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- 5 Exploring potentials and constraints of H_2O_2 water disinfection for household settings
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- 12 **Abstract:** Poor sanitation facilities and deficiencies in infrastructure lead to a scenario of waterborne
- diseases, particularly in low-income regions. Point-of-use (POU) and point-of-entry (POE) solutions
- may be potential interventions for a positive impact in public health, complying with the United
- Nations Sustainable Development Goal of safe and affordable water for all (SDG 6). Chlorination is
- 16 a common POU practice, thus benchmarking disinfection against it could be beneficial for finding
- 17 alternative household-scale approaches. Here we explored hydrogen peroxide, a well-known and
- commercially available oxidant, as a standalone disinfectant targeting *Escherichia coli* and Phi X174
- bacteriophage as a model of enteric viruses, common pathogens found in source waters. Oxidation of
- 20 natural organic matter (NOM) was also assessed by photometric assays. A 30-minute exposure to
- 21 H_2O_2 at 0.3% provided >6.5 log_{10} -inactivation of phage, whereas chlorine reached approximately
- 22 3.0. When exclusively targeting bacteria, both disinfectants were considered efficient, but, when Phi
- 23 X174 was included, only H₂O₂ satisfied criteria. Chlorine oxidation performance was considered
- sufficient, however, NOM variations obtained by H₂O₂ treatments should be further assessed.
- 25 Though some limitations are discussed, particularly considering residuals, these are taken as
- 26 directions for investigating practical applications. Overall, results suggest H₂O₂ is a potential
- 27 standalone POU disinfectant, encouraging research on context-specific household settings or
- 28 emergency scenarios.
- 29 **Keywords:** hydrogen peroxide; Phi X174; bacteriophage; oxidation; point-of-use disinfection;
- 30 indicator bacteria

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Introduction

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- Water quality compliance in most low-income countries is insufficient due to a lack of government commitment to supply and infrastructure, thus leading to poor access to safe water and an improved service delivery (Okoro et al. 2021). Even in some urban settings, where there are centralized treatment systems, some studies report measurable levels of pathogens in drinking water (Subbaraman et al. 2013). As for self-supply, water quality is largely heterogeneous, and research has shown these systems are significantly more likely to be contaminated (Genter et al. 2021). This scenario poses a risk to human health and a threat to achieving the United Nations Sustainable
- In light of this, point-of-use (POU) and point-of-entry (POE) solutions, which are both householdscale water treatments, have been rising as a promising strategy to control the waterborne diseases burden in developing countries (Ehdaie et al. 2020), by filling the service gap when piped water is not available or not considered safe for potability (Brown and Sobsey 2010). These are on-site treatment systems, capable of reducing pathogen levels in water sources prior to consumption (Pooi and Ng 2018).

Development Goal of safe and affordable water for all (SDG 6) by 2030 (WHO; UNICEF 2020).

- An example of a simple and suggestively well-known POU disinfection technique for drinking water is chlorination followed by safe storage. However, chlorine in the presence of natural organic matter (NOM) is associated to the formation of disinfection by-products (DBPs) (Hu et al. 2018; Mazhar et al. 2020). Thus, investigating alternative disinfectant products that could be potentially applied at the household level would avoid such concern, whereas leading to satisfactory pathogen inactivation.
- 60 In this sense, hydrogen peroxide (H₂O₂) is a potential candidate, as it has been widely employed in 61 surface disinfection (Brauge et al. 2020; Hayrapetyan et al. 2020; Yamasaki et al. 2020). Although 62 there are also reports of its application (both standalone and combined use) in disinfection of water 63 sources (Guimarães et al. 2014; Karel 2018), recreative water (Rosende et al. 2020) and wastewater 64 (Formisano et al. 2016; Guadagnini et al. 2013; Koivunen and Heinonen-Tanski 2005), to our 65 knowledge, research has not focused on individual use of liquid H₂O₂ at the household level for 66 either POU/POE applications, nor humanitarian emergency water supply. Most of the data on its use 67 as a disinfectant is concentrated in decontamination (particularly in hospital settings) (Oon et al. 68 2020; Romeu et al. 2020; Totaro et al. 2020), food industry and agricultural applications (Melo et al. 69 2019; Motola et al. 2020; Ortiz-Solà et al. 2020; S. Wang et al. 2020; Wlazlo et al. 2020). The way it 70 is applied also depends on the goal and the matrix, so information from the literature refers to H₂O₂

not only in its liquid form, but also spray and aerosolized hydrogen peroxide.

