Chemosphere 361 (2024) 142421

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Wastewater disinfection with photodynamic treatment and evaluation of its ecotoxicological effects

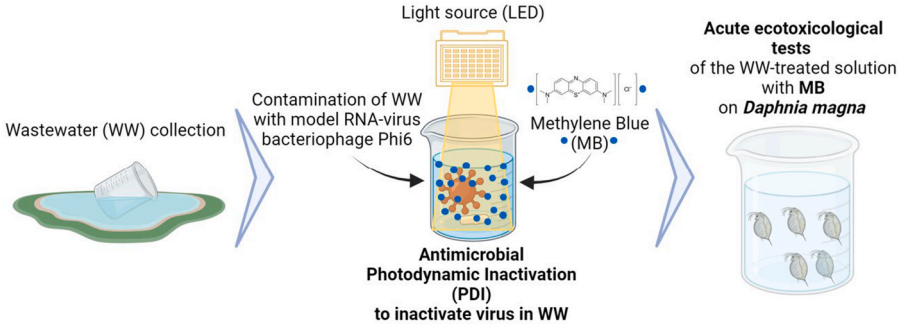
Maria Bartolomeu [a,b], Thierry J. Gomes [a], Fábio Campos [a], Cátia Vieira [a], Susana Loureiro [a], M. Graça P.M.S. Neves [c], M. Amparo F. Faustino [c], Ana T.P.C. Gomes [b], Adelaide Almeida [a,*]

[a] Centre for Environmental and Marine Studies (CESAM) and Department of Biology, University of Aveiro, 3810-19, Aveiro, Portugal
[b] Universidade Católica Portuguesa, Faculty of Dental Medicine (FMD), Center for Interdisciplinary Research in Health (CIIS), 3504-505, Viseu, Portugal
[c] LAQV-REQUIMTE and Department of Chemistry, University of Aveiro, 3810-193, Aveiro, Portugal

HIGHLIGHTS

- The study proposes PDI as a promising approach to overcome the limitations of the conventional wastewater treatment methods.
- The photosensitizers Methylene Blue and cationic porphyrin TMPyP showed efficacy in inactivating the enveloped RNA-virus.
- The survival of *Daphnia magna* increased when exposed to the PDI-treated WW with MB. • The fading of toxic effects are related to the MB irradiation and the dilution effect upon WW release into environment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: S Garcia-Segura

Keywords:
Antimicrobial photodynamic inactivation
Enveloped RNA viruses
Methylene blue
Porphyrin
Wastewater disinfection
Ecotoxicological evaluation
Daphnia magna

ABSTRACT

Research has demonstrated the presence of viruses in wastewater (WW), which can remain viable for a long period, posing potential health risks. Conventional WW treatment methods involving UV light, chlorine and ozone efficiently reduce microbial concentrations, however, they produce hazardous byproducts and microbial resistance that are detrimental to human health and the ecosystem. Hence, there is a need for novel disinfection techniques. Antimicrobial Photodynamic Inactivation (PDI) emerges as a promising strategy, utilizing photosensitizers (PS), light, and dioxygen to inactivate viruses. This study aims to assess the efficacy of PDI by testing methylene blue (**MB**) and the cationic porphyrin **TMPyP** as PSs, along a low energy consuming white light source (LED) at an irradiance of 50 mW/cm^2, for the inactivation of bacteriophage Phi6. Phi6 serves as an enveloped RNA-viruses surrogate model in WW. PDI experiments were conducted in a buffer solution (PBS) and real WW matrices (filtered and non-filtered). Considering the environmental release of the treated effluents, this research also evaluated the ecotoxicity of the resulting solution (post-PDI treatment effluent) on the model organism *Daphnia magna*, following the Organisation for Economic Cooperation and Development (OECD) immobilization technical 202 guideline. Daphnids were exposed to WW containing the tested PS at different concentrations and dilutions (accounting for the dilution factor during WW release into receiving waters) over 48 h. The results indicate that PDI with **MB** efficiently inactivated the model virus in the different aqueous matrices, achieving reductions superior to 8 $\log_{10}$ PFU/mL, after treatments of 5 min in PBS and of *ca.* 90 min in WW. Daphnids

* Corresponding author.
E-mail addresses: maria.bartolomeu@ua.pt (M. Bartolomeu), aalmeida@ua.pt (A. Almeida).

https://doi.org/10.1016/j.chemosphere.2024.142421
Received 13 February 2024; Received in revised form 6 May 2024; Accepted 22 May 2024
Available online 24 May 2024

survival increased when subjected to the PDI-treated WW with **MB**, considering the dilution factor. Overall, the effectiveness of PDI in eliminating viruses in WW, the fading of the toxic effects on daphnids after **MB**' irradiation and the rapid dilution effect upon WW release in the environment highlight the possibility of using **MB** in WW PDI-disinfection.

1. Introduction

Viruses are obligate intracellular parasites with a protective protein capsid that may contain a linear, segmented, or circular genome (single- or double-stranded RNA or DNA). This capsid may or may not be enveloped by a lipid envelope (Verbyla and Mihelcic, 2015; Tchobanoglous et al., 2003). Within the spectrum of virus categories, RNA-viruses typically have a higher propensity for mutation. The elevated mutation rate contributes to the emergence of resistance against conventional antiviral treatments and ingrains this resistant characteristic within virus populations. Consequently, this phenomenon reduces their vulnerability to pharmaceutical interventions (Peck and Lauring, 2018; Cann, 2012; Barr and Fearns, 2016; Wargo and Kurath, 2012).

Viruses are ubiquitous and persistent in raw and treated wastewater (WW) and the receiving water bodies (Corpuz et al., 2020). One of the main sources of viruses, including viral pathogens in WW, is human fecal matter, particularly that from infected persons (Corpuz et al., 2020). An infected person can shed 10^5 to 10^{12} viral particles *per* gram of fecal matter, belonging to more than one hundred known species (Verbyla and Mihelcic, 2015; Courault et al., 2017; Corpuz et al., 2020). Enteric viruses, such as Norovirus, Adenoviruses, Hepatitis viruses, Rotavirus, Enterovirus, Poliovirus, and Coronavirus, are the most detected in WW (Verbyla and Mihelcic, 2015; Courault et al., 2017; Corpuz et al., 2020). After undergoing treatment in WW treatment plants, various viruses can still persist in the treated WW effluent. The water bodies that receive these treated WW are often used for recreational activities and agriculture and as a source of raw water for drinking water production (Helmecke et al., 2020; Bhagwat, 2019).

Due to the lack of water resources or implemented water reuse policies, many countries use this treated WW only for irrigation of agricultural land, while the resultant sludge is used as fertilizer (Courault et al., 2017; Radi et al., 2019). The viruses present in these waters will, therefore, be deposited in agricultural areas, where they can survive due to their resistance capacities, thus becoming an increased risk to public health, either by the migration of viruses in the soil and consequent contamination of water bodies, or even by the consumption of contaminated agricultural products (Gholipour et al., 2022; Lahrich et al., 2021).

The presence of a wide diversity of viruses and their high concentrations highlights the importance of viruses control and removal in WW treatment (Li et al., 2023; Kilaru et al., 2023; Lanrewaju et al., 2022). Although tertiary-phase disinfection treatments may be already used in some circumstances, they can incur high costs (*e.g.*, ozonation) (Chavoshani et al., 2020), prove harmful to aquatic life, and trigger genetic alterations to microorganisms (*e.g.*, chlorination and UV treatments) (Pang et al., 2016). Hence, it is crucial to consider the advancement of novel and secure technologies for the disinfection of WW.

Photodynamic Inactivation (PDI) stands out as an alternative therapy, using photosensitizers (PSs) able to target specific microorganisms and consequently limiting the toxic effect on their cellular components (Dai et al., 2012). PDI has proven effective in destroying microorganisms, namely viruses (Costa et al., 2008; 2012; Carvalho et al., 2007), having as targets the microbial external structures by irreversible damage (Alves et al., 2014).

The purpose of the presented study was to evaluate if PDI can be considered an adequate approach for the inactivation of RNA-viruses in WW, using the bacteriophage Phi6 as a surrogate model in WW. Bacteriophage Phi6, a virus belonging to the *Cystoviridae* family, has proven to be a good surrogate model for enveloped RNA-viruses, including Coronavirus (Gonzalez et al., 1977; Lytle et al., 1991).

The PDI assays were performed with a low-energy consuming light source, a light-emitting diode (LED), and using two structurally different positively charged PSs: the phenothiazine methylene blue (**MB**) and the porphyrin derivative 5,10,15,20-tetrakis(1-methylpyridinium-4-yl) porphyrin tetraiodide (**TMPyP**). The PDI tests were performed in different aqueous matrices with different composition complexity: phosphate buffered-saline (PBS), filtered WW and non-filtered WW. These positively charged PSs were selected for their proven efficacy in inactivating different microorganisms, including viruses. Additionally, the potential toxic effects of the resulting PDI-treated WW were assessed using *Daphnia magna*, a well-established model organism for ecotoxicity testing. This evaluation aimed to assess the treated effluent's safety considering its potential environmental discharge.

