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ESCOLA SUPERIOR DE BIOTECNOLOGIA
PORTO

STUDY OF THE APPLICATION OF ULTRAVIOLET RADIATION TECHNOLOGY IN WATER TREATMENT PROCESSES

By

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Training Placement Report /Dissertation presented to *Escola Superior de Biotecnologia* of the *Universidade Católica Portuguesa* to fulfill the requirements of Master of Science degree in Applied Microbiology

By
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Aos meus pais, família, irmão e amigos, um obrigado especial.

"A dúvida é o princípio da sabedoria", Aristóteles

Resumo

Neste estudo foram avaliados os efeitos de diferentes métodos de tratamento para produzir água de consumo, relativamente a diferentes parâmetros, incluindo a sobrevivência de uma bactéria patogénica oportunista. O trabalho incluiu duas abordagens: a análise de dados de monitorização obtidos ao longo da linha de tratamento, incluindo radiação ultravioleta (UV), disponibilizados pela empresa Águas do Norte, e ensaios experimentais para avaliar o efeito de radiação UV na remoção de *Klebsiella pneumoniae*, resistente a carbapenemos e alguns genes relacionados com resistência a antibióticos.

Compararam-se características microbiológicas, químicas e físicas de água bruta, filtrada, tratada com cloro e tratada com cloro/UV, com base em análise estatística. Esta avaliação mostrou que o tratamento é particularmente eficaz na remoção de microrganismos e turbidez. O tratamento adicional com radiação UV, em comparação com o tratamento com cloro, não mostrou diferenças significativas na melhoria da maior parte dos parâmetros analisados.

Estudou-se ainda, à escala laboratorial, a sobrevivência de duas estirpes de *K. pneumoniae* de origem clínica (KP2-448) e ambiental (SM1). O PCR quantitativo (qPCR) foi utilizado para avaliar a variação dos genes de resistência a antibióticos (*bla*$_{\text{KPC}}$, *bla*$_{\text{OXA-1}}$, e *sul1),* e outros relacionados *(intI1*, *qacEΔ1).* Os resultados mostraram respostas variadas relativas à exposição da radiação UV e subsequente recuperação. Os ensaios de sobrevivência à radiação ultravioleta (UV) reforçam o impacto da radiação UV-C na persistência e viabilidade bacteriana. A análise da razão *bla*$_{\text{KPC}}$/*phoE* mostrou um aumento superior a 10 vezes na abundância relativa do gene *bla*$_{\text{KPC}}$ na estirpe clínica de *K. pneumoniae* após recrescimento.

Nas duas abordagens, foi ainda feita a pesquisa de 10 biomarcadores de resistência a antibióticos, estando na sua maioria ausentes, com exceção dos genes *intI1*, *qacEΔ1* e *sul1.*

Este estudo contribuiu para uma compreensão mais profunda sobre o impacto e eficácia da radiação UV-C no tratamento de água e como otimizar este processo.

Palavras-chave: Tratamento de água, *Klebsiella pneumoniae*, Radiação UV-C, Resistência a antibióticos

Abstract

This study investigated the effects of different treatment methods for producing drinking water on various parameters, including the survival of an opportunistic pathogenic bacteria. The work included two approaches: monitoring data analysis obtained along the treatment line, including ultraviolet (UV) radiation, provided by the company Águas do Norte, and experimental assays to evaluate the effect of UV radiation on the removal of carbapenem-resistant *Klebsiella pneumoniae* and some genes related with antibiotic resistance.

Microbiological, chemical, and physical characteristics of raw, filtered, treated with chlorine, and treated with chlorine/UV water were compared based on statistical analysis. This evaluation showed that the treatment is particularly effective in the removal of microorganisms and turbidity. The additional treatment with UV radiation, compared to chlorine treatment, did not show significant differences in the improvement of most of the analyzed parameters.

The survival of two strains of *K. pneumoniae* of clinical (KP2-448) and environmental (SM1) origin was studied at the laboratory scale. Quantitative PCR (qPCR) was used to evaluate the variation of antibiotic resistance genes (*bla*$_{\text{KPC}}$, *bla*$_{\text{OXA-1}}$, and *sul1),* and others related (*intl1*, *qacEΔ1)*. The results showed varied responses regarding UV-C exposure and subsequent recovery. Ultraviolet (UV) radiation survival assays reinforced its impact on the persistence and bacterial viability. The analysis of the *bla*$_{\text{KPC}}$/*phoE* ratio showed a tenfold increase in the relative abundance of the *bla*$_{\text{KPC}}$ gene in the clinical strain of *K. pneumoniae* after the regrowth phase.

In both approaches, It was also done a search for 10 antibiotic resistance biomarkers, that were most of them absent, with exception of the *intl1*, *qacEΔ1* e *sul1* genes.

These collective insights offer a deeper understanding of antibiotic resistance dynamics and water treatment efficacy, guiding future research directions for optimized strategies.

Keywords: Water treatment, *Klebsiella pneumoniae*, UV-C radiation, Antibiotic resistance

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1. Introduction

Water is a vital resource for all life on Earth, so it has become the main essential resource for human survival, whether it is used for drinking, domestic use, or food production. According to the World Health Organization (WHO), safe water is defined as the water which during its consumption doesn’t express any significant risk to human life. The WHO also estimates that 80% of diseases worldwide are waterborne, in developing countries. Likewise, it is necessary to supply water in adequate quantities and hygienic conditions, since there is a wide variety of pathogenic organisms transmitted through it which can cause serious public health problems. These pathogenic organisms comprise viruses, bacteria, protozoa, fungi and toxic substances, such as heavy metals (WHO, 2022).

More than ever, water pollution has become a global concern since contaminants are rising from all kinds of sectors, jeopardizing human and wildlife health. Untreated water is a great threat, engaging several waterborne diseases such as cholera, diarrhea, typhoid fever, leptospirosis, respiratory tract infection, kidney and endocrine damages, becoming very risky and lastly leading to death (WHO, 2022). However, water is only the passive carrier of infectious agents. In accordance with Centers for Disease Control and Prevention (CDC), the principal pathogens that have been shown to cause diseases are *Salmonella* spp., *Shigella* spp*., Campylobacter* spp., *Escherichia coli*, *Cryptosporidium*, *Vibrio cholerae*, *Giardia duodenalis* and influenza virus. The spread of these microbiological hazards is related to the ingestion of unsafe water, poor sanitation, and lack of clean water supply (Patel, 2019).

To better understand the correlation between the microorganisms and the poor quality of water an overview of waterborne pathogens, route of transmission and sites of infection are represented in Figure 1.

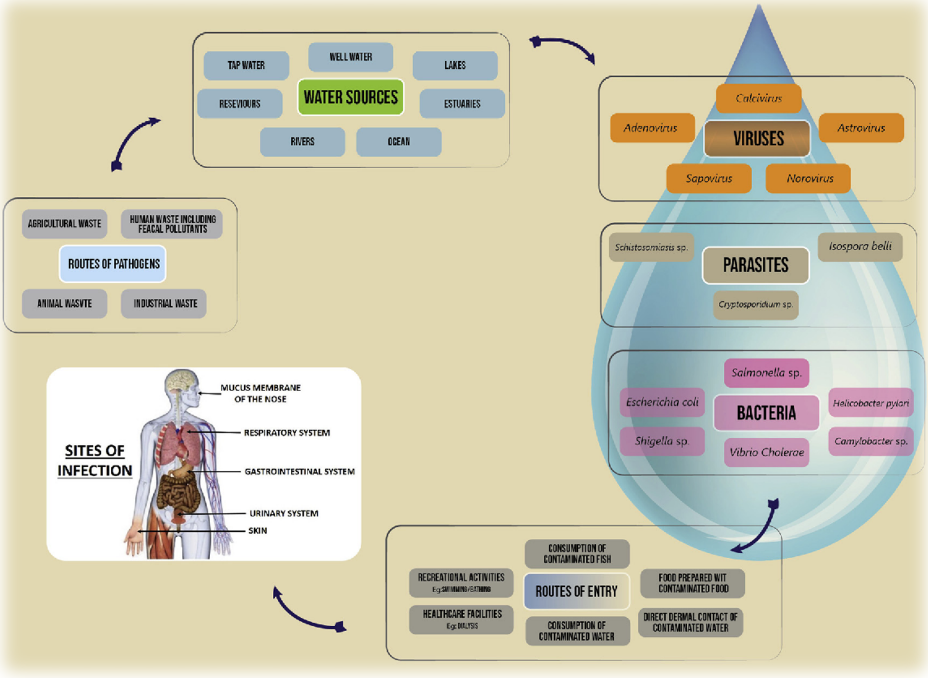


Figure 1. Overview of waterborne pathogens, route of transmission and sites of infection (Adapted from Magana-Arachchi and Wanigatung, 2020).

To overcome these issues and to gain access to improved sources of water these microbial contaminants must be removed, so regular monitoring of water sources is essential to minimize the severe effects from these waterborne pathogens and improve the water quality. Nowadays, there are many water treatment processes available, involving steps such as coagulation and flocculation, used to separate small particles from raw water; the next steps are sedimentation of the particles and filtration to remove the microorganisms and finally, the last one is disinfection (CDC, 2022). Disinfection can be referred to as the removal or deactivation of harmful microorganisms and it has been considered a more appropriate process when compared with sterilization, since the last one not only kills harmful microorganisms but also the harmless ones (Saqib Ishaq *et al*., 2019). Within the process of disinfection, there are also many ways to perform it. The most commonly used are chlorination, ozonation, ultrafiltration (UF) and UV irradiation (Cao *et al.,* 2009).

Chlorination was the first great achievement of water disinfection, being widely used due to its low cost and high efficiency. This process takes part in the cellular oxidation of the microorganisms and ends up killing them. However, after its introduction some studies have shown that this method is more likely to form chlorination by-products (CBPs) due to a reaction between the chlorine and the natural organic matter present in the water (Richardson *et al.,* 2007). Those CBPs are said to be genotoxic and carcinogenic to humans (Richardson *et al.,* 2007) and some of them, such as trihalomethanes (THMs), may increase the risk of adverse reproductive outcomes. And besides leaving harmful residues in the water, this method can also change the organoleptic properties of drinking water becoming a great disadvantage for its use (Akçaalan *et al.,* 2022).

Another used method is ozonation. Its mode of action is the same as chlorination with the difference that has greater oxidizing power. Furthermore, it has a short reaction time and can kill the pathogenic agents and remove organic matter (*Figure 2*) in just a few seconds (Morrison *et al.,* 2022). Another benefit is that it has no effect in the taste or color of the treated water. However, some flaws about this process have been detected, such as limited residual disinfection, high initial cost, energy-intensive process, limited effectiveness against certain contaminants, and potential formation of harmful disinfection byproducts. Plus, ozone is unstable at atmospheric pressure, becoming toxic at high concentrations as it is a greenhouse gas. So, it is a demanding method in terms of handling requiring extreme care, since it can cause acute damage to humans – such as skin, eye and respiratory tract injuries and allergic reactions (Chavoshani *et al.,* 2020).

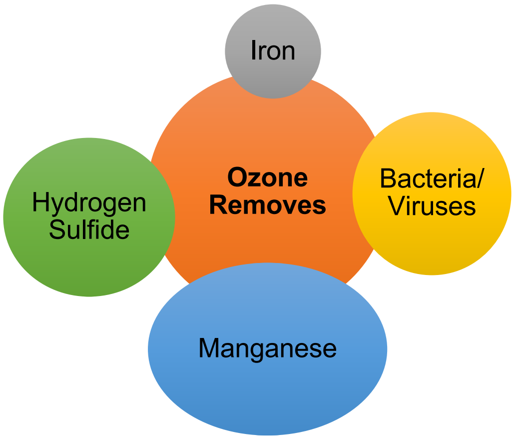


Figure 2. Some substances that can be removed from water through ozonation processes.

In this regard, ultrafiltration (UF) has also been classified as an advanced method for drinking water disinfection with a high removal of microorganisms from contaminated water and can provide proper water for human consumption (Chen *et al.,* 2023). In addition, UF has an easy operation system and has been considered one of the most economical technique for drinking water treatment being used as a suitable choice for safe water supply (Li *et al.,* 2018). This method works through a reuse water treatment plant (WTP) and is mainly designed to remove physical solids from water by passing it through a semi-permeable membrane to turn raw water into drinking water. The biggest barrier to a broader application of this technique is the fact that bacteria and other pathogens can be occasionally detected in the membrane permeate, especially in long stop periods in full-scale system operation (Li *et al.,* 2018).

With the variety of disadvantages found in all these techniques, new ones are constantly arising to ensure safety issues associated with water contamination. Nowadays, ultraviolet (UV) light disinfection has experienced an increasing use as a water treatment system able to remove the most resistant microbiological contaminants from water, aiming to eliminate any potential pathogen that threatens human life (Li *et al.,* 2019). This method uses UV radiation that penetrate the cell wall of microorganisms causing damage to their DNA/RNA making them inactive. This genetic alteration interrupts their reproduction systems preventing them from multiplying (Srivastav *et al.,* 2020).

There are three types of UV irradiation that can be applied. UV-A, with a wavelength between 320 to 400 nanometers; UV-B, with a wavelength between 280 - 320 nm; and UV-C, with a wavelength <280 nm. In general, UV-C rays are the most used because of its energetic power and germicidal properties breaking even the most powerful chemical bonds present in the microorganism genetic material (Hofman-Caris, 2017). This process is only able to occur by using lamps capable of emitting UV-C rays. The UV system transfers electromagnetic energy to the organism's DNA through a low-pressure arc lamp composed by elemental mercury and an inert gas (argon, for example) capable of producing UV light in the wavelength needed (<280 nm) to break the nucleic acids bonds and consequently, kill the microbes (Hofman-Caris, 2017). Normally, quartz sleeves are used to contain and protect the lamps (Figure 3).

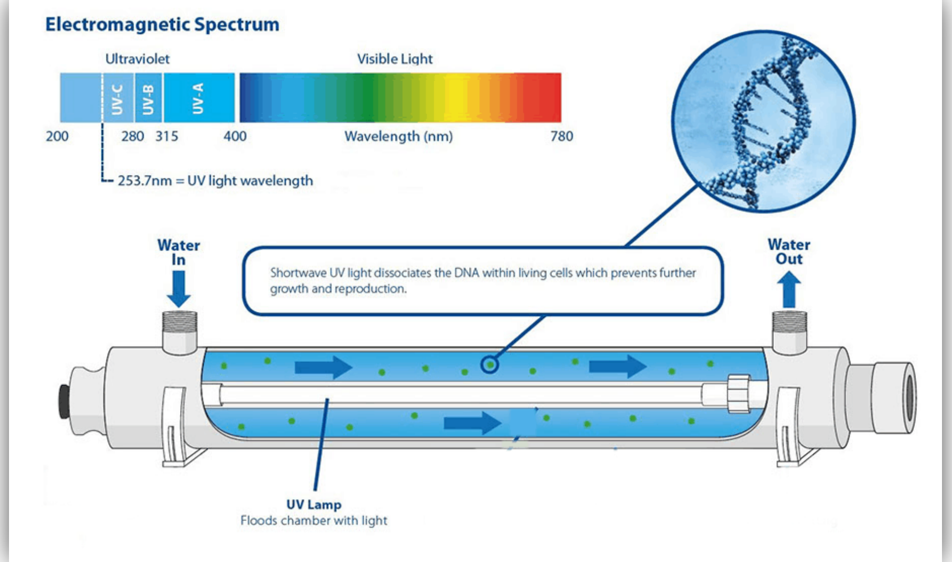


Figure 3. UV disinfection system (adapted from Alfaa UV, 2021).

Also, the effectiveness of the method depends on the exposure time and the intensity of the radiation that microorganisms are submitted. The UV dosage must be applied according to the objective of the method and bacterial load. If the dosage is poorly calculated, it can only cause damage allowing the microorganisms to regenerate. In UV disinfection, the fraction of surviving microorganisms will decrease exponentially with increasing UV dose (Gibson *et al.,* 2017). For UV at a wavelength minor than 280 nm is usually needed a dose in the range of 40 mJ/cm^2, but it can be calculated using the following equation (Saguti *et al.,* 2022):

$$\boxed{\boldsymbol{UV\ dose = UV\ intensity\ \left(\frac{\mu W}{cm^2}\right)\ .\ Exposure\ Time\ (seconds)}}$$

It should also be noted that UV disinfection is mostly preferred over other techniques because of its many advantages. Besides its effectiveness, it's also a cheap and safer process for the environment involving no use of chemical agents and consequently, avoiding toxicity problems (Saguti *et al.,* 2022). In this way, the physicochemical characteristics of the water are maintained and there's no production of disinfection by-products (DBPs) – contrary to chlorination (Ao *et al.,* 2020). It's important to mention that it is a physical process which also allows to minimize those residues from water. Finally, this process has a lower maintenance cost and lower energy consumption since it is a minimally fast process (Saguti *et al.,* 2022). However, this method also has its challenges. UV light is only able to eliminate microorganisms from water. Contaminants such as heavy metals, salts, chlorine or artificial substances such as petroleum products or pharmaceuticals should be eliminated through other filtration methods combined with UV technology. In recent years, UV-Light Emitting Diode (UV-LED) has been employed as an alternative to reduce such weaknesses (Saguti *et al.,* 2022).

This work aims to focus on the use of UV light disinfection in drinking water treatment, but it goes beyond this application.

Regarding the subject of drinking water treatment, it is also important to address that the analysis of pathogenic microorganisms in order to monitor water quality implies significant costs, especially when a large number of parameters are involved. Furthermore, analyzes for some pathogens are fallible and may require a long incubation period, which is unacceptable for the rapid responses required. Thus, according to Decree-Law 2020/2184 proposed by the European Parliament and the Council of the European Union, the introduction of new flexible monitoring methods presents a potential opportunity to reduce these costs and risks, without compromising the protection of public health. Although, all the parametric values set out in this Directive are based on current scientific knowledge in order to ensure the safety of human water consumption (Official Journal of the European Union, L 435/3, 2020). In this directive, it is also mentioned that safe water intended for human consumption presupposes not only the absence of microorganisms but also the presence of certain amounts of natural and essential minerals that benefits human health (Official Journal of the European Union, L 435/4, 2020).

Therefore, the concept of indicator organism is used in order to denounce the possible presence of disease-causing elements. The use of intestinal organisms as indicators of faecal pollution, rather than pathogens themselves, is universally accepted for monitoring and assessing microbiological safety. When these faecal bacteria are detected in the water source, it is possible that the water source is contaminated and needs treatment. There are several proposed characteristics that make it possible to identify this indicator organism, such as being present in polluted water and absent from drinking water, being present in water when pathogens are present, being absent in water when pathogens are absent, and their number should correlate with the value of pollution (Motlagh et al., 2019).

In order to assess the microbiological parameters relating to water quality, total counts of microorganisms at 22 ºC, counts of coliform bacteria and counts of *Escherichia coli* (EN ISO 9308-1) must be performed. Then, intestinal *Enterococcus* counts (EN ISO 7899-2), *Clostridium perfringens* counts (EN ISO 14189) and *Legionella* counts (EN ISO 11731) are also important to monitor water quality (Official Journal of the European Union, L 435/50, 2020). To verify whether the analyzed water sample is suitable for human consumption, it is necessary to compare the values obtained with the reference values proposed by the European drinking water legislation (Table 1).

Table 1. Reference values of microbiological parameters, expressed as Number/100 mL (number of microorganisms per 100 milliliters) and CFU/L (colony forming units per liter). Retrieved from: Official Journal of the European Union, L 435/50, 2020).

Parameter	Parametric Value	Units
Number of colonies at 22ºC	No abnormal change.	-
Coliform bacteria	0	N/100 mL
Escherichia coli	0	N/100 mL
Intestinal *Enterococcus*	0	N/100 mL
Clostridium perfringens	0	N/ 100 mL
Legionella	< 1000	CFU/L

To conclude, there are several types of microbiological hazards associated with water contamination. The adoption of preventive measures seems to be the main tool available to preserve and treat water sources, considerably reducing the risk of pathologies that affect human life (Salamandane *et al*., 2021).

2. Objectives

The major objective of this thesis was to study the effects of different treatment methods for producing drinking water, mainly UV-C radiation, on various quality parameters and on the survival of an opportunistic bacterium. Specifically:

i) understand the effect of the different treatment steps, including the ultraviolet radiation, on the improvement of the drinking water parameters, both physicochemical and microbiological (Chapter 1),

ii) evaluate the effect of UV radiation on the removal of carbapenem-resistant *Klebsiella pneumoniae* and some antibiotic resistance-related genes from water (Chapter 2).

3. Chapter 1. Analysis of real data from the functioning of a drinking water treatment plant

With the aim of evaluate the effect of UV-C radiation in the physicochemical and microbiological characteristics of water for human consumption, a dataset provided by the water treatment company (Águas do Norte) was analysed. The study consisted of doing the data analysis, that is routinely collected by the treatment plant to monitor its functioning. The treatment system adopted by this WTP is schematized in Figure 4.

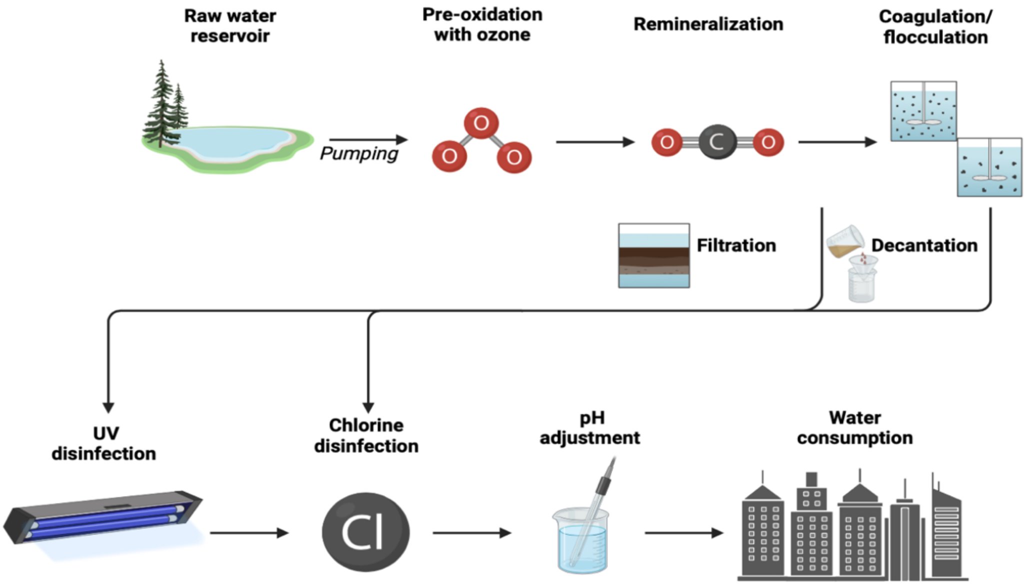


Figure 4. Treatment system of the Water treatment plant (Águas do Norte). Image created in Biorender.

The data was for the period between January 2022 and February 2023. The analysis compared average values of the parameters registered for raw water (herein designated raw), after pre-oxidation, remineralization, coagulation/flocculation and sand filtration (herein designated filtered), after disinfection with chlorine (herein designated treated other) and after UV radiation combined with chlorine (herein designated UV treated). The data provided included the following parameters (n=42): 1,2-dichloroethane, acrylamide, alkalinity, aluminium, antimony, ammoniacal nitrogen, coliform bacteria, benzene, bromates, calcium, solvent organic carbon, total organic carbon, scent, free residual chlorine, *Clostridium perfringens*, conductivity at 20 ˚C, color, *Cryptosporidium*, total hardness, enterococci, *Escherichia coli*, phytoplankton cyanobacteria, total phytoplankton, *Giardia* spp., polyaromatic hydrocarbons, *Legionella pneumophila*, magnesium, manganese, nitrates, nitrate 0.50 mg/L + nitrite/0.30 mg/L, nitrites, total heterotrophs count at 22 ˚C and 36 ˚C, oxidability, pH, taste, sodium, sum of tetrachloroethene, temperature, trihalomethanes, turbidity, and UV at 254 nm.

