RESEARCH ARTICLE

ELIMINATION OF *PSEUDOMONAS AERUGINOSA* IN DRINKING WATER BY ULTRAVIOLET – C (UV-C) RADIATION

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ABSTRACT

As far as the consumer is concerned presence of pathogenic bacteria in drinking water is of great health concern. Ubiquitous bacteria like *Pseudomonas aeruginosa* present in water are opportunistic pathogens that affect immunocompromised individuals when contaminated water is consumed. Drinking water already treated with UV radiation at the factory before bottling have been reported to contaminated with *Pseudomonas aeruginosa*. This controversy caught our attention as UV-treatment is intended to eradicate *Pseudomonas aeruginosa* in drinking water. This study aimed to systematically evaluate the efficacy of UV irradiation in eliminating *P. aeruginosa* in water. The proof of concept was first established by exposing sterile distilled water spiked with *P. aeruginosa* to varying intensities of UV-C irradiation over different exposure intervals: 10,350, 17,300 and 24,500 µW cm^{-2} for 5, 10, 20 and 60 second durations and bacterial viability was tested post exposure to UV-C radiation. Further, three randomly picked bottled water brands were first analyzed for the presence of *P. aeruginosa* by the membrane filtration technique, using cetrimide agar supplemented with 15 mg/l of nalidixic acid and all three brands tested were positive for *P. aeruginosa*. Total elimination of *P. aeruginosa* was observed with UV-C doses of approximately 17,300 µW/cm^2 and 24,500 µW/cm^2 just with 5 seconds exposure in spiked water samples, while 10,350 µW cm^{-2} showed 4-fold reduction of bacterial counts. Each of the three UV doses resulted in complete eradication of *P. aeruginosa* in bottled water just with 5 seconds exposure. The presence of *P. aeruginos*a in drinking water is not acceptable and it could raise health concerns, hence regular monitoring of the purification process and disinfection of the drinking water by using appropriate exposure levels to UV irradiation is highly recommended to safeguard the health of the consumer.

Keywords: *Pseudomonas aeruginosa, UV-c radiation, Drinking water,*

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1. INTRODUCTION

Pseudomonas species like *Pseudomonas aeruginosa*, are ubiquitous in various environments such as soil, water, and plants. *Pseudomonas aeruginosa*, a common bacterium in tropical regions [1], is considered opportunistic pathogens in humans. *P. aeruginosa,* hence their presence in drinking water is unacceptable for consuming, and it has been associated with waterborne and food borne diseases, and is also considered a primary infectious agent [2]. The World Health Organization (WHO) recommends that safe drinking water should contain less than 20 colony-forming units per milliliter (CFU/mL) of heterotrophic bacteria, and should be free from coliform bacteria, fecal coliforms, *E. coli*, and *P. aeruginosa* [3]. In immunocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections. *P. aeruginosa* expresses virulence factors such as, exotoxins, a phagocytosis resistant slime layer, and various enzymes and hemolysins that degrade host tissues [4].

P. aeruginosa is often found in carbon filters, cooling towers, drinking water dispensers, bottled water, and of course water taps. [5] Anyhow, outbreaks of infection caused by *P. aeruginosa* in water are common [6, 7]. This species can grow even in low-nutrient water [8] and therefore, can colonize bottled waters and survive for long starvation periods [9]. Many studies have reported that bottled water is contaminated with *P. aeruginosa*. Herath *et al*., in 2014 [10] reported *P. aeruginosa's* presence in bottled water. A study done in China, reported that 24.5 % of the collected samples were contaminated with *P. aeruginsa* [11]. *P. aeruginosa* has been detected in 36.7% of all bottled water samples examined in Iran [12]. Further, it has been reported that biofilm formation of *P. aeruginosa* in water is alarming and it may trigger a waterborne outbreak in the future [13]. Further, *P. aeruginosa* has been found in some mineral waters in various countries such as Brazil, Canada, France, Germany, Spain, and United States and others [14]. In addition, bottled water in many countries has been found to be contaminated with different bacterial species [10, 15-22] Moreover, Herath, (2021) [23] revealed that spring water in locations where certain bottled water companies are situated was also found to be contaminated. Further, a study suggests that the need to consider *P. aeruginisa*, as a quality parameter for swimming pool water should be mandatory [24].