2. Material and methods

In the present study, conducted to assess the efficacy of PDI against an RNA-model virus, bacteriophage Phi6, three different types of matrices were used: (1) PBS solution, used as a standard solution, but crucial for understanding the performance and effectiveness of the PS in a medium without organic matter; (2) filtered WW; and (3) raw WW (non-filtered). The assays with the two WW matrices (filtered and non-filtered) were performed to approach the reality of a WW treatment plant (WWTP) and to evaluate the impact of particulate and dissolved organic matter on the efficiency of PDI. For the WW filtration, 0.22 μm porosity filters were used to reduce the amount of particulate organic matter and the number of natural microorganisms present in the WW, focusing on the inactivation of the selected virus, the bacteriophage Phi6. Ecotoxicity tests were performed on the model organism *Daphnia magna*, to investigate the safety of PDI-treated WW concerning the remaining presence of PS in the WW after PDI treatment, according to the Organisation for Economic Cooperation and Development (OECD) guideline 202 (OECD, 2004).

2.1. Wastewater samples collection and preparation

The WW samples were collected at a WWTP after undergoing a secondary treatment phase. The chosen WWTP serves a wide geographical area, receiving WW from domestic, hospital, and industrial environments, located in the littoral centre of Portugal. The 24 h composite samples, representative of the entire working day (representing a 24-h variation of the WW composition), were collected on different days, in the morning at the same time on the day, around 10 a.m. During transport and laboratory processing, samples were protected from light and refrigerated at 4 °C until further use (within a maximum period of one week). Before each assay, the WW samples were let warm up to room temperature, and, in the case of PDI assays in the WW filtered matrice, the collected secondary treated WW samples were filtered by 0.22 μm pore mixed cellulose ester (MCE) membrane (Millipore, Bedford, MA, USA).

2.2. Biological entities and culture conditions

2.2.1. Bacterial strain and growth conditions

Pseudomonas sp. DSM 21482 (Leibniz-Institute DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) was used as the bacterial host of phage Phi6.

Pseudomonas sp. cells were cultivated under controlled and optimum growth conditions, at 25 °C for 18 h, under 120 rpm orbital shaking, in medium Tryptic Soy Broth (TSB; Liofilchem, Roseto degli Abruzzi, TE, Italy). After overnight growth, bacterial glycerol stocks (10% glycerol) were prepared and stored at −80 °C. Before each experiment, a bacterial stock was aseptically inoculated into 30 mL of fresh TSB. The sample was incubated overnight as described above, until reaching a viable cell density of approximately 10^8–10^9 colony-forming units per milliliter (CFU/mL).

2.2.2. Phage Phi6 preparation and enrichment

Phage Phi6 DSM 21518 (Leibniz-Institute DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) suspensions were obtained from a phage stock prepared in SM buffer (0.1 M NaCl, 8 mM $MgSO_4$, 20 mM Tris-HCl, 2% (w/v) gelatin, pH 7.5; Sigma, St. Louis, MO, USA) using the bacterium *Pseudomonas* sp. as host: aliquots of Phi6 phage stock (2.0 mL) and overnight grown bacteria suspension of *Pseudomonas* sp. (1.0 mL) were added to 50 mL of SM buffer and incubated under orbital shaking (60 rpm), overnight, at 25 °C; the preparation was centrifuged at 12,000×*g* for 10 min, and the obtained supernatant was filtered through a membrane with a pore size of 0.22 μm (Merck Millipore, Darmstadt, Germany) to remove the remaining bacterial debris or intact bacteria.

The Phi6 phage titer was determined by the double-layer agar method and the phage suspension was maintained at 4 °C: successive dilutions of the phage suspension were made in PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4{\cdot}2H_2O$, 1.76 mM KH_2PO_4, pH 7.4; Sigma, St. Louis MO, USA), and to 5.0 mL of the TSB 0.6% top agar layer were added 500 μL of the Phi6 phage and 200 μL of *Pseudomonas* sp. suspensions, which were placed on a Petri plate with Tryptic Soy Agar (TSA; Liofilchem, Roseto degli Abruzzi, TE, Italy). The plates were incubated for 18 h at 25 °C and formed plaques expressed as plaque-forming units per milliliter (PFU/mL); phage stock suspension was calculated to be 10^{10} PFU/mL.

A spot test was performed to confirm the Phi6 phage stock purity: 5.0 mL of the TSB 0.6% top agar layer with 200 μL of bacteria suspension was added to a plate with TSA, and after, 20 μL of the phage stock was added. The plate was incubated as described above.

2.3. Photodynamic Inactivation (PDI) treatments

The first assays concerning the efficiency of Phi6 phage photoinactivation with **MB** and **TMPyP** were assessed at a PS concentration of 5.0 μM in PBS. Then, solely **MB** was used to further PDI assays (also at a concentration of 5.0 μM) in both filtered- and non-filtered (raw) WW. The PDI efficiency was assessed by quantification of viral particles (PFU/mL) along the time of PDI assays.

Suspensions of Phi6 phage at a concentration of *ca.* 10^9 PFU/mL were prepared in the three aqueous matrices used (PBS, filtered WW, or raw WW) from the previously prepared phage stock suspension (at a concentration of 10^{10} PFU/mL). The appropriate volume of this newly prepared phage suspension was added to 6-well plates. In each assay, the samples (Phi6 phage + PS + light), light controls (Phi6 phage + light), and dark controls (Phi6 phage + PS – light) were prepared in parallel at a final volume of 5.0 mL per well. Appropriate volumes of **MB** or **TMPyP** were added to the samples and dark controls to reach a final concentration of 5.0 μM. The plates were then incubated in the dark for 10 min, and magnetically stirred to enable the interaction of the PS with the viral particles. After this incubation period, the 6-well plates containing samples and light controls were exposed to white light at 50 mW/cm^2 irradiance and kept under agitation during the PDI assays. Dark controls were protected from light. After predetermined assay times (0, 5, 10, 15, 30, 45, 60, and 90 min), aliquots of 100 μL were collected from each well and ten-fold diluted in PBS. Then, for Phi6 phage survival monitoring, aliquots of the previous suspension (10 μL) were drop-plated in triplicate in Petri dishes, previously prepared with TSA and a layer of TSB 0.6% top agar layer with the phage host *Pseudomonas* sp. (detection limit of the platting method to 2.0 $\log_{10}$ PFU/mL). At least three independent assays were performed for each condition.

2.3.1. Photosensitizers

A stock solution of methylene blue (**MB,** CAS 122965–43–9, Sigma-Aldrich/Merck KGaA, Darmstadt, Germany) (Fig. 1A) was prepared at 500 μM in PBS, while for the tetracationic porphyrin 5,10,15,20-tetrakis (1-methylpyridinium-4-yl)porphyrin tetraiodide (**TMPyP**) (Fig. 1B), a stock solution at 500 μM was prepared in dimethylsulfoxide (DMSO). The preparation and purification of **TMPyP**were conducted following the methods outlined in the literature (Simões et al., 2016), and its purity was confirmed through thin-layer chromatography and NMR analysis (Simões et al., 2016). Both PS stock solutions were stored in the dark, at room temperature, until further use. Before each assay, each stock solution was sonicated for 5 min at room temperature (ultrasonic bath, Nahita 0.6 L, 40 kHz).

2.3.2. Irradiation conditions in the PDI assays

In the PDI assays, after the pre-incubation period of the PS in the dark (10 min), the samples were exposed to white light (400–700 nm) provided by LED lamps (EL®MARK, 20 W, ~230 V and ~50 Hz) at an irradiance of 50 mW/cm^2, measured with a power meter (model FieldMaxII-Top, Coherent, Santa Clara, CA, USA) connected to a high-sensitivity thermopile sensor (model PS19Q, Coherent, Santa Clara, CA, USA).

2.4. Ecotoxicity assays on Daphnia magna

2.4.1. Test organism and culture conditions

The model organism *D. magna* Straus clone K6 (originally from Antwerp, Belgium) was obtained from a maintained continuous culture and was cultured in the American Society for Testing and Materials (ASTM) moderated-hard-water medium (ASTM medium) within a temperature range of 20 ± 1 °C, and a 16-h light – 8-h dark photoperiod. Twenty daphnids were maintained in 1.0 L glass beakers containing 0.8 L of ASTM culture medium. The culture medium was renewed three times a week. Daphnids were fed with *Raphidocelis subcapitata* at a concentration of 3×10^5 cells/mL and supplemented with the Marinure seaweed extract (Glenside Organics Ltd). The acute tests were performed with *D. magna* neonates from the third to fifth broods.