In addition to the comprehensive water treatment process, a more detailed analysis was conducted by comparing the data on a daily basis for the period in which the UV treatment was functioning, between the months of September to November 2022. This analysis aimed to assess the variation of the parameters analysed before and after UV water treatment, to gain deeper insights into its effect on water quality.

Furthermore, water samples were collected before and after UV disinfection at the water treatment plant (WTP) for the evaluation of specific biomarkers of antibiotic resistance contamination. The quantification of 16S rRNA gene and 10 selected biomarkers was conducted using quantitative PCR (qPCR) to monitor and assess the effectiveness of the UV treatment process in the removal of genes associated with antibiotic resistance from anthropogenic origin, that are typically rare in clean environments. The genes analysed belong to three categories (Teixeira *et al.,* 2023):

- Class 1 integrons integrase (*intI1)* – associated with mobile genetic elements and the transference of genetic material by horizontal gene transfer, a process through which genetic material can be transferred among different microorganisms, potentially leading to the spread of antibiotic resistance genes,
- *sul1, ermB, ermF, aph (3'')-ib, qacEΔ1, mefC* and *tetX – genes* associated with antimicrobial resistance, indicating the potential presence of antibiotic-resistant bacteria,
- *uidA* and crAssphage – associated with fecal contamination, where *uidA* indicates the presence of fecal bacteria like *Escherichia coli* while crAssphage serves as a viral indicator of human fecal contamination.

3.1. Materials and Methods

3.1.1. Data Analysis

The water treatment company (Águas do Norte) provided a complete dataset with all the parameters that were determined for the WTP under study. The initial dataset with 42 parameters (listed above) was trimmed to select the parameters that supported a reliable statistical analysis. The criteria used was the availability of data for more than one sampling point and corresponding to a period of at least three months. The parameters that fulfilled these criteria were (n=24): aluminium, ammoniacal nitrogen, calcium, *Clostridium perfringens*, coliform bacteria, color, conductivity, total organic carbon, enterococci, *Escherichia coli*, free residual chlorine, nitrates, nitrites, oxidability, pH, scent, taste, temperature, total heterotrophs count at 22 ˚C and 36 ˚C, total organic carbon, trihalomethanes, turbidity and UV at 254 nm. The selected data was, whenever necessary, converted to the same units within each parameter and values below the Limit of Quantification (LOQ) were considered to be one decimal unit below LOQ. For each parameter, for each month were determined the average and standard deviation values (Appendix 2). These data were used to perform the statistical analyses, aiming to compare the water quality across the treatment process. The statistical analyses were preceded by the assessment of data normality and homogeneity as a support for the decision of the use of parametric or non-parametric tests. All analyses used SPSS (IBM SPSS Statistics, Version 28, 2021). For the daily analysis, the parameters analysed were (n=10) aluminium, *Clostridium perfringens*, coliform bacteria, color, conductivity, nitrates, pH, temperature, total organic carbon and UV at 254 nm, the ones for which it was possible to have data for the different points (raw, filtered, treated (other) and UV treated water) simultaneously for the same day. In this case, for each parameter was calculated the variation or removal ($\Delta = Filtered - UV\ Treated$)).

3.1.2. Genes quantification using qPCR

Three samples were collected, weekly (24 and 30 of June 2023 and 07 of July 2023), from the water treatment plant (WTP) before and after UV disinfection. The samples were filtered in triplicate through polycarbonate membranes (0.22 μm porosity, Whatman, UK) using a volume of 650 mL for each replica. Membranes were stored at -80 ˚C until DNA extraction. The remaining volume of the samples were incubated in the dark, at room temperature, for a period of 3 days to evaluate the possible bacterial regrowth. After this period the samples were filtered as described above, in duplicate. Total DNA extraction was carried out using the NZY Tissue gDNA Isolation kit (NZYTech, Portugal). The recommended protocol was modified, being used the double volume of the Buffer NT1, Proteinase K solution, Buffer NL and ethanol solution, first to guarantee that the membrane was totally immersed in the buffer and then the proportionality. After the filtration in the NZYSpin Tissue column, the protocol was performed according to the manufacturer instructions. The DNA concentration was measured using Qubit with the dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). Quantitative PCR assays were performed to target 16S rRNA gene and 10 specific biomarkers (*intI1, sul1, ermB, ermF, aph (3'')-ib, qacEΔ1, mefC, tetX, uidA,* and crAssphage). Quantifications were carried out using the Standard Curve method described in Brankatschk *et al.,* (2012) on a StepOne^{TM} Real-Time PCR System (Life Technologies, Carlsbad, USA), using the protocols described by Teixeira *et al.,* 2023. Three independent DNA extracts per sample were analyzed. The results were evaluated based on quality criteria from Rocha *et al.,* (2020), including standard curve efficiency, CT value interpolation, consistent melting curve, single and correct melting point observation and absence of shoulders.

3.2. Results and Discussion

3.2.1. Global and monthly data analysis

The statistical analysis showed the variations observed across the treatment line and highlighted the possible effect of some treatment processes. The treatment involves steps such as pre-oxidation with ozone, remineralization and coagulation/flocculation. After this, the water undergoes filtration followed by disinfection using chlorine and, in some periods, UV-C radiation is also applied for additional disinfection. The impact of UV radiation was evaluated through a statistical analysis. The treatment with UV was applied in some periods (01 to 21 of January/22, 23 to 29 of May/22, and 12 of September until 18 of November/22) as an extra step in the treatment.

In a first approach all the data, for the period January 2022 – February 2023, was analysed to understand the effect of the treatment in a group of 24 physical-chemical and microbiological parameters. Some parameters (n=18) were excluded from the analysis because were just sporadically measured or were not measured for more than one of the sampling points.

Graphics and Boxplots

- **Aluminium (µg/L Al)**

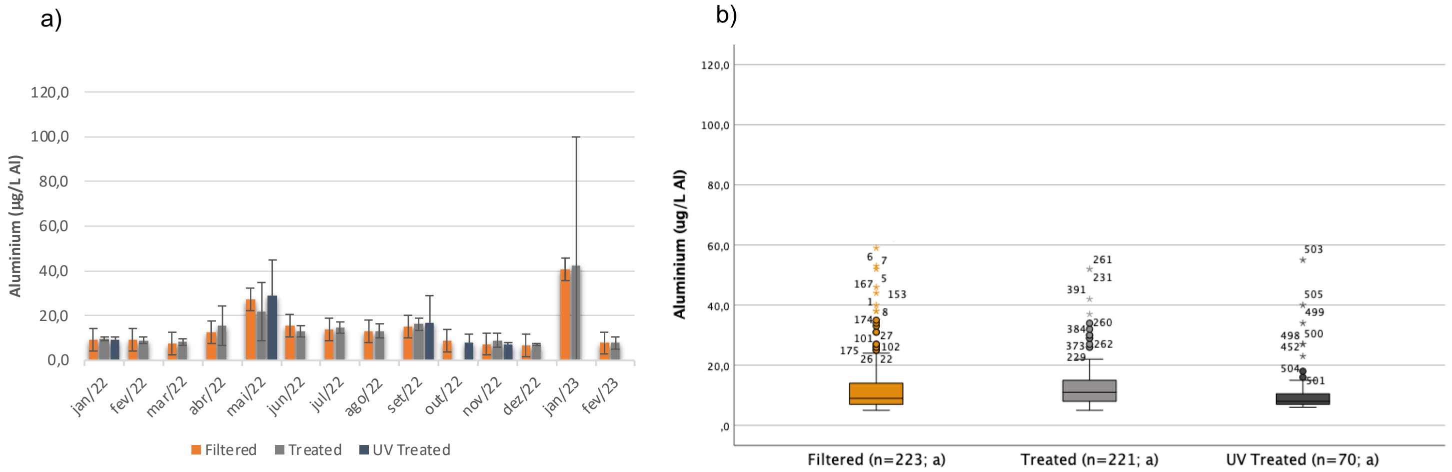


Figure 5. a) Concentration of aluminium (µg/L) measured on ICP-MS method and graphite atomic absorption spectrophotometry for filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 514 samples. b) Boxplot representation of the distribution of determinations for each type of water – for filtered , treated (other) and UV treated water (p=0.242).

- **Ammoniacal nitrogen (mg/L)**

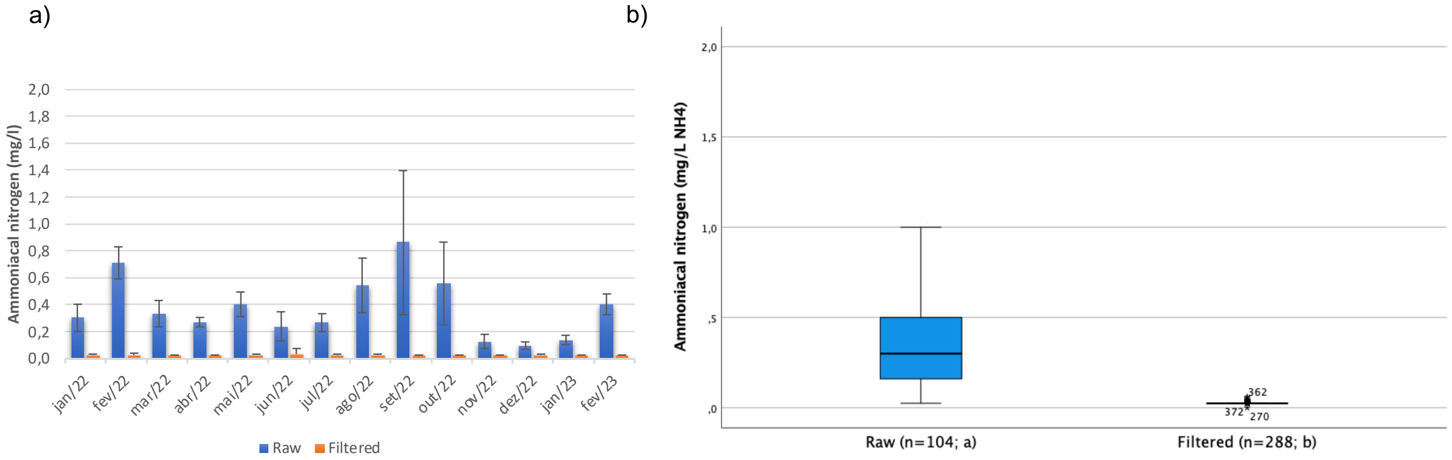


Figure 6. a) Concentration of ammoniacal nitrogen (mg/L) measured based on molecular absorption spectrophotometry for raw and filtered water between the period of January/22 and February/23, in 392 samples. b) Boxplot representation of the distribution of determinations for each type of water – for raw and filtered water (p<0.001).

- **Calcium (mg/L Ca)**

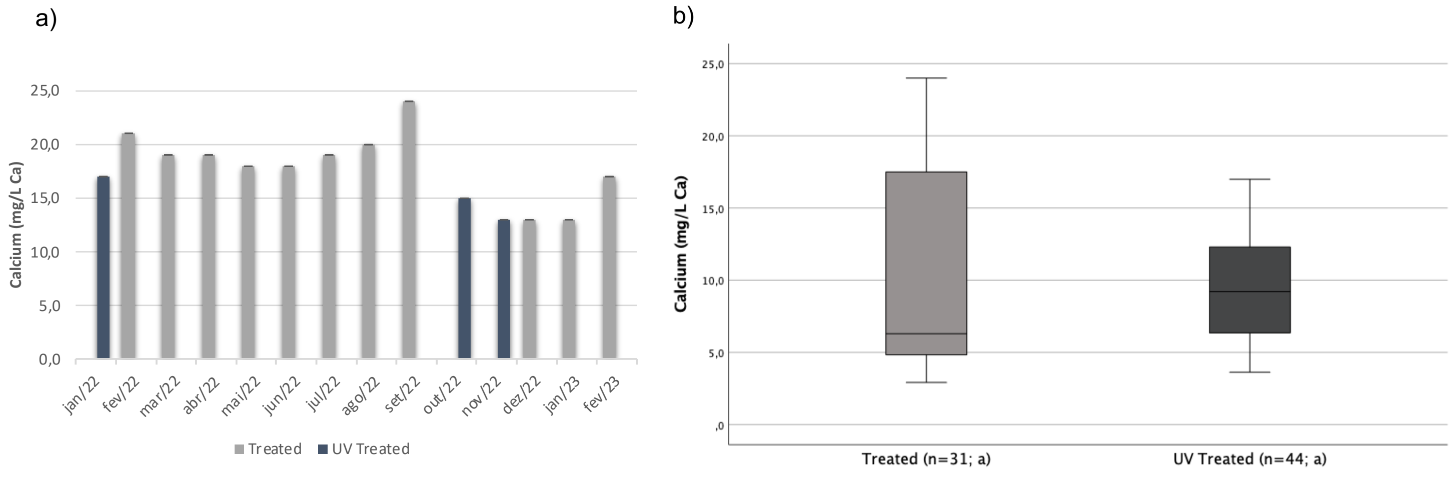


Figure 7. a) Concentration of calcium (mg/L) measured based on flame atomic absorption spectrophotometry for treated (other) and UV treated water between the period of January/22 and February/23, in 75 samples. b) Boxplot representation of the distribution of determinations for each type of water – for treated (other) and UV treated water (p=0.123).

- ***Clostridium perfringens* (N/100mL)**

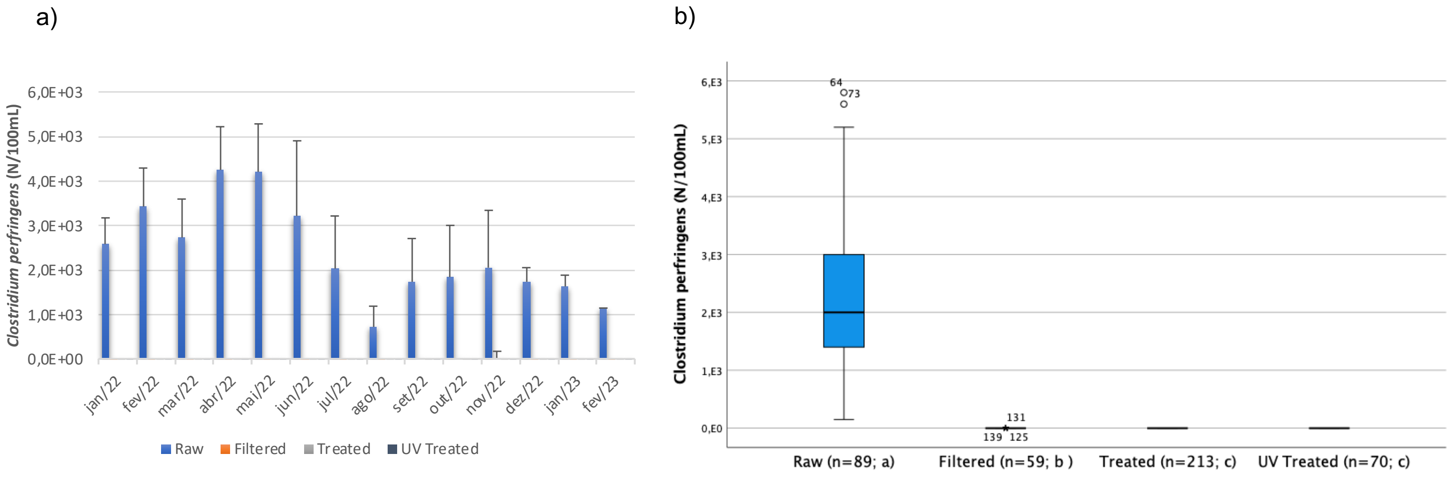


Figure 8. a) Abundance of *Clostridium perfringens* (N/100 mL) measured based on membrane filtration for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 431 samples. b) Boxplot representation of the distribution of determinations for each type of water – for raw, filtered, treated (other) and UV treated water (p<0.001).

- **Coliform bacteria (N/100mL)**

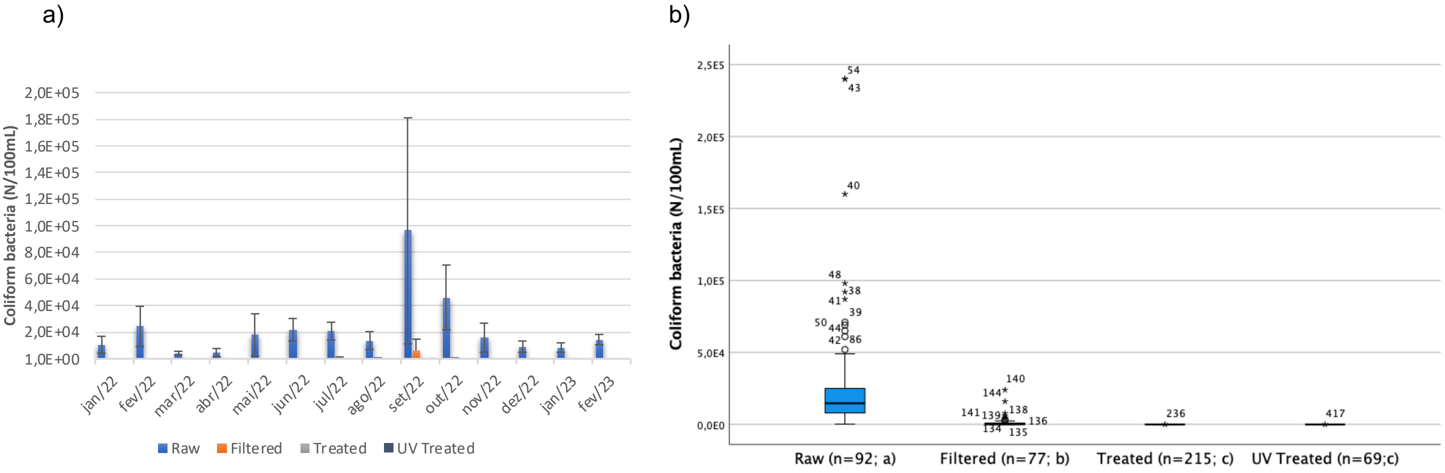


Figure 9. a) Abundance of coliform bacteria (N/100mL) measured based on membrane filtration and multiwells method for raw, filtered, treated (other) and UV treated between the period of January/22 and February/23, in 453 samples. b) Boxplot representation of the determinations for each type of water – for raw, filtered, treated (other) and UV treated water (p<0.001).

- **Color (mg/L PtCo)**

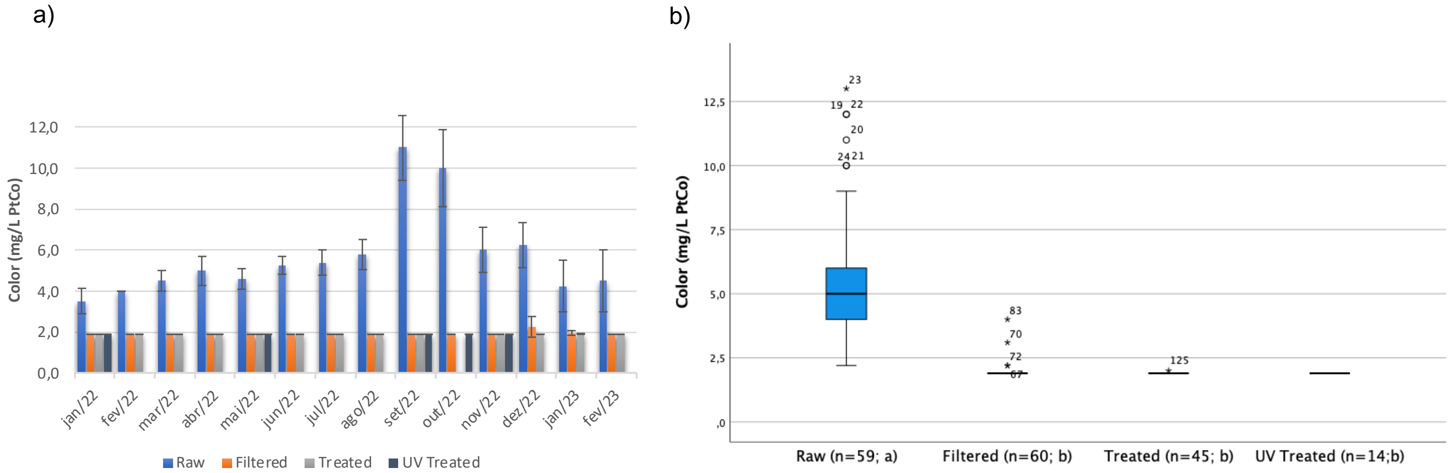


Figure 10. a) Color (mg/L PtCo) measured based on molecular absorption spectrophotometry for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 184 samples. b) Boxplot representation of the distribution of determinations for each type of Water - for raw, filtered, treated (other) and UV treated water (p<0.001).

- **Conductivity at 20 ºC (µS/cm)**

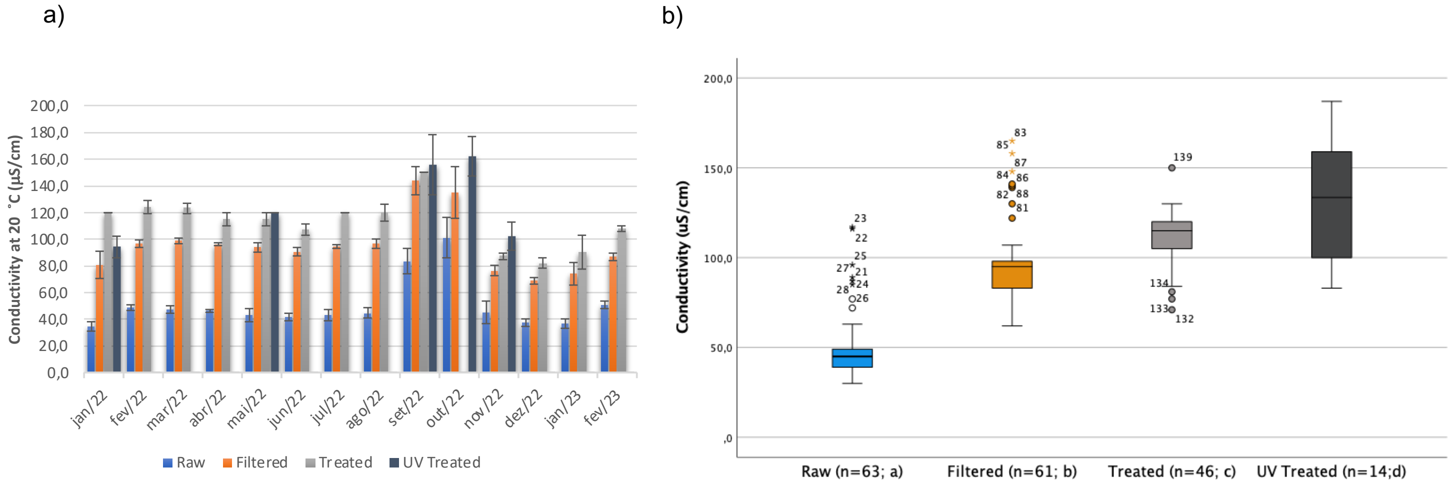


Figure 11. a) Conductivity (µS/cm) measured based on conductimetry for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 184 samples. b) Boxplot representation of the distribution of determinations for each type of Water – for raw, Filtered, treated (other) and UV treated water (p<0.001).