- As much of the effective application of chlorine can be limited by uncertainties regarding the
- determination of initial dose (Wu and Dorea 2021), such difficulty also applies to hydrogen peroxide
- disinfection, which lacks straight-forward information for household-scale treatments. In order to
- shed light onto the possible application of H₂O₂ as a POU sole disinfectant for drinking water, it is
- 76 important to initially evaluate its performance in laboratory-controlled settings, contemplating
- 77 different microbial contamination scenes.
- 78 It should be noted that, from a research standpoint, probabilities of infection risk statistically increase
- when survival information for different microorganisms are used comparatively to indicator species
- data (Mraz et al. 2021). In other words, relying on indicator bacteria alone for assessing treatment
- 81 efficiency may underestimate the health risk to consumers (Mraz et al. 2021).
- 82 Recent studies have underscored effluents as sources of viral contamination (Yang et al. 2021) and
- numerous reports have dedicated to the detection of viruses in surface water (Guo et al. 2018; Hata et
- al. 2014), freshwater (Masachessi et al. 2020), groundwater (Emelko et al. 2019; Ji et al. 2020) and
- 85 even drinking water (Wang et al. 2020). However, most household purification systems (and that
- 86 includes chlorination) are characterized by their efficiency in removing bacteria, but not viruses in
- general (Lugo et al. 2021). Timely, bacteriophages that infect coliform bacteria have been considered
- as possible surrogates for enteric viruses in surface and groundwater, as well as disinfected samples
- 89 (Lau et al. 2020; Savichtcheva and Okabe 2006). Hence, simulating contamination with
- bacteriophages as enteric viruses' models should be a suitable complementary analysis to standard
- 91 indicator organisms, particularly because coliform bacteria and E. coli are not necessarily
- 92 representative markers for viral contamination (Pang et al. 2021).
- 93 Considering this, the aim of this study was to assess the performance of hydrogen peroxide as a
- 94 standalone disinfectant for potential point-of-use applications, considering a water source with low
- 95 levels of natural organic matter, but high microbial load. This was achieved by a comparison to
- 96 conventional chlorine disinfection, considering a microbiological contamination simulated by seeded
- 97 Escherichia coli as an indicator from the bacterial group, and Phi X174 bacteriophage as a virus
- 98 model. We also aimed to make some preliminary considerations on H₂O₂ effects on organic matter,
- in order to shed light onto challenges and perspectives from the oxidation standpoint.

Materials and methods

101 Experimental procedure

Disinfection tests were carried out in reagent glass bottles previously disinfected. These were wrapped in aluminum foil, in order to avoid photo-degradation of hydrogen peroxide, particularly. Reactional conditions were provided by slow magnetic stirring. Raw and treated samples were characterized in terms of pH, temperature, and conductivity, as well as chemical parameters that required analytical methods further detailed.

Specific volumes of disinfectant stock solutions (sodium hypochlorite 10-15 % and hydrogen peroxide 30 %, both purchased from Sigma-Aldrich, USA) were added into 500 mL of artificially contaminated test water to achieve the desired initial doses, listed in table 1. The selected concentrations for chlorine disinfection referred to preliminary demand tests carried out using seeded GTW. In short, the 1.5 mg L⁻¹ dose was motivated considering that typical chlorine doses in final treated water range from 0.2–2.0 mg L⁻¹ of free chlorine (Brandt et al. 2017; Government of Sudan 2017). The demand assay indicated 0.2 mg L⁻¹ free chlorine even at an initial concentration as low as 0.5 mg L⁻¹ (supplementary material). This concentration was therefore reproduced here, though at a shorter contact time (15 min), so that a critical scenario could be explored.

As for the chosen doses for hydrogen peroxide, this research considered information from literature, mainly on inactivating microorganisms' suspensions, which often require higher concentrations and exposure times. Thus, we started from 3 % H₂O₂ (Choi and Lee 2020; Kolar et al. 2015; Scano et al. 2019; Tuvo et al. 2020), then tested lower doses laid out in table 1, which were explored stepwise, based in the obtained results. It should be noted that hydrogen peroxide concentrations are present in % (v v⁻¹) for practical convenience, considering common ground in their commercial applications. However, concentrations in mg L⁻¹ were checked prior to every test, considering stock solutions, initial dose, and residuals, so that coherence was obtained throughout this assessment.

