Photolysis of sodium chloride and sodium hypochlorite by ultraviolet light inactivates the trophozoites and cysts of *Acanthamoeba castellanii* in the water matrix

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ABSTRACT

The present study aimed to investigate an effective, sustainable and accessible way to inactivate chlorine-resistant microorganisms, such as *Acanthamoeba castellanii*, through the photolysis of sodium chloride (NaCl) and sodium hypochlorite (NaOCl) in the water matrix. The trophozoites and cysts (2×10^7 per 8 mL) were exposed for 30, 60, 90, 120 and 150 min to the photolysis effect of NaOCl (1.0, 2.0, 4.0 and 8.0 mg/L) or NaCl (5.0, 10, 20 and 40 g/L) by ultraviolet light C (243 μW·cm^2), then the viability was analyzed. The inactivation of all trophozoites was achieved by exposure to the photolysis effect of 2.0 mg/L of NaOCl or 20 g/L of NaCl, in 150 or 120 min, respectively. Inactivation of all cysts was achieved by double exposure to the photolysis effect of 1.0 mg/L NaOCl or 5.0 g/L NaCl from 90 min of each exposure round. The exposure time was a strong determinant in the inactivation of *A. castellanii* trophozoites or cysts. The photolysis of NaOCl or NaCl is an effective method to eliminate *A. castellanii* in water. These findings expand the list of chlorine-resistant microorganisms that can be inactivated by NaOCl photolysis and show that NaCl photolysis is a new and promising method for treating swimming pool water and wastewater.

Key words | *Acanthamoeba castellanii*, advanced oxidation processes, disinfection, free-living amoebae, photolysis

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HIGHLIGHTS

- Photolysis of NaCl/NaOCl by UV-C in water inactivated 6 logs of *A. castellanii* trophozoites.
- Double exposure to the effect of NaCl/NaOCl photolysis by UV-C was necessary to inactivate cysts.
- The exposure time to UV-C is strongly correlated with inactivation of cysts.
- The NaCl photolysis is a new promising method for treating swimming pool water and wastewater.

GRAPHICAL ABSTRACT
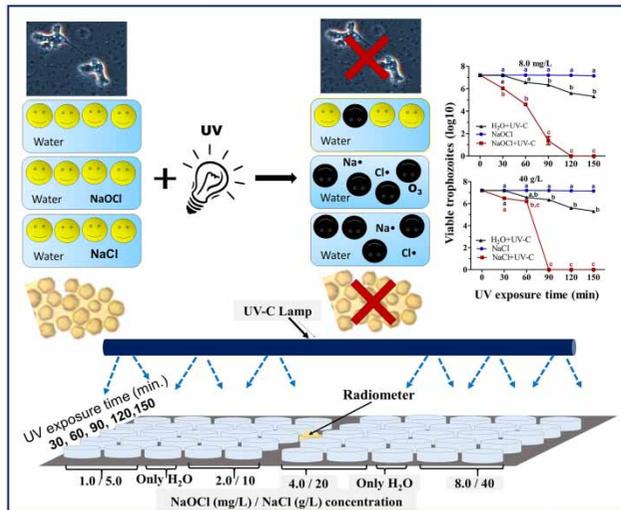
INTRODUCTION

Free-living amoebae (FLA) stand out among cosmopolitan protozoa, as they have a high environmental prevalence, having been isolated from almost all natural environmental matrices, such as soil, air, water, dust and sediments (Rodriguez-Zaragoza 1994; Koyun *et al.* 2020). They have been also isolated from sewage, drinking water treatment plants (Rodriguez-Zaragoza 1994; Thomas *et al.* 2008), hospital water reticulation systems (Rohr *et al.* 1998), public swimming pool (Paknejad *et al.* 2019) domestic water networks (Kilvington et al. 2004) and cooling towers (Barbaree *et al.* 1986).

Many species among the FLA have been described as pathogens or opportunists, and amoebae of the genus *Acanthamoeba* are one of the most frequently reported in human infections (Visvesvara 2013) and cause diseases such as granulomatous amoebic encephalitis, skin infections and amoebic keratitis (AK). AK is the most prevalent and affects immunocompetent individuals (Nagington *et al.* 1974; Galarza *et al.* 2008; Fabres *et al.* 2018; Lau *et al.* 2019; Haddad *et al.* 2019; Nakagawa *et al.* 2019).

Amoebae exist as trophozoites, which are the active forms that feed and reproduce, or as cyst, which is the environmentally resistant form (Garajová *et al.* 2019). Cysts are formed in adverse environmental conditions, such as food shortages, extreme temperatures and desiccation, as well

as survive to chemical changes in the environment, such as the presence of high concentrations of drugs and disinfectants, including chlorine (Neff *et al.* 1964; Byers *et al.* 1991; Thomas *et al.* 2004).

Cysts have been reported to be resistant to a variety of harsh conditions such as pH 2.0, freezing, gamma radiation, moist heat or 24 years in the water at a temperature of 4 °C, UV-B radiation and desiccated environments for more than 20 years (Mazur *et al.* 1995; Aksozek *et al.* 2002; Sriram *et al.* 2008; Cervero-Aragó *et al.* 2013). It was also reported that they survived exposure to UV-C, despite a reduction in the viability of about 4 logs of *Acanthamoeba castellanii* trophozoites and cysts by exposure to a fluence of 100 and 800 J/m^2 of UV-C (253.7 nm), respectively (Cervero-Aragó *et al.* 2014). The extreme resistance of *Acanthamoeba* cysts is attributed to its wall, which is composed of two layers consisting of cellulose and other components (Garajová *et al.* 2019).