In addition to being a primary infectious agent, *P. aeruginosa* is an indicator of other opportunistic pathogens [25]. Although, inspection of drinking water for the presence of *P. aeruginosa* is not usually recommended as a routine procedure, it can be used as an indicator of good manufacturing processes and suitability for drinking water [3]. The drinking water must be free from *P. aeruginosa* as its presence constitutes a health risk to the general public. *P. aeruginosa* is used as an indicator organism in water contamination [26]. In Sri Lanka, although *P. aeruginosa* is tested as a quality parameter for natural mineral water standards, it is not included in bottled water standards. However, according to the drinking water criteria of the European Union, *P. aeruginosa* should be absent in 250 mL of bottled water [27].

Use of UV radiation to eliminate *P. aeruginosa* in drinking water is the mostly used method in the industry. UV radiation does not inactivate micro-organisms by chemical action but the released energy interacts with nucleic acids and other vital cellular components, damaging or killing exposed cells. The plethora of knowledge in this field confirms that ultraviolet radiation acts fast, efficiently, and safely, and offers a cheap environmentally friendly technology [28]. Ultraviolet-C (UV-C) (100-280 nm) radiation has been suggested as one of the successful disinfection practices for water treatment. Therefore, UV-C treatment has become a practical and attractive solution for safe disinfection of water. Ultraviolet sanitizing units are used in many water purification systems to control bacteria and have certain applications in animal drinking water systems [29].

UV units can be effective water treatment tools, nevertheless, drinking water already treated with UV radiation by the manufacturer before bottling have been reported to be contaminated with *Pseudomonas aeruginosa*. This controversy caught our attention as UV-treatment is intended to eradicate *Pseudomonas aerugin*osa in drinking water. This study aimed to systematically evaluate the efficacy of UV irradiation in eliminating *P. aeruginosa* in water.

2. MATERIAL AND METHODS

2.1 Elimination of Pseudomonas aeruginosa by UV- C radiation in spiked water samples

A UV-C germicidal lamp (COLE PARMER, VC-215.G, France), consisting of two 15 W low-pressure UV-C bulbs was used for irradiation as described by Hayes et al, 2008 [30] with modifications. Different UV doses were obtained by changing the distance between the UV – C lamps and the sample and UV-C exposure time. A radiometer (ST 512 Sentry Optronic Crop. Taiwan) was used to measure the irradiance in microwatts per square centimeter (µW cm^{-2}). Exposures spanned a UV-C dose range of approx. 10,350 – 24,500 µW cm^{-2}. 5.0 mL of sterile distilled water was spiked with *P. aeruginosa* (ATCC 27853) to obtain an optical density of 0.132 at 600 nm (0.5 McFarland standards – 1x 10^8 cfu/mL). This spiked water sample was serially diluted to obtain a 1 x 10^4 cfu/mL suspension of *P. aeruginosa*, of which 5 mL was transferred to a sterile petri dish (90 mm). 5.0 mL aliquots were separately irradiated with UV-C doses of 10350, 17,300 and 24,500 µW cm-2 for 5, 10, 20 and 60 seconds for each dose. Different UV-C doses were obtained by changing the distance between UV-C lamps and the bacterial suspension. Non-irradiated samples served as controls.

2.1.1. Detection of *P. aeruginosa* in irradiated-spiked-water samples

The irradiated samples were serially diluted and plated on tryptic soy agar (TSA) using Miles and Misra method [31] as follows. Here, the suspension was serially diluted (10^{-1} to 10^{-6}). Each TSA plate was divided into 6 equal sectors and the sectors were labeled with the dilutions. In each sector, two of 20 µl-drops of the appropriate dilution was placed on the surface of the agar and the drops were allowed to spread naturally. The plates were left upright on the bench to dry. Subsequently, the plates were inverted and incubated at 37°C for 24 h. Following incubation, each sector was observed for growth. Number of colonies in sectors containing 2-20 colonies was counted. The following equation was used to calculate the number of colonies forming units (CFU) per mL from the original suspension.

CFU per mL = Average number of colonies per dilution x 50 x dilution factor

The non-irradiated samples were plated as controls. Duplicate plate counts were made at each dilution in three replicate trials.