2.4.2. Safety PDI-treated samples evaluation

2.4.2.1. Sample conditions

2.4.2.1.1. MB and TMPyP solutions at 10 μM. In order to evaluate the safety of PDI-treated samples, the first ecotoxicity assays were performed using each PS (**MB** or **TMPyP**) at a concentration two times higher (10 μM) than the one used in PDI assays for the inactivation of the viral particles (5.0 μM); these samples were prepared in the daphnids medium culture ASTM. The experimental design in this primary phase included the following PDI-treated samples and controls:

(i) Negative control (ASTM)
(ii) Solvent (DMSO) control (2%)
(iii) **MB** in ASTM (10 μM) (non-irradiated)
(iv) **TMPyP** in ASTM (10 μM) (non-irradiated)
(v) PDI treatment with **MB** (10 μM) (irradiated)
(vi) PDI treatment with **TMPyP** (10 μM) (irradiated)

2.4.2.2. MB solutions at 5.0 μM with consequent dilutions. In order to meet a realistic scenario of treated WW discharge into the natural receiving waters, the concentration of **MB** in each PDI-treated sample was posteriorly reduced to 5.0 μM using raw WW and then further diluted in ASTM medium (1:2, 1:10, 1:100; and 1:1000) immediately

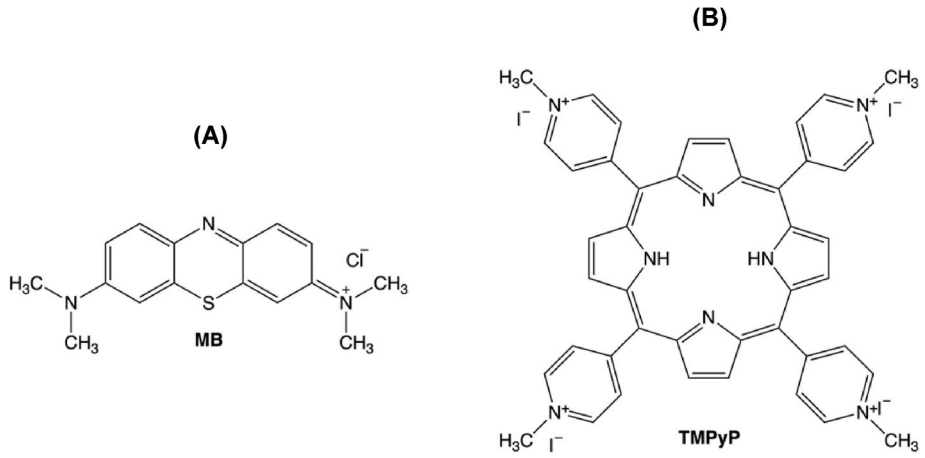


Fig. 1. Photosensitizers assessed in this study.

before the beginning of the acute immobilization tests (*section 2.4.4*). This dilution series allowed to simulate the potential dilution upon effluent discharge in an aquatic ecosystem. The PDI-treated experimental setup obtained in this secondary phase considered:

(i) Negative control (ASTM)
(ii) Wastewater (WW)
(iii) **MB** in WW (5.0 μM) 1:1 (non-irradiated)
(iv) **MB** in WW (5.0 μM) 1:2 (non-irradiated)
(v) **MB** in WW (5.0 μM) 1:10 (non-irradiated)
(vi) **MB** in WW (5.0 μM) 1:100 (non-irradiated)
(vii) **MB** in WW (5.0 μM) 1:1000 (non-irradiated)
(viii) PDI treatment with **MB** in WW (5.0 μM) 1:1 (irradiated)
(ix) PDI treatment with **MB** in WW (5.0 μM) 1:2 (irradiated)
(x) PDI treatment with **MB** in WW (5.0 μM) 1:10 (irradiated)
(xi) PDI treatment with **MB** in WW (5.0 μM) 1:100 (irradiated)
(xii) PDI treatment with **MB** in WW (5.0 μM) 1:1000 (irradiated)

2.4.3. Irradiation conditions

The prepared samples were exposed to solar irradiation for 3 h, with light irradiance ranging from 75.0 to 95.0 mW/cm^2, and UV index between 3.5 and 6 – the irradiated samples and controls. Solar irradiance and the UV index were monitored through the meteorology website CliM@UA of the University of Aveiro, where the tests were carried out. In parallel, samples and controls were prepared at the same conditions as the irradiated samples but kept in the dark during the above-described procedure – the non-irradiated samples and controls. After the irradiation period, the samples were frozen at −20 °C until further use.

2.4.4. Ecotoxicity acute tests

The acute immobilization tests were performed according to the OECD 202 guideline (OECD, 2004). *D. magna* neonates with less than 24 h were used to initiate the tests. The experimental setup consisted of three replicates of five neonates for every treatment and control. Neonates were exposed to previously prepared PDI-treated samples and control solutions for 48 h with no food supplied. Daphnids were observed for immobilization at 24 h and 48 h after the test start, and the number of immobilized organisms was recorded. In these assays, organisms that did not respond to gentle agitation of the test beakers were considered immobile.

2.5. Statistical analysis

Statistical analysis of the data was performed using GraphPad Prism software. The normal distribution of the data was assessed by Shapiro-Wilk test. The significance of viral particle concentration between treatments and across experiments was assessed using ANOVA analysis of variance and Tukey's multiple comparisons test. A value of $p < 0.05$ was considered significant. Three independent experiments were performed in duplicate for each test.

3. Results

3.1. PDI assays

3.1.1. PDI assays in PBS

The results obtained in the PDI assays towards bacteriophage Phi6, using PBS as the aqueous matrice, and with both PSs, **MB** and **TMPyP**, at a concentration of 5.0 μM, are shown in Fig. 2. In the PDI assays performed with **MB**, a decrease in the concentration of the bacteriophage (*ca.* 9.0 log PFU/mL) up to the detection limit of the platting method (2.0 log PFU/mL) was attained after *ca.* 5 min of treatment ($p < 0.05$) (Fig. 2A). When **TMPyP** was tested as PS, the detection limit of the method for Phi6 phage content was reached within 10 min of treatment (Fig. 2B).

The bacteriophage concentration remained constant throughout the experiment in light (LC) and dark (DC) controls. This indicates that the viral particles were not affected when exposed to white light in the absence of PSs (LC) or in the presence of PS without light irradiation (DC) ($p > 0.05$).

3.1.2. PDI assays with MB in filtered and raw (non-filtered) WW

Fig. 3A presents the results of PDI assays conducted with **MB** in WW samples collected on different days and subsequently filtered. The findings reveal that the use of white light (LED) at an irradiance of 50 mW/cm^2 in combination with **MB** at a concentration of 5.0 μM, resulted in the inactivation of at least 6.0 $\log_{10}$ PFU/mL in bacteriophage content after 90 min of treatment ($p < 0.05$).

Again, the bacteriophage content on light and dark controls showed no significant changes ($p > 0.05$), confirming that the bacteriophage viability was not directly affected by light nor by **MB** in the absence of light.

Fig. 3B represents the outcomes from PDI assays conducted in non-filtered wastewater. These results show that after 90 min of sample exposition to white light (LED) at an irradiance of 50 mW/cm^2) in the presence of **MB** at a concentration of 5.0 μM, there was a reduction in bacteriophage content up to the detection limit of the method, approximately 8.0 log PFU/mL ($p < 0.05$).

In the case of light and dark controls, there was no significant decrease in bacteriophage content. This outcome confirms that phage viability was not directly affected by light nor by the presence of **MB** (at

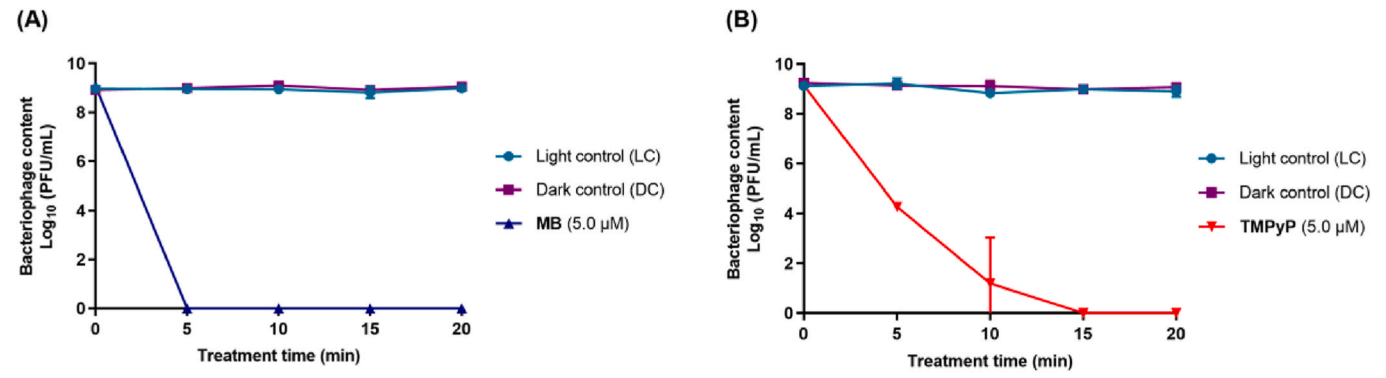


Fig. 2. Inactivation of bacteriophage Phi6 in PBS using (A) **MB** and (B) **TMPyP** at a concentration of 5.0 μM, exposed to white light (LED) at an irradiance of 50 mW/cm^2. Data points represent the average of three independent experiments in duplicate and error bars represent the standard deviation. Lines just combine experimental points.