Table 1 – Experimental conditions tested for *Escherichia coli* inactivation in test water

Disinfectant	Exposure time	Dose
Chlorine	30 min	1.50 mg L ⁻¹
Cilionnie	15 min	0.50 mg L^{-1}
	60 min	3.00%
		3.00%
	30 min	0.30%
Hydrogen peroxide		0.10%
		0.05%
		0.03%
		0.01%

Note: Hydrogen peroxide concentrations in mg L⁻¹ were confirmed prior to each assay. The same applies to chlorine, obtained by sodium hypochlorite, diluted into working solutions also tested for active disinfectant in terms of mg L⁻¹ Cl₂.

After the contact time was completed, the residual concentration of the disinfectant under test was assessed according to analytical methods commercially available. Physicochemical characterization was performed, and disinfectant residuals were quenched by sodium metabisulfite (Neon, Brazil), as recommended by contemporary literature (Moore et al. 2021). Microbiological examinations were carried out immediately afterwards, so that any residual activity regarding slow action of the quencher (Wang et al. 2019) would be avoided. Inactivation was calculated according to equation 1.

- 135 $Y = -log_{10}(\frac{N}{N_0})$ Equation 1
- Experiments described in table 1 were brought about considering E. coli as a target organism. After
- data analysis, Phi X174 inactivation was evaluated for the chlorine treatment that led to the highest
- 138 log₁₀-inactivation of E. coli. As for experiments targeting the bacteriophage, efficacy criteria
- considered no E. coli CFU mL⁻¹ found in prior tests, as well as statistically similarity of means
- 140 compared to chlorine treatment.
- 141 Controlled samples were kept for: test water without inoculum nor disinfectant (negative control),
- seeded test water without disinfectant (positive control), test water without inoculum but subjected to
- treatment. The latter was a reference for microbiological demand, when comparing residuals to the
- treated samples, whereas the positive control indicated the microbial input.
- 145 Test water
- Study water was prepared based on the recommendation of the World Health Organization for the
- validation of household treatment technologies (WHO 2014). An adaptation of general test water
- 148 (GTW), which is not technology-specific and represents high-quality groundwater or rainwater
- (WHO 2014), was produced in order to simulate a matrix suitable for disinfection. In short, total
- organic carbon (TOC) from GTW derived from tannic acid (Sigma-Aldrich, USA) and sodium
- carbonate (Qhemis, Brazil) provided alkalinity input. pH was adjusted with sulfuric acid (Sigma-
- 152 Aldrich, USA). Test water characterization, prior to microorganism inoculum, consisted of TOC
- 153 (TOC-LCPN, Shimadzu, Japan), alkalinity and pH (APHA et al. 2012). UV absorbance at 254 nm
- and 274 nm wavelengths were also measured, as described in the analytical methods section.
- 155 Target organisms and microbiological analyses
- 156 In order to allow evaluating disinfection efficiency, although a high-quality water was tested,
- 157 microbial load was added to the GTW. This scenario could simulate on-site contamination, for
- instance.

- 159 A lyophilized *Escherichia coli* strain (ATCC® 11229[™]) was activated, replicated, and cultivated in
- nutrient medium. Aliquots leading to an approximate concentration of 10⁷ to 10⁸ CFU 100 mL⁻¹ were
- spiked into test water for artificial contamination. After treatments were performed, detection was
- 162 carried out by the membrane filtration technique and E. coli colonies were grown in Chromocult®
- 163 Coliform Agar medium (Merck, USA). Petri dishes were kept at 37 °C for 18-24 hours of
- incubation, and counts were performed in terms of CFU 100 mL⁻¹.
- 165 This study has used bacteriophage Phi X174 (ATCC[®] 13706-B1[™]) as a virus model and *Escherichia*
- 166 coli (ATCC® 13706TM) as its host. Seeding of test water was done with an approximate order of
- magnitude of 10⁶ to 10⁸ PFU mL⁻¹. Phi X174 was counted by the double-layer agar method (Kim et
- al. 2017; USEPA 2001). Tryptone soya agar (Oxoid[™], USA) was used as culture media and Tryptone
- soya agar (Oxoid[™], USA) and bacteriological agar (Sigma-Aldrich, USA) consisted of the top agar.
- 170 Considering these were non-selective media, samples were filtered in 0.2 µm membranes coupled to
- sterile syringes. Filtered samples were added to top agar together with the same volume of host E.
- 172 coli suspensions and then overlayed onto the culture media. Plates were incubated at 37 °C for
- 173 18–24 hours and enumerated in terms of PFU mL⁻¹, according to equation 2.
- $(\frac{PFU}{mL}) = \frac{1000 \times average\ PFUs\ on\ plates}{volume\ of\ sample\ added\ (\mu L)} \times serial\ dilution\ PFUs\ were\ counted\ at\quad Equation\ 2$
- 175 Analytical methods
- 176 Free chlorine concentrations, as well as residual hydrogen peroxide were measured by colorimetric
- assays using a DR 3900 spectophotometer (Hach, USA). The former was carried out by the USEPA
- 178 DPD (N,N-diethyl-p-phenylenediamine) method using powder pillows (Hach, USA) of immediate
- reaction analyzed at $\lambda = 530$ nm. The latter was performed by the ferric thiocyanate method, using
- the Vacu-vials® kit (Chemetrics, USA) analyzed at 470 nm wavelength.
- Total organic carbon was not measured in artificially contaminated test water, nor treated samples.
- 182 Instead, spectrophotometric methods were used to assess organic matter after experiments were
- performed, using one-centimeter quartz cuvettes (Nanocolor UV/vis II, Macherey-Nagel, Germany).
- Absorbance was measured at 254 nm, representing dissolved organic carbon. The relationship
- between UV absorbance and tannic acid concentration was established by equation 3 ($r^2 = 0.9984$,
- detection limit of 0.09 mg L^{-1} and limit of quantification of 0.30 mg L^{-1}). Thus, the 274 nm
- wavelength was additionally measured, in order to indirectly monitor organic matter derived from
- the tannic acid, main source of organic carbon from the test water. Details are provided in the