2.2 Detection of *Pseudomonas aeruginosa* in bottled drinking water samples

Concerns have been raised about the contamination of drinking water, which has undergone UV radiation treatment at the factory prior to bottling, with *Pseudomonas aeruginosa*. This controversy caught our attention, given that UV treatment is designed to eliminate *Pseudomonas aeruginosa* in drinking water. In order meet the objective of this study, which is to comprehensively assess the effectiveness of UV irradiation in eradicating *P. aeruginosa* in water, first we randomly sampled drinking water bottles from the market.

2.2.1 Sample collection

Three randomly selected bottled water-brands were used to determine the occurrence of *P. aeruginosa* in bottled water in Sri Lanka. Five bottles (500 mL) from each brand were purchased from local markets.

2.2.2 Detection of P. aeruginosa

Membrane filtration was used to detect *P. aeruginosa* in water samples. 100.0 mL volumes of each sample were allowed to pass through the membrane filtration apparatus (Pyrex, Germany) using sterilized membrane filters (Sartorius, Germany, 0.45 µm). Filters were incubated on Cetrimide agar plates supplemented with 15 mg/l of nalidixic acid at 37 °C for 44±2 h. Colonies that showed a bluish/greenish pigmentation or that were fluorescent when examined under UV light (364 nm) were selected as presumptive *P. aeruginosa* strains. Pure cultures made on nutrient agar plates were incubated at 37 °C for 22±2 h [32]. *P. aeruginosa* (ATCC 27853) was used as a reference control in all the assays performed in the study. Three bottles from each brand were analyzed in duplicate, resulting in six replicates per brand. Stock cultures of all strains were maintained for further identification.

2.3 Elimination of *Pseudomonas aeruginosa* by UV- C radiation in bottled water samples

Five milliliter aliquots from each brand were separately irradiated with UV-C doses of 10350, 17,300 and 24,500 µW cm^{-2} for 5, 10, 20 and 60 seconds for each dose. The presence of *P. aeruginosa* in these irradiated samples was detected using the method described in 2.1.1.

3. RESULTS AND DISCUSSION

We hypothesized that *P. aeruginosa* could be eliminated upon exposure to UV-C radiation. To test and gauge the dosage of UC radiation required to eliminate the bacteria, water samples spiked with *P. aeruginosa* were exposed to varying intensities of UV-C light.

3.1 Elimination of *Pseudomonas aeruginosa* by UV- C radiation in spiked water samples

Sterile distilled water was spiked with the *P. aeruginosa* followed by exposure to 3-UV doses for varying time intervals as summarized in Table 1. When the water was tested for the presence of the bacteria post-UV treatment, it was observed that UV doses of 24,500 and 17,300 µW/cm^2 were very effective against *P. aeruginosa*, eliminating all the bacteria within 5 seconds of exposure time (bacteria count - 1x 10^4 cfu/mL). 4-fold reduction in bacterial count was observed even at the lower UV doses (approx. 10,350 µW/cm^2) within the lowest exposure time of 5 seconds. The controls, the spiked water samples, which were not exposed to UV did not show any reduction of bacterial growth (Table 1 and Figure 1). This strongly establishes the fact that the UV-C irradiation could effectively eliminate *P. aeruginosa* in water.

Table 1: Bacterial plate counts of *P. aeruginosa* following exposure to various doses of UV irradiation.

Time (sec.)	UV dose (µW /cm^2)			
	24,500	17,300	10,350	Control
5	0	0	0.25 x 10^4	TNTC
10	0	0	0.62 x 10^4	TNTC
20	0	0	0.25 x 10^4	TNTC
60	0	0	0.25 x 10^4	TNTC

Initial bacterial count (CFU/ml) = 1 × 10^4

**All samples were run in duplicate with triplicate trials; the number of colony-forming units per milliliter (CFU/mL) indicates the average bacterial counts of replicates. The abbreviation is as follows: TNTC = too numerous to count*