- **Enterococci (N/100mL)**

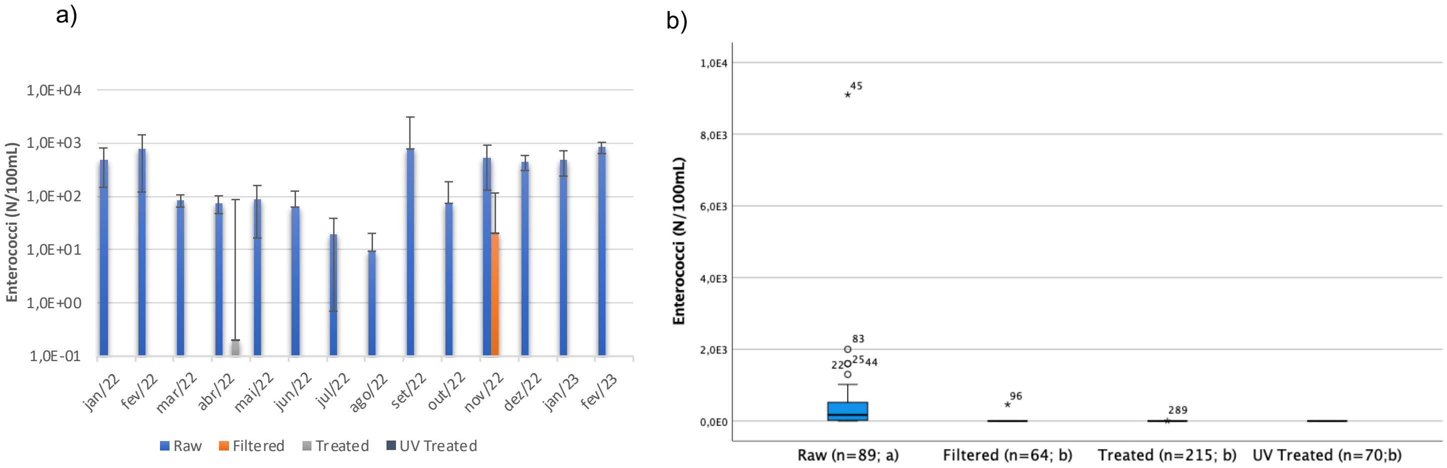


Figure 12. a) Abundance of enterococci (N/100mL) measured based on membrane filtration method for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 438 samples. b) Boxplot representation of the distribution of determinations for each type of water- for raw, filtered, treated (other) and UV treated water (p<0.001).

- ***Escherichia coli*** **(N/100mL)**

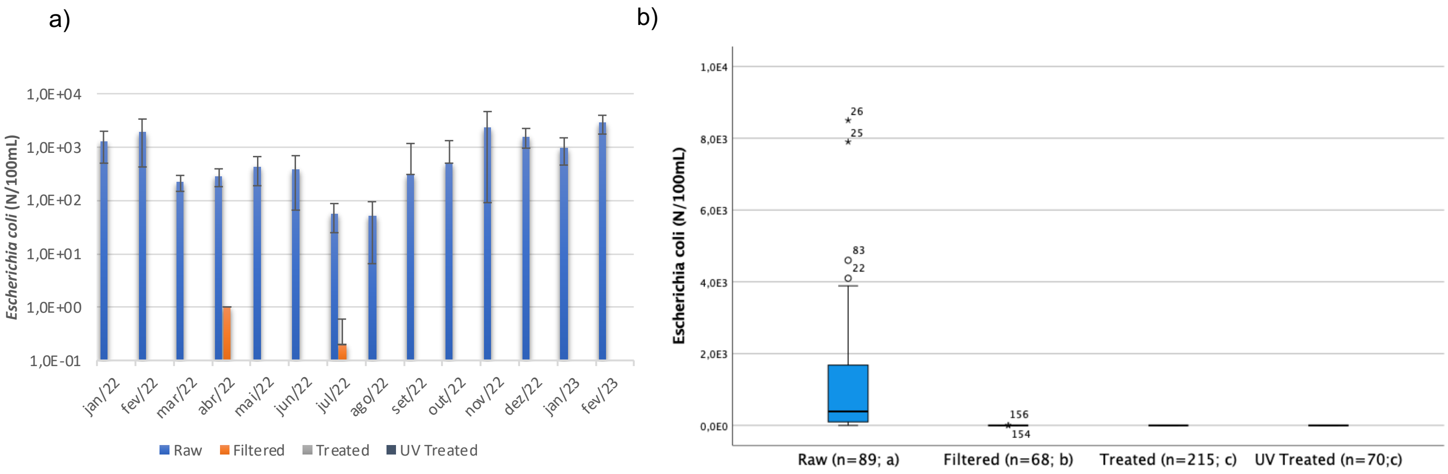


Figure 13. a) Abundance of *Escherichia coli* (N/100 mL) measured based on membrane filtration and multiwells method for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 442 samples. b) Boxplot representation of the distribution of determinations for each type of water – for raw, filtered, treated (other) and UV treated water ($p<0.001$).

- **Free residual chlorine (mg/L Cl_2)**

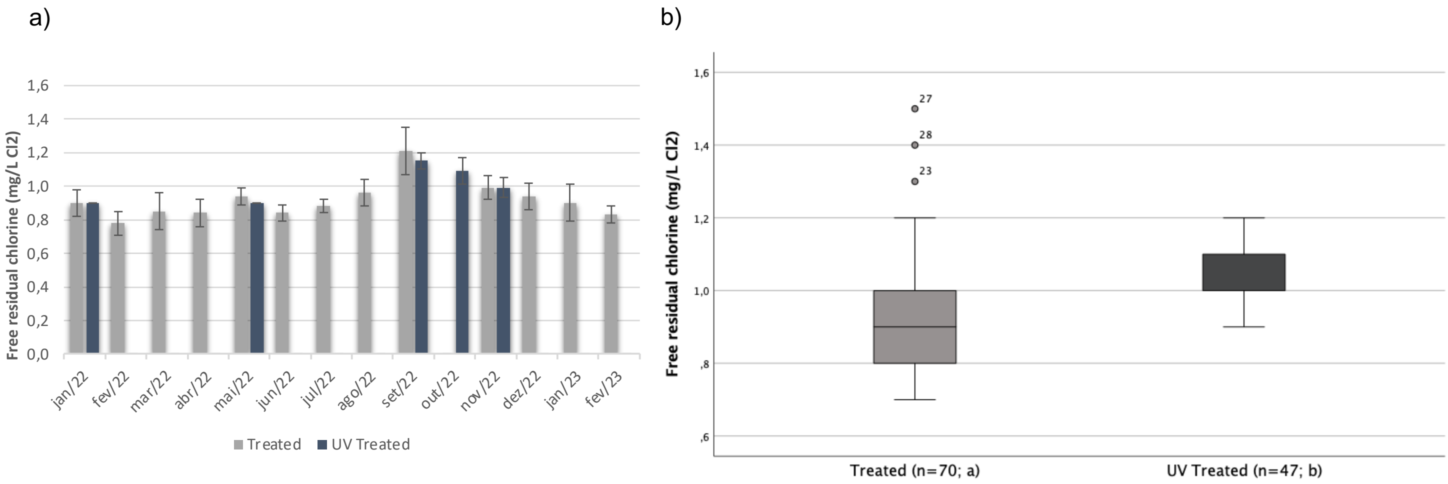


Figure 14. a) Free residual chlorine (mg/L Cl2) measured based on colorimetry method for treated (other) and UV treated water between the period of January/22 and February/23, in 117 samples. b) Boxplot representation of the distribution of determinations for each type of water – for Treated (other) and UV treated water ($p<0.001$).

- **Nitrates (mg/L NO_3)**

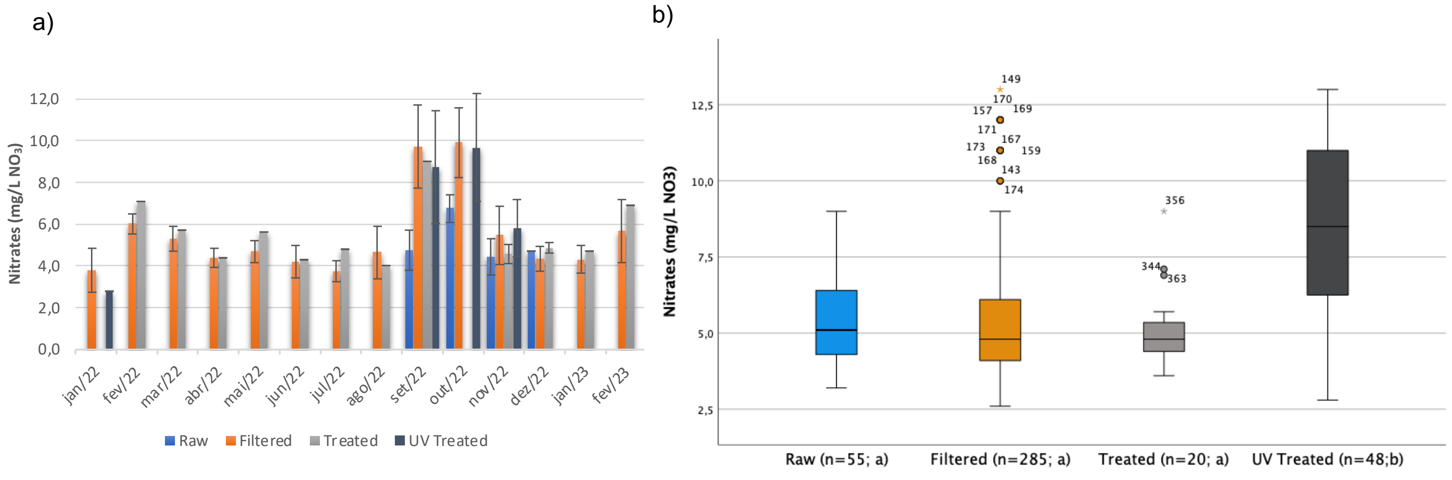


Figure 15. a) Concentration of nitrates (mg/L NO_3) measured based on ion chromatography for raw, filtered, treated (other) and UV treated water between the January/22 and February/23, in 408 samples. b) Boxplot representation of the distribution of determinations for each type of water – for raw, filtered, treated (other) and UV treated water ($p<0.001$).

- **Nitrites (mg/L NO_2)**

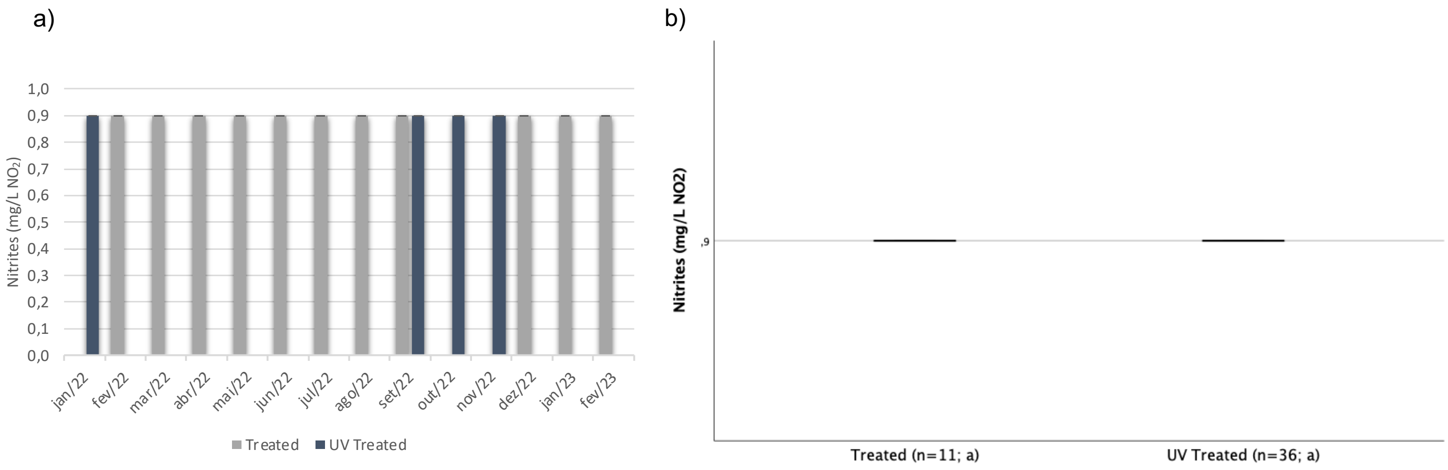


Figure 16. a) Concentration of nitrites (mg/L NO_2) measured based on ion chromatography for treated (other) and UV treated water between the period of January/22 and February/23, in 47 samples. b) Boxplot representation of the distribution of determinations for each type of water – for treated (other) and UV treated water ($p=1.000$).

- **Oxidability (mg/L O_2)**

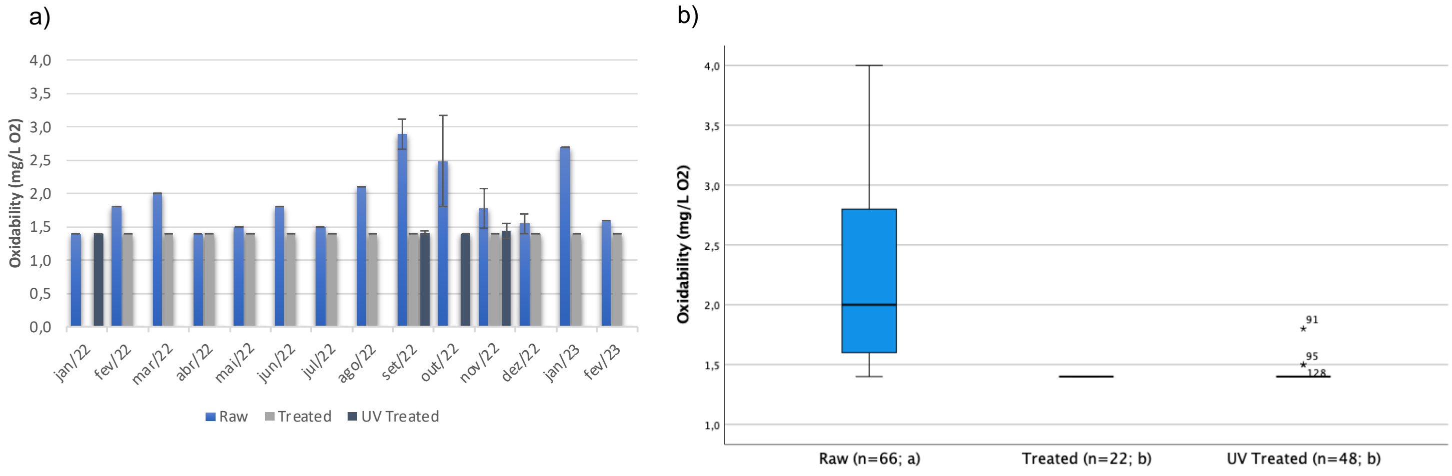


Figure 17. a) Oxidability (mg/L O_2) measured based on titration for raw, treated (other) and UV treated water between the period of January/22 and February/23, in 136 samples. b) Boxplot representation of the distribution of determinations for each type of Water - raw, treated (other) and UV treated water ($p<0.001$).

- **pH (Sorensen scale)**

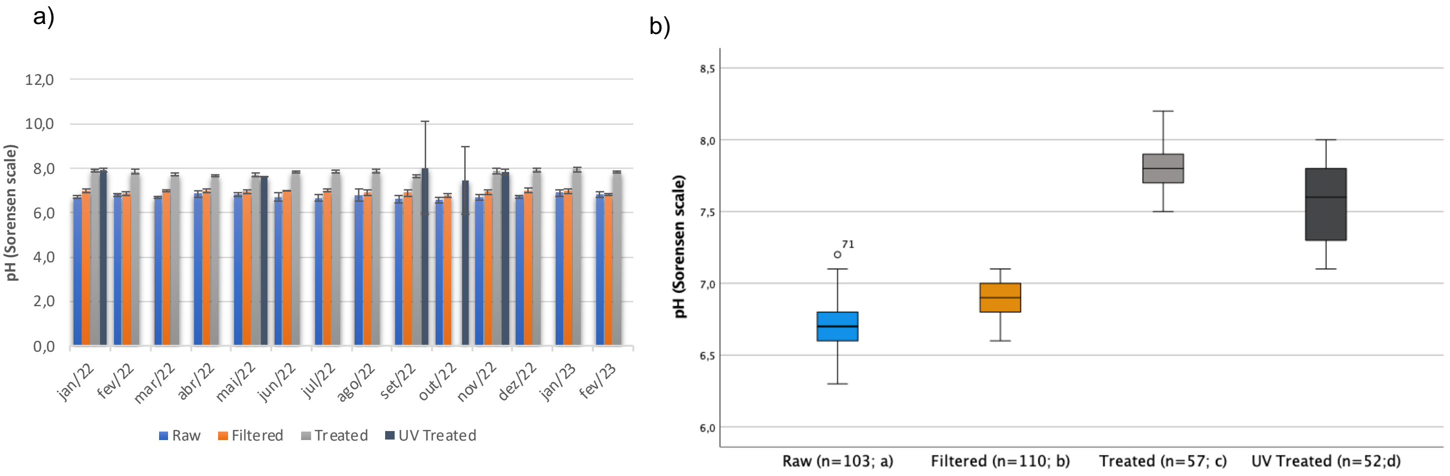


Figure 18. a) pH (Sorensen scale) measured based on potentiometry for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 322 samples. b) Boxplot representation of the distribution of determinations for each type of water – for raw, filtered, treated (other) and UV treated water ($p<0.001$).

- **Scent (Dilution factor)**

a) b)

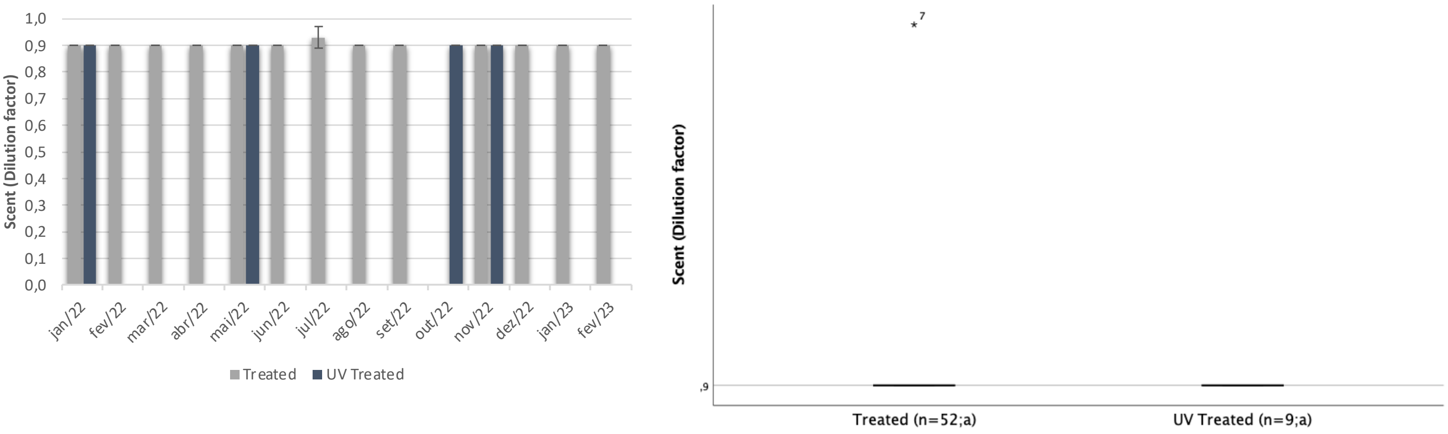


Figure 19. Scent (dilution factor) measured based on successive dilutions for treated (other) and UV treated water between the period of January/22 and February/23, in 61 samples. b) Boxplot representation of the distribution of determinations for each type of water – for treated (other) and UV treated water (p=0.677).

- **Taste (Dilution factor)**

a) b)

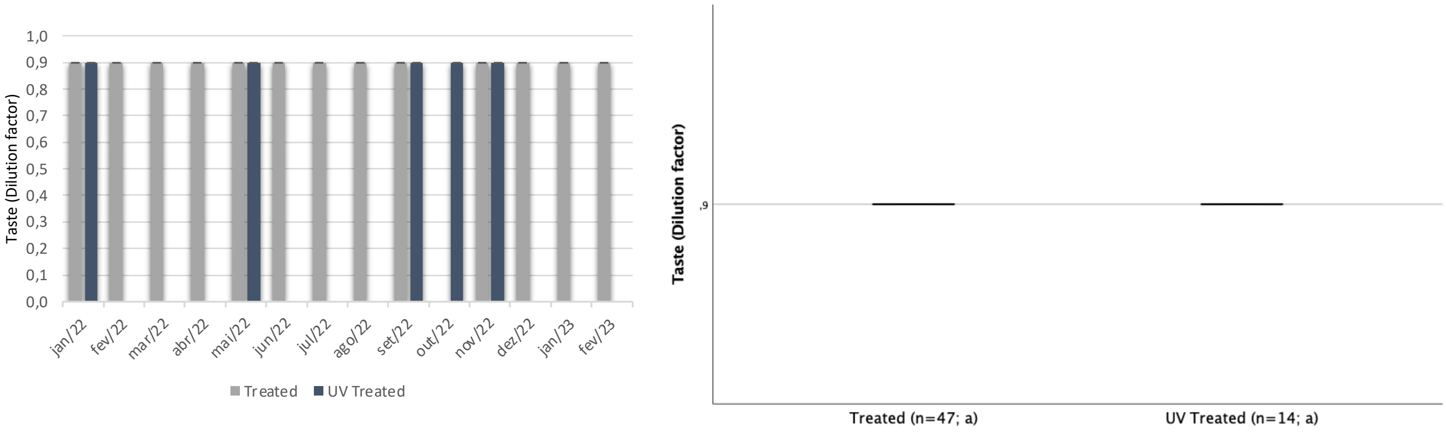


Figure 20. Taste (dilution factor) measured based on successive dilutions for treated (other) and UV treated water between the period of January/22 and February/23, in 61 samples. b) Boxplot representation of the distribution of determinations for each type of Water – for treated (other) and UV Treated water (p=1.000).

- **Temperature (˚C)**

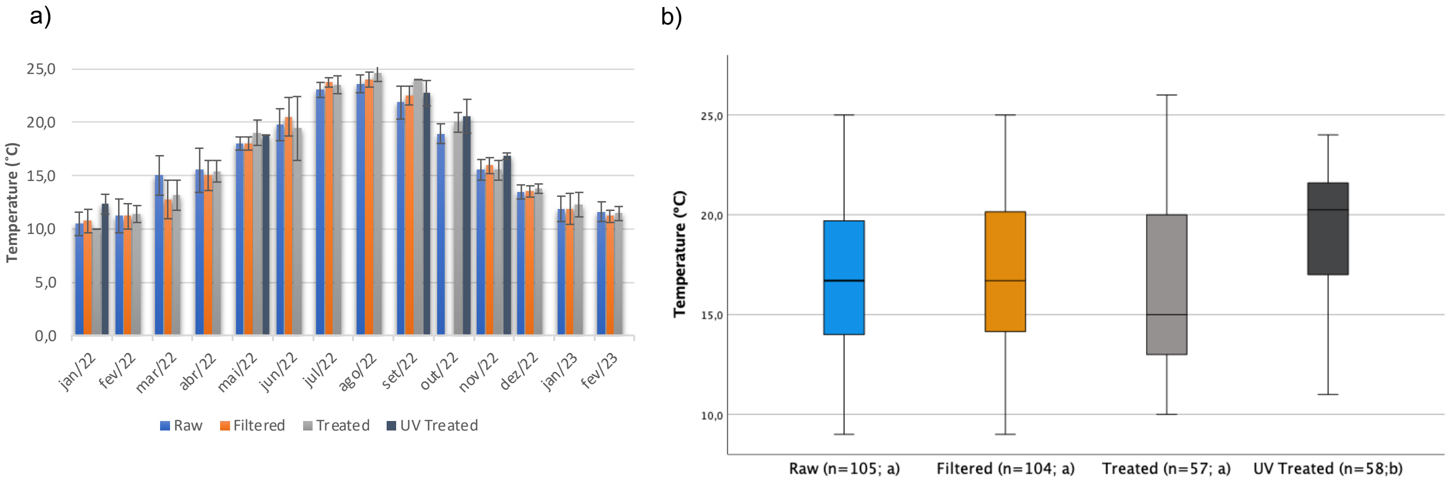


Figure 21. a) Temperature (˚C) measured based on thermometry for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 324 samples. b) Boxplot representation of the distribution of determinations for each type of water – for raw, filtered, treated (other) and UV treated water (p<0.001).