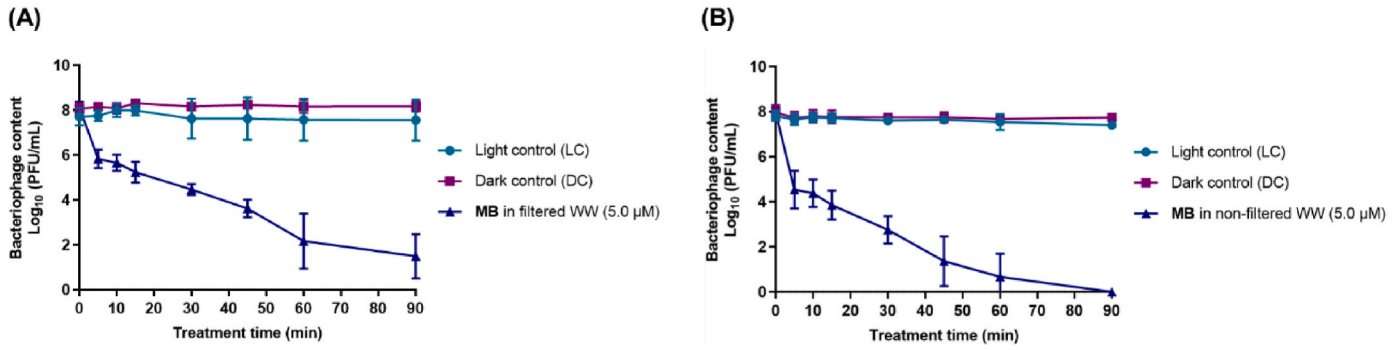


Fig. 3. Inactivation of bacteriophage Phi6 in 0.22 μm pore size filtered WW (A), and in non-filtered WW (B) samples collected in different days using **MB** at a concentration of 5.0 μM, and exposed to white light (LED) at an irradiance of 50 mW/cm^2. Data points represent the average of three independent experiments in duplicate and error bars represent the standard deviation. Lines just combine experimental points.

5.0 μM) under dark conditions ($p > 0.05$).

3.2. Ecotoxicity of PDI-treated WW in Daphnia magna

3.2.1. Acute toxicity assays with PSs in ASTM

Fig. 4 shows the survival of *Daphnia magna* after being exposed to PS solutions containing the two PS under study, **MB** and **TMPyP**. The results indicate that both irradiated and non-irradiated solutions in the presence of **TMPyP** at 10 μM exhibited toxic effects on daphnids, leading to a 100% mortality rate after 24 h of exposure to the **TMPyP** solutions (respectively Fig. 4B and A). To account for potential solvent effects originating from the **TMPyP**stock solution (2%), an experiment was conducted using 2% DMSO under similar conditions. The results indicated that DMSO at 2% concentration did not exhibit toxicity to daphnids.

The results obtained with **MB** showed that the daphnids survival rate was influenced by PS irradiation (PDI simulation) (Fig. 4). After 24 h of exposure to previously irradiated **MB** solution, the survival rate of the daphnids was *ca.* 87%, reaching 20% after 48 h (Fig. 4B). Consequently, under these conditions, **MB** seems to be less toxic than **TMPyP**. However, under no irradiation, the **MB** solution presented similar toxicity to the **TMPyP,** where total mortality was observed after 24 h of exposure (Fig. 4A).

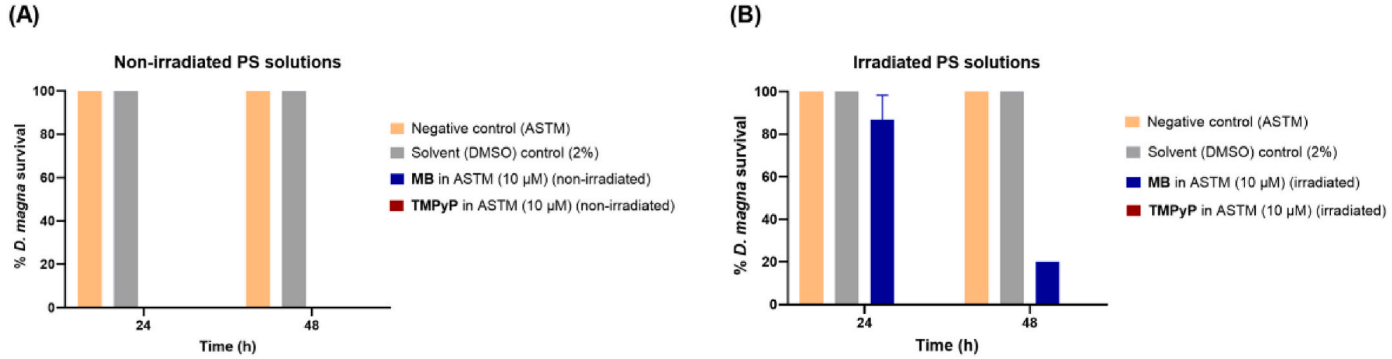


Fig. 4. Survival of Daphnia magna exposed for 48 h to (A) non-irradiated (B) irradiated PS solutions of **MB** and **TMPyP** at a concentration of 10 μM. D. magna survival was also assessed in DMSO at the same concentration used on the **TMPyP** assays (2%). Data represent the average of three independent assays with five daphnids per experiment.

3.2.2. Acute toxicity assays with PDI-treated WW with MB and dilution effect

The toxicity of untreated WW as well as PDI-treated WW at a concentration of 5.0 μM (1:1) and subsequent dilutions (1:2, 1:10, 1:100, and 1:1000) of this initial **MB** solution were tested on daphnids (Fig. 5). Regarding WW toxicity *per se* towards the daphnids, no toxic effect was detected after 48 h of exposure, remaining a survival rate of 100%, which indicates that WW by itself was non-toxic to the neonates. The results from the non-irradiated **MB** solution at 5.0 μM (1:1) and after 48 h of exposure (Fig. 5A) showed similar toxicity (0% survival rate) to that observed in the previous assays with **MB** at a concentration of 10 μM in ASTM medium. However, after the same exposure period (48 h), the toxicity was lower for the treatments where the initial **MB** solution was diluted. While the survival rate was *ca.* 7% for the 1:2 dilution, it increased to 93% for the 1:10 dilution and to 100% for the dilutions 1:100 and 1:1000. At 24 h, the survival rates of daphnids exposed to non-irradiated **MB** solutions were 100% from the 1:10 to the 1:1000 dilutions.

When exposed to irradiated **MB** solutions simulating PDI-treated WW (Fig. 5B), a high toxicity towards the daphnids was still observed, particularly in the less diluted conditions: 1:1 and 1:2 dilution, with survival rates of *ca.* 13% and 33%, respectively, after 48 h of exposure. On the other hand, in the case of daphnids' exposure to irradiated **MB** solutions simulating PDI-treated WW, survival rates were consistently 100% from the 1:10 to the 1:1000 dilutions for both time points.

4. Discussion

The rising worry about antibiotic resistance and viral mutation rates demands the development of alternate WW disinfection techniques. When viruses are introduced into natural water sources for drinking, recreational, or irrigation, they can cause serious public health risks. Photodynamic inactivation appears as an appealing technique due to its wide efficiency against bacteria, viruses, and other pathogens. This study investigates the environmental and public health implications of PDI used for WW disinfection, including its potential for viral inactivation during WW treatment before discharge into natural water bodies.

WW disinfection plays a vital role in protecting public health by eliminating harmful pathogens before their release into the environment. However, the methods currently employed often have unintended consequences, raising concerns about their ecotoxic effects. Chlorination remains the most widely used disinfection method due to its effectiveness and cost-effectiveness. However, its reaction with organic matter naturally present in WW produces known disinfection by-products like trihalomethanes and haloacetic acids (Nieuwenhuijsen et al., 2009). These disinfection by-products are suspected carcinogens and have been linked to adverse health effects in humans (Nieuwenhuijsen et al., 2009). Alternative disinfectants like ozone and UV irradiation offer advantages over chlorine in terms of disinfection by-products formation. However, they are not without their drawbacks: ozone, though a powerful disinfectant, can decompose into oxygen rapidly, reducing its effectiveness in large treatment facilities (Epelle et al., 2023). While UV irradiation has limited penetration, potentially leaving pathogenic microorganisms viable (Kauser et al., 2019). The environmental hazard of current disinfection methods extend beyond by-products formation: chlorine disinfection can lead to the discharge of chlorine residuals or resistant microorganisms, bringing toxicity to aquatic life, impacting sensitive aquatic species and, consequently, disrupting the ecological balance. The quest for an environmentally friendly and efficient WW disinfection method continues. Promising alternatives like membrane filtration and PDI are gaining traction. Membrane filtration physically removes pathogens without chemical byproducts, while PDI utilizes light and PSs to inactivate microorganisms, offering broad-spectrum efficacy and, from what is known to date, minimal negative environmental impact.