Exposed to UV-c radiation Control

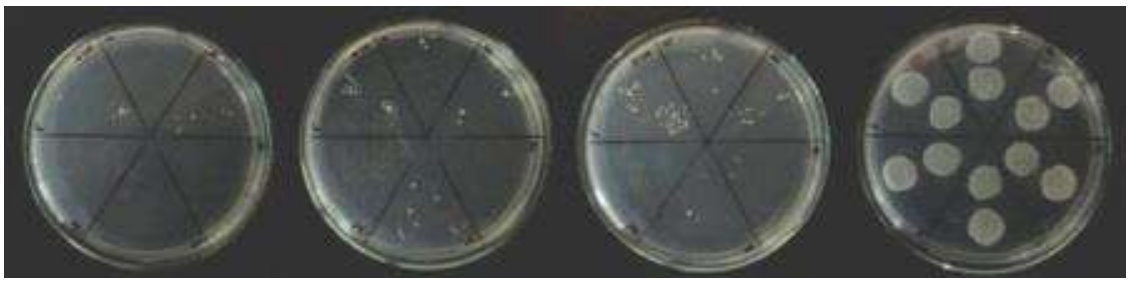

Figure 1 - Bacterial plate counts of *P. aeruginosa* following exposure to various doses of UV irradiation – Miles and Misra Method

3.2 Detection of *Pseudomonas aeruginosa* in bottled drinking water samples

Having established an effective UV-based method to eliminate the *P. aeruginosa*, we then turned to the test this method on bottled water. First, we had to detect the presence of *P. aeruginosa* in bottled water sold in the local market. Three randomly selected bottled water brands were analyzed for the presence of *P. aeruginosa* and the results are summarized in Table 2 and Figure 2.

Numerous studies have reported the contamination of bottled water with *P. aeruginosa* [33, 15, 10]. Manaia *et al*. (1990) [34] reported that 83% of carbonated bottled water samples tested in their study were contaminated by *Pseudomonas* spp., while [35] Hunter *et al*. (1990) found 29% of the *Pseudomonas* spp. detected from bottled water to be *P. aeruginosa*. In Greece, *P. aeruginosa* is one of the undesirable microbiological criteria in bottled water and is used as a process management indicator in production. Its presence means contamination during the bottling process or that the source has become polluted by organic material [36, 37]. Moreover, it has been documented that if these microorganisms are not adequately removed during processing and bottling, bacterial multiplication may occur within 1–3 weeks after bottling, and the bacterial count can reach up to 10^3–10^4 bacteria mL^{-1} at 37 ºC [35, 38]. The storage temperature of bottled water has also been demonstrated to affect the rate of multiplication and survival of microorganisms [39].

P. aeruginosa was detected in all three brands tested in this study (Table 2 and Figure 2).

Table 2: Number of bacterial isolates detected in bottled drinking water samples

Bottled water brands	**The average number of bacterial colonies isolated per 100 mL**
Brand 1	128± 4
Brand 2	74 ± 9
Brand 3	95 ± 18

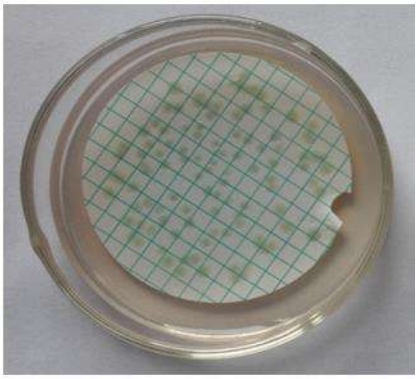

Figure 2: Presumptive *P. aeruginosa* isolates on membrane filter placed on cetrimide agar

3.3 Elimination of *Pseudomonas aeruginosa* by UV- C radiation in bottled water samples

The same treatment done on the spiked water samples was repeated on the bottled water samples to test the effectiveness of the UV-C based *P. aeruginosa* elimination method. As it was observed with the spiked experiment, UV doses of 24,500, 17,300 and 10,350 $\mu W/cm^2$ were seemed very effective against *P. aeruginosa* in bottled water samples, indicating the elimination all the bacteria within 5 seconds of exposure time (Table 3). However, the controls, which were not exposed to UV-irradiation also did not show any colony formation when tested according to the Miles and Misra method. This might be attributed to the initial bacterial count, which was a mere 128 in 100 mL, proving insufficient for detection using the Miles and Misra method.

Subsequently, it was decided to employ the membrane filtration technique as described in 2.2.2 with slight modification to assess the bacterial count after UV exposure. Here the 100 mL of the bottled water was exposed to each UV-radiation dose and analyzed by membrane filtration technique. Results revealed that the UV-irradiated samples did not have a single colony while the controls, which were not exposed to UV radiation had comparable count of 113 to the original-128 in100 mL.