- **Total heterotrophs count at 22 ˚C (N/mL*)***

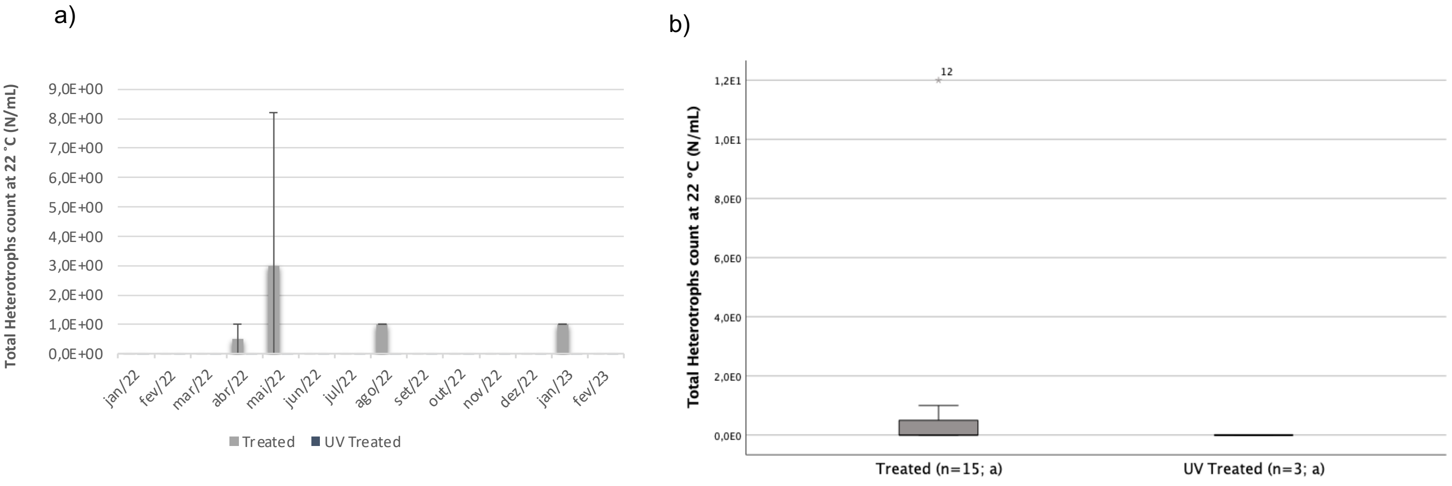


Figure 22. a) Abundance of total heterotrophs count at 22 ˚C (N/mL) measured based on embedding plate methods for treated (other) and UV treated water between the period of January/22 and February/23, in 18 samples. b) Boxplot representation of the distribution of determinations for each type of water – for treated (other) and UV treated water (p=0.327).

- **Total heterotrophs count at 36 ˚C (N/mL*)***

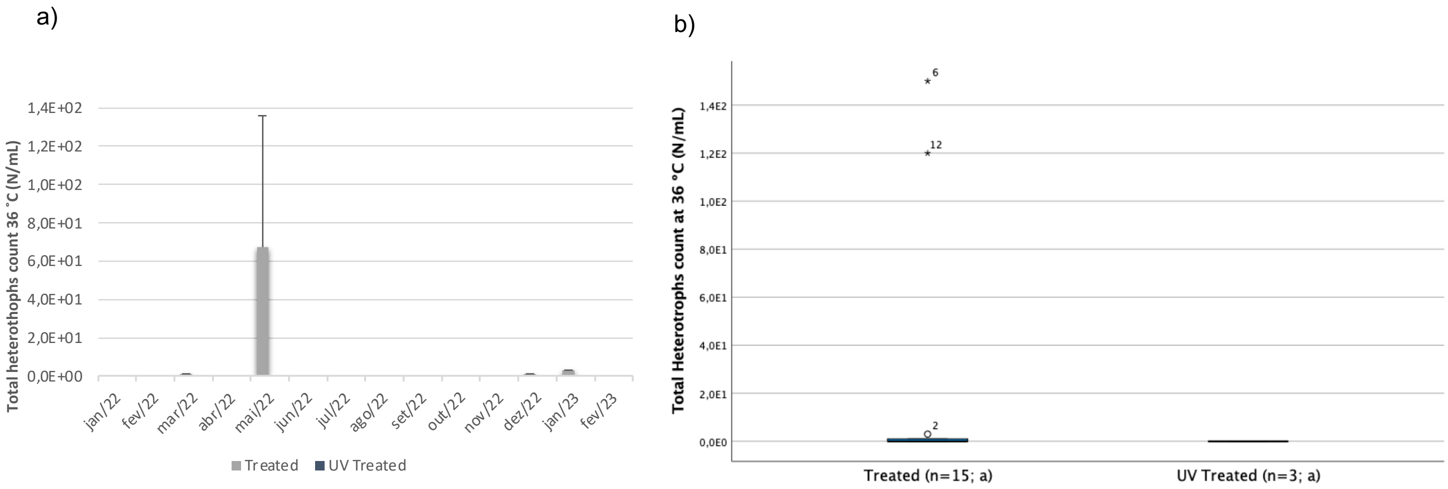


Figure 23. a) Abundance of total heterotrophs count at 36 ˚C (N/mL) measured based on embedding plate methods for treated (other) and UV treated water between the period of January/22 and February/23, in 18 samples. b) Boxplot representation of the distribution of determinations for each type of water – for treated (other) and UV treated water (p=0.260).

- **Total organic carbon (mg/L)**

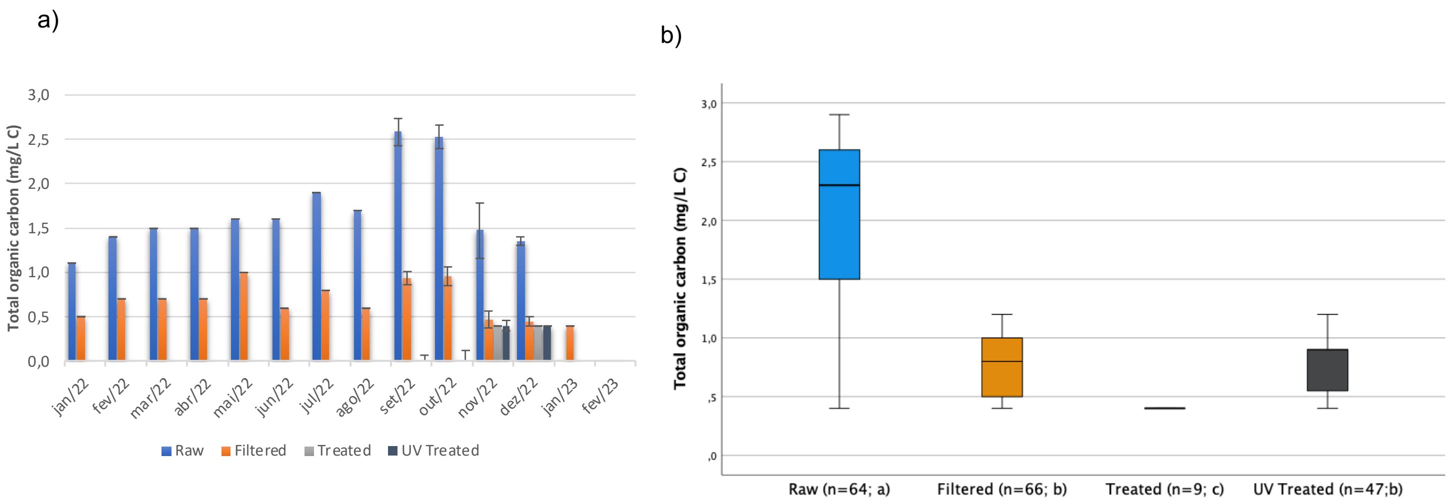


Figure 24. a) Total organic carbon (mg/L C) measured based on high temperature combustion for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 186 samples. b) Boxplot representation of the distribution of determinations for each type of water – for raw, filtered, treated (other) and UV treated water (p<0.001).

- **Trihalomethanes (µg/L)**

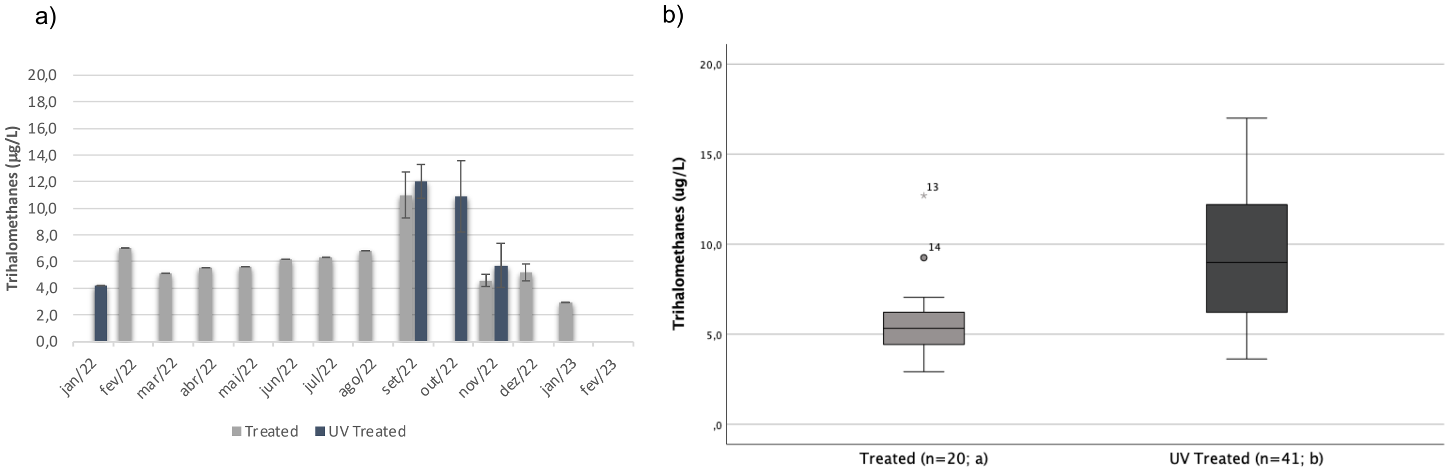


Figure 25. a) Concentration of Trihalomethanes (µg/L) measured based on subcontracted methods for treated (other) and UV treated water between the period of January/22 and February/23, in 61 samples. b) Boxplot representation of the distribution of determinations for each type of water – for treated (other) and UV treated water (p<0.001).

- **Turbidity (NTU)**

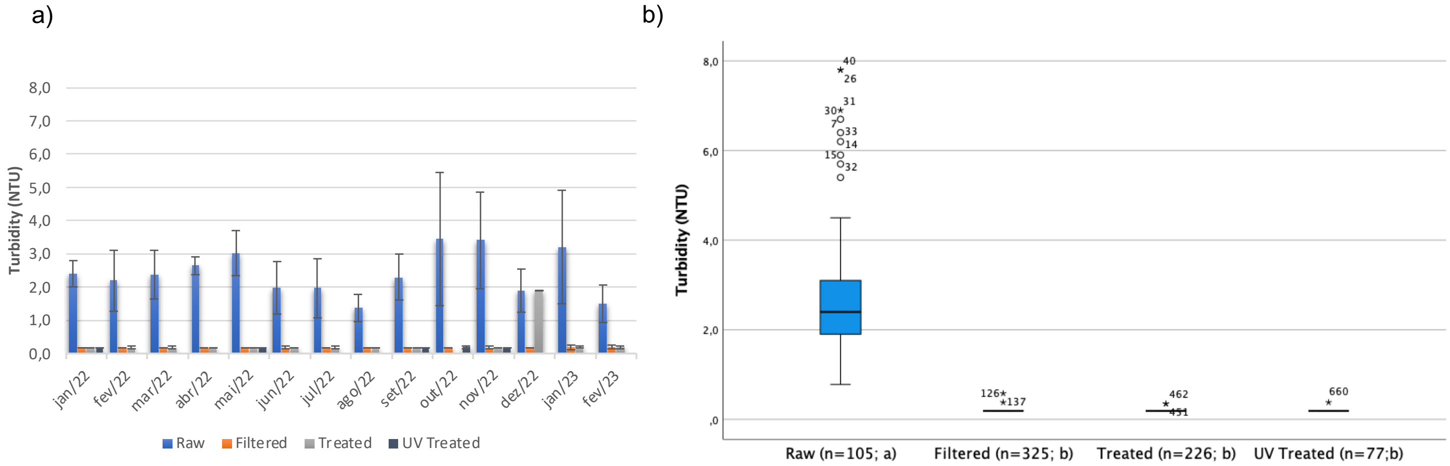


Figure 26. a) Turbidity (NTU) measured based on turbidimetry for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 733 samples. b) Boxplot representation of the distribution of determinations for each type of water – for raw, filtered, treated (other) and UV treated water (p<0.001).

- **UV at 254 nm**

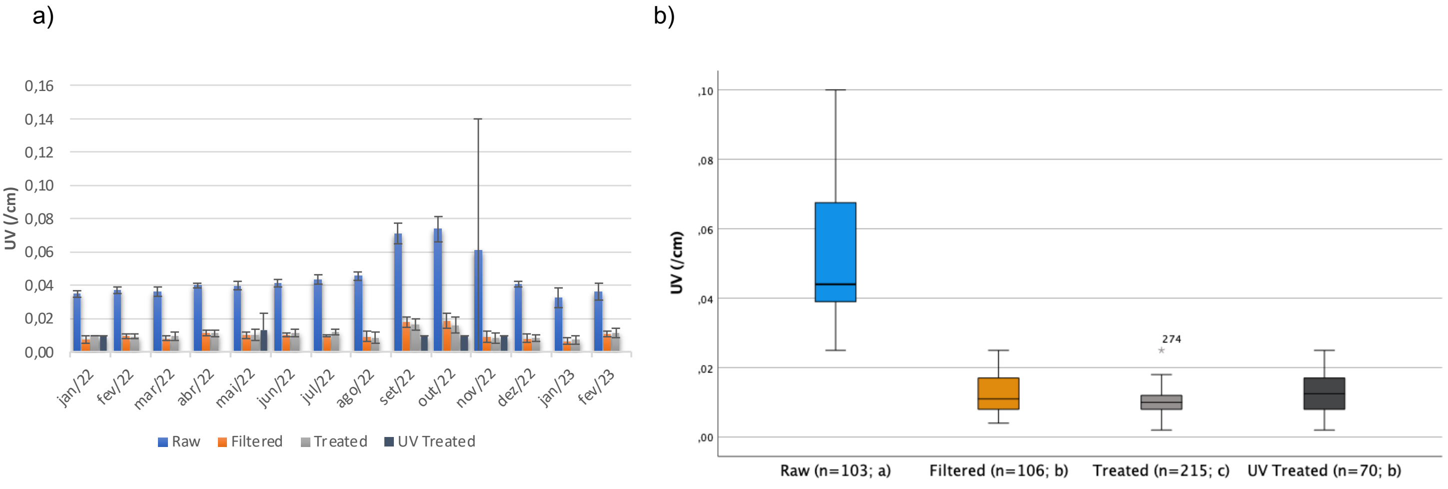


Figure 27. a) UV (/cm) measured based on molecular absorption spectrophotometry for raw, filtered, treated (other) and UV treated water between the period of January/22 and February/23, in 494 samples. b) Boxplot representation of the distribution of determinations for each type of water – for raw, filtered, treated (other) and UV treated water ($p<0.001$).

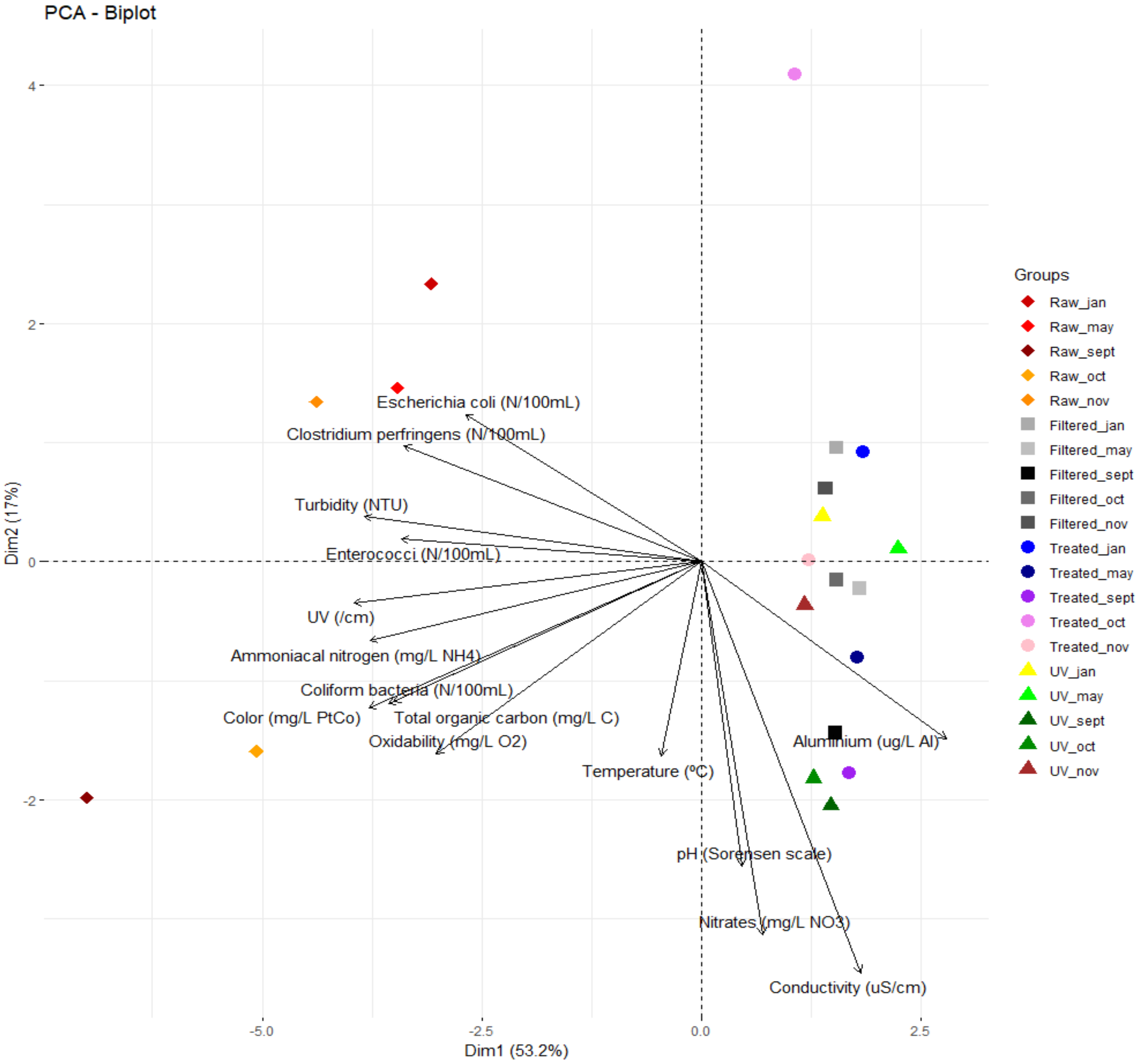


Figure 28. Principal component analysis with the distribution of the samples, for the months for which were sampled the four points.

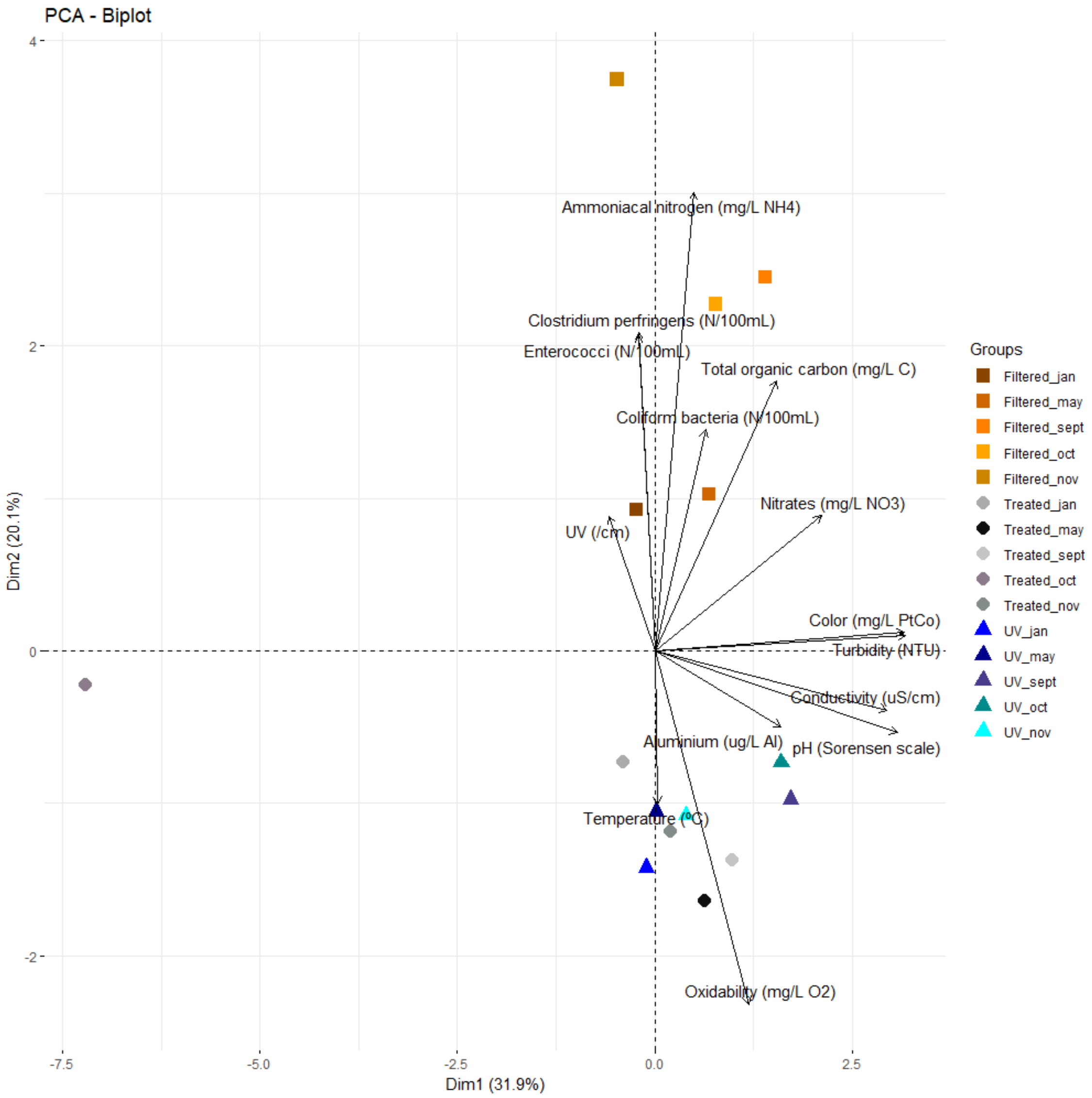


Figure 29. Principal component analysis with the distribution of the filtered and treated (other) and UV treated water samples.