Acanthamoeba is an opportunistic pathogen that has been increasingly reported in human pathologies, as in the case of AK, which is favored by the increase in contact lens wearers associated with some risky behavior (Nagington *et al.* 1974; Dos Santos & Rott 2017; Fabres *et al.* 2018). In addition, *Acanthamoeba* can harbor inside and spread a

wide variety of pathogenic bacterial, viral and eukaryotic species in the environment (Greub & Raoult 2004).

About 19% of the 539 bacterial species described as pathogens to humans and animals have been reported to be amoeba-resistant microorganisms (ARMs), which are able to resist and potentially proliferate after ingestion by trophozoites and remain viable even when amoebae encyst (Thomas *et al.* 2010). ARMs include *Vibrio cholerae*, *Escherichia coli* O157, *Acinetobacter*, *Legionella pneumophilla*, *Enterobacter*, *Pseudomonas* sp., *Serratia* sp., *Listeria monocytogenes* and various Mycobacteria (Ly & Muller 1990; Thomas *et al.* 2010; Balczun & Scheid 2017). This interaction potentially results in the development, acquisition or increased pathogenicity of amoeba (Dubois *et al.* 2019; Nakagawa *et al.* 2019) or bacteria as a result of the activation of silenced genes or genetic recombination (Berk *et al.* 2008; Van der Henst *et al.* 2018).

The interaction of FLA and ARM allows many non-amoebic pathogens to present a 'pseudo-resistance' to the processes and products applied in the disinfection of water, including chlorine and UV-C (Thomas *et al.* 2004; Cervero-Aragó *et al.* 2014). It occurs because they are protected inside the trophozoite or the cyst that is why the search for mechanisms to inactivate trophozoites and especially FLA cysts in water remains necessary. Despite the understanding of the risk of the presence of FLA in water, procedures or disinfectants that are capable of inactivating FLA in water intended for drinking, recreation or other activities in which the presence of FLA represents a health risk remain insufficient.

One of the potential ways to achieve inactivation of FLA in water is the use of advanced oxidation processes, such as photolysis (Remucal & Manley 2016). In the photolysis of disinfectants such as sodium hypochlorite (NaOCl), hypochlorous acid (HOCl) or hypochlorite ($ClO^−$), several reactive oxidants are produced, including OH^-, O_3 and chlorine radical (Cl^-), that have biocidal effect (Buxton & Subhani 1972; Remucal & Manley 2016; Astuti *et al.* 2017). The formation of transient forms of chlorine, including Cl_2, $Cl_3^−$, $HClO^−$, $ClO^−$ and $Cl^−$, has also been observed during the exposure of highly concentrated NaCl solutions to ionizing radiation, such as gamma radiation (Büppelmann *et al.* 1988; Paviet-Hartmann *et al.* 2002). NaCl photolysis has also been reported due to exposure to solar UV radiation in the upper stratosphere (Rowland & Rogers 1982). The transient forms of chlorine and oxidants react non-selectively with many organic contaminants and biomolecules (e.g. proteins and nucleic acids from the FLA) (Buxton *et al.* 1988; Bossmann *et al.* 1998) compromising their biological functions, which can therefore lead to death cell. The biocidal effect of oxidizing radicals such as OH^- and O_3 produced during photolysis of available free chlorine (FAC) by UV has been demonstrated for chlorine-resistant microorganisms, including *Cryptosporidium parvum* oocysts and *Bacillus subtilis* spores (Forsyth *et al.* 2013; Zhou *et al.* 2014; Remucal & Manley 2016).

In the present study, we evaluated the effect of sodium chloride (NaCl) and NaOCl photolysis by ultraviolet C radiation on the viability of trophozoites and cysts of *A. castellanii* and demonstrated that the photolysis of NaCl or NaOCl in the water matrix is able to inactivate trophozoites and cysts.

MATERIALS AND METHODS

Chemicals and materials

The growth media and reagents were purchased from commercial suppliers. Deionized water by reverse osmosis was used to prepare the growth media, which were autoclaved before use.

The standardization of solutions with different concentrations of FAC prepared from NaOCl – 12% (JT Baker) was performed by the method *N,N*-diethyl-*p*-phenylenediamine (DPD) (APHA 2005). Solutions were prepared with 1.0, 2.0, 4.0 and 8.0 mg of FAC per liter. Solutions were also prepared with 5.0, 10, 20 and 40 g of NaCl per liter of water. Before preparing the solutions, the water had a pH value of 6 ± 0.5 and was kept at a temperature of $25 \pm 2\,°C$ during the experiments.

Cultivation of *A. castellanii* trophozoites and cysts

Trophozoites and cysts of *A. castellanii*, Neff strain, ATCC 30010, were used in the experiments. Trophozoites were grown axenically in PYG medium (2% proteose peptone, 0.2% yeast extract and 1.8% yeast extract, and 1.8% glucose)

at 30 °C for 7 days. The adhered trophozoites were washed twice with deionized and autoclaved water before being used in the experiments. The cysts were obtained from trophozoites incubated for 7 days at 30 °C in Neff's encystment solution (0.1 M KCl, 0.02 M Trisamine, 8 mM $MgSO_4$, 0.4 mM $CaCl_2$ and 1 mM $NaHCO_3$). The PYG medium and the Neff's encystment solution were supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin during the cultivation of trophozoites and production of cysts. The cysts were collected by centrifugation (2,800 rpm for 5 min) and suspended in the encystment solution, being maintained at 4 °C until use. The viability of the cysts was confirmed by exclusion staining with 0.4% trypan blue before use.

