Journal of Water & Health

IWA PUBLISHING

Journal of Water and Health Vol 20 No 7, 1084 doi: 10.2166/wh.2022.055

Phosphate drinking water softeners promote *Legionella* growth

Gregor Jereb[a,b], Ivan Eržen[b,c], Martina Oder[a] and Borut Poljšak[a,*]

[a] Faculty of Health Sciences, University of Ljubljana, Zdravstvena pot 5, 1000 Ljubljana, Slovenia
[b] Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia
[c] The National Institute of Public Health, Trubarjeva 2, 1000 Ljubljana, Slovenia
*Corresponding author. E-mail: borut.poljsak@zf.uni-lj.si

GJ, 0000-0003-0687-4094; BP, 0000-0003-3598-3468

ABSTRACT

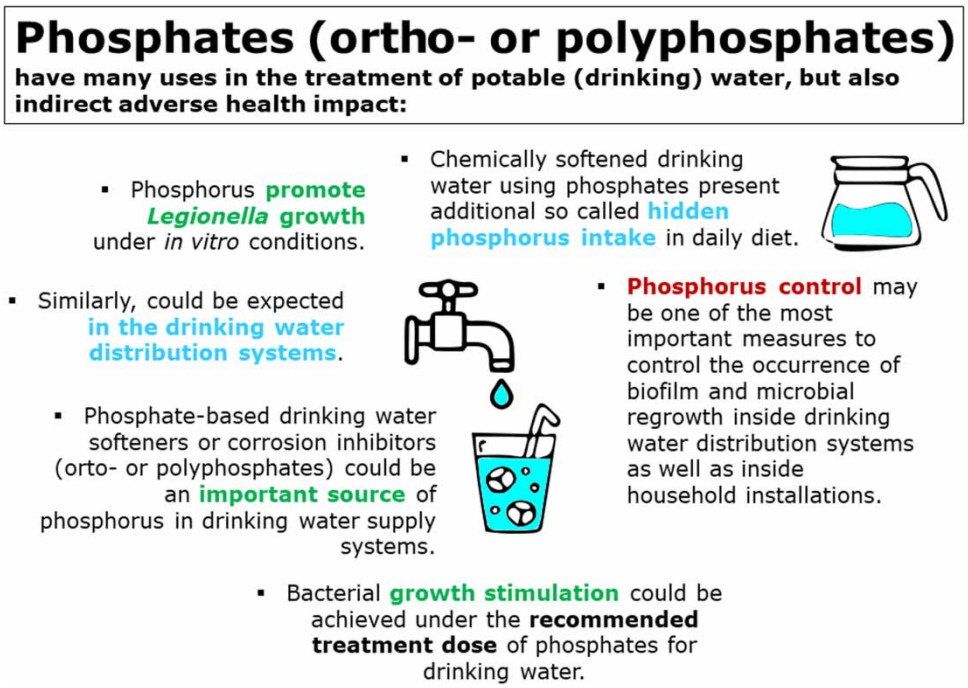
Phosphate-based drinking water softeners are commonly used to prevent scale formation in drinking water distribution infrastructure. The main reason for drinking water softening is primarily economic (protection of pipes and extension of equipment life), while the health aspect of such treatment is usually neglected. The aim of this work is to investigate the effects of phosphate-based drinking water softeners on growth stimulation of *Legionella pneumophila*. Bacterial growth was observed at two different phosphate concentrations. On average, an increase in growth of 1.19–1.28 log CFU/mL was observed in selected samples with added phosphates compared with the control. The results of the *in vitro* experiment confirmed that the added phosphates stimulate the growth of *L. pneumophila*; growth stimulation could therefore be expected in drinking water distribution systems (DWDS) when phosphates are used as well. The availability of phosphorus in DWDS may be a crucial limiting factor for biofouling control. Consequently, phosphate-based chemicals for drinking water should be avoided or used with prudence, especially in drinking water with high concentrations of other nutrients.