In this sense, the principal aims of this study were: i) to evaluate the efficiency of PDI in the inactivation of viruses, using the RNA-virus model phage Phi6, in WW using as PS the phenotiazinium dye **MB** and the tetracationic porphyrin **TMPyP**; and ii) to estimate the hazard associated to the release of PS solutions subjected to the previous PDI protocol by evaluating their ecotoxicity to the model organism *D. magna*.

The results obtained with the preliminary PDI tests performed in PBS demonstrated that both PSs can photoinactivate the phage Phi6 until the detection limit of the method, but the fastest response was obtained with **MB** (Fig. 2). This fact prompted us to select the phenotiazinium dye **MB** for the additional PDI-treatments performed in filtered and non-filtered WW (Fig. 3A and B). Even so, in the preliminary ecotoxicity evaluation using the model organism *D. magna*, the tests were performed with both PSs, **MB** and **TMPyP,** and, to achieve robust results, these assays were performed with the PS concentration two times higher than the one used in PDI experiments. Since the irradiated PS-treated effluent with **TMPyP** exhibited higher toxicity levels towards daphnids than **MB,** acute toxicity tests at different dilution levels were conducted exclusively with the phenotiazinium **MB**. This will allow an estimation of effects under an expected dilution upon the release of WW effluents into aquatic systems.

Despite confirming the presence of various RNA viruses in WW (Corpuz et al., 2020; Lahrich et al., 2021; Okoh et al., 2010; Courault et al., 2017; Ali et al., 2021), studies of their survival time and infectiousness in this type of environment are still lacking. So, prevention is the best strategy to be adopted to reduce the risks of exposure and transmissibility by this route into receiving waters. Although currently used disinfection processes, such as chlorination, are capable of inactivating viruses in WW, it may imply the overdosage of chlorine, and the generation of high levels of toxic by-products causing risks to human health and the environment (Gerba et al., 2013).

The hypothesis that PDI can be effective against viruses in WW

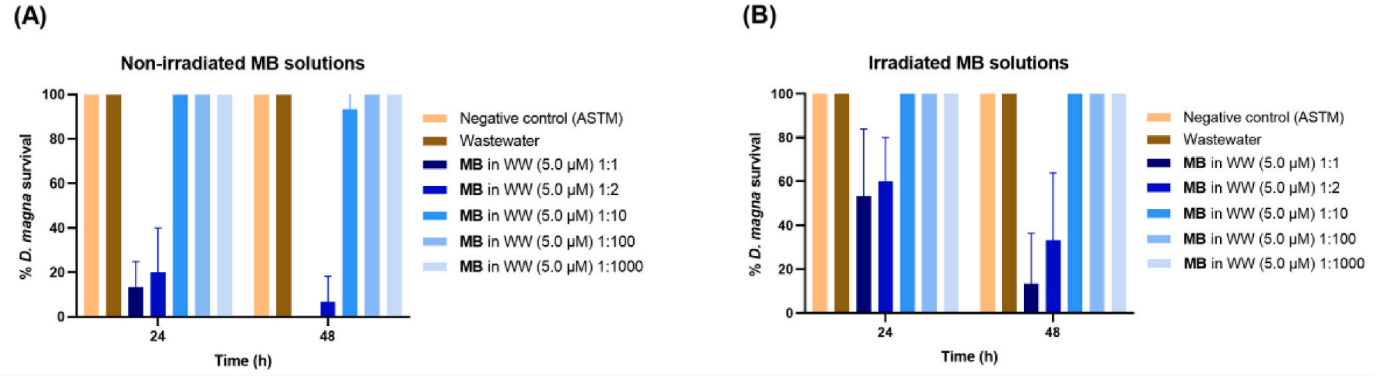


Fig. 5. Survival of Daphnia magna exposed for 48 h to (A) non-irradiated and (B) irradiated **MB** solutions at the concentration of 5.0 μM and its dilutions of 1:2, 1:10, 1:100, and 1:1000. Data represent the average of three independent assays with five daphnids per experiment.

treatment has recently gained support (Bartolomeu et al., 2022; Vieira et al., 2019; Bartolomeu et al., 2021). The efficiency of viral PDI, as in the inactivation of other microorganisms, has already been shown to be effective in inactivating microorganisms in WW. The efficient application of PDI in the inactivation of viruses in WW has been demonstrated using cationic porphyrins against T4-like bacteriophage (used as a model of non-enveloped mammalian DNA-viruses) in WW (Costa et al., 2012, 2011). Non-enveloped viruses have been shown to be more difficult to inactivate than their enveloped counterparts [48].

Studies carried out with **MB** have shown that this PS can also be effective against viruses in different and complex matrices, namely in blood and their components (Floyd et al., 2004; Wainwright and McLean, 2017; Faddy et al., 2019). The few studies available in the literature on the application of PDI in WW with **MB**, include its potential against fecal bacteria (*e.g., Enterococcus* sp., *E.coli)*, proving the ability of **MB** to inactivate efficiently bacteria in WW (Sabbahi et al., 2010; Jemli et al., 2002).

A study conducted by Gerba et al. (1977) assessed the ability of **MB** to inactivate *Poliovirus* in WW. This study showed that **MB** was able to inactivate *Poliovirus*. The authors tested **MB** at a concentration of 13 μM with red light (670 nm) at an irradiance of 2.0 mW/cm^2. A reduction of more than 90% (>1 log of reduction) in the virus titer was achieved after approximately 5 min of light exposure. However, this result was obtained with a significantly extended pre-incubation period (~18 h) with **MB** in the darkGerba et al., 1977).

These results agree with the present study regarding the inactivation of viruses in WW by PDI with **MB**, in which this PS proved to be efficient in inactivating the bacteriophage at a concentration of 5.0 μM. After 5 min of white light irradiation, the inactivation of the Phi6 phage was superior to 90% (>1 log of reduction) in filtered WW and higher than 99.9% (>3 log reduction) in non-filtered WW. In both WW matrices, extending the PDI-treatment to 90 min resulted in the inactivation of bacteriophage to the detection limit of the method, reaching an inactivation of 8.0 log PFU/mL. It is important to highlight that, in this study, a lower concentration of **MB** was used (5.0 μM), which predicts a reduction of the environmental risk.

As it was already described, viruses with a lipidic envelope (enveloped viruses) tend to be more susceptible to PDI than their non-enveloped counterparts, suggesting that the external structures, such as the envelope, are the main targets of PDI. Damage to these external structures promotes the leakage of viral content (Ayala et al., 2008; Costa et al., 2011; Ke et al., 2014; Wu et al., 2015). Ye et al. (2018) also showed that enveloped viruses are more susceptible to PDI (Ye et al., 2018), and Korneev et al. (2019) demonstrated that PDI-induced damage on surface glycoproteins from the influenza *H5N8* virus (an enveloped RNA-virus like bacteriophage Phi6), making it non-infectious and inactivating the virus completely (Korneev et al., 2019).

The higher efficiency of **MB** in the controlled aqueous matrice (PBS) used to establish the PDI protocol, when compared with its efficiency in the WW matrices (filtered and non-filtered) to simulate the environment in a WWTP, can be associated with the presence of organic matter in these WW matrices. Alves et al. (2014) suggested that the diffusion of singlet oxygen ($^1O_2$) or other reactive oxygen species (ROS), produced upon irradiation in the presence of **MB,** depends on the type of environment/matrice in which the PDI treatment is being performed (Alves et al., 2014). Therefore, the amount of organic matter in the WW can influence the $^1O_2$ diffusion rate. Also, we have previously highlighted that organic matter particles can quench the ROS or even adsorb the PS (Bartolomeu et al., 2021). As a consequence, the availability of PS to generate ROS can decrease, potentially leading to less efficient inactivation of viruses in WW. However, we have also demonstrated that multiple factors influence the efficiency of PDI-treatment in WW, such as pH, dissolved dioxygen, electrical conductance, or total dissolved solids (Bartolomeu et al., 2023), among others (Almeida et al., 2014). Although the presence of particulate organic matter in suspension in raw WW (non-filtered) could conduct to adsorption of the **MB** molecules, it is also known that some organic matter present in WW can improve the generation of ROS (Filipe et al., 2017). Therefore, PDI-treatment may be more efficient in raw WW matrices than in less complex matrices (Bartolomeu et al., 2023).

Direct comparison of the results obtained in this study with those from previously mentioned studies is not possible due to differences in the tested PDI conditions and the models of the microorganisms used. However, studies on PDI against enveloped RNA-viruses have demonstrated that photoinactivation can be a successful approach, regardless of the PS or the light source used (Costa et al., 2012). This strongly suggests that this approach can be effective in the inactivation of RNA-viruses, alongside other microorganisms.