Disinfection procedures

The experiments were carried out in duplicates with three replications. Water with different doses of NaOCl and NaCl, previously contaminated with 2×10^7 trophozoites/8 mL or 10^7 cysts/8 mL of water, was exposed to ultraviolet C light ($\lambda = 254$ nm). About 8 mL of water was placed in glass plates measuring 27 cm^2 of the surface area, and the body of water had a thickness of ~1 cm. The plates were placed in a biological safety cabinet equipped with a UV-C lamp (11-W low-pressure Hg UV lamp with a wavelength of 254 nm, 4P-SE, Philips). The mean intensity of the measured UV-C was 243 µW·cm^2using a UV radiometer (lux meter X1-1 UV-C radiometer, UV-3726 model) at 254 nm. The plates were placed at a distance of 60 cm from the lamp. The lamp was previously turned on for 15 min before the start of the experiment. The exposure time was 60, 90, 120 and 150 min for treatment with NaOCl and 30, 60, 90, 120 and 150 min for treatment with NaCl.

In the experiments with trophozoites, two control approaches were implemented: (i) the effect of NaOCl/NaCl only and (ii) the effect of UV-C only. In experiments with cysts, a single control approach was performed, evaluating the effect of NaOCl/NaCl only. In our previous tests, we found that *A. castellanii* cysts were resistant to the isolated effect of UV-C, even after being exposed for 120 consecutive minutes (our unpublished data). However, a reduction of 6 logs was achieved when the water temperature was raised and kept at 55 °C, then exposed to UV-C

for 10 min, using procedures described in the literature (Heaselgrave & Kilvington 2011).

In the second stage of the experiment, the cysts were subjected to double exposure to the effect of NaOCl/NaCl photolysis in the water matrix by UV-C. After the first exposure, the cysts were kept in water and in the dark for 72 h, then the water was exposed to UV-C again. The water containing NaOCl received new doses of chlorine before the second exposure. In this stage, treatments such as 1.0, 2.0 and 4.0 mg/L for NaOCl and 5.0, 10 and 20 g/L for NaCl were implemented, with the same contact times as mentioned above.

Viability analysis of trophozoites and cysts

The entire volume of treated water was centrifuged (1,500 rpm for 5 min), discarding the supernatant. The pellet was washed twice with deionized water previously autoclaved (to remove residues of NaOCl or NaCl) and then resuspended in 100 µL. The viability analyses of trophozoites and cysts were performed using two different techniques.

Trophozoites

About 10 µL of 0.4% trypan blue dye was added to 100 µL of the suspension, then incubated for 5 min, and viable trophozoites were counted using a Fuchs Rosenthal counting chamber in an inverted optical microscope with phase contrast. Although the culture was also considered for viability analysis, it was not considered adequate, because during the exposure of trophozoites to the effect of NaOCl/NaCl photolysis, it was observed that the cells were fragmented. It was observed that the longer the exposure time to UV-C radiation of solutions with increasing concentrations of NaOCl/NaCl, less or no whole trophozoite cell was observed. For this reason, it was not possible to count the portion of inactivated trophozoites.

Cysts

Spots of 50 µL of cyst suspension were seeded in the center of each of the two quadrants of the fresh NNA plate on which a heat-inactivated *Escherichia coli* layer was previously spread. After spots drying, the plates were

incubated for 9 days at 30 °C and checked after 1, 2, 3, 6 and 9 days of incubation. Cysts exposed twice to the effect of NaOCl/NaCl photolysis in the water matrix by UV-C were checked for up to 12 days.

Five randomized microscopy fields (100×) were found on each NNA agar quadrant, in the area where the spot was deposited, to check for the absence or presence of trophozoites. If present, the number of trophozoites observed per microscopy field was counted and an arithmetic mean was calculated. Microscopy fields with higher density and homogeneous distribution of cysts or trophozoites were considered.

No mathematical model was used to relate the number of trophozoites observed per microscopic field, with the initial number of cysts. Our aim was to find out whether the cysts were viable after treatment and whether the number of trophozoites recovered from the cysts decreased with an increasing NaOCl/NaCl concentration and the time of exposure to UV-C. We also aim to determine the minimum necessary concentration of NaOCl/NaCl and the exposure time to UV-C required to inactivate 10^6 cysts/8 mL in the water matrix.

DATA ANALYSIS

The two-tailed paired *t*-test was used to determine the significance of the differences between the data using the BioEstat 5.0 software. A value of $p < 0.05$ was considered significant. The GraphPad prism 8.02 program was used to plot graphs.