Key words: drinking water softening, growth stimulation, *Legionella*, phosphorus

HIGHLIGHTS

- Phosphorus promotes the growth of *Legionella* under *in vitro* conditions; growth stimulation could be expected also in DWDS.
- Stimulation of bacterial growth could be achieved at proposed treatment doses of phosphates for drinking water.
- Phosphate-based drinking water softeners and/or corrosion inhibitors (ortho- or polyphosphates) could be an important source of phosphorus in DWDS.

GRAPHICAL ABSTRACT



INTRODUCTION

The main aspect of drinking water softening is to prevent scale formation and pipe corrosion and to reduce the leaching of copper and lead from pipe materials. Another reason for drinking water softening is also consumer convenience. In many households and also in some central drinking water treatment plants, drinking water is softened by using various softening techniques (precipitation, ion exchange, or membrane separation) (Casale 2001). Phosphate salts and polyphosphate solutions are often used as drinking water softeners (Casale 2001; Batté *et al.* 2003a).

According to SIST EN 1212 (2005), the treatment dose of phosphates for drinking water treatment should not exceed 5 mg P_2O_5 per liter of drinking water. The Slovenian National Institute of Public Health (NIJZ 2019) and the German Federal Environmental Agency – UBA (UBA 2020) also recommend up to 2.2 mg of phosphates per liter as P (i.e., 5.04 mg as P_2O_5 or 6.74 mg as PO_4). For corrosion inhibition and scale prevention in drinking water systems, EPA (2016) has issued similar guidelines with somewhat lower concentrations of residual phosphates up to 1.0 mg/L as P (i.e., 3.0 mg/L as PO_4). However, according to EPA, a 2- to 3-fold higher initial dose is recommended if so required, followed by a lower maintenance dose.

The EU Directive on the quality of water intended for human consumption (Directive (EU) 2020/2184) does not regulate phosphate concentration or drinking water softening techniques as such. Consequently, the phosphate (or phosphorus) concentration in drinking water is not controlled. Therefore, it is not surprising that the phosphate concentration in drinking water samples in Slovenia, where phosphate softeners are used on a large scale, varies widely, from 0.2 to 24.6 mg PO_4/L (Jereb *et al.* 2017).

When it comes to drinking water quality, the control of not only chemical hazards but also microbiological hazards is of great importance. Management of biofouling in the drinking water distribution system (DWDS) is an important strategy to ensure safe drinking water. Phosphate treatment of drinking water carries the risk of promoting the growth of microorganisms, which could be described as a side effect of chemical softening. According to various researchers (Miettinen *et al.* 1997; Sathasivan *et al.* 1997; Sathasivan & Ohgaki 1999; Batté *et al.* 2003a; Park *et al.* 2008; Wen *et al.* 2014), the availability of phosphorus in the water environment may be crucial for microbial growth. Therefore, the addition of a scarce micronutrient (such as phosphorus) to water as part of drinking water treatment could contribute significantly to the growth of various bacterial species. One of them is *Legionella pneumophila*, pathogenic bacteria commonly found in water supply

systems (Toplitsch *et al.* 2018). *Legionella* is a ubiquitous microorganism found in the natural aquatic environment (Bonetta & Bonetta 2020), where it does not normally pose a health risk due to its low concentrations. A health risk arises when the bacteria occur in high concentrations in DWDS (Barskey *et al.* 2020; ECDC 2021), where they can cause Legionnaires' disease and/or Pontiac fever (Prussin *et al.* 2017). Among the various members of the genus *Legionella*, *L. pneumophila* is the most confirmed pathogen (Harrison *et al.* 2009; ECDC 2021).

The objective of the present study was to investigate the effects of phosphate-based drinking water softeners on the growth and reproduction of *L. pneumophila* to provide additional data for informed decision making in case of using phosphate-based drinking water softeners.