- supplementary material, including peaks at 274 nm obtained by spectrum scanning and relationships to tannic acid concentrations and TOC.
- Abs 274 nm = $0.0423 \times \text{tannic acid concentration (mg } L^{-1}) + 0.0026 \text{ Equation } 3$
- 192 Any hydrogen peroxide interferences in photometric assays were accounted for using blank
- standardized curves, considering found residuals. These are provided in the supplementary material.
- 194 Data analysis

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- Descriptive and inferential statistics was performed using PAST 3.2 software (Hammer et al. 2001).
- 196 Probability distribution of the samples was verified by Shapiro-Wilk normality test under a 95%
- 197 confidence interval. Normally distributed data was tested by one-way ANOVA and the *post hoc*
- Tukey's test. For two-sample tests, Student's t test was used.

Results and discussion

Matrix characterization

Table 2 displays the physicochemical characteristics of the test water as a function of the seeded microorganisms used in this study. Therefore, test water used in this research was trusted as similar to matrices considered compatible to disinfection (apart from the microbial load, intended to be high) (WHO 2014). That is because these matrices present low concentrations of organic carbon, thus not requiring separation treatments. Disinfection, instead of the removal of microorganisms, results in their inactivation.

Table 2 – Physicochemical characterization of general test water (GTW) and effects of microbial load

Parameter	Unit	GTW	GTW + E. coli	GTW + Phi X174
Temperature	°C	25.0 ± 1.0	23.2 ± 1.0	21.2 ± 0.4
pН	-	7.07 ± 0.05	7.09 ± 0.16	6.62 ± 0.00
Conductivity	μS cm ⁻²	232.1 ± 17.8	215.2 ± 18.3	305.2 ± 3.9
TOC	mg L ⁻¹	1.186 ± 0.191	NM	NM
Abs 274 nm	-	0.106 ± 0.013	0.097 ± 0.003	0.082 ± 0.001
Abs 254 nm	-	0.064 ± 0.006	0.063 ± 0.006	0.055 ± 0.003
Alkalinity	mg L ⁻¹ CaCo ₃	55.81 ± 4.33	NM	NM

Notes: NM = not measured. TOC = total organic carbon. All the displayed values consist of average from the replicates and respective standard deviation. All repetitions referred to genuine replicates (different samples).

Replicates for GTW characterization: n = 7, except for TOC and alkalinity, which n = 3. Samples inoculated

with E. coli: n = 3. Samples inoculated with Phi X174: n = 2.