RESULTS

Kinetics of NaOCl photolysis by UV-C radiation

The photolysis of NaOCl by UV-C and the consequent reduction of FAC to undetectable levels by the method used for concentrations of 1.0 and 2.0 mg/L occurred after 30 and 60 min, respectively, and for concentrations of 4.0 and 8.0 mg/L occurred after 90 min (Figure 1).
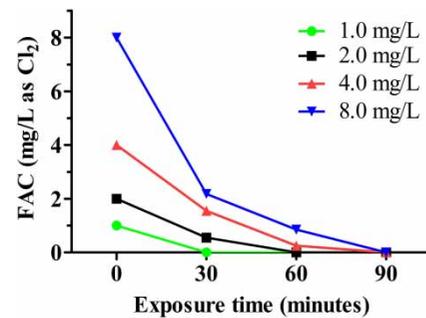

Figure 1 │ Concentration of FAC in water during NaOCl photolysis by UV-C (243 μW·cm²/ $\lambda = 254$ nm).

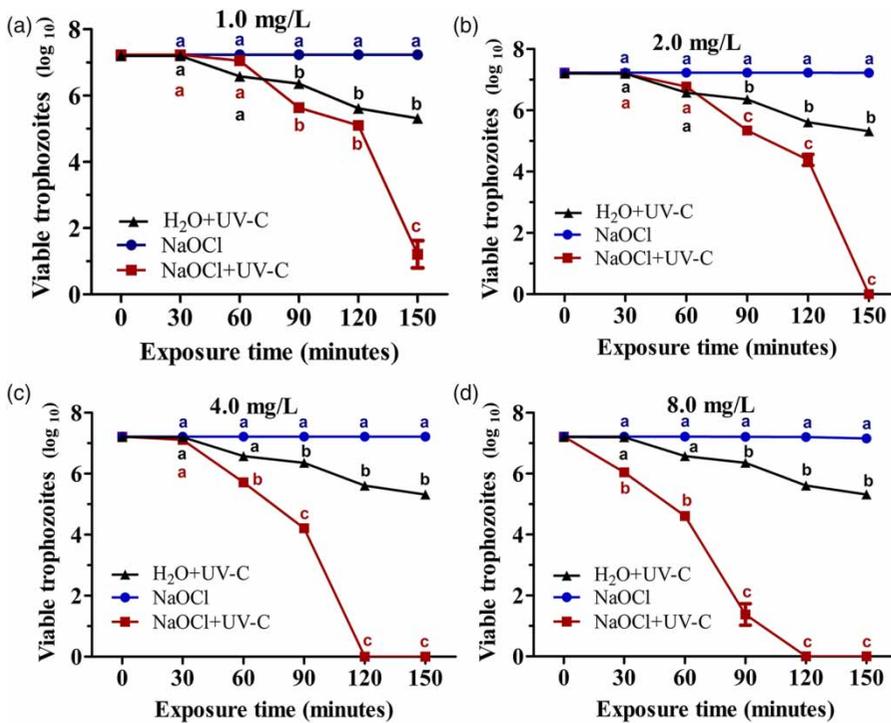### Effect of UV-C only on the viability of *A. castellanii* trophozoites

A slight reduction in the viability of trophozoites was observed, and after 150 min of exposure, a reduction of up to 1.7 $\log_{10}$ was obtained (Figure 2).

Effect of NaOCl photolysis against *A. castellanii* trophozoites

A reduction in the number of viable trophozoites was obtained in all approaches for each treatment variation (Figure 3). The average numbers of viable trophozoites in concentrations of 1.0 and 2.0 mg/L differed significantly from the corresponding controls (NaOCl only) after 90 min of exposure time ($p < 0.05$). In concentrations of 4.0 and 8.0 mg/L, the same result was obtained after 60 min of exposure time. The difference between the
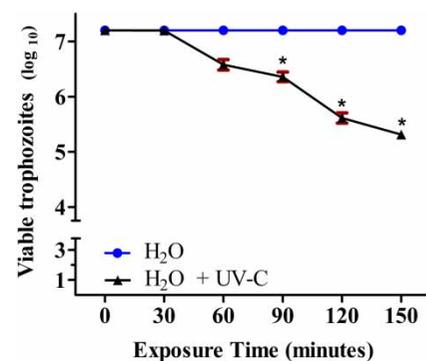
Figure 2 │ Effect of UV-C (243 μW·cm²/$\lambda = 254$ nm) only on the viability of *A. castellanii* trophozoites suspended in water. The results are expressed as the average number of trophozoites per mL of sample. *Significantly different from the corresponding controls ($p < 0.05$).

Figure 3 │ Effect of photolysis of different doses of NaOCl by UV-C radiation (243 μW·cm^2/λ = 254 nm) on the viability of *A. castellanii* trophozoites. The results are expressed as the average number of trophozoites per mL. Points with different letters indicate significant differences ($p < 0.05$).

average number of viable trophozoites in NaOCl + UV-C and H$_2$O + UV-C treatments was significant only after 150 min at 1.0 mg/L and after 90 min at 2.0 mg/L ($p < 0.05$). The death of all trophozoites was obtained from 2.0 mg/L (Figure 3).

Effect of NaCl photolysis against *A. castellanii* trophozoites

The average numbers of viable trophozoites counted in treatments 5.0 and 10 g/L differed significantly from the corresponding controls (NaCl only) after 150 min of exposure time ($p < 0.05$). In the 20 and 40 g/L treatments, the same result was obtained after 60 and 30 min of exposure time, respectively (Figure 4).

In the NaCl + UV-C treatment, the reduction in the number of viable trophozoites in the concentration of 5.0 g/L was lower than in H$_2$O + UV-C, and in the concentration of 10 g/L, it was greater only after 150 min of exposure. The difference between the average number of viable trophozoites in the NaCl + UV-C and H$_2$O + UV-C treatments was significant after 90 min of exposure in all

treatments ($p < 0.05$). The death of all trophozoites was obtained in the treatments of 20 and 40 g/L with minimum exposure times of 120 and 90 min, respectively.