MATERIALS AND METHODS

The standard strain of *Legionella pneumophila* subsp. *pneumophila* ATCC 33152 (acquired from the Czech Collection of Microorganisms – CCM) was used. For the study of the growth curve under two different phosphate concentrations, the bacteria were incubated at 36 °C (SIST EN ISO 11731 2017) in microtiter plates. To ensure reproducibility, the experiment was performed with liquid yeast extract broth (Sigma-Aldrich, 92144-500G-F) containing 400 mg/L L-cysteine HCl (*Legionella* BCYE α-growth supplement, Biolife, 423210). An overnight culture with an initial bacterial cell concentration of 6.06 ($\pm$0.23) log CFU/mL was used as the starter concentration in all experiments. Since bacteria of the genus *Legionella* are known to have slower growth and longer regeneration time (SIST EN ISO 11731 2017), bacterial growth was monitored up to 144 h after the start of the experiment. Bacterial concentration was monitored at time 0 and after 24, 48, 72, 96, 120, and 144 h. After the incubation period, the tenfold dilution approach was performed (SIST EN ISO 8199 2007) and the method of counting bacterial colonies on solid medium (*Legionella* BCYE Agar Base, Biolife, 4015822) was used. Three experimental replicates were performed for each phosphate concentration, as well as for the control, resulting in a total of seven sample replicates. Due to an error in the incubation process, one sample with the highest phosphate concentration was withdrawn from further analysis after 48 h of incubation. Thus, a total of 146 samples were analyzed. Results are presented in logarithmic values as log CFU/mL for each concentration and incubation time.

A commercial drinking water softener with a nominal concentration of 7 $\pm$ 0.2% P_2O_5 was used to observe the effect of added phosphates on *L. pneumophila* growth. Two stock solutions with concentrations of 1 and 10 g/L were prepared from the commercial softener (basic phosphate solutions A and B). In both cases, 0.24 mL of phosphate stock solution A or B (or distilled water in the control) was added to a total volume of 48.6 mL of medium containing liquid yeast extract broth, cysteine, and bacterial starter culture. The pH of the medium did not change. The control in the experiment consisted of the bacteria *L. pneumophila* in yeast extract without added phosphates.

To determine the phosphate concentration in each sample, equal amounts of stock solutions A and B were added to 48.6 mL of distilled water. The phosphate concentration in the samples was measured according to the standard ISO (SIST EN ISO 6878 2004). The method of chemical detection is described in detail in Jereb *et al.* (2017). A phosphate solution was prepared with a concentration of 3.6 mg PO_4/L and 39.5 mg PO_4/L. With the lower concentration chosen (3.6 mg PO_4/L), we aimed to achieve the average concentrations of added phosphate in Slovenian drinking water systems reported in previous research (Jereb *et al.* 2017). A tenfold higher concentration was prepared for a higher test concentration.

RESULTS

The number of CFU for the two phosphate concentrations tested and for the control differed across all time points of the experiment (Figure 1). Nevertheless, increased growth of *Legionella* was observed in the samples with added phosphates compared with the control.

The highest difference in the number of bacteria in the samples with phosphates compared with the control (1.76 log CFU/mL) was observed at time t_{120} at lower phosphate concentration (3.6 mg PO_4/L). In the time intervals 72–120 h after incubation, the difference in the number of organisms in the samples with phosphates compared with the control was 1.40 log CFU/mL or more for both phosphate concentrations. Even after 144 h of incubation, the number of bacteria was still significantly higher in the case of added phosphates (7.82 log CFU/mL and 7.58 log CFU/mL) compared with the control (6.63 log CFU/mL), with a difference of 1.19 and 0.95 log CFU/mL, respectively.