According to the data, the raw water can be distinguished from the chlorine treated and UV treated water, being positively correlated with several parameters (e.g., bacterial counts, turbidity, total organic carbon) that are affected (usually decreased) with the treatment (Fig. 28), underscoring the efficiency of these methods in enhancing water quality.

The filtered water, the water treated with chlorine and the water treated with UV radiation showed significantly lower values for ammoniacal nitrogen, *Clostridium perfringens,* coliform bacteria, color, enterococci, *Escherichia coli*, oxidability, total organic carbon, turbidity and UV at 254 nm, and higher values of conductivity and pH (Figs. 6; 8; 9; 11; 12; 13; 17; 18; 24; 26; 27, Appendix 3). After filtration, the additional steps of treatment (chlorination and pH adjustment with lime water) promoted a significant decrease in parameters such as *Clostridium perfringens*, coliform bacteria and *Escherichia coli* to non-detection levels ensuring the microbial quality of the water. However, the biggest removal was obtained with the filtration process, that is described as extremely effective in removing contaminants and microbes from water (Cescon *et al.,* 2020). The chlorination and UV treatment further contribute to microbial inactivation and pollutant removal (Cescon *et al.,* 2020). Conductivity and pH were significantly higher after the treatment (in chlorine treated and UV treated water samples). This rise could be attributed to various factors related to the treatment methods employed, such as the removal of acidic substances during conventional treatment, possible elimination of certain organic and inorganic compounds or higher temperature during UV treatment. The pH adjustment by addition of calcium hydroxide, a common practice in the water treatment plant, may also justify the pH elevation (O'Donnel, 2022). Differences between the chlorine treated water and the UV treated water were not found in parameters such as color, enterococci and turbidity (Appendix 1). This result implies that both chlorine and UV treatments can adequately manage color related constituents, possible by targeting organic compounds responsible for coloration. Furthermore, the lack of distinction in enterococci and turbidity reduction aligns with the broader understanding that both treatments are established disinfection methods, targeting microbial pathogens and suspended particles. It suggests that either treatment can be adopted with confidence to ensure microbial safety and water clarity (Popescu *et al.* 2017). Regarding temperature, differences were found between both treatments (higher values during UV treatment). The heat of the season might have affected the temperature levels since UV treatment was mostly applied during summer. In terms of nitrates, it was also expected a decrease, which has not happened maybe due to the filters used and the composition of raw water, demonstrating the importance of considering specific filter characteristics (Archdall, 2022).

The treatment with UV was applied in some periods (01 to 21 of January/22, 23 to 29 of May/22 and 12 of September until 18 of November/22) as an extra step in the treatment. To try to understand the effect of this extra treatment the samples treated with chlorine and UV treated were compared. The parameters calcium*, Clostridium perfringens*, coliform bacteria, color, enterococci, *Escherichia coli,* nitrites, oxidability, scent, taste, turbidity and total heterotrophs count at 22 ˚C and 36 ˚C were the parameters less affected showing no significant differences between the two groups (Appendix 1). The lack of significant differences in these parameters implies that both chlorine treatment and the combination of UV and chlorine were comparably effective in managing these aspects of water quality. Although, a study from Shekhawat *et al.,* (2021) implies that the highest elimination of bacterial species is achieved in chlorine/UV treatment. Several parameters, including conductivity, free residual chlorine, nitrates, pH, temperature, total organic carbon, trihalomethanes and UV were the main ones that seem to differ within the chlorine treated water and water treated with UV radiation. In particular, pH levels exhibited noticeable increases following the UV treatment (Fig. 18). This variations likely stem from the diverse mechanisms of the treatments. The differences observed in free residual chlorine levels can be attributed to the additional chlorine dose introduced during UV treatment to counteract the potential higher decay. The principal component analysis revealed a clear separation between the raw and treated water samples, primarily attributed to the removal of microorganisms and turbidity during the treatment process – key indicators of treatment efficacy

(Fig. 28). The PCA's ability to visually differentiate between raw and treated water reinforces the substantial alterations introduced by the treatment methods. There is also a significant differentiation between the filtered and treated water samples, with the majority of the treated samples grouping closely (Fig. 29). This clustering pattern implies that the combination of UV treatment and chlorine treatment does not introduce significant differences compared to chlorine treatment alone. Since both treatments showed similar results, some authors defend that UV disinfection should be adopted as an alternative over chemical disinfection, as it is a safer and a long-term cost-effective method (Pereira *et al.* 2023).

3.2.2. Daily variation analysis

The daily variation analysis conducted on the water treatment process from September to November 2022 revealed interesting results on the removal (filtered water - water treated with UV) of some parameters.

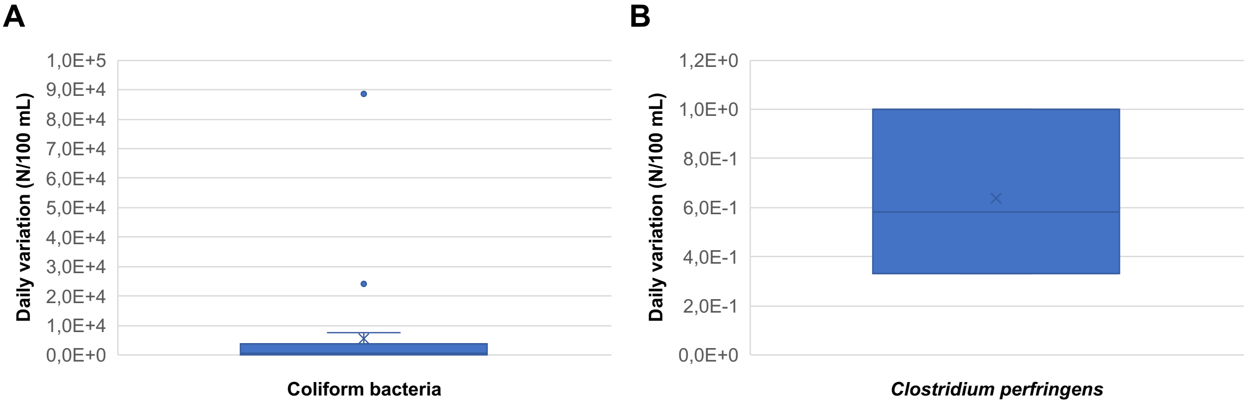


Figure 30. Boxplot representation of **A)** the Coliform bacteria (N/100 mL) and **B)** the *Clostridium perfringens* (N/100 mL) daily variation (difference between filtered and UV treated water), for the period of September/22 to November/22. **A)** Data collected for 30 days, 29 of which were below the limit of detection – showing a positive removal after UV treatment. **B)** Data collected for 29 days, 6 of which were below the limit of detection – showing a positive removal after UV treatment.

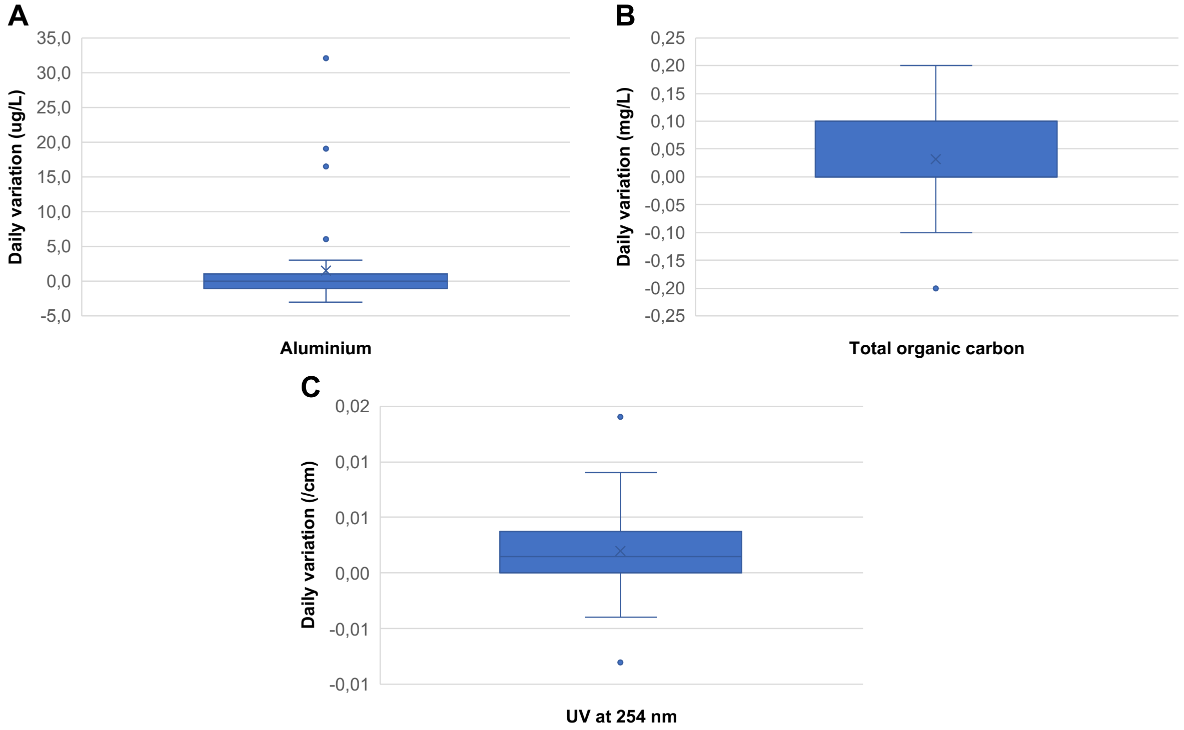


Figure 31. Boxplot representation of **A)** the aluminium (μg/L), **B)** total organic carbon (mg/L) and **C)** UV at 254 nm (/cm) daily variation (difference between filtered and UV treated water), for the period of September/22 to November/22. **A)** Based on 44 determinations, corresponding to 44 days, 17 of which showed no removal and 27 a removal after UV treatment. **B)** Based on 44 determinations, corresponding to 44 days, 29 of which showed no removal and 15 a removal after UV treatment. **C)** Based on 44 determinations, corresponding to 44 days, 16 of which showed a negative variation and 28 a positive variation after UV treatment.

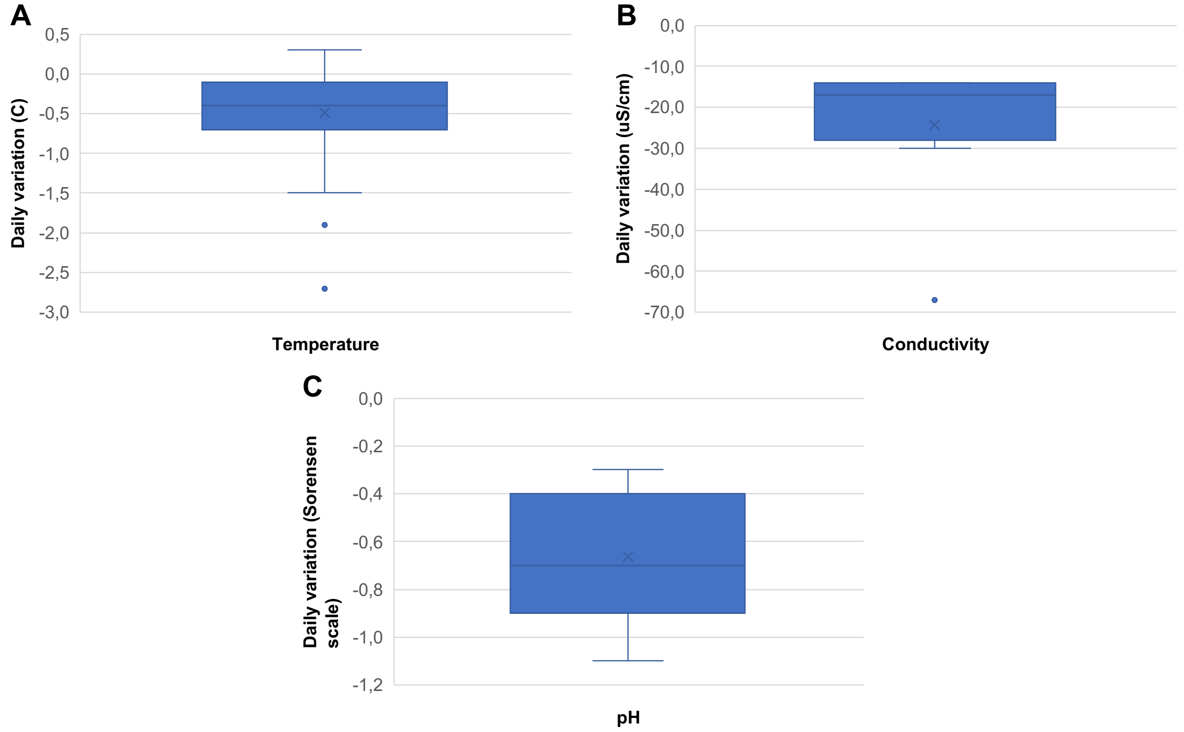


Figure 32. Boxplot representation of **A)** Temperature (˚C), **B)** Conductivity (μS/cm) and **C)** pH (Sorensen scale) daily variation (difference between filtered and UV treated water), for the period of September/22 to November/22. **A)** Data collected for 38 days, with 31 days showing an increase of the temperature after UV treatment. **B)** Data collected for 8 days, with all showing an increase after the UV treatment. **C)** Data collected for 44 days, with 44 showing an increase of the pH after UV treatment.

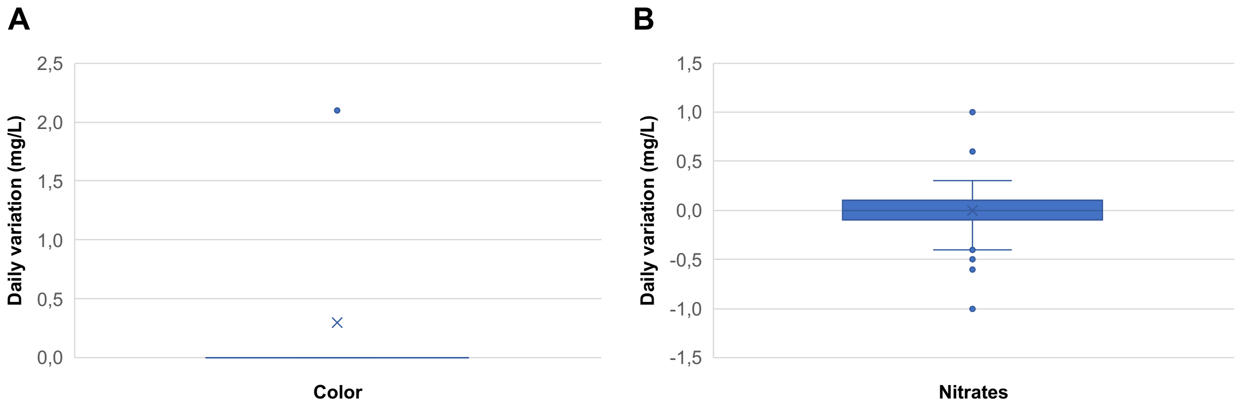
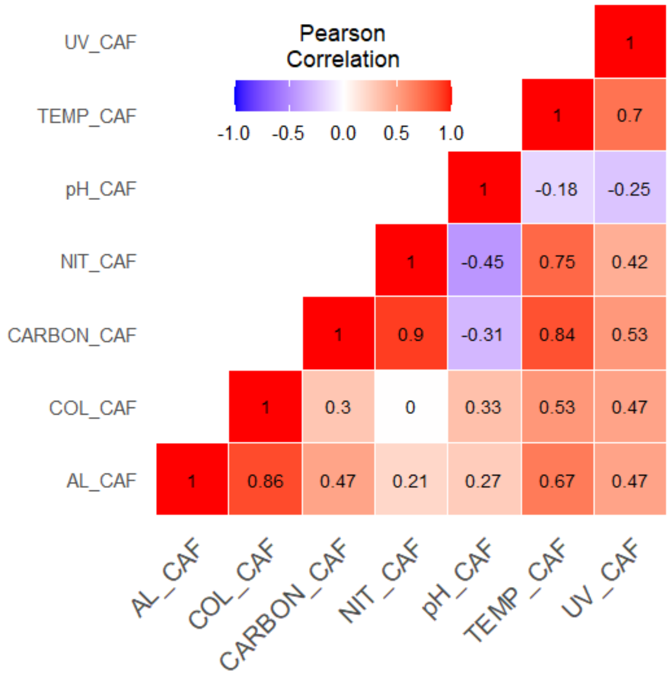


Figure 33. Boxplot representation of the **A)** color (mg/L) and **B)** nitrates (mg/L) daily variation (difference between filtered and UV treated water), for the period of September/22 to November/22. **A)** Data collected for 7 days, with 1 day showing a decrease after UV treatment. **B)** Based on 45 determinations, corresponding to 45 days, 13 of which showed no removal and 32 a removal after UV treatment.

3.2.3. Pearson Correlation

a)



	AL_CAF	COL_CAF	CARBON_CAF	NIT_CAF	pH_CAF	TEMP_CAF	UV_CAF
UV_CAF							1
TEMP_CAF						1	0.7
pH_CAF					1	-0.18	-0.25
NIT_CAF				1	-0.45	0.75	0.42
CARBON_CAF			1	0.9	-0.31	0.84	0.53
COL_CAF		1	0.3	0	0.33	0.53	0.47
AL_CAF	1	0.86	0.47	0.21	0.27	0.67	0.47

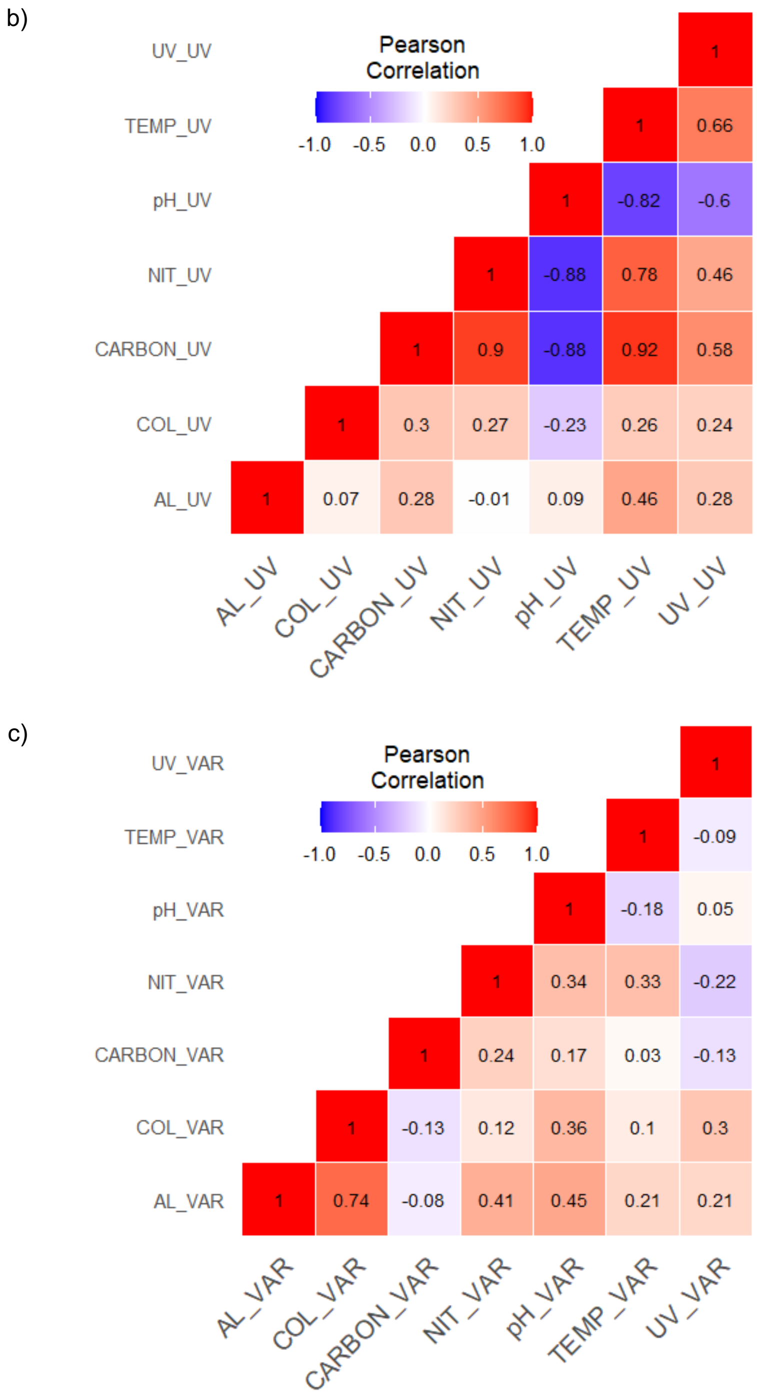


Figure 34. Pearson correlation for the daily data collected in the period of UV treatment (September/22 to November/22) for seven parameters (aluminium, Clostridium perfringens, coliform bacteria, nitrates, pH, temperature and UV at 254 nm) for the **(a)** filtered water (CAF), **(b)** UV treated water, and **(c)** the daily variations calculated by the difference between filtered and UV treated water.

The daily variation analysis conducted on the water treatment process from September to November 2022 revealed consistent results on the removal (filtered water - water treated with UV) for microbiological parameters. Specifically, UV system demonstrated effective reduction of coliform bacteria and *Clostridium perfringens* counts, with a higher impact in coliform bacteria that were frequently more abundant in the water after filtration. *Clostridium perfringens* were only sporadically detected (six times in the period of three months) in filtered water. In both cases, the UV treatment was almost always able to reduce to non-detection values (Fig. 30). For parameters such as aluminium, total organic carbon and UV at 254 nm, the impact of UV treatment was variable but predominantly positive, leading to a reduction – showing the potential of UV in mitigating the presence of organic and inorganic compounds (Fennel *et al.,* 2022) (Fig. 31). Regarding temperature, after the UV treatment it was frequently observed an increase. The same was observed for conductivity and pH (Fig. 32). On the other hand, UV treatment did not affect parameters such as color, or the nitrates concentration (Fig. 33). By a correlation analysis, we observed that in filtered water the concentration of aluminium and of coliforms is positively correlated, as well as a positive correlation between higher temperatures and a higher concentration of nitrates, total organic carbon or UV at 254nm (Fig. 34 (a)). After the treatment this correlation with the temperature persist, but it is possible to observe some negative correlations between the pH, and the temperature, total organic carbon and nitrates concentration (Fig. 34 (b)). In terms of removal, it was only observed a positive correlation between the removal of coliforms and of aluminium (Fig. 34 (c)). These correlations may be limited by the low number of coincident data available for the period of analysis. These findings collectively emphasize the nuanced nature of UV water treatment's impacts. While its prowess in microbial reduction is evident, its influence on other parameters is modulated by multifaceted interactions, including temperature-related dynamics (the correlation analysis proves that temperature influences various factors). This analysis emphasizes UV treatment's effectiveness in addressing water quality issues and the need for a comprehensive evaluation to understand its broader impact on water quality dynamics.