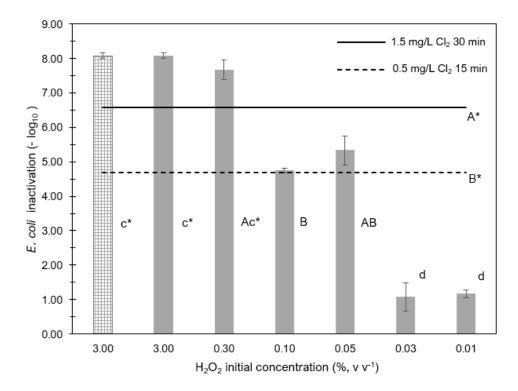
212 Disinfection

213 Inactivation of indicator bacteria obtained for different treatments (table 1) is exhibited in Fig. 1.

Baselines indicate the log₁₀-reductions obtained by chlorine disinfection at different concentrations

and exposure times. The $0.5 \text{ mg L}^{-1} \text{ Cl}_2$ concentration was intentionally low, in order to simulate free residual concentrations within storage tanks. During 15 min exposure time, this dose provided a $4.69 \pm 0.54 \log_{10}$ -inactivation of *E. coli*. Although recommended in the literature as an adequate residual for water in pipelines, it is most likely not sufficient for storing water at home (Lantagne and Clasen 2009) or providing treatment *per se*. As for $1.5 \text{ mg L}^{-1} \text{ Cl}_2$ in contact with contaminated water for 30 min, no colony forming units were found, providing a $>6.58 \log_{10}$ of inactivation. These are promising results, as they are refer to lower chlorine concentrations, as in some recommendations of dosing at 5 mg L^{-1} , which is likely to exceed the taste acceptability threshold (Lantagne and Clasen 2009).

Fig. 1 Mean \log_{10} -reductions of *Escherichia coli* as a function of disinfectant dose after 60-min exposure for grid-patterned columns and 30-min for solid-filled ones. Note: Baselines refer to \log_{10} -reduction by chlorine disinfection. Letters denote statistically significant differences (Tukey's pairwise; $\alpha = 0.05$). Error bars indicate standard deviation (n = 3). Asterisks indicate conditions in which *E. coli* (CFU 100mL⁻¹) was not detected in one or more replicates of treated samples.

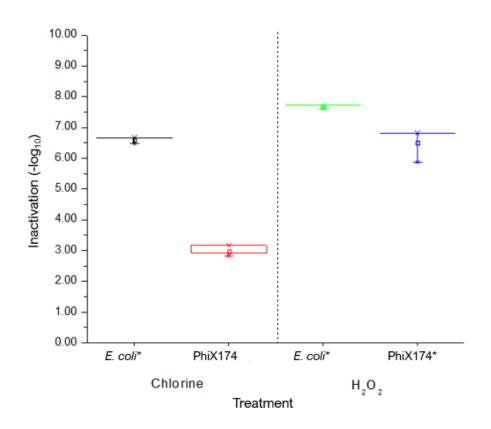


Results obtained from hydrogen peroxide disinfection displayed in Fig. 1 support that, as a standalone disinfectant, H_2O_2 requires high doses and a long exposure time (Wagner et al. 2012). An assessment of disinfection performance in pool water artificially contaminated with *E. coli* and *Pseudomonas aeruginosa* concluded that hydrogen peroxide was not effective as a biocide at 1.2 mg L^{-1} (Rosende et al. 2020), which is a compatible disinfectant concentration to reports of pools in use, but much lower than other H_2O_2 applications. Taking other studies into account, the 3 % (v v⁻¹)

concentration provided limited effect in shock disinfection followed by 1 hour flushing of dental settings (Tuvo et al. 2020), suggesting exposure time is also an important parameter. Decontamination of footbath for ovine footrot, targeting the bacteria *Dichelobacter nodosus* led to a 7.2 \log_{10} -reduction, but dosing was as high as 5 % (v v⁻¹) (Hidber et al. 2020). In the present research, results showed limited *E. coli* inactivation at lower doses (0.03 and 0.01 %), but 0.05 % and higher concentrations of H_2O_2 for 30 min led to statistically similar or greater \log_{10} -removals to chlorine treatments.

As *E. coli* is considered a suitable model organism for disinfection studies, particularly when fecal contamination of drinking water is assessed (WHO 2011), the highest values obtained for its inactivation were picked for the following test runs. These were carried out targeting Phi X174 and Fig. 2 illustrates bacteriophage inactivation in a boxplot graph. For this assay, the selected chlorine concentration vs time (CT) values were 1.5 mg L⁻¹ for 30 minutes, while 0.3 % for 30 min was the chosen CT for hydrogen peroxide. The latter referred to a more conservative approach, as its choice was based on similarity to chlorine disinfection ($\alpha = 0.05$) and lower standard deviation (SD = 0.29) compared to the log₁₀-inactivation obtained by 0.05 % H₂O₂ for 30 min (SD = 0.42).