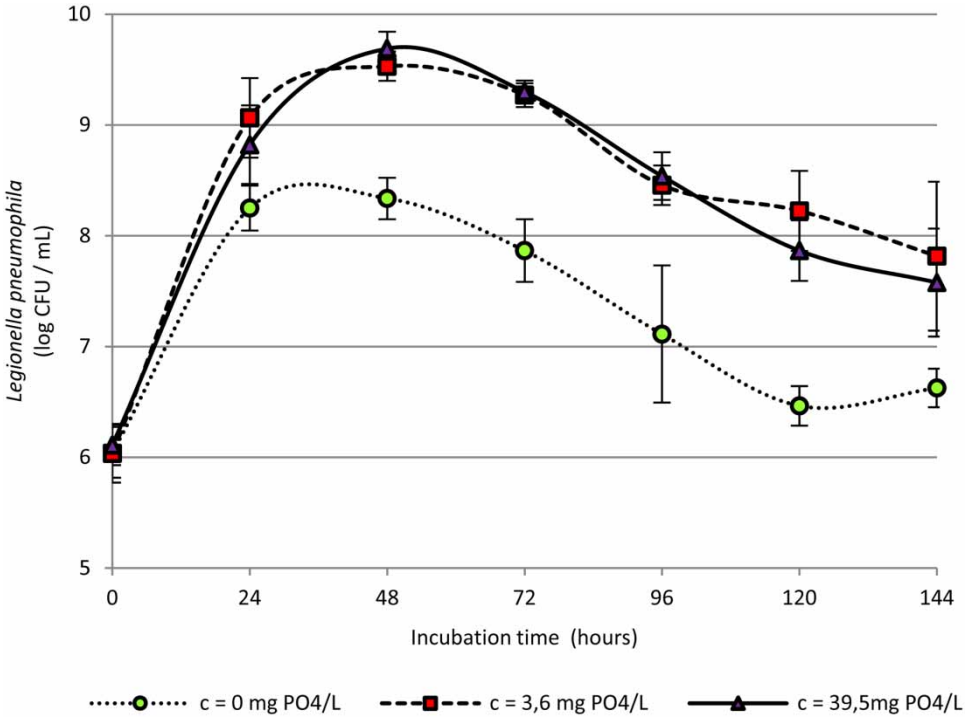


Figure 1 | Influence of a phosphate-based water softener on the growth of *L. pneumophila* – growth curve of *L. pneumophila* subsp. *pneumophila* ATCC 33152 with added phosphates (c = 3.6 and 39.5 mg PO_4/L) compared with control.

A nonparametric Mann–Whitney test was used to calculate the differences in the number of CFUs between the two phosphate concentrations tested and the control group at different time points (Table 1). At the beginning of the experiment (t_0), there were no statistically significant differences between the groups (all $p > 0.05$). Over time, the groups with added phosphates differed significantly from the control group (all $p < 0.01$) in the number of CFUs. The increased growth confirms the positive effect of phosphates on the growth of *L. pneumophila* subsp. *pneumophila* ATCC 33152. However, there were no statistically significant differences in the number of CFUs observed when comparing high and low phosphate concentrations (except at t_{48}, where the difference is at a level of 0.05). The later results suggest no additional growth potential when phosphate concentration increases.

Table 1 | Statistical difference in the number of colonies grown – comparison of different phosphate concentrations to control

	Comparison					
	C_0 vs. $C_{3.6}$		**C_0 vs. $C_{39.5}$**		**$C_{3.6}$ vs. $C_{39.5}$**	
Time	***z***	***p***	***z***	***p***	***z***	***p***
t_0	0.000	1.000	0.948	0.382	0.738	0.460
t_{24}	3.258	0.001**	2.941	0.003**	1.156	0.248
t_{48}	3.130	0.002**	3.000	0.003**	2.143	0.032*
t_{72}	2.882	0.004**	2.882	0.004**	0.561	0.575
t_{96}	3.003	0.003**	3.130	0.002**	0.831	0.406
t_{120}	2.882	0.004**	2.882	0.004**	1.922	0.055
t_{144}	3.134	0.002**	3.134	0.002**	0.192	0.848

Note: Mann–Whitney U-test pairwise comparisons between groups.
C_0 – control without added phosphate.
$C_{3.6}$ – sample with 3.6 mg PO_4/L.
$C_{39.5}$ – sample with 39.5 mg PO_4/L.
*$p < 0.05$.
**$p < 0.01$.