3.2.4. Antibiotic resistance biomarkers

After this data analysis, we decided to collect some samples before and after the UV treatment to evaluate the effect that UV may have in terms of abundance of antibiotic resistance biomarkers.

Table 2. Quantification of the 16S rRNA gene and 10 other genes classified as antibiotic resistance biomarkers in filtered (NT) and UV treated (UV) water samples and in the same samples after 3 days of regrowth (R), for three independent samples collected in three consecutive weeks. Results are expressed on log-units (gene copy number/mL of sample).

SAMPLE/gene	**16S rRNA**	***intI1***	***uidA***	***ermB***	***aph(3")-ib***	**crAssphage**	***ermF***	***qacEdelta1***	***sul1***	***tetX***	***mefC***
LOQ	**1.57**	**0.19**	**0.15**	**0.69**	**1.69**	**0.49**	**0.49**	**0.69**	**0.31**	**0.69**	**0.64**
NT.1.	4.82±0.05	1.52±0.09	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.39±0.29	<LOQ*	<LOQ	<LOQ
NT.1.R.	5.33±0.10	2.33±0.66	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	3.45±0.16	<LOD	<LOQ	<LOD
UV.1.	4.51±0.10	1.56±0.05	<LOQ*	<LOQ*	<LOQ	<LOQ	<LOQ*	<LOQ*	<LOD	<LOQ	<LOQ
UV.1.R.	4.53±0.01	1.14±0.15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.23±0.06	<LOD	<LOQ	<LOD
NT.2.	4.87±0.05	2.06±0.08	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.98±0.44	<LOQ*	<LOQ	<LOQ*
NT.2.R.	5.52±0.01	2.49±0.30	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	3.44±0.02	<LOD	<LOQ	<LOD
UV.2.	4.50±0.22	2.35±0.17	<LOQ*	1.48±0.74	<LOQ	<LOQ*	2.36±0.71	<LOQ*	2.71±0.08	<LOQ*	<LOQ*
UV.2.R.	5.17±0.12	0.79±0.17	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.94±0.06	<LOD	<LOQ	<LOD
NT.3.	4.43±0.24	1.02±0.28	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	1.38±0.11	<LOD	<LOQ	<LOQ
NT.3.R.	5.73±0.35	3.82±0.24	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	4.47±0.31	<LOD*	<LOQ	<LOD
UV.3.	3.81±0.25	0.68±0.34	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ*	<LOD	<LOQ	<LOD
UV.3.R.	5.56±0.38	0.85±0.04	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	1.19±0.26	<LOD	<LOQ	<LOD

*absence of reproducibility among replicas.

In terms of total bacterial load, measured by the quantification of the 16S rRNA gene, the filtered water varies between 4.43 to 4.87 log units/mL sample and the UV treated water significantly decreases ($p<0.001$) these values to 3.81 - 4.51 log units/mL (Table 2, Fig. 35; Fig. 36A). After regrowth the bacterial load significantly and proportionally increased for the filtered and UV treated samples (Fig 36A). The decrease in 16S rRNA gene levels post UV treatment reflects successful disinfection, aligning with the treatment's intended purpose. The variations observed during regrowth underline the complex response of microorganisms to treatment and subsequent regrowth conditions.

Regarding the antibiotic resistance biomarkers, only *intI1* was detected in all the samples (Table 2, Fig. 35). The *intI1* biomarker was detected in all tested samples, with the filtered water ranging from 1.02 to 2.06 log units/mL of sample and the UV treated water ranging from 0.68 to 2.35 log units/mL. The *qacEΔ1* was quantifiable for all tested samples, except for UV treated samples. For this biomarker, the filtered water ranged from 1.38 to 1.98 log units/mL sample (Table 2, Fig. 35). The UV treatment had no significant impact on the quantification of the *intI1* biomarker but did affect the *qacEΔ1* gene levels, positively (Fig 36B and C). Regrowth was observed in the filtered water samples, with a significant increase for both biomarkers. It is not surprising that the two genes are detected in close concentrations since they can co-occur in class 1 integrons (Gillings *et al.* 2014). The presence of these biomarkers across most of the samples suggests that these genetic elements were probably less affected by the treatment and also the potential occurrence of horizontal gene transfer mechanisms, which facilitate the spread of genetic material between microorganisms contributing to the evolution and adaptation of microbial communities (Piergiacomo *et al.* 2020). Also, the quantification of the *qacEΔ1* gene, associated with resistance to disinfectants (Shafaati et al. 2016), implies that some microorganisms could be harboring this gene, impacting treatment effectiveness. The observed regrowth indicates that certain microorganisms have the ability to rebound. However, the observation of a higher regrowth for the filtered water samples, in comparison to the UV treated, shows that the UV treatment may contribute to reduce the bacterial regrowth after treatment.

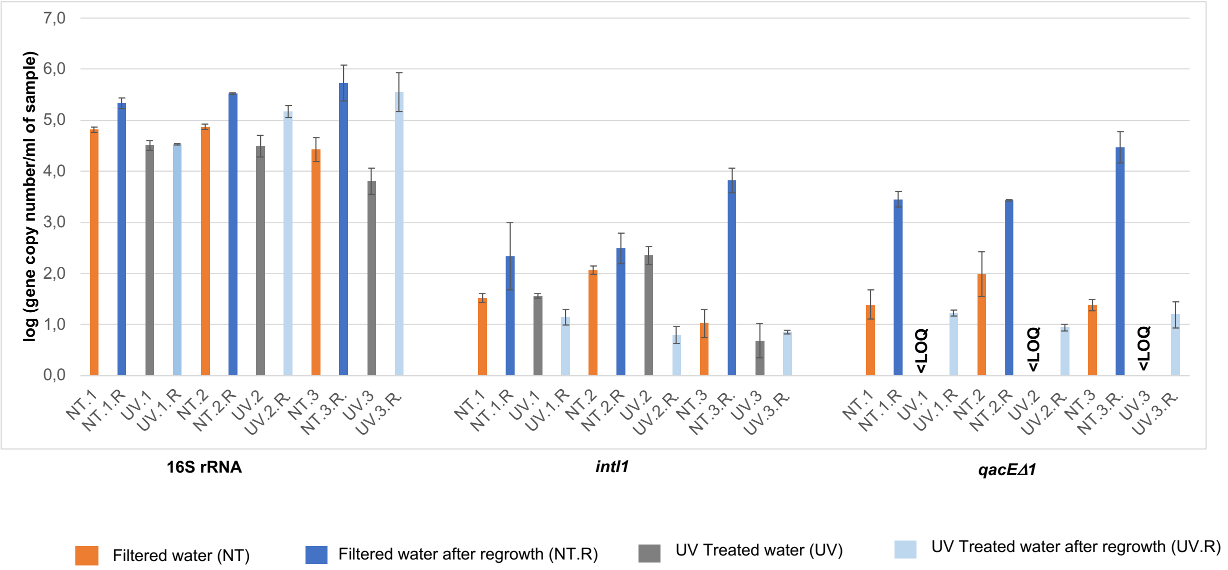
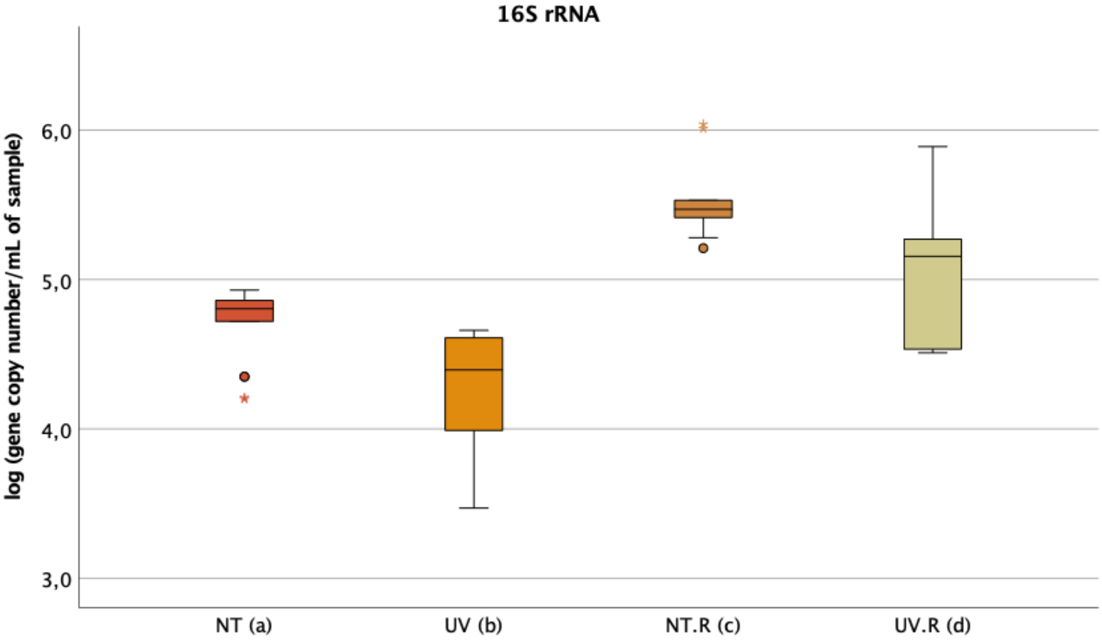
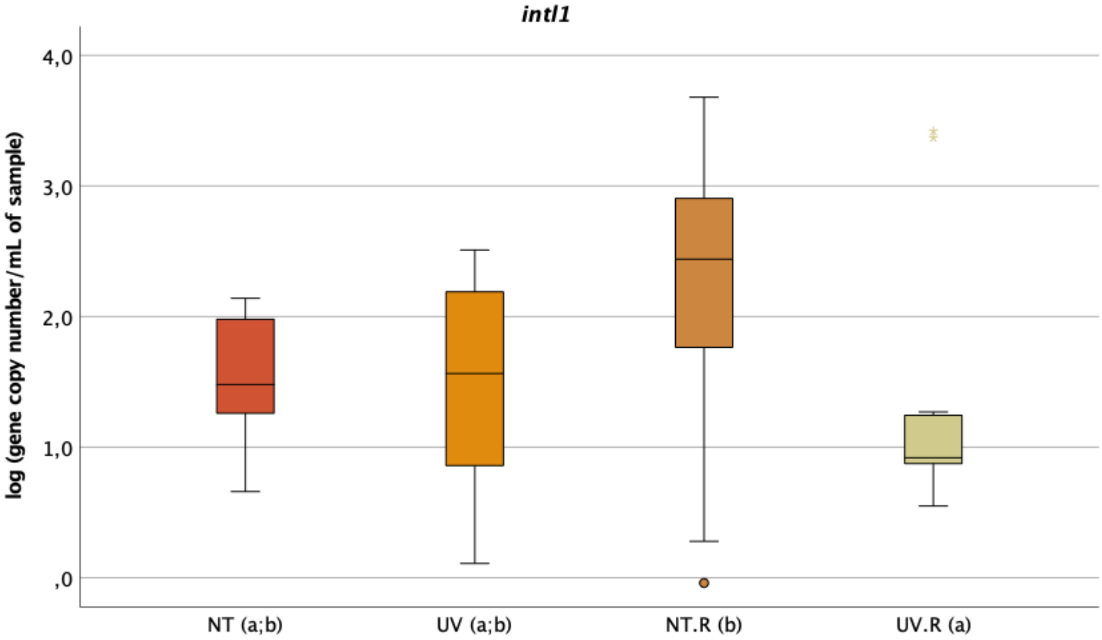


Figure 35. Quantification of the 16S rRNA gene, *intI1* and *qacEΔ1* biomarkers quantified in filtered (NT) and UV treated (UV) water samples and in the same samples after 3 days of regrowth (R). Results are expressed on log-units (gene copy number/mL of sample).

A


16S rRNA
log (gene copy number/mL of sample)
6,0
5,0
4,0
3,0
NT (a)
UV (b)
NT.R (c)
UV.R (d)

B


intI1
log (gene copy number/mL of sample)
4,0
3,0
2,0
1,0
,0
NT (a;b)
UV (a;b)
NT.R (b)
UV.R (a)

C

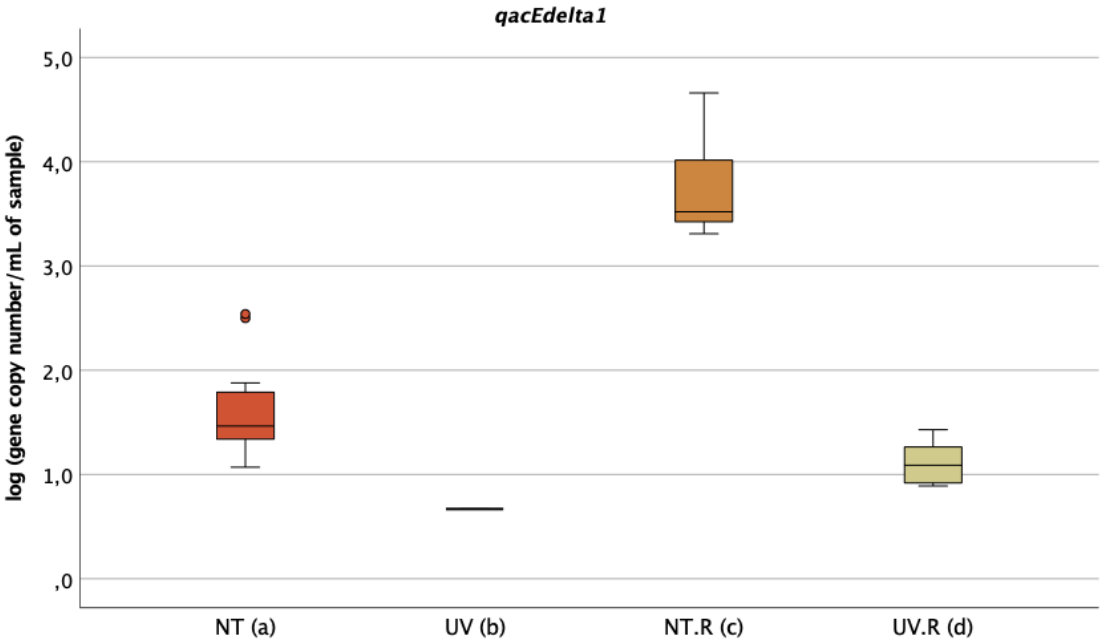


Figure 36. Boxplot representation of the quantification of the **A)** 16S rRNA, **B)** *intI1* and **C)** *qacEΔ1* biomarker, for filtered (NT) and UV treated (UV) water samples and in the same samples after 3 days of regrowth (R) ($p<0.001$). Results are expressed on log-units (gene copy number/mL of sample).

The other antibiotic resistance biomarkers were just sporadically quantified (Table 2). For the *sul1* biomarker, most of the samples had undetectable levels or were below the limit of quantification, except for UV.2 sample ranging 2.71 log units/mL (Table 2). The quantification results for *ermB* and *ermF* genes were similar – levels below the quantification limit, except for UV.2 sample showing detectable levels. The *aph(3'')-ib*, *uidA* and *tetX* biomarkers were below the quantification limit in all tested samples (Table 2). For the crAssphage and *mefC* biomarkers all samples were below quantification and detection limits (Table 2). The identification of these genes, often linked to antibiotic resistance, fecal contamination, or horizontal gene transfer, is not customary in drinking water and could indicate possible contamination from human or animal sources, thereby posing health concerns. Despite occasional gene quantifications, their generally limited presence corresponds with the projected low levels in treated drinking water since drinking water treatment processes are unable to completely remove antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Destiani & Templeton, 2019).

Overall, while these biomarkers are unexpected in drinking water, their monitoring is important to optimize the treatment processes and mitigate risks associated with gene transfer and microbial regrowth.

3.3. Conclusions

The drinking water treatment (pre-oxidation with ozone, remineralization, coagulation/flocculation and filtration) are responsible for the significative reduction of most of the parameters analyzed. It effectively reduced *Clostridium perfringens*, coliform bacteria and *Escherichia coli* to non-detection levels, resulting in a decrease of 2-3 logs, 4 logs and 3 logs, respectively.

The UV treatment was applied in samples with levels of conductivity, nitrates and trihalomethanes significantly higher than those observed in other dates, however, the treatment did not alter those parameters significantly as it could be suggested.

After UV treatment was also observed an increase in free residual chlorine, due to an increased chlorine dose during UV treatment, in anticipation of higher chlorine decay. It should be noted that this observation was based on a group of 47 samples.

The temperature was higher during UV treatment, it can be attributed to the general increase in temperature during the time of September, associated with the heat of the season.

The impact of UV treatment in these specific water quality parameters remains uncertain. However, it indicates that UV treatment is equally effective as chlorine treatment. Further studies are required to fully evaluate the effectiveness of this treatment, given the relatively short validation period of the data available.

The daily analysis conducted from September to November 2022 demonstrated effective reduction of microbiological parameters, which suggests that the UV system implemented in the water treatment process was successful in reducing these harmful microorganisms. It also had a mostly positive effect on parameters such as aluminium, total organic carbon and UV at 254 nm, which indicates a beneficial role in mitigating the presence of these substances in the treated water. However, UV treatment did not have a significant impact on parameters such as color, conductivity, nitrates, and pH.

In summary, the analysis of various biomarkers provided insights into the microbial composition and antibiotic resistance profiles of the tested samples. The 16S rRNA gene was quantifiable, indicating the presence of bacteria and its bacterial load – showing a significant reduction after UV treatment. The presence of *intI1 and qacEΔ1* biomarkers suggest potential prevalence of class 1 integrons in the water, that are mobile genetic elements that facilitate the acquisition and dissemination of antibiotic resistance genes.

These findings enhance a better comprehension of the microbial composition and the intricate dynamics of antibiotic resistance within the tested samples.

4. Chapter 2. Assays of *Klebsiella pneumoniae* survival with UV radiation

Klebsiella pneumoniae is a common Gram-negative bacterium widely distributed in various environmental niches and is commonly associated with hospital-acquired infections (Le *et al.,* 2021). This study focused on evaluating the survival in tap water of *Klebsiella pneumoniae* strains isolated from clinical (KP2-448) and environmental (SM1) sources in tap water samples. These assays aimed to investigate the bacteria's resistance to UV-C radiation and evaluate its survival and regrowth capabilities under controlled exposure conditions. By quantifying the surviving of the bacterial colonies on selective media after UV exposure, it was possible to determine the reduction in colony-forming units (CFUs) and assess the strains susceptibility to UV-induced damage.

In addition to the culture-based survival assays, this study also employed culture-independent methods, specifically quantitative polymerase chain reaction (qPCR), to further evaluate the presence and abundance of *Klebsiella pneumoniae* in water samples. Specific genetic markers were targeted, including the 16S rRNA gene for bacterial quantification, the *phoE* gene for *Klebsiella* spp. quantification, and the *bla*$_{\text{KPC}}$ (harboured by the two strains) and *bla*$_{\text{OXA-1}}$ (harboured by the clinical strain) genes associated with antibiotic resistance.

The study expanded by testing additional antibiotic resistance biomarkers (the same tested in chapter 1: *intI1*, *sul1*, *ermB*, *ermF*, *aph (3")-ib*, *qacEΔ1*, *mefC*, *tetX*, *uidA* and crAssphage) through qPCR to obtain information of the tap water bacterial community in terms of antibiotic resistance and how that can affect the *K. pneumoniae* survival.

The combination of both analyses allowed the evaluation of the bacterium's prevalence, behavior and resistance traits in the water samples.

4.1. Methodology

4.1.1. Strains

Two strains of *Klebsiella pneumoniae*, designated as KP2-448 and SM1, were selected for this study due to their distinct origins and shared presence of the *bla*$_{\text{KPC}}$ gene, which confers resistance to carbapenem antibiotics. These strains were chosen to explore potential differences in their genetic behavior and whether they react equivalently to UV exposure. Their origin, genetic traits and phenotypic attributes are summarized in Table 3.

Clinical and environmental *Klebsiella pneumoniae* strains were retrieved from frozen stocks and revived on Plate Count Agar (PCA) and incubated at 37 °C to allow bacterial growth. The authenticity of the strains was verified by the antibiotic resistance phenotypes against gentamicin (GEN) and sulfamethoxazole-trimethoprim (SXT) that allow to distinguish the two strains (Table 3), tested by disk diffusion method (CLSI).

Table 3. Phenotypic and genotypic characteristics and genetic determinants associated with antibiotic resistance of isolates of *Klebsiella pneumoniae* KP2-448 and SM1 (adapted from Ferreira et al., 2023).

Characteristic	**KP2-448**	**SM1**
Origin	Clinical (urine)	Environmental (river sediment)
Identification (16S rRNA)	*K. pneumoniae* subsp. pneumoniae (99.86 %; AJJI01000018)	*K. pneumoniae* subsp. ozaenae (99.90 % ; Y17654)
Phenotypes of Antibiotic Resistance	Resistant: Amoxicillin (25 µg), ticarcillin (75 µg), cephalothin (30 µg), ceftazidime (30 µg), meropenem (10 µg), ciprofloxacin (5 µg), sulfamethoxazole (25 µg), Gentamicin (10 µg), streptomycin (10 µg), sulfamethoxazole + trimethoprim (23.75 + 1.25 µg)	Resistant: Amoxicillin (25 µg), ticarcillin (75 µg), cephalothin (30 µg), ceftazidime (30 µg), meropenem (10 µg), ciprofloxacin (5 µg), sulfamethoxazole (25 µg)
	Susceptible: Colistin (50 µg), tetracycline (30 µg) – Susceptible	Susceptible: Gentamicin (10 µg), streptomycin (10 µg), sulfamethoxazole + trimethoprim (23.75 + 1.25 µg), Colistin (50 µg), tetracycline (30 µg)
Tolerance to	50 °C (30 min) – No UV-C radiation (30 s) – Yes 400 mM H_2O_2 (15 min) – Yes	50 °C (30 min) – Yes UV-C radiation (30 s) – No 400 mM H_2O_2 (15 min) – No
Multilocus sequence type	ST-147	ST-147
Assembly acc. number	GCF_019093695.1	GCF_024179105.1
Antibiotic resistance genes	***bla_{KPC}; bla_{OXA-1}; qacE; sul1; intI1*** *(class 1 integrase)*	***bla_{KPC}***

4.1.2. Survival assays

To prepare the bacterial suspensions, a single, well-isolated colony from each strain was aseptically transferred into separate test tubes containing 9 mL of sterile saline solution (0.85% (w/v) NaCl). The optical density (OD) of each bacterial culture was measured at 610 nm using a spectrophotometer to achieve a standardized OD of 0.1, corresponding to approximately $10^{\wedge 8}$ CFU/mL. Tap water samples were directly collected from the laboratory tap to represent our water control. Two separate sterile Schott flasks with a capacity of 1 L were used for the collection, and each flask was filled with a final volume of 990 mL of tap water – one flask for each strain. 10 mL of the bacterial suspension, both clinical and environmental, was added to each flask and thoroughly homogenized to ensure even distribution of the bacteria, simulating a realistic water environment contaminated with 10^6 CFU/mL concentration of *K. pneumoniae*. Before inoculation, a volume of 200 mL was previously distributed in a cylinder with a capacity of 250 mL to serve as the baseline condition without bacterial inoculation (control). After homogenization, 200 mL of the bacterial-inoculated tap water was carefully distributed into three different cylinders – one to serve as the initial condition of the bacterial suspension at time zero (T0) to compare the subsequent changes in bacterial populations and behavior after exposure to UV radiation, and the other two were exposed to UV radiation for 30 seconds using a UV lamp with the following specifications:

11W power, UV-C radiation type, made of quartz glass, submersible, and measuring 20 x 240 mm (8 x 9.45 inches). After exposure, one of the UV-exposed cylinders was incubated at room temperature for 24 hours to evaluate bacterial regrowth. All cylinders were covered with aluminium foil to avoid additional light exposure. Afterward, all samples were serially diluted and the dilutions spread on Tryptone Bile X-glucuronide Agar (TBX), a selective and differential culture media for coliforms, and the general culture medium Reasoner´s 2A (R2A) agar using sterile beads for bacterial enumeration. TBX agar allowed the assessment of the total coliform counts, as it is selective for coliforms, including *Klebsiella* species. This choice was made due to the expectation that only *Klebsiella* bacteria would grow on this medium, given the absence of coliforms in tap water. Meanwhile, R2A agar allowed to enumerate total heterotrophic bacteria. Plates were incubated at 37 °C (TBX) or 30 °C (R2A) for 24 hours and 7 days to allow the enumeration of the slow growing bacteria.