In order to develop a safe WW disinfection protocol, it is critical to investigate if PDI-treated effluents would affect aquatic organisms from the receiving WW. In the present study, the acute toxicity of the two PSs, **MB** and **TMPyP,** was performed in WW after being subjected to a PDI-treatment, using *D. magna*, a recognized model species in ecotoxicological evaluations. For these preliminary tests on the toxicity of both PSs (**MB** and **TMPyP**), the assays were performed at higher concentrations than the one used in the photodynamic treatment tests for two reasons: (i) to obtain more robust results on the level of toxicity of the PSs tested; (ii) eventually consider testing these PSs at higher concentrations.

The acute toxicity tests of this study revealed high levels of toxicity when both PSs were tested at the higher concentration of 10 μM, with the porphyrin **TMPyP** demonstrating to be more toxic than **MB** towards the daphnids, principally in the assays performed in the irradiated samples (Fig. 4).

The WW previously subjected to secondary-phase treatment in a WWTP tested in the daphnids acute test showed a 100% survival rate after 24 h and 48 h exposure, indicating a lack of toxicity of the secondarily-treated WW. These results are in line with a previous study carried out by Serra (2019), where the potential of the crustacean *D. magna* as a bioindicator of toxicity for domestic WW to be treated with another testing procedure (peracetic acid) was also evaluated (Serra, 2019). In that study, toxicity tests were carried out also on secondarily treated WW, pre-treated in a WWTP (Serra, 2019). The results showed that the exposure of the organism to the secondary effluent, without further disinfection procedures, did not result in mortality or immobility of the daphnids.

The lower toxicity of **MB** relative to the **TMPyP** after the irradiation protocol can be associated with its photodegradation. Kurşun et al. (2019) evaluated the acute toxicity of WW with **MB** towards *D. magna* in a study aiming to promote **MB** degradation in water samples. These authors obtained similar results regarding the **MB** toxicity at 10 μM, and the diminishing toxicity after photocatalytic degradation of **MB** (Kurşun et al., 2019). Also, Çifçi (2016) had previously demonstrated decreased toxic effects on daphnids exposed to **MB** solutions after **MB** degradation. However, when daphnids were exposed to the PSs **MB** and **TMPyP** in the dark, some toxicity was observed with both PSs. As the PSs were not activated by light, the resulting toxic effects towards daphnids should be related to the physicochemical characteristics of the PSs, and the sensitivity of the daphnids to these chemicals. The amphiphilic properties of the PSs, especially of **TMPyP**, can lead to interactions and eventual cell membrane disruption in daphnia tissues, originating cellular contents leakage and electrolyte imbalance. Also, the PSs can bind to cellular components, including DNA, enzymes, and proteins involved in essential processes (Ramos et al., 2021; Almeida et al., 2015; Alves et al., 2014), disrupting normal cellular activities and potentially leading to negative effects on growth and reproduction. Additionally, PSs can accumulate in Daphnia's gills, interfering with the diffusion of dioxygen across the gill membranes hindering respiration.

Regarding the exposure of the daphnids to the previously irradiated WW-PDI treated, toxicity is dependent of time and concentration of exposure. Therefore, it is expected that toxicity increases from the 24 h period to the 48 h period. The increased toxicity of **MB** towards

daphnids after 48 h compared to 24 h exposure may involve a combination of factors, such as the bioaccumulation of **MB** and potentially generated degradation products, since daphnids continuously filter water, potentially accumulating **MB**, leading to higher internal concentrations of **MB** after 48 h compared to 24 h. Also, exposure to **MB** and its effects over 24 h might weaken daphnids' physiological state, and a weakened state of the daphnids may lead to a more susceptible state to further damage from **MB** and its potential degradation products, resulting in a more pronounced toxic effect after 48 h of exposure. Nevertheless, it is worth noting that the toxicity of the irradiated **MB** solution demonstrated to be much lower for the daphnids compared to the non-irradiated **MB** solution at both time points.

Furthermore, it is important to focus on the pronounced dilution effect that treated effluents suffer immediately after their release into receiving water bodies. The ecotoxicity results show that at a dilution of 1:10 and higher dilutions (till 1:1000), no ecotoxic effect was observed as the concentration of **MB** in the diluted samples was much lower.

The mixing behaviour of the effluent during the wastewater discharge in the receiving environment is highly dependent on the water depth, current, effluent vs. receiving water densities, and also on the design of the outfall (Campos et al., 2022). When the effluent release occurs in freshwater bodies, such as rivers, the mixing process is also influenced by the morphology of the stream channel and the river flow rate (Abily et al., 2021; Campos et al., 2022). Given the high number of variants influencing the prediction of the dilution factor of the effluent in freshwater, we will rely on a modelling regarding the marine environment by Ramos and Neves (Ramos and Neves, 2009). These authors monitored the WW discharge occurring through an underwater sewage outlet (in the central coastal region of Portugal), and presented predictive mathematical models based on the effluent behavior close to the source of flow and the behavior of the effluent far from the source of flow, being able to map the plume' dispersion and its dilution (Ramos and Neves, 2009). Moreover, ocean currents play an important role in the effluents' dispersion of in receiving waters: in the given submarine WW pipe, at a depth of *ca*. 15 m, the average discharge flow rate is about 0.8 m^3/s, resulting in an estimated dilution rate greater than 30 at the initial mixing zone (between 15 and 11 m depth), with the plume dilution being estimated to be higher than 300 with the rise in the water column up to 8 m depth (Ramos and Neves, 2009).

Overall, the combined results of (i) PDI-treatment efficiency in the elimination of viruses in WW and (ii) the reduction of toxic effects on daphnids of WW with irradiated **MB** and the dilution effect upon WW release, highlight the potential use of **MB** in WW PDI-disinfection.

5. Conclusions

In contrast to traditional tertiary WW treatments, PDI efficiently eradicates microorganisms in WW without producing harmful byproducts or genetic damage to microorganisms, curbing the emergence of resistance. Yet, more research is needed to gauge PDI's practicality in WWTPs. Tests on *D. magna* and effluent disinfected with **MB** highlighted that the recorded ecotoxicity is due to the presence of the active PS. The decrease of toxicity after the irradiation of WW with **MB** also indicates that this PS, once photodegraded, does not lead to the formation of more toxic by-products. Moreover, considering the potential dilution effect of treated effluents upon discharge in receiving water bodies, a low to no hazard can be predicted regarding the PS treatment used with **MB**.

Funding

This work received support and help from FCT/MCTES through financial support for the LAQV-REQUIMTE (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) and CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020) research units, and, where applicable, it was co-financed by the FEDER—Operational Thematic Program for Competitiveness and Internationalization—COMPETE 2020 within the PT2020 Partnership Agreement and the Portuguese NMR Network. The Center for Interdisciplinary Research in Health (CIIS) was funded by FCT through the Strategic Project UIDB/04279/2020 and UIDP/04279/2020€. The doctoral grants to C.V. and F.C. were funded by FCT (SFRH/BD/150358/2019 and 2021.07099.BD, respectively).

CRediT authorship contribution statement

Maria Bartolomeu: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Thierry J. Gomes:** Writing – original draft, Methodology, Investigation, Formal analysis. **Fábio Campos:** Methodology, Investigation, Formal analysis. **Cátia Vieira:** Investigation. **Susana Loureiro:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Data curation, Conceptualization. **M. Graça P.M.S. Neves:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **M. Amparo F. Faustino:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Data curation, Conceptualization. **Ana T.P.C. Gomes:** Writing – original draft, Supervision, Conceptualization. **Adelaide Almeida:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

The authors are grateful to the University of Aveiro and the Departments of Biology and Chemistry, where the experiments were carried out. The authors are also grateful to CESAM , LAQV-REQUIMTE and CIIS, and their respective funding sources. C.V. and F.C. thank the Fundação para a Ciência e a Tecnologia (FCT) for their doctoral grants (SFRH/BD/150358/2019 and 2021.07099.BD, respectively).

References

Abily, M., Acuña, V., Gernjak, W., Rodríguez-Roda, I., Poch, M., Corominas, L., 2021. Climate change impact on EU rivers' dilution capacity and ecological status. Water Res. 199, 117166 https://doi.org/10.1016/j.watres.2021.117166.

Ali, W., Zhang, H., Wang, Z., Chang, C., Javed, Ali, K., Du, W., Niazi, N.K., Mao, K., Yang, Z., 2021. Occurrence of various viruses and recent evidence of SARS-CoV-2 in wastewater systems. J. Hazard Mater. 414, 125439 https://doi.org/10.1016/j.jhazmat.2021.125439.

Almeida, A., Faustino, M.A.F., Tomé, J.P.C., 2015. Photodynamic inactivation of bacteria: finding the effective targets. Future Med. Chem. 7 (10), 1221–1224. https://doi.org/10.4155/FMC.15.59.

Almeida, J., Tomé, J.P.C., Neves, M.G.P.M.S., Tomé, A.C., Cavaleiro, J.A.S., Cunha, Â., Costa, L., Faustino, M.A.F., Almeida, A., 2014. Photodynamic inactivation of multidrug-resistant bacteria in hospital wastewaters: influence of residual antibiotics. Photochem. Photobiol. Sci. 13 (4), 626–633. https://doi.org/10.1039/c3pp50195g.