DISCUSSION

The present study confirms the effect of added phosphates on *Legionella* growth under *in vitro* conditions and similar impact on growth could be expected in DWDS. Stimulation of bacterial growth was achieved at the proposed treatment doses of phosphates for drinking water. Drinking water softeners or phosphate-based corrosion inhibitors (ortho- or polyphosphates) could therefore be an important source of phosphorus in DWDS. The number of *Legionella* increased significantly compared with the control at a concentration of 3.6 mg PO_4/L, while at a higher concentration of added phosphorus (c = 39.5 mg PO_4/L), the growth remained the same as at a lower concentration. On average, 1.19–1.28 log CFU/mL higher growth was observed as compared with the control group.

Some other researchers also emphasize the importance of inorganic nutrients in drinking water as a key factor in controlling the growth of microorganisms, especially in aquatic environments with high levels of available carbon. Batté *et al.* (2003a) reported that the addition of phosphorus affects the growth of bacteria of the genus *Legionella*. Sathasivan *et al.* (1997) documented increased bacterial growth when phosphorus was added to the water medium. Even when the carbon concentration in the water was high, no increase in microbial growth was observed when the phosphorus content was inadequate. Similarly, Park *et al.* (2008) demonstrated that the addition of phosphorus had no effect on microbial growth at low concentrations of available carbon, while at high carbon concentrations the addition of phosphorus significantly stimulated microbial growth. Stimulation of growth was measured by increased number (CFU/cm^2) and weight (μg/cm^2), both again suggesting the role of available phosphorus in bacterial proliferation. Sathasivan & Ohgaki (1999) also identified phosphorus (0.3 mg PO_4/L) as the major growth-limiting factor in samples from the Tokyo DWDS. Miettinen *et al.* (1997) confirmed comparable results in drinking water samples from Finland. Despite the relatively high concentrations of naturally occurring organic carbon in the water, they observed a noticeable increase in the growth of microorganisms only when phosphorus was added. According to the study by Polanska *et al.* (2005), phosphorus in Belgian waters (which are also rich in carbon) also proved to be an important limiting growth factor for microorganisms in their drinking water. A similar conclusion was reported by Wen *et al.* (2014) for northeast China in the city of Harbin.

It can be concluded that the availability of phosphorus may be a crucial limiting factor for bacterial growth in DWDS. Since various phosphorus formulations (from phosphoric (V) acid to various ortho- and polyphosphates) are commonly used to soften drinking water or to inhibit corrosion (Casale 2001; Batté *et al.* 2003a), additional caution is needed. This is especially important since the effects of phosphorus on *Legionella* proliferation are often ignored. Although researchers reported high phosphorus (phosphate) concentrations in their studied DWDS, they did not attribute the proliferation of microorganisms to phosphates (phosphorus), but to a lower disinfection effect. For example, Girolamini *et al.* (2019) reported phosphorus concentrations in observed hospital hot water pipes ranging from 1.68 to 3.52 mg/L as P_2O_5. However, in their study, phosphorus was measured solely to monitor the maintenance of anti-scale and corrosion treatment. Although a high percentage of positive samples (95%) and high *Legionella* concentrations were detected in the observed DWDS, the authors did not investigate the cause of *Legionella* proliferation. Similarly, Song *et al.* (2021) studied the effect of phosphate in DWDS on reducing the biocidal effect of copper ions (phosphate as a corrosion inhibitor) but neglected its growth stimulation on *Legionella*. Cullom *et al.* (2020) also mentioned phosphorus in DWDS only as an inhibitor of copper ions. Likewise, they did not correlate higher numbers of microorganisms with growth stimulation by phosphate, but rather with its reduction of copper ions (biocidal effect) due to the reduction of leaching from copper pipes. Martin *et al.* (2020) reached the same conclusions in the case of a high concentration (CFU) of *Legionella* in Flint DWDS.