For total DNA extraction, 200 mL of tap water samples (non-inoculated and inoculated) were filtered through polycarbonate membranes (0.22 μm porosity, Whatman, UK). Membranes were stored at -80 ˚C until DNA extraction (same protocol used in chapter 1). Three assays were performed, separately for both strains, three times each (Table 4). The irradiation system can be observed in Figure 37.

Table 4. Microcosm assays, sampling dates and analysed parameters.

Microcosm number	**Microcosm starting date**	**UV exposure times (samples)**	**Regrowth**
M1	24.02.23	0 sec (TW_C) 30 sec (TW_K; TW_E)	1h (TW_K_R; TW_E_R)
M2	08.03.23	0 sec (TW_C) 30 sec (TW_K; TW_E)	1h (TW_K_R; TW_E_R)
M3	29.03.23	0 sec (TW_C) 30 sec (TW_K; TW_E)	24h (TW_K_R24; TW_E_R24)

TW_C, non-inoculated microcosm assay; TW_K microcosm assay inoculated with the *K. pneumoniae* KP2-448; TW_E microcosm assay inoculated with the *K. pneumoniae* SM1; R, regrowth.

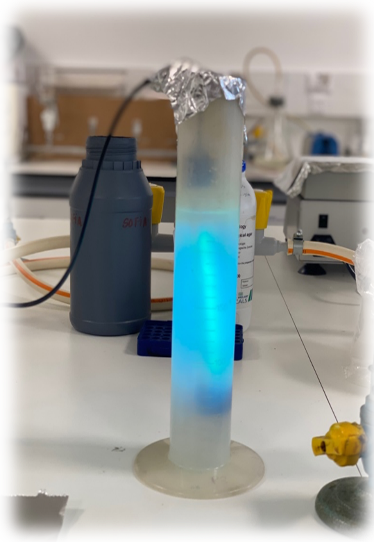


Figure 37. UV irradiation system.

4.1.3. Genes quantification using qPCR

Real-time quantitative PCR (qPCR) was used to quantify housekeeping genes (16S rRNA, *phoE)*, antibiotic resistance genes harbored by the strains used as inoculum (*bla*$_{\mathrm{KPC}}$ and *bla*$_{\mathrm{OXA\text{-}1}}$), and genes described as antibiotic resistance biomarkers (*intl1*, *sul1*, *ermB*, *ermF*, *aph(3'')-ib*, *qacEΔ1*, *mefC*, *tetX*, *uidA* and crAssphage) (Teixeira et al., 2023). Some of these biomarkers, such as *intl1* and *sul1*, were identified within the genomes of the strains used as inoculum (Table 3).

For the quantification of biomarkers, and to avoid limitations in terms of DNA extract quantity, the three replicas of the DNA extracts were pooled to create a single DNA extract that represents the individual samples.

Quantifications were carried out using the Standard Curve method described in Brankatschk *et al.* (2012) on a StepOne$^{\mathrm{TM}}$ Real-Time PCR System (Life Technologies, Carlsbad, CA, USA), using the qPCR conditions listed in Appendix 4 and the protocols described by Teixeira *et al.,* 2023. The results were evaluated based on quality criteria from Rocha *et al.,* (2020), including standard curve efficiency, CT value interpolation, consistent melting curve, single and correct melting point observation and absence of shoulders. Data was expressed as absolute abundance (gene copy number/mL of sample).

4.2. Results and Discussion

4.2.1. Survival of *K. pneumoniae* and persistence of their genes

The survival assays were conducted to investigate the response of *Klebsiella pneumoniae* strains to UV radiation exposure. The results were analyzed by assessing bacterial growth, log(CFU/mL), with the mean values of the M1, M2 and M3 assays (each performed in triplicate) presented for both TBX and R2A culture media, after 7 days of incubation (Fig. 38).

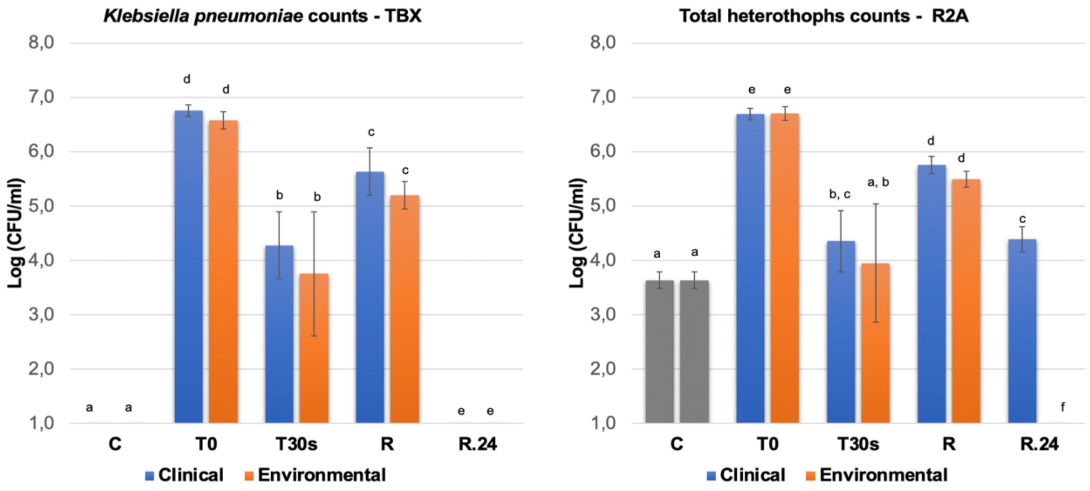


Figure 38. Representation of average colony counts of clinical and environmental strains of *Klebsiella pneumoniae* in TBX (A) and R2A (B) culture media of the 3 assays (each performed in triplicate), after exposure to UV-C radiation for varying durations (before UV exposure (T0); after 30 seconds of UV exposure (T30); and after 1h and 24h of regrowth (R)). The distinctive color coding represents the control – non-inoculated tap water (grey), and the clinical (blue) and environmental (orange) strains. a, b, c and d represent samples that were significantly different at a value of $p<0.001$.

Within the TBX culture medium, selective for coliforms and where it is expected that grows mostly the inoculated *K. pneumoniae* strains, the average colony counts showed a significative reduction of 2 log-units post 30 seconds of UV-C exposure for both strains. Following the subsequent incubation period, both the clinical and environmental strain demonstrated a recovery (regrowth), as indicated by increased colony counts (~1 log units) at the 30-second exposure time (T30). It's noteworthy that significant differences were observed between T0 (before UV-C exposure) and T30, as well as between T30 and regrowth for both strains (Fig. 38). However, no significant disparities were found in T0, T30 and regrowth between strains, indicating a consistent pattern of behavior in response to UV-C exposure (Fig.38).

For the R2A culture medium, where total heterotrophic bacteria can grow, similar results were observed. This is mostly due to the fact that the inoculated *K. pneumoniae* are in high abundance in comparison to the native tap water microbiota (average 3 log-units). Overall, this reduction in colony counts after UV-C exposure serves as a confirmation, aligning with findings in the study by Pullerits *et al.* (2020), that UV radiation is an effective method for reducing bacterial viability, thereby demonstrating its potential as a water disinfection technique. It also aligns with the well-known antimicrobial properties of UV radiation, which can damage bacterial DNA and inhibit replication (Abdullatif and Al-Askar, 2022). Nevertheless, the similar trend of regrowth in both TBX and R2A media supports the notion that UV radiation may not entirely eliminate all bacterial cells, allowing for recovery under favorable conditions. Furthermore, the consistent response of both strains across different culture media highlights the broad applicability of UV treatment, although it might be influenced by the initial bacterial load or time of UV exposure warranting further optimization for real-world applications. Some studies showed that *Mycobacterium avium* needs a UV dose of 128 J/m^2, while a cultivated environmental isolate of *Escherichia coli* requires a dose of 81 J/m^2 for equivalent reduction (Abdullatif and Al-Askar, 2022). As implied by these references, this variance underscores de possibility that *Klebsiella* strains may also require distinct UV doses to achieve successful inactivation.

The investigation into *K. pneumoniae* persistence genes involved the analysis of the 16S rRNA, *phoE*, *bla*$_{\text{KPC}}$ and *bla*$_{\text{OXA-1}}$ genes, as illustrated in Figure 39.

The 16S rRNA gene served as a valuable indicator of the overall bacterial population. The initial gene levels observed in the non-inoculated water control (C), clinical strain (K) and environmental strain (E) demonstrated the inherent presence of bacteria in all samples. The inoculated assays were approximately 1log-unit above the non-inoculated control (Fig. 39). The subsequent exposure to UV radiation for 30 seconds resulted in a reduction of the 16S rRNA gene abundance, but with no significant differences (Fig. 39). Interestingly, while both the clinical and environmental inoculated assays displayed reduced 16S rRNA gene levels after UV exposure, only the environmental strain exhibited significant differences (Fig. 39). During the regrowth phase (R) after 1-hour or 24-hours of storage, the clinical inoculated assay gene levels remained higher compared to the initial levels, suggesting potential recovery.

However, statistical significance was achieved only with 24-hour regrowth, suggesting a delayed regrowth process. In contrast, the environmental inoculated assay demonstrated a significant regrowth at both time points (Fig. 39). The comparison between the 16S rRNA gene and cultivable colonies in R2A medium provided insights into the microbial response to UV-C radiation. The 16S rRNA gene indicated a reduction in the bacterial abundance after radiation exposure, with partial recovery during regrowth. Similarly, cultivable colonies also decreased after radiation exposure but showed complete recovery during regrowth, suggesting UV-C radiation affects both cultivable and non-cultivable species alike.

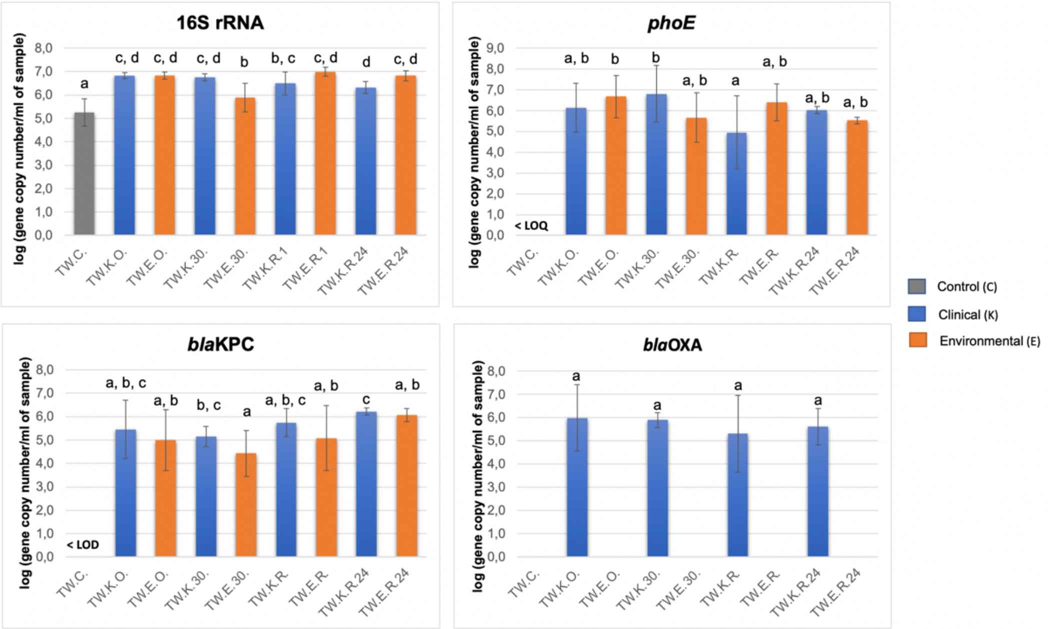


Figure 39. Quantification of the 16S rRNA, *phoE, bla*$_{\text{KPC}}$ and *bla*$_{\text{OXA-1}}$ genes for the clinical strain designated as "K" and the environmental strain labeled as "E". The designation tap water (TW) correspond to the assays M1, M2 and M3 conducted in triplicate (average values), while 0, 30, and R and R.24 represent the UV radiation exposure times (time zero, 30 seconds, and regrowth 1h and 24h) for the experimental conditions. Results are expressed on log-units (gene copy number/mL of sample). a, b, c and d represent samples that were significantly different at a value of $p<0.001$.

The *phoE* gene encodes an outer membrane porin protein responsible for phosphate ion transport across bacterial cell membranes and the presence of this gene is consistent among *Klebsiella pneumoniae* strains (Hu *et al.* 2022). As expected, in the control (non-inoculated) samples, the *phoE* gene levels were below the limit of quantification (<LOQ), confirming its absence in this context. In both clinical (K) and environmental (E) strain inoculated assays, the quantifiable *phoE* gene levels at time zero (0), in a concentration of 10^6 gene copy/mL of sample, highlighted its inherent presence as a housekeeping gene in *Klebsiella* strains. However, after a 30-second UV radiation exposure (T30), divergent responses emerged between the clinical and environmental assays. The clinical inoculated assay displayed an increase in *phoE* gene levels, whereas the environmental inoculated assay exhibited a reduction, suggesting a distinct response to the same stressor. Nevertheless, these differences were not statistically significant when compared to the levels at time zero (Fig. 39). During the regrowth phase (R), the clinical

inoculated assay showed a decrease in *phoE* gene levels and the environmental inoculated assay exhibited an increase, yet once again, these differences were not statistically significant (Fig. 39). Comparing the results of the *phoE* gene and cultivable colonies in TBX medium, the *phoE* gene levels exhibited a reduction post-exposure, reflecting the impact on the overall *Klebsiella* population. In contrast, cultivable colonies displayed a decrease in numbers after UV-C exposure, followed by recovery during regrowth. This divergence suggests that UV-C radiation may influence the viability of different bacterial species within *Klebsiella* population.

The bla_{KPC} gene is a type of carbapenemase-encoding gene associated with antibiotic resistance in *K. pneumoniae*. The gene is often carried in plasmids, which can be transferred between bacterial cells and contribute to the spread of antibiotic resistance (Ghasemnejad, 2019). In the control samples, the bla_{KPC} gene levels were below the limit of detection (<LOD), in line with its absence in tap water. As anticipated, both clinical (K) and environmental (E) assays, exhibited detectable bla_{KPC} gene levels at time zero (0), due to its inherent presence within their genomes. Upon 30-second UV radiation exposure, the clinical strain assay displayed a slight reduction in gene levels, whereas the environmental assay exhibited a more pronounced decrease – though statistically insignificant for both of them. Impressively, regrowth in both the clinical and environmental assays demonstrated an increase in gene levels, but once again without significant differences. A study by Serna-Galvis et al. (2020) noted that UV-C light effectively reduces the percentage of resistance genes only after an exposure of 600 seconds (10 minutes). This suggests that the duration of UV-C exposure in our study might not have been optimal for the efficient removal of the bla_{KPC} gene.

For bla_{OXA-1}, associated with antibiotic resistance (Nitz *et al.,* 2021), the control samples exhibited non-detectable gene levels. Furthermore, the environmental strain (SM1) assay demonstrated non-quantifiable levels across all exposure times, highlighting its baseline absence. This outcome aligns with the expectations since the environmental strain was not expected to harbor the bla_{OXA-1} gene (Table 3), highlighting the genetic divergence between the two strains. In the clinical strain assay, following the 30-second UV radiation exposure (T30), was observed a reduction in gene levels – although this was not statistically significant (Fig. 39). During the regrowth phase, the gene levels remained diminished and with no statistical significance observed (Fig. 39). This reduction suggests a potential impact on bacterial viability induced by UV-C exposure.

The calculation of the bla_{KPC}/*phoE* gene ratio aimed to determine whether the *Klebsiella* strains may have lost the plasmid carrying the bla_{KPC} gene. Similarly, the bla_{OXA-1}/*phoE* gene ratio was computed to assess the potential loss of the bla_{OXA-1} gene in the clinical strain.

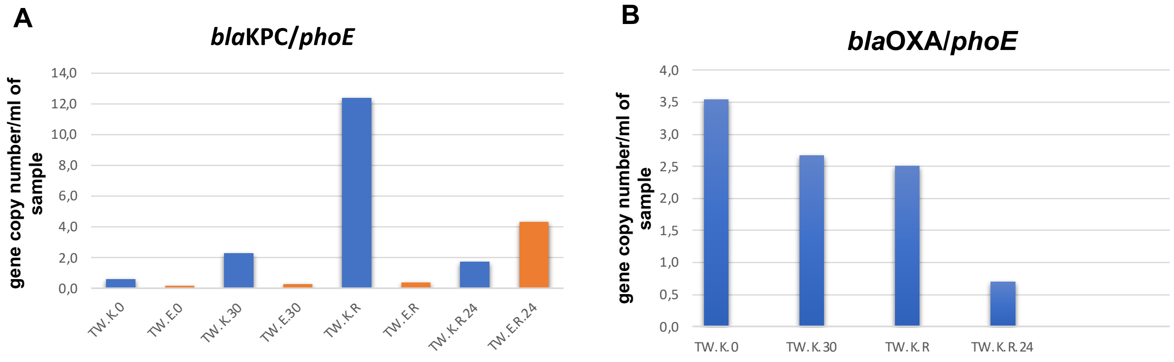


Figure 40. Calculation of the *bla*$_{\mathrm{KPC}}$/*phoE* (A) and the *bla*$_{\mathrm{OXA-1}}$/*phoE* (B) gene ratios.

The *bla*$_{\mathrm{KPC}}$/*phoE* ratio post-UV exposure indicates a slight increase of the ratio, suggesting that UV-C radiation might have little effect on the stability or replication of the plasmid harbouring the bla$_{\mathrm{KPC}}$ gene. However, the subsequent increase of the ratio during the regrowth phase implies that the strains were able to replicate the plasmid, indicating a degree of resilience or adaptation. The differential increased rates between strains may suggest varying abilities to replicate the plasmid or to answer the stress. Alternatively, it's possible that strains carrying the plasmid are more adept at recovering and subsequently dominating the regrowth phase (Fig.40.A). Plasmid quantification could validate this hypothesis.

Indeed, the ratio of *bla*$_{\mathrm{OXA-1}}$ to *phoE* gene levels showed a slight decrease of the prevalence of the *bla*$_{\mathrm{OXA-1}}$ from 3.5 to 0.5 after the exposure of the clinical strain to UV-C radiation (Fig.40.B). The declining ratio following UV exposure implies a reduction in the abundance of the *bla*$_{\mathrm{OXA-1}}$ compared to the housekeeping gene *phoE*. However, the reduction observed is small to be considered relevant.

4.2.2. Antibiotic resistance biomarkers

10 selected biomarkers were quantified in the assays (Table 5, Fig.41).

Table 5. Quantification of 10 other genes classified as antibiotic resistance biomarkers for a clinical strain designated as "K" and an environmental strain labeled as "E". The designation tap water (TW) correspond to the M1, M2 and M3 assays conducted in triplicate (average values), while 0, 30, and R represent the UV radiation exposure times (time zero, 30 seconds, and regrowth *1h and 24h*) for the experimental conditions. Results are expressed on log-units (gene copy number/mL of sample). For each gene is indicated the limit of quantification (LOQ) of the method, considering a volume of 200 ml of sample.

SAMPLE/gene	***intI1***	***uidA***	***ermB***	***aph3-ib***	**crAssphage**	***ermF***	***qacEdelta1***	***sul1***	***tetX***	***mefC***
LOQ	**0.7**	**0.66**	**1.2**	**2.2**	**1.01**	**1.01**	**1.2**	**0.82**	**1.2**	**1.15**
TW.C	2.20±1.55	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	2.90±2.22	3.60±1.14	<LOQ	<LOQ
TW.K.0	5.18±1.19	<LOQ	<LOD	<LOQ	<LOD	<LOQ	6.69±0.07	6.61±0.13	<LOQ	<LOQ
TW.E.0	3.57±1.68	<LOQ	<LOD	<LOQ	<LOD	<LOQ	4.58±1.47	4.39±1.38	<LOQ	<LOQ
TW.K.30	5.89±0.22	<LOQ	<LOQ	<LOD	<LOD	<LOQ	6.93±0.26	6.71±0.22	<LOQ	<LOD
TW.E.30	2.83±0.13	<LOQ	<LOQ	<LOD	<LOD	<LOQ	3.93±0.18	5.27±1.24	<LOQ	<LOQ
TW.K.R	5.72±0.03	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	5.97±0.66	4.57±2.09	<LOQ	<LOD
TW.E.R	2.37±0.13	<LOQ	<LOD	<LOD	<LOD	<LOQ	2.81±0.41	3.51±0.39	<LOQ	<LOQ
TW.K.R.24	5.85±0.01	<LOQ	<LOQ	<LOD	<LOD	<LOQ	5.72±0.18	6.74±0.03	<LOQ	<LOD
TW.E.R.24	2.28±0.00	<LOQ	<LOD	<LOD	<LOD	<LOQ	1.63±0.05	3.34±0.02	<LOQ	<LOQ

Table 5 presents all biomarkers results. The study of *uidA*, *ermF* and *tetX* biomarkers within *Klebsiella pneumoniae* assays has yielded consistent outcomes. Across all samples and conditions, all results for these genes fell below the limit of quantification suggesting its low prevalence within the examined microbial population (Table 5). These genes are associated with specific functions: *uidA* is related to β-glucuronidase production, *ermF* is linked to macrolide-lincosamide-streptogramin B (MLSB) resistance, and *tetX* associated with tetracycline resistance (Bryan *et al.* 2004). The absence of these genes signifies a low occurrence of these genetic traits in the microbial community present in these samples. For the *ermB*, *aph (3``)-ib,* crAssphage and *mefC* most of the samples were below quantification and detection limits, indicating their relatively low abundance or sporadic occurrence, aligning with the initial expectations (Table 5). *ermB* and *mefC* genes are associated with MLSB resistance, *aph(3``)-ib* encodes an aminoglycoside phosphotransferase enzyme, and crAssphage is a bacteriophage associated with human fecal contamination (Teixeira *et al.,* 2023). The absence of these genes shows a favorable scenario from a public health perspective, indicating a reduced potential for antibiotic resistance dissemination and human fecal contamination.

Interestingly, despite these low quantification rates, it's crucial to recognize instances where quantification was achieved – *intl1*, *qacEΔ1* and *sul1*. These three genes are commonly part of the conserved structure of class 1 integrons, potentially influencing the observed gene levels. Since only the clinical strain possesses *intl1* in its genome, these instances underscore the potential influence of the water source's microbial diversity on these biomarker levels. This suggests that the sampled water contains a mixture of microbial entities, some of which may carry the quantified biomarkers, potentially contributing to the observed levels (Fig. 41).