Effect of NaOCl photolysis against *A. castellanii* cysts

Single exposure

The results show that there was a slight reduction in the viability of cysts exposed to the isolated effect of NaOCl, but a drastic reduction was observed when they were subjected to the effect of NaOCl photolysis by UV-C in all treatments. The average number of trophozoites recovered from the cysts differed significantly from the corresponding controls (NaOCl only) ($p < 0.05$) in most counts in all treatments. However, inactivation of all cysts was not achieved (Figure 5).

Double exposure

The results show that there was inactivation of all cysts in almost all treatments. The recovery of few trophozoites

from the treated cysts occurred only in the 1.0 mg/L treatment with 60 min of exposure time (Figure 6).

Effect of NaCl photolysis against *A. castellanii* cysts

Single exposure

The data show a strong similarity with the data obtained in the tests with NaOCl. The means of trophozoites recovered from the treated cysts differed significantly from the means of the corresponding controls ($p < 0.05$) in most counts in all treatments. The inactivation of all cysts was not achieved in any of the treatments (Figure 7).

Double exposure

The data also closely resemble the data previously presented in the double exposure tests using NaOCl, showing the inactivation of all cysts in almost all treatments, through double exposure to the effect of NaCl photolysis. The recovery of a few trophozoites from the treated cysts occurred only in the 5.0 g/L treatment with 60 min of exposure time (Figure 8).

Our findings (Figures 5 and 6) show that the number of trophozoites recovered from treated cysts is more strongly influenced by the exposure time to the effect of NaOCl and NaCl photolysis by UV-C than by the concentration of FAC or NaCl in the water matrix (Tables 1 and 2).

DISCUSSION

To verify the effect of NaCl and NaOCl photolysis by UV on the viability of *A. castellanii* trophozoites and cysts in the water matrix, solutions containing with 0.5, 1.0, 2.0 and 4.0% of NaCl and NaOCl, previously contaminated by trophozoites or cysts, were exposed to UV-C for different contact times.

The results show that the *A. castellanii* trophozoites and cysts are resistant to the isolated effects of different doses of chlorine, including the doses used in water treatment for different purposes (Cimetiere & Laat 2014), as well as to different levels of salinity.

The data (Figure 1) are in agreement with the findings of other researchers. Although the drop to undetectable levels
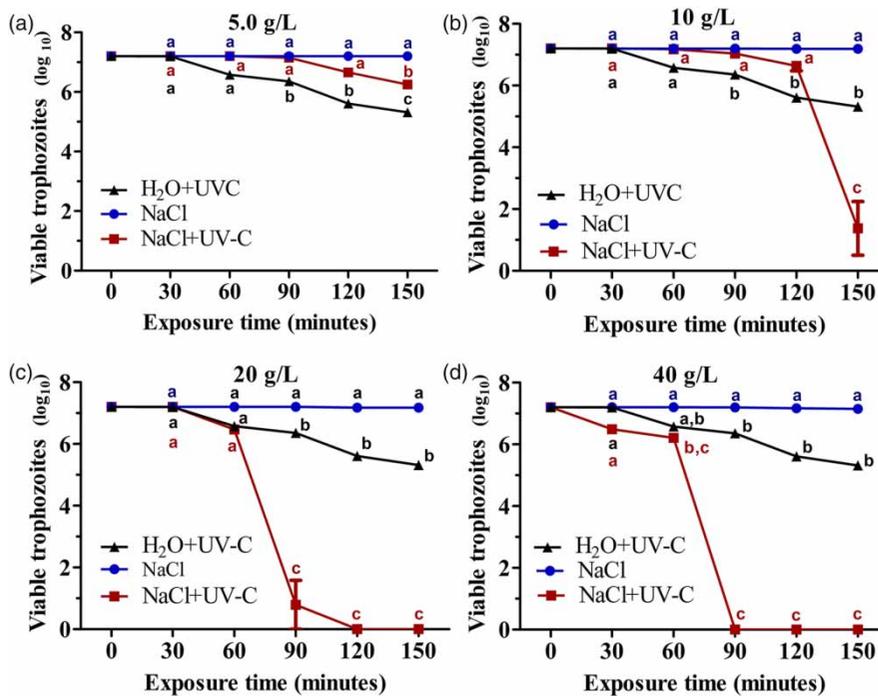
Figure 4 | Effect of photolysis of different concentrations of sodium chloride by UV-C radiation (243 μW·cm²/λ = 254 nm) on the viability of *A. castellanii* trophozoites. The results are expressed as the average number of trophozoites per mL. Points with different letters indicate significant differences ($p < 0.05$).
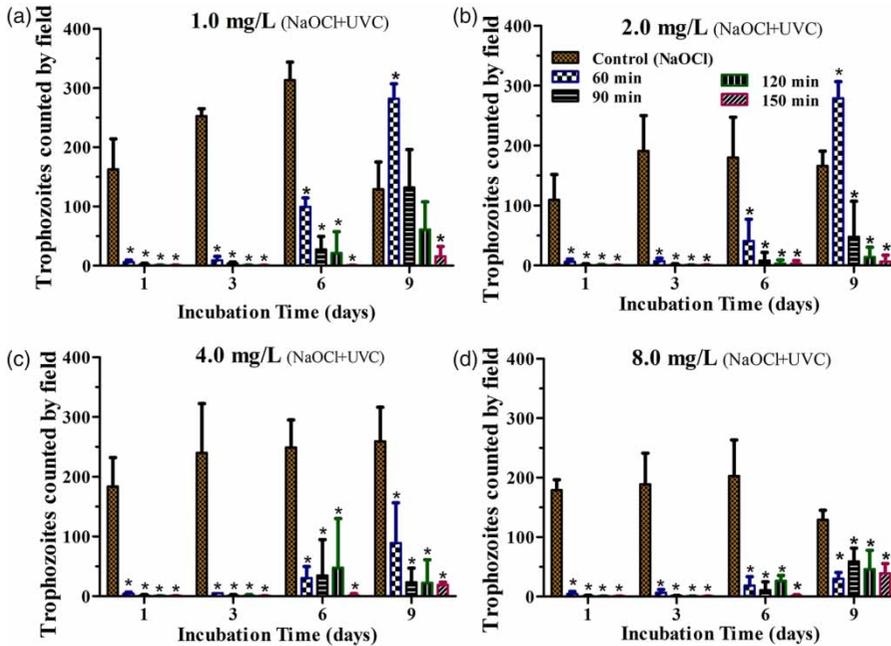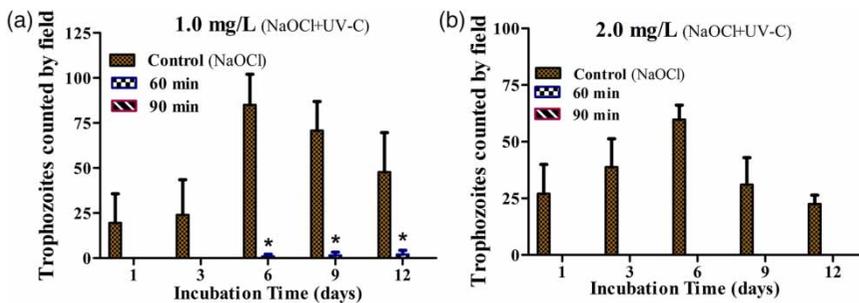
Figure 5 | Susceptibility of *A. castellanii* cysts to the effect of single exposure to photolysis of different doses of NaOCl by UV-C (243 $\mu W \cdot cm^2/\lambda = 254$ nm). The results are expressed as the average number of trophozoites per field (100×). *Significantly different from the corresponding controls ($p < 0.05$).