Most microorganisms in drinking water are heterotroph and require adequate concentrations of organic and inorganic nutrients for their proliferation. One of these nutrients is phosphorus (or phosphates). Phosphorus is an essential element required for cell growth as part of DNA/RNA, phospholipids, and many enzymes, and therefore plays an important role in cell metabolism, some structural functions, and cell response to stress (Brown & Kornberg 2008). Because the bacterial system of phosphate transport across membranes is not efficient when the phosphate concentration in water is low but becomes energy efficient when the concentration increases, typical concentrations of phosphate corrosion control agents or drinking water softeners greatly exceed the nutrient requirements of bacterial cells and can promote bacterial proliferation (Batté *et al.* 2003b).

According to the above studies, phosphorus is often neglected as an important nutrient in DWDS. Even more, it seems that the most important nutrients needed for microbial growth in the drinking water system (organic carbon and phosphorus) interact according to the principle of Liebig's law of minimum. Since phosphorus concentrations are rather low in the vast majority of drinking water sources (Batté *et al.* 2003a), phosphate-based drinking water softeners could be an important

source of additional phosphorus. In such cases, phosphorus control may be one of the most important measures to control the occurrence of biofilm and microbial regrowth in DWDS, especially in waters rich in other nutrients.

It should be emphasized that the presented experiment was performed under laboratory conditions in yeast extract broth with a standardized strain of *Legionella pneumophila* subsp. *pneumophila* ATCC 33152 without other microorganisms present. *L. pneumophila* was added at higher concentrations than normally found in the water supply systems, so it is possible that the bacteria would react differently under real (*in vivo*) conditions. To confirm the effect of phosphate-based drinking water softeners on *Legionella* growth in DWDS, further experiments should be conducted in the future using different water samples with different (controlled) levels of assimilable organic carbon and phosphorus. Nevertheless, the results of the experiment in culture media indicated that the added phosphorus significantly affected the growth of *L. pneumophila*. Therefore, a similar effect could be expected in DWDS when phosphates are used as drinking water softeners.

In the future, any drinking water treatment that could promote microbial growth in DWDS should be avoided or used judiciously. In addition, it is strongly recommended that at least a chemical analysis of the raw water be performed before phosphate-based softeners or corrosion inhibitors are used (and when the phosphorus concentration is low and the concentration of other nutrients high, phosphate softeners should be avoided).

CONCLUSION

Since the number (concentration) of *L. pneumophila* present in drinking water is a key factor in the occurrence of infections, any promotion of *Legionella* growth in DWDS poses an unnecessary health risk. The results of this study provide a deeper understanding of the previously neglected risk of bacterial growth when drinking water is treated with phosphates as water softeners or corrosion inhibitors. Future risk assessment of phosphate-based drinking water softeners and/or corrosion inhibitors should therefore also consider the indirect effect of promoting bacterial growth. Especially in drinking water rich in other nutrients, the (non)availability of phosphorus may be an important growth-limiting factor. Controlling phosphorus could therefore be one of the most important measures to control the biofouling and microbial regrowth in DWDS.

ACKNOWLEDGEMENTS

For the English proofreading, Tina Levec, an English language instructor, is gratefully acknowledged. Additionally, this work is a part of Ph.D. thesis which was supported by a grant (Jereb G.; 322/2012) from the European Social Fund (ESF) and the Ministry of Education, Science and Sport of the Republic of Slovenia.

AUTHOR CONTRIBUTIONS

All authors participated in the design of the study. Gregor Jereb conducted the literature review, prepared the samples, performed all analyses, and conceptualized the manuscript. Martina Oder was involved in the laboratory analyses. Borut Poljšak, Ivan Eržen, and Martina Oder contributed to the interpretation of results and manuscript revisions. All authors reviewed and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Barskey, A., Lackraj, D., Tripathi, P. S., Cooley, L., Lee, S., Smith, J. & Edens, C. 2020 *Legionnaires' Disease Surveillance Summary Report, United States–2016 and 2017*. Centers for Disease Control and Prevention (CDC), Washington. Available from: https://www.cdc.gov/legionella/health-depts/surv-reporting/2016-17-surv-report-508.pdf (accessed 19 July 2021).