Alves, E., Faustino, M.A.F., Neves, M.G.P.M.S., Cunha, A., Tomé, J.P.C., Almeida, Adelaide, Cunha, Â., Tomé, J.P.C., Almeida, A., 2014. An insight on bacterial cellular targets of photodynamic inactivation. Future Med. Chem. 6 (2), 141–164. https://doi.org/10.4155/fmc.13.211.

Ayala, F., Grimaldi, E., Perfetto, B., Donnarumma, M., De Filippis, A., Donnarumma, G., Tufano, M.A., 2008. 5-Aminolaevulinic acid and photodynamic therapy reduce HSV-1 replication in HaCat cells through an apoptosis-independent mechanism.

Photodermatol. Photoimmunol. Photomed. 24 (5), 237–243. https://doi.org/10.1111/j.1600-0781.2008.00367.x.
Barr, J.N., Fearns, R., 2016. Genetic instability of RNA viruses. In: Genome Stability. Elsevier, pp. 21–35. https://doi.org/10.1016/B978-0-12-803309-8.00002-1.
Bartolomeu, M., Oliveira, C., Pereira, C., Neves, M.G.P.M.S., Faustino, M.A.F., Almeida, A., 2021. Antimicrobial photodynamic approach in the inactivation of viruses in wastewater: influence of alternative adjuvants. Antibiotics 10 (7). https://doi.org/10.3390/antibiotics10070767.
Bartolomeu, M., Gomes, M., Vieira, C., Gomes, A.T.P.C., Faustino, M.A.F., Neves, M.G.P.M.S., Almeida, A., 2022. Photoinactivation of phage Phi6 as a SARS-CoV-2 model in wastewater: evidence of efficacy and safety. Microorganisms 10 (3), 659. https://doi.org/10.3390/microorganisms10030659.
Bartolomeu, M., Monteiro, C.J.P., Fontes, M., Neves, M.G.P.M.S., Faustino, M.A.F., Almeida, A., 2023. Photodynamic inactivation of microorganisms in different water matrices: the effect of physicochemical parameters on the treatment outcome. Sci. Total Environ. 860, 160427 https://doi.org/10.1016/j.scitotenv.2022.160427.
Bhagwat, V.R., 2019. Safety of water used in food production. In: Food Safety and Human Health. Elsevier, pp. 219–247. https://doi.org/10.1016/B978-0-12-816333-7.00009-6.
Campos, C.J.A., Morrisey, D.J., Paul, B., 2022. Principles and technical application of mixing zones for wastewater discharges to freshwater and marine environments. Water 14 (8), 1201. https://doi.org/10.3390/w14081201.
Cann, A.J., 2012. Genomes. In: Principles of Molecular Virology. Elsevier, pp. 55–101. https://doi.org/10.1016/B978-0-12-384939-7.10003-1.
Carvalho, C.M.B., Gomes, A.T.P.C., Fernandes, S.C.D., Prata, A.C.B., Almeida, A., Cunha, A., Tomé, J.P.C., Faustino, M.A.F., Neves, M.G.P.M.S., Tomé, A.C., Cavaleiro, J.A.S., Lin, Z., Rainho, J.P., Rocha, J., 2007. Photoinactivation of bacteria in wastewater by porphyrins: bacterial β-galactosidase activity and leucine-uptake as methods to monitor the process. J. Photochem. Photobiol. B. 88, 112–118. https://doi.org/10.1016/j.jphotobiol.2007.04.015.
Chavoshani, A., Hashemi, M., Amin, M.M., Ameta, S.C., 2020. Pharmaceuticals as emerging micropollutants in aquatic environments. In: Micropollutants and Challenges. Elsevier, pp. 35–90. https://doi.org/10.1016/B978-0-12-818612-1.00002-7.
Çifçi, D.İ., 2016. Decolarization of methylene blue and methyl orange with Ag doped TiO2 under UV-A and UV-visible conditions: process optimization by response surface method and toxicity evaluation. Global NEST J. 18 (2), 371–380. https://doi.org/10.30955/gnj.001899.
Corpuz, M.V.A., Buonerba, A., Vigliotta, G., Zarra, T., Ballesteros, F., Campiglia, P., Belgiorno, V., Korshin, G., Naddeo, V., 2020. Viruses in wastewater: occurrence, abundance and detection methods. Sci. Total Environ. 745, 140910 https://doi.org/10.1016/j.scitotenv.2020.140910.
Costa, L., Tomé, J.P.C., Neves, M.G.P.M.S., Tomé, A.C., Cavaleiro, J.A.S., Faustino, M.A.F., Cunha, Â., Gomes, N.C.M., Almeida, A., 2011. Evaluation of resistance development and viability recovery by a non-enveloped virus after repeated cycles of APDT. Antivir. Res. 91 (3), 278–282. https://doi.org/10.1016/j.antiviral.2011.06.007.
Costa, L., Tomé, J.P.C., Neves, M.G.P.M.S., Tomé, A.C., Cavaleiro, J.A.S., Cunha, Â., Faustino, M.A.F., Almeida, A., 2012. Susceptibility of non-enveloped DNA- and RNA-type viruses to photodynamic inactivation. Photochem. Photobiol. Sci. 11 (10), 1520–1523. https://doi.org/10.1039/c2pp25156f.
Costa, L., Alves, E., Carvalho, C.M.B., Tomé, J.P.C., Faustino, M.A.F., Tomé, A.C., Cavaleiro, J.A.S., Cunha, Â., Almeida, A., 2008. Sewage bacteriophage photoinactivation by cationic porphyrins: a study of charge effect. Photochem. Photobiol. Sci. 7, 415–422. https://doi.org/10.1039/b712749a.
Costa, L., Faustino, M.A.F., Neves, M.G.P.M.S., Cunha, Â., Almeida, A., 2012. Photodynamic inactivation of mammalian viruses and bacteriophages. Viruses 4, 1034–1074. https://doi.org/10.3390/v4071034.
Courault, D., Albert, I., Perelle, S., Fraisse, A., Renault, P., Salemkour, A., Amato, P., 2017. Assessment and risk modeling of airborne enteric viruses emitted from wastewater reused for irrigation. Sci. Total Environ. 592, 512–526. https://doi.org/10.1016/j.scitotenv.2017.03.105.
Dai, T., Fuchs, B.B., Coleman, J.J., Prates, R.A., Astrakas, C., Denis, T.G.S., Ribeiro, M.S., Mylonakis, E., Hamblin, M.R., Tegos, G.P., 2012. Concepts and principles of photodynamic therapy as an alternative antifungal discovery platform. Front. Microbiol. 3 https://doi.org/10.3389/fmicb.2012.00120.
Epelle, E.I., Macfarlane, A., Cusack, M., Burns, A., Okolie, J.A., Mackay, W., Rateb, M., Yaseen, M., 2023. Ozone application in different industries: a review of recent developments. Chem. Eng. J. 454, 140188 https://doi.org/10.1016/j.cej.2022.140188.
Faddy, H.M., Fryk, J.J., Hall, R.A., Young, P.R., Reichenberg, S., Tolksdorf, F., Sumian, C., Gravemann, U., Seltsam, A., Marks, D.C., 2019. Inactivation of yellow fever virus in plasma after treatment with methylene blue and visible light and in platelet concentrates following treatment with ultraviolet C light. Transfusion 59 (7), 2223–2227. https://doi.org/10.1111/trf.15332.
Filipe, O.M.S., Mota, N., Santos, S.A.O., Domingues, M.R.M., Silvestre, A.J.D., Neves, M.G.P.M.S., Simões, M.M.Q., Santos, E.B.H., 2017. Identification and characterization of photodegradation products of metoprolol in the presence of natural fulvic acid by HPLC-UV-MSn. J. Hazard Mater. 323, 250–263. https://doi.org/10.1016/j.jhazmat.2016.05.072.
Floyd, R.A., Schneider, J.E., Dittmer, D.P., 2004. Methylene blue photoinactivation of RNA viruses. Antivir. Res. 61 (3), 141–151. https://doi.org/10.1016/j.antiviral.2003.11.004.
Gerba, C.P., Craig, W., Melnick, J.L., 1977. Application of photodynamic oxidation to the disinfection of tapwater, seawater, and sewage contaminated with Poliovirus. Photochem. Photobiol. 26 (5), 499–504. https://doi.org/10.1111/j.1751-1097.1977.tb07521.x.
Gerba, C.P., Kitajima, M., Iker, B., 2013. Viral presence in waste water and sewage and control methods. In: Viruses in Food and Water. Elsevier, pp. 293–315. https://doi.org/10.1533/9780857098870.3.293.
Gholipour, S., Ghalhari, M.R., Nikaeen, M., Rabbani, D., Pakzad, P., Miranzadeh, M.B., 2022. Occurrence of viruses in sewage sludge: a systematic review. Sci. Total Environ. 824, 153886 https://doi.org/10.1016/j.scitotenv.2022.153886.
Gonzalez, C.F., Langenberg, W.G., Van Etten, J.L., Vidaver, A.K., 1977. Ultrastructure of bacteriophage Phi 6: arrangement of the double stranded RNA and envelope. J. Gen. Virol. 35 (2), 353–359. https://doi.org/10.1099/0022-1317-35-2-353.
Helmecke, M., Fries, E., Schulte, C., 2020. Regulating water reuse for agricultural irrigation: risks related to organic micro-contaminants. Environ. Sci. Eur. 32 (1), 4. https://doi.org/10.1186/s12302-019-0283-0.
Jemli, M., Alouini, Z., Sabbahi, S., Gueddari, M., 2002. Destruction of fecal bacteria in wastewater by three photosensitizers. J. Environ. Monit. 4 (4), 511–516. https://doi.org/10.1039/B204637G.
Kauser, I., Ciesielski, M., Poretsky, R.S., 2019. Ultraviolet disinfection impacts the microbial community composition and function of treated wastewater effluent and the receiving urban river. PeerJ 7, e7455. https://doi.org/10.7717/peerj.7455.
Ke, M.-R., Eastel, J.M., Ngai, K.L.K., Cheung, Y.-Y., Chan, P.K.S., Hui, M., Ng, D.K.P., Lo, P.-C., 2014. Oligolysine-conjugated zinc(II) phthalocyanines as efficient photosensitizers for antimicrobial photodynamic therapy. Chem. Asian J. 9 (7), 1868–1875. https://doi.org/10.1002/asia.201402025.
Kilaru, P., Hill, D., Anderson, K., Collins, M.B., Green, H., Kmush, B.L., Larsen, D.A., 2023. Wastewater surveillance for infectious disease: a systematic review. Am. J. Epidemiol. 192 (2), 305–322. https://doi.org/10.1093/aje/kwac175.
Korneev, D., Kurskaya, O., Sharshov, K., Eastwood, J., Strakhovskaya, M., 2019. Ultrastructural aspects of photodynamic inactivation of highly pathogenic avian H5N8 influenza virus. Viruses 11 (10), 955. https://doi.org/10.3390/v11100955.
Kurşun, G., Pala, A., Canpolat, Ö., Çelik, E., 2019. Evaluation of K2La2Ti3O10-based coatings for Daphnia magna acute toxicity test of methylene blue degradation in water. J. Aust. Ceram. Soc. 55 (1), 289–296. https://doi.org/10.1007/s41779-018-0234-z.
Lahrich, S., Laghrib, F., Farahi, A., Bakasse, M., Saqrane, S., El Mhammedi, M.A., 2021. Review on the contamination of wastewater by COVID-19 virus: impact and treatment. Sci. Total Environ. 751, 142325 https://doi.org/10.1016/j.scitotenv.2020.142325.
Lanrewaju, A.A., Enitan-Folami, A.M., Sabiu, S., Edokpayi, J.N., Swalaha, F.M., 2022. Global public health implications of human exposure to viral contaminated water. Front. Microbiol. 13 https://doi.org/10.3389/fmicb.2022.981896.
Li, J., Hosegood, I., Powell, D., Tscharke, B., Lawler, J., Thomas, K.V., Mueller, J.F., 2023. A global aircraft-based wastewater genomic surveillance network for early warning of future pandemics. Lancet Glob. Health 11 (5), e791–e795. https://doi.org/10.1016/S2214-109X(23)00129-8.
Lytle, C.D., Budacz, A.P., Keville, E., Miller, S.A., Prodouz, K.N., 1991. Differential inactivation of surrogate viruses with merocyanine 540. Photochem. Photobiol. 54, 489–493. https://doi.org/10.1111/j.1751-1097.1991.tb02047.x.
Nieuwenhuijsen, M.J., Martinez, D., Grellier, J., Bennett, J., Best, N., Iszatt, N., Vrijheid, M., Toledano, M.B., 2009. Chlorination disinfection by-products in drinking water and congenital anomalies: review and meta-analyses. Environ. Health Perspect. 117 (10), 1486–1493. https://doi.org/10.1289/ehp.0900677.
OECD, 2004. Test No. 202: Daphnia sp. Acute immobilisation test. In: Organisation for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris. https://doi.org/10.1787/9789264069947-en.
Okoh, A.I., Sibanda, T., Gusha, S.S., 2010. Inadequately treated wastewater as a source of human enteric viruses in the environment. Int. J. Environ. Res. Publ. Health 7 (6), 2620–2637. https://doi.org/10.3390/ijerph7062620.
Pang, Y., Huang, J., Xi, J., Hu, H., Zhu, Y., 2016. Effect of ultraviolet irradiation and chlorination on ampicillin-resistant Escherichia coli and its ampicillin resistance gene. Front. Environ. Sci. Eng. 10 (3), 522–530. https://doi.org/10.1007/s11783-015-0779-9.
Peck, K.M., Lauring, A.S., 2018. Complexities of viral mutation rates. J. Virol. 92 (14) https://doi.org/10.1128/JVI.01031-17.
Radi, S., Abiad, C., Moura, N.M.M., Faustino, M.A.F., Neves, M.G.P.M.S., 2019. New hybrid adsorbent based on porphyrin functionalized silica for heavy metals removal: synthesis, characterization, isotherms, kinetics and thermodynamics studies. J. Hazard Mater. https://doi.org/10.1016/j.jhazmat.2017.10.058.
Ramos, C.I.V., Monteiro, A.R., Moura, N.M.M., Faustino, M.A.F., Trindade, T., Neves, M.G.P.M.S., 2021. The interactions of H2TMPyP, analogues and its metal complexes with DNA G-Quadruplexes—an overview. Biomolecules 11 (10), 1404. https://doi.org/10.3390/biom11101404.
Ramos, P., Neves, M.V., 2009. Monitorização de Descargas de Águas Residuais No Mar Através de Veículos Submarinos Autónomos (VSAs). Ingeniería Del Agua 16 (3), 201–216. https://doi.org/10.4995/ia.2009.2954.
Sabbahi, S., Alouini, Z., Ayed, L.B., Jemli, M., 2010. Inactivation of faecal bacteria in wastewater by methylene blue and visible light. Desalin. Water Treat. 20 1-3, 209–219. https://doi.org/10.5004/dwt.2010.1171.
Serra, A.M.C., 2019. Assessment of Daphnia Magna as a Toxicity Bioindicator for Wastewaters. Universidade Nova de Lisboa, Lisboa.
Simões, C., Gomes, M.C., Neves, M.G.P.M.S., Cunha, Â., Tomé, J.P.C., Tomé, A.C., Cavaleiro, J.A.S., Almeida, A., Faustino, M.A.F., 2016. Photodynamic inactivation of Escherichia coli with cationic meso-tetraarylporphyrins – the charge number and charge distribution effects. Catal. Today 266, 197–204. https://doi.org/10.1016/j.cattod.2015.07.031.