Fig. 2 Boxplot of log_{10} -reductions obtained for *Escherichia coli* and Phi X174 for different disinfectants during 30 min contact time. Note: Dashed line separates results obtained for chlorine at 1.5 mg L⁻¹ Cl₂ and H₂O₂ 0.3%. Asterisks denote treatments in which there was absence of microorganisms in treated samples.



Comparison between chlorine and H_2O_2 treatments in test water contaminated with Phi X174 lead to a statistically significant difference in mean inactivation (p < 0.001; t-Student's test for chlorine against viral average log_{10} -inactivation as a given mean). Hydrogen peroxide was considered a better disinfectant alternative when virus are targets, achieving $>6.505 \pm 0.450$ log_{10} -inactivation, whilst chlorine led to 2.914 ± 0.147 .

Analyzing the performance on different target organisms (Fig. 2), chlorine reached a higher log-inactivation for E. coli compared to virus (p < 0.001; t-Student's test for two samples). This result endorses that studies relying on indicator bacteria alone may overestimate treatment efficiency (Mraz et al. 2021), which poses a risk to its prompt application in POU settings without considering different pathogen groups. That is because chlorine disinfection under the concentration versus time evaluated in this research was not deemed safe in scenarios of virus contamination, even if the literature has considered this concentration of free chlorine "good" for virus inactivation, in a scale from "excellent" to "poor" (Gray 2013). Disinfection treatments that lead to a minimum 4-log₁₀ virus reduction are considered justifiable for matrices as in groundwater in absence of more detailed information in virus occurrence, enumeration, and dose-response (Emelko et al. 2019). This threshold was not achieved by chlorine at the CT under study.

Although, apparently, the same outcome ($E.\ coli\ log_{10}$ -inactivation > Phi X174's) was found for H_2O_2 disinfection (p=0.0014; Student's t test for chlorine against viral average log_{10} -inactivation as a given mean), in this comparison, no FPU mL^{-1} were detected in treated samples. The log_{10} -inactivation obtained for virus (>6.505), lower than the one reached for $E.\ coli\ (>7.678)$, may be explained by variations in the order of magnitude of the inoculum. Hence, hydrogen peroxide disinfection was considered efficient within the scope of the present work. However, further research comprising other groups of microorganisms e. g. protozoa and helminths is recommended.

Oxidation

Table 3 exhibits the physicochemical characterization of disinfected samples (targeting *E. coli*), as a function of contact time and concentration of both chlorine and hydrogen peroxide. Similarly, table 4 displays these characteristics for GTW spiked with Phi X174.

Table 3 – Physicochemical characterization of treated samples and residual disinfectant concentration for treatments targeting *E. coli*

			treati	mems target	ing L. con				
		ne (mg	Hydrogen peroxide (%)						
Parameter	30 min	15 min	60 min			30 mi	n		
	1.5	0.5	3.00	3.00	0.30	0.10	0.05	0.03	0.01

Temperature (°C)	23.9	25.1	22.1	22.1	22.5	22.8	22.5	23	22.5
pН	7.52	7.73	5.59	5.13	7.24	7.21	7.07	7.04	7.07
Conductivity (μS cm ⁻²)	308.4	308.4	253.1	231.2	221.2	221.2	225.6	225.4	223.5
Abs 274 nm	0.018	0.020	NA	NA	NA	0.120	0.118	0.100	0.111
Abs 254 nm	0.030	0.010	NA	2.203	0.414	0.163	0.021	NA	NA
Mean residual (mg L^{-1}) \pm SD	0.54 ± 0.02	0.25 ± 0.03	31955.88 ± 2363.30	35931.49 ± 1373.66	3811.55 ± 2.18	1059.88 ± 50.45	627.07 ± 0.94	364.94 ± 34.73	114.63 ± 0.08

Notes: NA = not available. SD = standard deviation. UV absorbance data for H_2O_2 treatments was corrected according to a second-order polynomial equations, adjusted to different hydrogen peroxide concentrations. Residual values were used as input, but if abs interference was superior to the obtained values or > 3.5, data was not considered and displayed as "NA". Residual concentrations of disinfectants were measured in duplicates.