Batté, M., Appenzeller, B. M. R., Grandjean, D., Fass, S., Gauthier, V., Jorand, F., Mathieu, L., Boualam, M., Saby, S. & Block, J. C. 2003a Biofilms in drinking water distribution systems. *Reviews in Environmental Science and Biotechnology* **2** (2), 147–168. https://doi.org/10.1023/B:RESB.0000040456.71537.29.

Batté, M., Koudjonou, B., Laurent, P., Mathieu, L., Coallier, J. & Prévost, M. 2003b Biofilm responses to ageing and to a high phosphate load in a bench-scale drinking water system. *Water Research* **37** (6), 1351–1361. https://doi.org/10.1016/S0043-1354(02)00476-1.

Bonetta, S. & Bonetta, S. 2020 Editorial comments to the special issue: '*Legionella* contamination in water environment'. *Pathogens* **9**, 1017. https://doi.org/10.3390/pathogens9121017.

Brown, M. R. & Kornberg, A. 2008 The long and short of it – polyphosphate, PPK and bacterial survival. *Trends in Biochemical Sciences* **33** (6), 284–290. https://doi.org/10.1016/j.tibs.2008.04.005.

Casale, R. J. 2001 Improving chemical handling procedures can help reduce associated treatment problems. *Journal/American Water Works Association* **93** (9), 95–106. https://doi.org/10.1002/j.1551-8833.2001.tb09290.x.

Cullom, A. C., Martin, R. L., Song, Y., Williams, K., Williams, A., Pruden, A. & Edwards, M. A. 2020 Critical review: propensity of premise plumbing pipe materials to enhance or diminish growth of *Legionella* and other opportunistic pathogens. *Pathogens* **9** (11), 957. http://dx.doi.org/10.3390/pathogens9110957.

Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption.

ECDC – European Centre for Disease Prevention and Control 2021 Legionnaires' disease. In: *ECDC. Annual Epidemiological Report for 2019*. ECDC, Stockholm. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/AER-legionnaires-2019.pdf (accessed 19 July 2021).

EPA – Environmental Protection Agency US 2016 *Optimal Corrosion Control Treatment Evaluation Technical Recommendations for Primacy Agencies and Public Water Systems*. Available from: https://www.epa.gov/dwreginfo/optimal-corrosion-control-treatment-evaluation-technical-recommendations (accessed 3 November 2021).

Girolamini, L., Dormi, A., Pellati, T., Somaroli, P., Montanari, D., Costa, A., Savelli, F., Martelli, A., Grottola, A., Fregni Serpini, G. & Cristino, S. 2019 Advances in *Legionella* control by a new formulation of hydrogen peroxide and silver salts in a hospital hot water network. *Pathogens* **8** (4), 209. http://dx.doi.org/10.3390/pathogens8040209.

Harrison, T. G., Afshar, B., Doshi, N., Fry, N. K. & Lee, J. V. 2009 Distribution of *Legionella pneumophila* serogroups, monoclonal antibody serogroups and DNA sequence types in recent clinical and environmental isolates from England and Wales (2000–2008). *European Journal of Clinical Microbiology* **28** (7), 781–791. https://doi.org/10.1007/s10096-009-0705-9.

Jereb, G., Poljšak, B. & Eržen, I. 2017 Contribution of drinking water softeners to daily phosphate intake in Slovenia. *International Journal of Environmental Research and Public Health* **14** (10), 1186. https://doi.org/10.3390/ijerph14101186.