of 8.0 mg/L of FAC occurred in 30 min at room temperature ($34 \pm 2\,°C$) using natural sunlight, but more than 60 min exposure time was required indoors using simulated sunlight at a temperature of $25\,°C$ (Zhou *et al.* 2014). In the present work, it occurred in 90 min using UV-C. The literature shows that the shorter the wavelength of the UV radiation used, the greater the quantum yield of the FAC decomposition (Remucal & Manley 2016). The higher relative speed of FAC degradation reported by Zhou *et al.* (2014) can be attributed to the combined effect of UV-A and UV-B, as well as the contribution of heat.

Although the reduction in the number of viable trophozoites in deionized water was observed by exposure to the isolated effect of UV-C radiation, in general this was considerably greater in the NaCl and NaOCl solutions exposed to UV-C. In the NaOCl+ UV-C treatment, at concentrations of 1.0 and 2.0 mg/L (Figure 3), the reduction in the number of viable trophozoites was unexpectedly smaller than in the $H_2O + UV\text{-}C$ treatment up to 60 min of exposure. It is important to note that the difference in the mean values of viable trophozoites was not statistically significant ($p < 0.05$). Despite the difference being statistically insignificant, these results seem to show that the addition of low doses of NaOCl (1.0 and 2.0 mg/L) to deionized water had an antagonistic effect to the biocidal activity of UV-C on the viability of the trophozoite in up to 60 min of