Table 4 – Physicochemical characterization of treated samples and residual disinfectant concentration for treatments targeting Phi X174 bacteriophage

Parameter	Chlorine 30 min	H ₂ O ₂ 30 min		
	1.5 mg L^{-1}	0.3%		
Temperature (°C)	21.9	21.6		
pH	6.72	6.62		
Conductivity (µS cm ⁻²)	309.4	309.1		
Abs 274 nm	0.077	0.083		
Abs 254 nm	0.088	0.092		
Mean residual (mg L^{-1}) \pm SD	0.04 ± 0.00	3763.28 ± 0.00		

Notes: SD = standard deviation. UV absorbance data for H_2O_2 treatments was corrected according to a second-order polynomial equations, adjusted to different hydrogen peroxide concentrations. Residual concentrations of disinfectants were measured in duplicates.

Chlorine treatments displayed in table 3 imply an oxidation of natural organic matter (NOM, simulated by tannic acid and represented by absorbance at 274 nm), as well as organic carbon in general, represented by the absorbance at 254 nm wavelength. This can be inferred by comparing such properties with the raw water (GTW spiked with *E. coli*, table 2). Assessing oxidation efficiency by chlorine, when water was contaminated with bacteriophage (table 4), however, did not meet expectations. Although there was a slight removal of abs 274 nm, suggesting oxidation of NOM, absorbance at 254 nm increased.

That said, evaluation of H_2O_2 oxidation performance was not considered fully reliable in this study. Tables 3 and 4 display the high residuals found, which may have hindered photometric assays, even though blank curves were prepared (supplementary material), and values displayed within these tables were corrected accordingly. This remaining interference was also endorsed by the increase in UV absorbance at 274 nm, which was supposed to have been associated exclusively to NOM (simulated by tannic acid), whereas 254 nm should had represented a broader perspective. Therefore,

- within the scope of our study, interpretations regarding release of intracellular organic matter and
- 313 oxidation of NOM were not made for hydrogen peroxide treatments.
- This issue has been reported for chemical oxygen demand (Wu and Englehardt 2012), but here we
- expand it to other photometric assays. It is suggested that any UV absorbance analyses are carried
- out after residual removal, so photolysis of hydrogen peroxide is avoided during measurements. If
- 317 quenching with catalase enzyme is performed (Arvin and Pedersen 2015; Flores et al. 2012), it is
- important to notice if there is any increase in the organic load of the samples. Further research is
- recommended, including total organic carbon as a parameter, not only to avoid H₂O₂ interference,
- 320 but especially because chlorine-based oxidation of NOM-enriched water may lead to the formation
- of disinfection byproducts (Goslan et al. 2009).
- 322 *General limitations and further research*
- Considering variations in water quality, disinfectant decay studies should be performed prior to any
- 324 implementation. It is recommended that these are carried out within different contamination
- scenarios (as in various organic loads, turbidities, and target microorganisms), in order to provide
- notions on required dose, as well as to assess the need of residual H_2O_2 neutralizing.
- 327 Similar research has considered hydrogen peroxide a promising alternative to chlorine-base
- disinfection, but also raised a concern towards performance in different community settings, as well
- as corrosion effects in pipelines (Marchesi et al. 2016). In this sense, though we present an overall
- assessment the performance of liquid H₂O₂ as a POU/POE disinfectant, case studies would allow
- exploring context-specific potentials and challenges for different source waters and household
- 332 settings.

Conclusions

- Results from this study reiterated that relying on indicator bacteria alone may be misleading or
- underestimate microbiological risk. This was inferred because chlorine disinfection and hydrogen
- peroxide were considered statistically similar targeting *Escherichia coli*, though the disinfectants
- 337 efficacy were dramatically different when Phi X174 bacteriophage was a target. In this scenario,
- hydrogen peroxide was more effective than chlorine, as the former led to an approximate $>6.5 \log_{10}$ -
- inactivation and the latter reached around 3.0 under the most ideal tested conditions.
- 340 Although a comparison of E. coli and Phi X174 was presented, a broader assessment of the H₂O₂
- disinfection effectiveness should be performed. It is recommended that inactivation efficiency
- evaluation is extended to different groups of pathogens, as well as different strains within each group

- prior to implementing hydrogen peroxide as a POU intervention. Residual decay assays, as well as
- 344 prediction models considering different contamination scenarios and hydrogen peroxide
- concentrations are also advised for future studies.
- Similarly, oxidation of natural organic matter should be studied considering total organic carbon as a
- parameter. That is because UV absorbance data (at 254 nm and 274 nm wavelengths) was not
- 348 considered consistent as an inference of organic load, even though effects from residuals were
- 349 accounted for.
- 350 This research suggests hydrogen peroxide may be promising as a point-of-use disinfectant aiming to
- 351 achieve SDG6, but further evaluations are required prior to any interventions. Additionally, though
- we presented a general perspective of some advantages and constraints, we recommend these are
- investigated within specific household settings.
- Data availability statement: the authors declare that all relevant data are included in the article and
- its supplementary information file.