Martin, R. L., Strom, O. R., Pruden, A. & Edwards, M. A. 2020 Interactive effects of copper pipe, stagnation, corrosion control, and disinfectant residual influenced reduction of *Legionella pneumophila* during simulations of the flint water crisis. *Pathogens* **9** (9), 730. http://dx.doi.org/10.3390/pathogens9090730.

Miettinen, I. T., Martikainen, P. J. & Vartiainen, T. 1997 Phosphorus and bacterial growth in drinking water. *Applied and Environmental Microbiology* **63** (8), 3242–3245. https://doi.org/10.1128/aem.63.8.3242-3245.1997.

NIJZ 2019. https://www.nijz.si/sites/www.nijz.si/files/uploaded/spremenjena_kopija_seznam_brez_vodn_tiska_14.6._2019_1.pdf (accessed 1 September 2021).

Park, S. K., Kim, Y. K. & Choi, S. C. 2008 Response of microbial growth to orthophosphate and organic carbon influx in copper and plastic based plumbing water systems. *Chemosphere* **72** (7), 1027–1034. https://doi.org/10.1016/j.chemosphere.2008.04.006.

Polanska, M., Huysman, K. & Van Keer, C. 2005 Investigation of microbially available phosphorus (MAP) in flemish drinking water. *Water Research* **39** (11), 2267–2272. https://doi.org/10.1016/j.watres.2005.04.019.

Prussin, A. J., Schwake, D. O. & Marr, L. C. 2017 Ten questions concerning the aerosolization and transmission of *Legionella* in the built environment. *Building and Environment* **123**, 684–695. https://doi.org/10.1016/j.buildenv.2017.06.024.

Sathasivan, A. & Ohgaki, S. 1999 Application of new bacterial regrowth potential method for water distribution system – a clear evidence of phosphorus limitation. *Water Research* **33** (1), 137–144. https://doi.org/10.1016/S0043-1354(98)00158-4.

Sathasivan, A., Ohgaki, S., Yamamoto, K. & Kamiko, N. 1997 Role of inorganic phosphorus in controlling regrowth in water distribution system. *Water Science and Technology* **35** (8), 37–44. https://doi.org/10.1016/S0273-1223(97)00149-2.

SIST EN 1212 2005 Chemicals Used for Treatment of Water Intended for Human Consumption – Sodium Polyphosphate.

SIST EN ISO 6878 2004 Water Quality – Determination of Phosphorus – Ammonium Molybdate Spectrometric Method.

SIST EN ISO 8199 2007 Water Quality – General Guidance on the Enumeration of Micro-Organisms by Culture.

SIST EN ISO 11731 2017 Water Quality – Enumeration of Legionella.

Song, Y., Pruden, A., Edwards, M. A. & Rhoads, W. J. 2021 Natural organic matter, orthophosphate, pH, and growth phase can limit copper antimicrobial efficacy for *Legionella* in drinking water. *Environmental Science and Technology* **55** (3), 1759–1768. https://doi.org/10.1021/acs.est.0c06804.

Toplitsch, D., Platzer, S., Pfeifer, B., Hautz, J., Mascher, F. & Kittinger, C. 2018 *Legionella* detection in environmental samples as an example for successful implementation of qPCR. *Water* **10**, 1012. https://doi.org/10.3390/w10081012.

UBA – Umweltbundesamt (German Environment Agency) 2020. https://www.umweltbundesamt.de/sites/default/files/medien/5620/dokumente/201208_22._bekanntmachung_der_liste_der_aufbereitungsstoffe_und_desinfektionsverfahren_gemaess_ss_11_der_trinkwv_unterzeichnet.pdf (accessed 1 September 2021).

Wen, G., Ma, J., Huang, T. L. & Egli, T. 2014 Using coagulation to restrict microbial re-growth in tap water by phosphate limitation in water treatment. *Journal of Hazardous Materials* **280**, 348–355. https://doi.org/10.1016/j.jhazmat.2014.08.020.

First received 14 February 2022; accepted in revised form 25 June 2022. Available online 5 July 2022