Figure 6 | Inactivation of *A. castellanii* cysts by double exposure to the effect of NaOCl photolysis by UV-C (243 $\mu W \cdot cm^2/\lambda = 254$ nm). The results are expressed as the average number of trophozoites per field (100×). *Differs significantly from the corresponding control ($p < 0.05$).
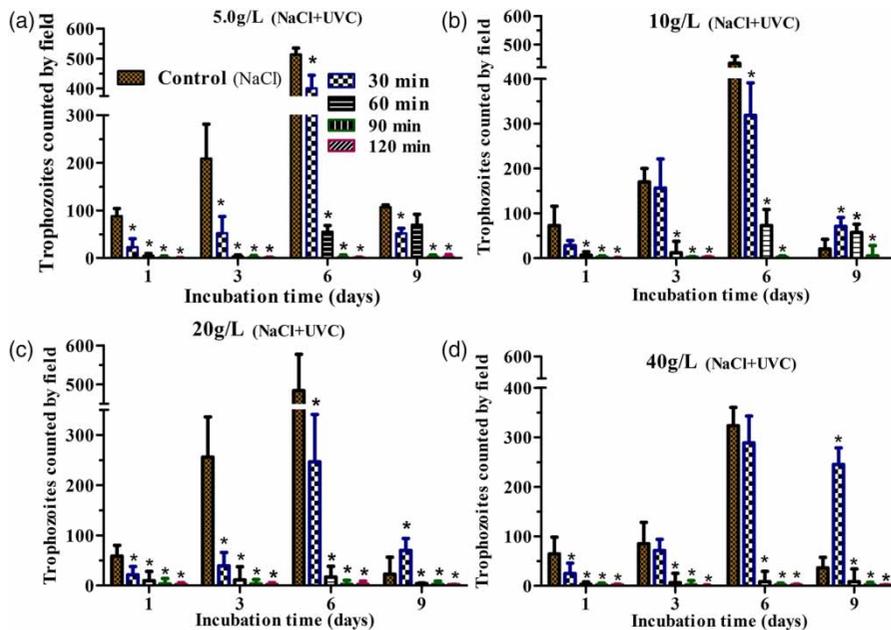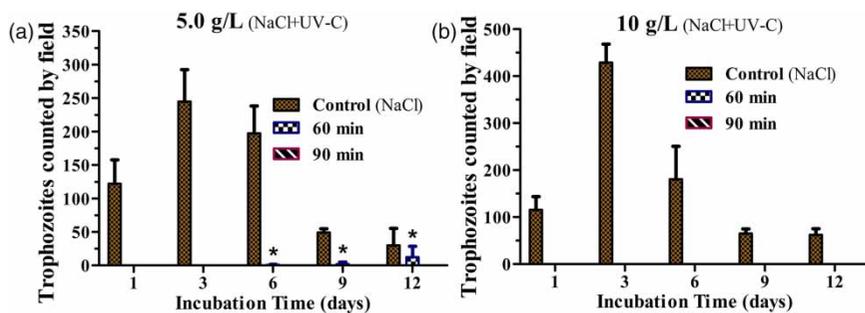
Figures 7 │ Susceptibility of *A. castellanii* cysts to the effect of single exposure to photolysis of different doses of NaCl by UV-C (243 μW·cm^2/λ = 254 nm). The results are expressed as the average number of trophozoites per field (100×). *Differs significantly from the corresponding control ($p < 0.05$).

exposure; however, a synergistic effect is observed after 90 min.

On the other hand, in the NaCl + UV-C treatment, at concentrations of 5.0 and 10 g/L, the reduction in the number of viable trophozoites was consistently less than in the H$_2$O + UV-C treatment (Figure 4). However, after 150 min of exposure, the reduction became considerably greater in the treatment NaCl + UV-C at 10 g/L concentration. The lower comparative efficacy of the NaCl + UV-C treatment can be attributed to the fact that the addition of low doses of NaCl in the deionized water alters the osmotic condition of the medium; in this case, the condition of the medium has changed from hypotonic to an almost isotonic condition. The results suggest that although the osmotic condition is not decisive for the inactivation of *A. castellanii*, the condition close to the isotonic favors resistance to UV-C, while the hypo- and hypertonic condition of the environment favors susceptibility to UV-C. Considering all this, the results show that the effect of the NaCl + UV-C treatment on the viability of *A. castellanii* is significantly greater; however, the amebicidal effect of this treatment is considerably higher when a minimum concentration of 10 g/L is used, and a minimum exposure time of 150 min.

Figure 8 │ Inactivation of *A. castellanii* cysts by double exposure to the photolysis effect of different doses of NaCl by UV-C (243 μW·cm^2/λ = 254 nm) in the water matrix. The results are expressed as the average number of trophozoites per field (100×). *Differs significantly from the corresponding control ($p < 0.05$).

Table 1 │ Pearson's correlation between the variables measured during the single exposure of the cysts to the NaOCl photolysis effect by UV-C (243 μW·cm^2/ $\lambda = 254$ nm)

	ET	TN	[FAC]
ET	1		
TN	-0.67188^*	1	
[FAC]	0	-0.3121	1

ET, exposure time; TN, trophozoite number; [FAC], free available chlorine concentration.
*Pearson's strong correlation ($p > 0.05$).

The inactivation of trophozoites can be attributed to the direct effect of UV-C radiation on the cell (Figure 2) and to the attack of transient species (Cl·, OH· and O$_3$) (Remucal & Manley 2016). These oxidants result from the photolysis of different forms of free chlorine, formed in the dissociation by hydrolysis and photolysis of NaOCl in water (Qu *et al.* 2014; Remucal & Manley 2016; Astuti *et al.* 2017).

The results show that the death rate of trophozoites is consistently proportional to the increase in UV-C exposure time; however, when the concentration of NaOCl and NaCl increases, less exposure time is necessary to achieve the same result. It is important to note that the reduction in the number of viable trophozoites (Figure 3) is consistent with the photolysis time until undetectable levels of free chlorine in the water (Figure 1). However, despite the cessation of NaOCl photolysis occurring after 60 min of exposure (Figure 1) in tests with lower doses of NaOCl (1.0 and 2.0 mg/L), inactivation of at least 1 log$_{10}$ of trophozoites was achieved after 90 min (Figure 3). The inactivation of about 6 log$_{10}$ and 7 log$_{10}$ of trophozoites was achieved in 150 min. This suggests that the amount of oxidative radicals resulting from FAC photolysis was not high enough to immediately inactivate the trophozoites, but the cell damage was severe enough to induce cell death. This may

Table 2 │ Pearson's correlation between the variables measured during the single exposure of the cysts to the photolysis effect of NaCl by UV-C (243 μW·cm^2/ $\lambda = 254$ nm)

	ET	TN	[NaCl]
ET	1		
TN	-0.55961^*	1	
[NaCl]	0	-0.01523	1

ET, exposure time; TN, trophozoite number; [NaCl], sodium chloride dose.
*Pearson's strong correlation ($p > 0.05$).

also suggest that trophozoites exposed to the synergistic effect of oxidizing radicals and UV-C during NaOCl photolysis become more sensitive to the isolated effect of UV-C.