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572	Supplementary material
573	Exploring potentials and constraints of $\mathrm{H}_2\mathrm{O}_2$ water disinfection for household settings
574	Water, Air & Soil Pollution
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579	

Fig. S1 Spectrum scanning between 190 to 700 nm considering tannic acid concentrations

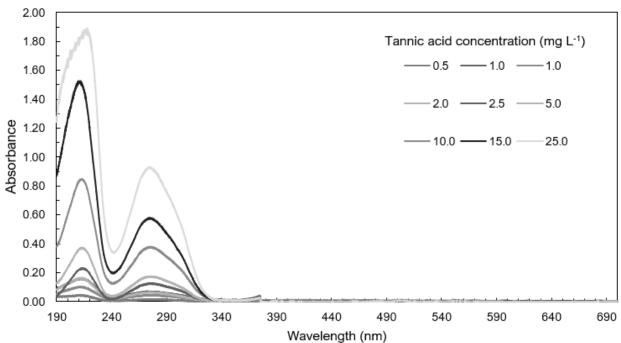
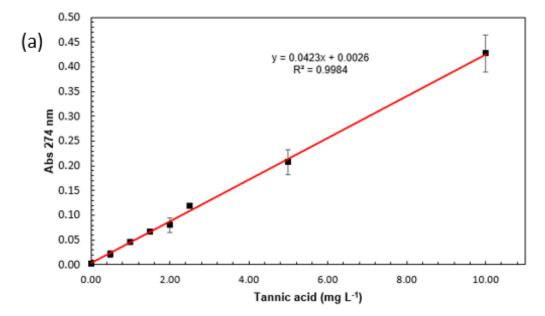


Fig. S2 Relationship between absorbance at 274 nm for low (a) and (b) high tannic acid concentrations. Error bars refer to standard deviation calculated for n=3 in low concentrations. Repetitions were not performed for high concentrations of tannic acid.



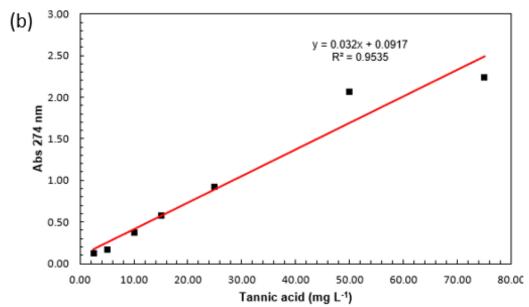


Fig. S3 Total organic carbon as a function of tannic acid concentration

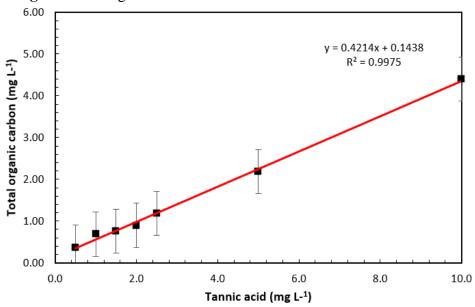


Fig. S4 Hydrogen peroxide contributions for absorbance at 254 and 274 nm

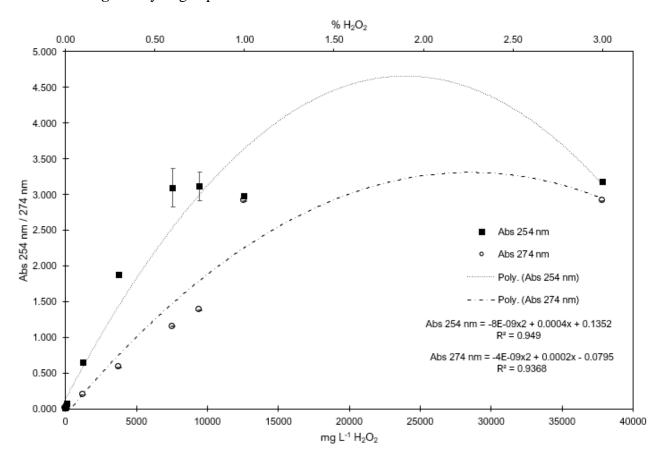


Fig. S5 Chlorine residuals obtained in artificially contaminated test water after an exposure time of 30 min. Samples were mixed at 700 s^{-1} for $\sim 7 \text{ s}$ at kept at 30 s^{-1} velocity gradient during contact time.

