**0.003**
Intact Cell Count (ICC), cells ml^{-1}	$8.36x10^4$ ($6.62x10^4$ - $1.87x10^5$)	$1.59x10^5$* ($7.11x10^4$)	**0.041**
Low Nucleic Acid content cells, % ICC	41.42* (4.47)	31.91* (4.28)	**0.004**
High Nucleic Acid content cells, % ICC	58.67* (4.41)	68.06* (4.16)	**0.004**

[a] 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.
Mean* (standard deviation) values are presented for normally distributed data or otherwise, **Median** (min-max) is used.