Our data show that the trophozoites were resistant to all concentrations of NaCl only at the different exposure times tested, although some slight reduction in viability was observed at higher concentrations (2.0 and 4.0% after 120 min). However, in solutions exposed to UV-C, a drastic reduction in the number of viable trophozoites was observed (Figure 4). The explanation for this reduction is a limitation for the present study. Although the formation of different transient forms of chlorine has been demonstrated, including Cl$_2$, Cl$_3^-$, HClO$^-$, ClO$^-$ and Cl$^-$, during the exposure of highly concentrated NaCl solutions to gamma radiation, the same was not confirmed during exposure to UV-C radiation (Büppelmann *et al.* 1988; Paviet-Hartmann *et al.* 2002). We hypothesize that the exposure of trophozoites to NaCl solutions makes them more sensitive to the biocidal effect of UV-C. Further studies are desirable to elucidate the physicochemical phenomena that occur when aqueous solutions of NaCl are exposed to UV-C and its implication in the viability of microorganisms.

The data show that the cysts were more resistant to the effect of NaOCl and NaCl photolysis by UV-C compared with trophozoites. The resistance of the *A. castellanii* cysts (Figures 5 and 7) is attributed to its double wall consisting of cellulose and other biomolecules in both layers (Garajová *et al.* 2019). The wall of the cysts is essentially opaque to radiation, which prevents the direct damage of intracellular biomolecules by radiation energy or by the intracellular formation of reactive oxygen species, as shown for *E. coli* (Castro-Alférez *et al.* 2016, 2017). The pathway of inactivation of *A. castellanii* cysts during the photolysis of NaOCl and NaCl in the water matrix is probably indirectly by the action of reactive oxidants that result from the photolysis of different forms of free chlorine formed by the hydrolysis or photolysis of NaOCl or NaCl (Astuti *et al.* 2017). These oxidants, such as OH· and O$_3$, react non-selectively with many cyst biomolecules, starting with the components of the ectocyst, substances present in the space between the layers of the wall, the endocyst, until reaching the intracellular biomolecules (Remucal & Manley 2016; Garajová *et al.* 2019). This process requires a longer exposure time and relatively greater amounts of reactive oxidants compared with what

would be needed to inactivate trophozoites. This may explain why the inactivation of all cysts was achieved only by double exposure to the effect of NaOCl (1.0 mg/L by 90 min) or NaCl (5.0 g/L by 90 min) photolysis (Figures 6 and 8).

The results (Figures 5 and 7) are in agreement with the findings of other researchers (Zhou *et al.* 2014) and suggest that *A. castellanii* cysts are more resistant than *C. parvum* oocysts.

Practical implications

Our data strongly suggest that the implementation of FAC photolysis through the use of UV-C lamps in drinking water treatment processes will ensure the inactivation of chlorine-resistant microorganisms, such as *A. castellanii* and also *C. parvum* oocysts and *B. subtilis* spores, as reported in the literature (Forsyth *et al.* 2013; Zhou *et al.* 2014). Achieving the inactivation of trophozoites and especially *Acanthamoeba* cysts is particularly desirable, as these microorganisms are recalcitrant, opportunistic pathogen and have been implicated in the persistence of pathogenic bacteria in chlorinated water and in the drinking water distribution network (Thomas *et al.* 2004; Bunsuwansakul *et al.* 2019).

Inactivation of cysts by NaCl photolysis in the water matrix is a promising way to eliminate chlorine-resistant microorganisms, for example *Acanthamoeba* spp., *Naegleria* spp. and *Cryptosporidium* spp., whose presence in swimming pool water represents a health risk (Zhou *et al.* 2014; Paknejad *et al.* 2019). This method is particularly desirable because it is inexpensive, as in addition to NaCl being relatively less expensive than other disinfectants such as chlorine, its use does not require the continued addition of new doses after each photolysis disinfection session. In addition, the only presence of NaCl in water exerts a biocidal influence on microorganisms, including to those that are chlorine-resistant, as shown to *Naegleria fowleri* (Lam *et al.* 2019), which is the etiologic agent of primary amoebic encephalitis, which is a disease of high mortality (Chen *et al.* 2019).

Our data also suggest that the photolysis of FAC and NaCl can also be applied in the treatment of wastewater, because, in addition to enabling the inactivation of microorganisms, it allows the indirect photodegradation of various chemical contaminants such as drug residues (Remucal &

Manley 2016; Patel *et al.* 2019). The need for post-treatment after disinfection of water by photolysis of NaCl in water should be assessed by measuring the concentration of Na^+ ions in the water. The use of ion exchange columns may be appropriate to reduce the Na^+ concentration to the desirable levels for each application of treated water (Flores 2014).

CONCLUSIONS

In this study, we evaluated the effect of NaCl or NaOCl photolysis by UV-C light on the viability of trophozoites and *A. castellanii* cysts in the water matrix. Our results showed that this process is capable of inactivating *A. castellani* trophozoites and cysts. The inactivation of *A. castellanii* was more favored by the time of exposure to UV-C than by the concentration of NaCl or NaOCl.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

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

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