Table 3: Bacterial plate counts of *P. aeruginosa* of bottled water samples following exposure to various doses of UV irradiation.

Time (sec.)	UV dose (µW /cm^2)			
	24,500	17,300	10,350	Control
5	0	0	0	0
10	0	0	0	0
20	0	0	0	0
60	0	0	0	0

**All samples were run in duplicate with triplicate trials; the number of colony-forming units per milliliter (CFU/mL) indicates the average bacterial counts of replicates*

Pseudomonas aeruginosa is also used as an indicator organism in water contamination and it has been suggested as a surrogate indicator for the presence of other opportunistic pathogens [40]. Therefore, *P. aeruginosa* is used as a parameter of bottled water standards in most countries. According to the European Union bottled water standards, *P. aeruginosa* should be absent in 250 ml of bottled water [27]. *P. aeruginosa* should be absent in 100 ml of bottled water in the United Kingdom [41], Canada [42], and East African Standards [43] for microbiological limits of bottled water. However, in Sri Lankan Standards, although *P. aeruginosa* is a criterion for natural mineral water standards, it is not included for bottled water standards, and this study could be a pivotal point for us to rethink not taking *P. aeruginosa* into account as far as the quality parameters of bottled water are concerned.

According to the results, UV irradiation is a very effective method of disinfection in the elimination of *P. aeruginosa* as it reduced the number of detectable *P. aeruginosa* to zero within 5 seconds of exposure time at UV doses 17,300 and 24,500 µW/cm^2, while it is reported by Eccleston (1998) [44] that *P. aeruginosa* could be eliminated by 10,500 µW/cm^2. However, in the current study, only 4-fold reduction of bacteria was obtained at a UV dose of 10,350 µW/cm^2 within 5 seconds of contact time.

Ultraviolet or UV energy is found in the electromagnetic spectrum between visible light and X-rays and can best be described as invisible radiation. To kill microorganisms, the UV rays must strike the cell. UV energy penetrates the outer cell membrane, passes through the cell body and disrupts its DNA preventing reproduction. However, Hijnen *et al*. (2006) [45] have noticed that bacteria (vegetative cells) are significantly more susceptible to UV radiation than viruses and also spores of *Bacillus subtilis* and *Clostridium perfringens* are notably less sensitive to UV than the vegetative bacterial cells and also most of the viruses and phages.

Under ideal conditions, a UV unit can provide a greater than 99% reduction of all bacteria [46]. Even with this performance, ultraviolet disinfection has some limitations: UV units only kill bacteria at one point in a watering system and do not provide any residual germicidal effect downstream. If just one bacterium passes through unharmed (100 % destruction of bacteria cannot be guaranteed), there is nothing to prevent it from attaching to downstream piping surfaces and proliferating. Further, bacteria cells are not removed in a UV unit but are converted into pyrogens. The killed microorganisms and any other contaminants in the water are a food source for any bacteria that survive downstream of the UV unit. Due to these limitations, the piping in a watering system treated by UV disinfection in the bottling plants will need to be periodically sanitized with a chemical disinfectant, lamp replacement, monitoring performance and monitoring UV dosage. The high number of *P. aeruginosa* in bottled water may be due to improper UV treatments, faulty UV systems, or cross-contamination. Nevertheless, this developed method is promising and better results in the elimination of *P. aeruginosa* are possible with proper implementation.

CONCLUSION

In summary, we have demonstrated that the use of UV-irradiation could eliminate *P. aeruginosa* effectively from water. Moreover, a few randomly picked bottled water brands, which are supposed to be in accordance with the WHO guidelines: potable water should have below 20 CFU/mL heterotrophic bacterial counts with no coliform bacteria, fecal coliforms, *E. coli*, enterococci, and *P. aeruginosa*. However, bottled water samples tested positive for *P. aeruginosa* and it is a threat to the health of the consumer.

However, it was shown this health threat can be eliminated or minimized when contaminated bottled water is exposed to appropriate UV irradiation over a sufficient time. We assert that achieving an appropriate dosage is crucial for the comprehensive elimination of *P. aeruginosa*, especially in an industrial setting, and we believe our discoveries could be advantageous in such contexts.

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