Tchobanoglous, G., Burton, F.L., Stencel, H.D., 2003. Wastewater Engineering: Treatment and Reuse, fourth ed. Metcalf & Eddy, Inc. Boston: McGraw-Hill.

Verbyla, M.E., Mihelcic, J.R., 2015. A review of virus removal in wastewater treatment pond systems. Water Res. 71, 107–124. https://doi.org/10.1016/j.watres.2014.12.031.

Vieira, C., Santos, A., Mesquita, M.Q., Gomes, A.T.P.C., Neves, M.G.P.M.S., Faustino, M. A.F., Almeida, A., 2019. Advances in APDT based on the combination of a porphyrinic formulation with potassium iodide: effectiveness on bacteria and fungi planktonic/biofilm forms and viruses. J. Porphyr. Phthalocyanines 23 (4–5), 534–545. https://doi.org/10.1142/S1088424619500408.

Wainwright, M., McLean, A., 2017. Rational design of phenothiazinium derivatives and photoantimicrobial drug discovery. Dyes Pigments 136, 590–600. https://doi.org/10.1016/j.dyepig.2016.09.015.

Wargo, A.R., Kurath, G., 2012. Viral fitness: definitions, measurement, and current insights. Curr. Opin. Virol. 2 (5), 538–545. https://doi.org/10.1016/j.coviro.2012.07.007.

Wu, J., Hou, W., Cao, B., Zuo, T., Xue, C., Leung, A.W., Xu, C., Tang, Q.-J., 2015. Virucidal efficacy of treatment with photodynamically activated curcumin on murine Norovirus bio-accumulated in oysters. Photodiagnosis Photodyn. Ther. 12 (3), 385–392. https://doi.org/10.1016/j.pdpdt.2015.06.005.

Ye, Y., Chang, P.H., Hartert, J., Wigginton, K.R., 2018. Reactivity of enveloped virus genome, proteins, and lipids with free chlorine and UV254. Environ. Sci. Technol. 52, 7698–7708. https://doi.org/10.1021/acs.est.8b00824.