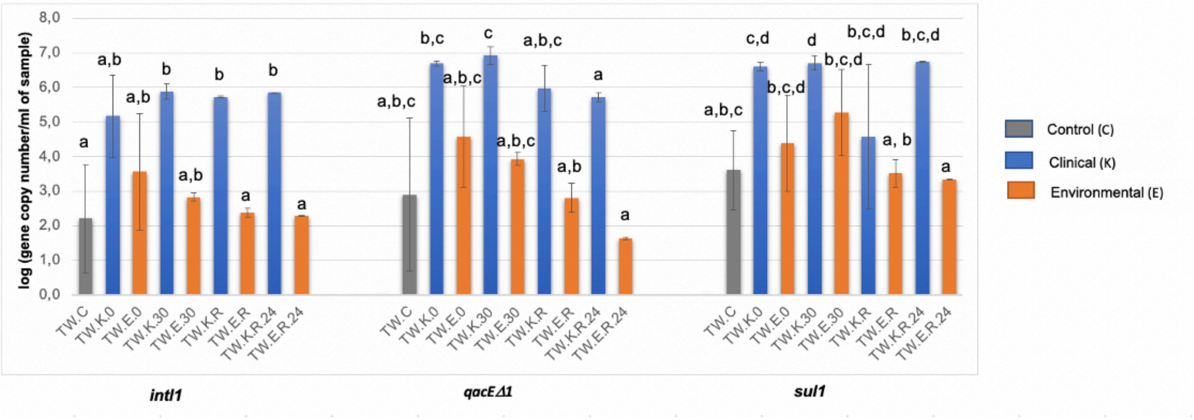


Figure 42. Quantification of the *intl1*, *qacEΔ1* and *sul1* genes for a clinical strain designated as "K" and an environmental strain labeled as "E". The designation tap water (TW) correspond to the assays conducted in triplicate (average values), while 0, 30, R and R.24 represent the UV radiation exposure times (time zero, 30 seconds, and regrowth 1h and 24h) for the experimental conditions. Results are expressed on log-units (gene copy number/mL of sample). a, b, c and d represent samples that were significantly different at a value of $p<0.001$.

The quantification of the *intl1* biomarker serves to assess the prevalence of integrons in the bacterial population (Ma *et al.,* 2017). Across all samples, *intl1* biomarker was quantifiable, ranging from 2.20 to 5.89 log units/mL sample, showing its consistent presence. In the clinical strain (K) assay, *intI1* levels increased post 30 seconds of UV exposure (from 5.18 to 5.89 log units/mL sample), approaching regrowth levels (5.85 log units/mL sample). In contrast, the environmental strain (E) assay experienced gradual *intI1* reduction throughout UV-C exposure (ranging from 3.57 to 2.28 log units/mL sample), but statistical analysis did not reveal significant differences between the conditions tested (Fig. 41). These different patterns between strain assays may be directly linked to the presence of this gene exclusively in the clinical strain's genome, explaining the higher values. Also, this genetic distinction can possibly impact on how these gene respond to UV-C exposure.

qacE∆1 gene levels, which encode an efflux transport protein conferring resistance to quaternary ammonium compounds (QACs) (Hrovat *et al.,* 2023), were quantifiable across all samples, including the non-inoculated control, emphasizing its ubiquitous presence (ranging from 1.63 to 6.74 log units/mL sample) and the significance of this gene in the microbial population. The clinical strain (K) inoculated assay displayed a small increase of this gene after UV-C exposure (from 6.69 to 6.93 log units/mL sample), followed by a decrease during regrowth (4.57 log units/mL sample). The environmental assay showed similar behavior – gene levels raised post-UV exposure and remained reduced during regrowth (varying from 4.58 to 3.34 log units/mL sample). Not significant differences were found, suggesting UV-C exposure seems to have a minimal impact in these gene levels (Fig. 41). Given that the clinical strain also possesses this gene in its genome, it's intriguing that both strains responded similarly. It may suggest that these strain assays might possess a mechanism to counteract the effect of the QACs, potentially enhancing their survival in environments where they are present, such as healthcare settings where disinfectants are commonly used (Nordholt *et al.,* 2021).

Quantifying the *sul1* gene can provide insights into the prevalence of resistance to sulfonamides (Ghasemnejad, 2019) in different strains of *Klebsiella pneumoniae* assays and how it might be affected by exposure to UV-C radiation and subsequent regrowth processes. *sul1* gene was detected in control samples, indicating its initial presence (ranging from 3.34 to 6.74 log units/mL sample). The clinical strain (K) assay showed minimal variation in gene levels post-UV exposure (from 6.61 to 6.71 log units/mL sample), but a notable decrease after regrowth (3.51 log units/mL sample). Also, the environmental strain (E) inoculated assay exhibited an increase after UV exposure and a subsequent decrease during regrowth. This suggests that even in the absence of the *sul1* gene in the environmental strain's genome, there may be interactions or responses to UV-C exposure that influence the prevalence of sulfonamide resistance, although to a lesser degree compared to the clinical strain.

This differential reactions between strains and assays highlight the complexity of microbial behavior under changing conditions and genetic differences. The responses of bacteria to stressors like UV-C radiation and subsequent regrowth are not uniform, even within the same species.

4.3. Conclusions

The survival assays demonstrated that UV radiation effectively reduced the viability of both clinical and environmental strains. The regrowth observed after UV exposure suggests that while UV-C radiation significantly diminishes bacterial populations, it may not completely eradicate all cells, inciting further considerations for optimal exposure times and conditions.

The analysis of the bla_{KPC}/*phoE* gene ratio illustrates the recovery of the clinical *Klebsiella pneumoniae* strain containing the plasmid that carries the bla_{KPC} gene during the regrowth phase. In the short-time regrowth (1h) was observed an increase in the bla_{KPC} gene prevalence, an effect that was reverted after 24h regrowth. UV-C exposure time may not have been optimally suited for the effective removal of the bla_{KPC} gene.

The results of the study indicated that while some genes, such as *uidA*, *ermB*, *aph (3``)-ib*, crAssphage*, ermF*, *tetX* and *mefC*, exhibited consistently low prevalence across all conditions – suggesting reduced prevalence or even absence of these specific resistance mechanism under UV-C stress, as expected; others like *intI1*, *qacEΔ1* and *sul1* demonstrated a more complex and dynamic response. The observed variations in gene levels across different strain assays could be linked to the adequacy of UV-C exposure times, tap water microbiota or the fact that these genes are only present in the clinical strain genome.

5. Future work

Additional research could focus on optimizing UV-C exposure times to maximize the efficacy of gene inactivation and assess the long-term effects on bacterial populations, contributing to the development of more efficient water disinfection strategies.

Explore the possible effect of the native microbiota or the presence of biofilms on the efficacy of the UV radiation to remove pathogenic bacteria from water.

Delve into the genetic mechanisms underlying the differential recovery rates of the *bla*$_{\text{KPC}}$ gene after UV-exposure, to uncover insights into plasmid stability and replication dynamics.

6. References

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7. Appendices

Appendix 3. List of parameters monitored per type of water - raw, filtered, treated (other) and UV treated and the number of determinations (n). a, b, c and d represent groups that were significantly different at a value of $p<0.001$.

Parameters	Raw	Filtered	Treated (other)	UV Treated	Statistical Method
Aluminium (μg/L Al; n=514)	---	a (n=223)	a (n=221)	a (n=70)	ANOVA One-Way (p=0.242)
Ammoniacal nitrogen (mg/L NH_4; n=392)	a (n=104)	b (n=288)	---	---	Kruskal-Wallis (p<0.001)
Calcium (mg/L Ca; n=14)	---	---	a (n=11)	a (n=3)	T-student test – independent samples (p=0.123)
Clostridium perfringens **(ufc/100 mL; n=60)**	a (n=14)	b (n=45)	--- (n=1)	---	Mann-Whitney (p<0.001)
Clostridium perfringens **(N/100 mL; n=431)**	a (n=89)	b (n=59)	c (n=213)	c (n=70)	Kruskal-Wallis (p<0.001); Mann-Whitney
Coliform bacteria (ufc/100 mL; n=59)	a (n=14)	b (n=44)	--- (n=1)	---	Kruskal-Wallis (p<0.001)
Coliform bacteria (N/100 mL; n=453)	a (n=92)	b (n=77)	c (n=215)	c (n=69)	Kruskal-Wallis (p<0.001); Mann-Whitney
Color (mg/L PtCo; n=184)	a (n=59)	b (n=60)	b (n=45)	b (n=14)	Kruskal-Wallis (p<0.001); Mann-Whitney
Conductivity (μS/cm; n=184)	a (n=63)	b (n=61)	c (n=46)	d (n=14)	Kruskal-Wallis (p<0.001); Mann-Whitney

Enterococci (ufc/100 mL; n=59)	a (n=14)	b (n=44)	--- (n=1)	---	ANOVA One-Way (p<0.001)
Enterococci (N/100 mL; n=438)	a (n=89)	b (n=64)	b (n=215)	b (n=70)	Kruskal-Wallis (p<0.001); Mann-Whitney
***Escherichia coli* (ufc/100 mL; n=59)**	a (n=14)	b (n=44)	--- (n=1)	---	Mann-Whitney (p<0.001)
***Escherichia coli* (N/100 mL; n=442)**	a (n=89)	b (n=68)	c (n=215)	c (n=70)	Kruskal-Wallis (p<0.001); Mann-Whitney
Free residual chlorine (mg/L Cl_2; n=117)	---	---	a (n=70)	b (n=47)	Mann-Whitney (p<0.001)
Nitrates (mg/L NO_3; n=408)	a (n=55)	a (n=285)	a (n=20)	b (n=48)	Kruskal-Wallis (p<0.001); Mann-Whitney
Nitrites (mg/L NO_2; n=47)	---	---	a (n=11)	a (n=36)	Mann-Whitney (p=1.000)
Oxidability (mg/L O_2; n=136)	a (n=66)	---	b (n=22)	b (n=48)	Kruskal-Wallis (p<0.001); Mann-Whitney
pH (Sorensen scale; n=322)	a (n=103)	b (n=110)	c (n=57)	d (n=52)	Kruskal-Wallis (p<0.001); Mann-Whitney
Scent (Dilution factor; n=61)	---	---	a (n=52)	a (n=9)	Mann-Whitney (p=0.677)
Taste (Dilution factor; n=61)	---	---	a (n=47)	a (n=14)	Mann-Whitney (p=1.000)
Temperature (˚C; n=324)	a (n=105)	a (n=104)	a (n=57)	b (n=58)	ANOVA (Post Hoc tests: Tukey, DMS, Bonferroni) (p<0.001)

Total Heterotrophs count at 22 ˚C (N/mL; n=18)	---	---	a (n=15)	a (n=3)	Mann-Whitney (p=0.327)
Total Heterotrophs count at 36 ˚C (N/mL; n=18)	---	---	a (n=15)	a (n=3)	Mann-Whitney (p=0.260)
Total organic carbon (mg/L C; n=186)	a (n=64)	b (n=66)	c (n=9)	b (n=47)	Kruskal-Wallis (p<0.001); Mann-Whitney
Trihalomethanes (µg/L; n=61)	---	---	a (n=20)	b (n=41)	Mann-Whitney (p<0.001)
Turbidity (NTU; n=733)	a (n=105)	b (n=325)	b (n=226)	b (n=77)	Kruskal-Wallis (p<0.001); Mann-Whitney
UV (/cm; n=494)	a (n=103)	b (n=106)	c (n=215)	b (n=70)	Kruskal-Wallis (p<0.001); Mann-Whitney

Appendix 4. Average and standard deviation for each month within each parameter.

		Samples							
		Raw		Filtered		Treated (other)		UV Treated	
Parameters	**Month**	Average	SD	Average	SD	Average	SD	Average	SD
Aluminium (µg/L Al)	Jan/22	-	-	9.00	1.40	9.67	0.75	9.35	1.10
	Feb/22	-	-	9.03	3.12	8.90	1.53	-	-
	Mar/22	-	-	7.40	1.43	8.20	1.37	-	-
	Apr/22	-	-	12.43	4.30	15.53	8.70	-	-
	May/22	-	-	27.36	19.77	21.56	13.01	28.80	16.02
	Jun/22	-	-	15.38	7.99	12.85	2.57	-	-
	Jul/22	-	-	13.83	2.64	14.57	2.46	-	-
	Aug/22	-	-	12.79	6.64	13.05	3.05	-	-
	Sept/22	-	-	15.18	12.44	16.14	2.70	16.68	12.17
	Oct/22	-	-	8.86	5.48	-	-	8.09	3.44
	Nov/22	-	-	7.24	1.61	8.88	3.02	7.08	0.83
	Dec/22	-	-	6.64	0.61	7.06	0.62	-	-
	Jan/23	-	-	40.70	62.45	42.17	57.47	-	-
	Fev/23	-	-	7.71	3.28	7.77	2.81	-	-
Ammoniacal nitrogen (mg/L NH$_4$)	Jan/22	0.30	0.10	0.03	0.00	-	-	-	-
	Feb/22	0.71	0.12	0.03	0.01	-	-	-	-
	Mar/22	0.33	0.10	0.02	0.00	-	-	-	-

	Apr/22	0.27	0.03	0.02	0.00	-	-	-	-
	May/22	0.40	0.09	0.03	0.00	-	-	-	-
	Jun/22	0.24	0.11	0.03	0.04	-	-	-	-
	Jul/22	0.27	0.07	0.03	0.00	-	-	-	-
	Aug/22	0.54	0.20	0.03	0.01	-	-	-	-
	Sept/22	0.86	0.54	0.02	0.00	-	-	-	-
	Oct/22	0.56	0.31	0.02	0.00	-	-	-	-
	Nov/22	0.13	0.05	0.02	0.00	-	-	-	-
	Dec/22	0.10	0.03	0.03	0.00	-	-	-	-
	Jan/23	0.14	0.04	0.02	0.00	-	-	-	-
	Fev/23	0.40	0.08	0.02	0.00	-	-	-	-
Calcium (mg/L Ca)	Jan/22	-	-	-	-	-	-	17.00	0.00
	Feb/22	-	-	-	-	21.00	0.00	-	-
	Mar/22	-	-	-	-	19.00	0.00	-	-
	Apr/22	-	-	-	-	19.00	0.00	-	-
	May/22	-	-	-	-	18.00	0.00	-	-
	Jun/22	-	-	-	-	18.00	0.00	-	-
	Jul/22	-	-	-	-	19.00	0.00	-	-
	Aug/22	-	-	-	-	20.00	0.00	-	-
	Sept/22	-	-	-	-	24.00	0.00	-	-
	Oct/22	-	-	-	-	-	-	15.00	0.00

	Nov/22	-	-	-	-	-	-	13.00	0.00
	Dec/22	-	-	-	-	13.00	0.00	-	-
	Jan/23	-	-	-	-	13.00	0.00	-	-
	Fev/23	-	-	-	-	17.00	0.00	-	-
Clostridium perfringens **(N/100mL)**	Jan/22	2.58E+03	5.97E+02	-	-	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Feb/22	3.44E+03	8.55E+02	-	-	0.00E+00	0.00E+00	-	-
	Mar/22	2.73E+03	8.79E+02	-	-	0.00E+00	0.00E+00	-	-
	Apr/22	4.25E+03	9.86E+02	-	-	0.00E+00	0.00E+00	-	-
	May/22	4.22E+03	1.07E+03	-	-	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Jun/22	3.23E+03	1.68E+03	-	-	0.00E+00	0.00E+00	-	-
	Jul/22	2.04E+03	1.18E+03	-	-	0.00E+00	0.00E+00	-	-
	Aug/22	7.18E+02	4.79E+02	-	-	0.00E+00	0.00E+00	-	-
	Sept/22	1.73E+03	9.87E+02	4.00E-01	8.00E-01	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Oct/22	1.84E+03	1.16E+03	5.56E-02	2.29E-01	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Nov/22	2.06E+03	1.29E+03	2.92E+01	1.37E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Dec/22	1.74E+03	3.14E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-	-
	Jan/23	1.64E+03	2.56E+02	-	-	0.00E+00	0.00E+00	-	-
	Fev/23	1.15E+03	2.54+02	-	-	0.00E+00	0.00E+00	-	-
Coliform bacteria (N/100mL)	Jan/22	1.04E+04	6.32E+03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Feb/22	2.45E+04	1.51E+04	-	-	0.00E+00	0.00E+00	-	-
	Mar/22	4.18E+03	1.58E+03	-	-	0.00E+00	0.00E+00	-	-

	Apr/22	4.60E+03	3.10E+03	1.00E+01	0.00E+00	0.00E+00	0.00E+00	-	-
	May/22	1.82E+04	1.58E+04	-	-	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Jun/22	2.18E+04	8.70E+03	-	-	0.00E+00	0.00E+00	-	-
	Jul/22	2.08E+04	6.61E+03	9.24E+02	6,78E+02	0.00E+00	0.00E+00	-	-
	Aug/22	1.36E+04	6.55E+03	5.01E+02	3,16E+02	0.00E+00	0.00E+00	-	-
	Sept/22	9.64E+04	8.49E+04	6.55E+03	8,16E+03	8.57E-01	2.10E+00	1.35E+01	5.04E+1
	Oct/22	4.59E+04	2.44E+04	4.97E+02	4,21E+02	-	-	4.76E-2	2.12E-01
	Nov/22	1.59E+04	1.10E+04	3.95E+00	3,93E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Dec/22	9.08E+03	4.11E+03	9.00E+00	0.00E+00	0.00E+00	0.00E+00	-	-
	Jan/23	8.34E+03	3.36E+03	2.00E+00	0.00E+00	0.00E+00	0.00E+00	-	-
	Fev/23	1.42E+04	3.79E+03	-	-	0.00E+00	0.00E+00	-	-
Color (mg/L PtCo)	Jan/22	3.53	0.61	1.90	0.00	1.90	0.00	1.90	0.00
	Feb/22	4.00	0.00	1.90	0.00	1.90	0.00	-	-
	Mar/22	4.50	0.50	1.90	0.00	1.90	0.00	-	-
	Apr/22	5.00	0.71	1.90	0.00	1.90	0.00	-	-
	May/22	4.60	0.49	1.90	0.00	1.90	0.00	1.90	0.00
	Jun/22	5.25	0.43	1.90	0.00	1.90	0.00	-	-
	Jul/22	5.40	0.62	1.90	0.00	1.90	0.00	-	-
	Aug/22	5.80	0.75	1.90	0.00	1.90	0.00	-	-
	Sept/22	11.00	1.58	2.43	0.91	1.90	0.00	1.90	0.00
	Oct/22	10.00	1.87	1.90	0.00	-	-	1.90	0.00

	Nov/22	6.00	1.10	1.90	0.00	1.90	0.00	1.90	0.00
	Dec/22	6.25	1.09	2.28	0.49	1.90	0.00	-	-
	Jan/23	4.24	1.26	1.96	0.12	1.92	0.04	-	-
	Fev/23	4.50	1.50	1.90	0.00	1.90	0.00	-	-
Conductivity (μS/cm)	Jan/22	34.75	3.42	80.75	10.06	120.00	0.00	94.33	8.01
	Feb/22	48.80	2.14	96.60	2.73	124.00	4.90	-	-
	Mar/22	47.50	2.60	98.75	2.17	123.25	4.09	-	-
	Apr/22	46.50	1.12	96.20	1.17	115.00	5.00	-	-
	May/22	43.40	4.92	94.00	3.29	115.00	5.00	120.00	0.00
	Jun/22	41.50	2.87	90.50	3.20	107.50	4.33	-	-
	Jul/22	43.00	4.30	94.50	1.66	120.00	0.00	-	-
	Aug/22	44.88	4.02	96.60	3.44	120.00	6.32	-	-
	Sept/22	83.50	9.50	143.75	10.40	150.00	0.00	155.75	22.61
	Oct/22	101.25	15.27	135.00	19.57	-	-	162.35	14.96
	Nov/22	45.28	8.15	76.50	4.09	87.50	2.50	102.33	10.40
	Dec/22	37.43	2.65	69.00	2.55	82.25	3.56	-	-
	Jan/23	36.80	3.31	74.20	8.63	90.20	12.61	-	-
	Fev/23	51.00	2.83	87.00	2.83	108.00	2.16	-	-
Enterococci (N/100mL)	Jan/22	4.85E+02	3.35E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Feb/22	7.84E+02	6.62E+02	-	-	0.00E+00	0.00E+00	-	-
	Mar/22	8.50E+01	2.20E+01	-	-	0.00E+00	0.00E+00	-	-

	Apr/22	7.40E+01	2.72E+01	0.00E+00	0.00E+00	2.00E-01	8.72E+01	-	-
	May/22	8.68E+01	7.06E+01	-	-	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Jun/22	6.30E+01	6.46E+01	-	-	0.00E+00	0.00E+00	-	-
	Jul/22	1.95E+01	1.88E+01	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-	-
	Aug/22	9.40E+00	1.05E+01	-	-	0.00E+00	0.00E+00	-	-
	Sept/22	7.77E+02	2.34E+03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Oct/22	7.51E+01	1.16E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Nov/22	5.19E+02	3.88E+02	2.00E+01	9.38E+01	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Dec/22	4.46E+02	1.45E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-	-
	Jan/23	4.74E+02	2.35E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-	-
	Fev/23	8.33E+02	1.98E+02	-	-	0.00E+00	0.00E+00	-	-
Escherichia coli **(N/100mL)**	Jan/22	1.26E+03	7.58E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Feb/22	1.91E+03	1.49E+03	-	-	0.00E+00	0.00E+00	-	-
	Mar/22	2.23E+02	7.60E+01	-	-	0.00E+00	0.00E+00	-	-
	Apr/22	2.83E+02	1.03E+02	1.00E+00	0.00E+00	0.00E+00	0.00E+00	-	-
	May/22	4.32E+02	2.46E+02	-	-	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Jun/22	3.80E+02	3.14E+02	-	-	0.00E+00	0.00E+00	-	-
	Jul/22	5.63E+01	3.11E+01	2.00E-01	4.00E-01	0.00E+00	0.00E+00	-	-
	Aug/22	5.08E+01	4.43E+01	-	-	0.00E+00	0.00E+00	-	-
	Sept/22	3.10E+02	8.61E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Oct/22	5.01E+02	8.10E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

	Nov/22	2.34E+03	2.25E+03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	Dec/22	1.59E+03	6.47E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-	-
	Jan/23	9.78E+02	5.15E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-	-
	Fev/23	2.87E+03	1.07E+03	-	-	0.00E+00	0.00E+00	-	-
Free residual chlorine (mg/L Cl_2)	Jan/22	-	-	-	-	0.90	0.08	0.90	0.00
	Feb/22	-	-	-	-	0.78	0.07	-	-
	Mar/22	-	-	-	-	0.85	0.11	-	-
	Apr/22	-	-	-	-	0.84	0.08	-	-
	May/22	-	-	-	-	0.94	0.05	0.90	0.00
	Jun/22	-	-	-	-	0.84	0.05	-	-
	Jul/22	-	-	-	-	0.88	0.04	-	-
	Aug/22	-	-	-	-	0.96	0.08	-	-
	Sept/22	-	-	-	-	1.21	0.14	1.15	0.05
	Oct/22	-	-	-	-	-	-	1.09	0.08
	Nov/22	-	-	-	-	0.99	0.07	0.99	0.06
	Dec/22	-	-	-	-	0.94	0.08	-	-
	Jan/23	-	-	-	-	0.90	0.11	-	-
	Fev/23	-	-	-	-	0.83	0.05	-	-
Nitrates (mg/L NO_3)	Jan/22	-	-	3.78	1.05	-	-	2.80	0.00
	Feb/22	-	-	6.02	0.49	7.10	0.00	-	-
	Mar/22	-	-	5.30	0.59	5.70	0.00	-	-

	Apr/22	-	-	4.40	0.45	4.40	0.00	-	-
	May/22	-	-	4.70	0.53	5.60	0.00	-	16.02
	Jun/22	-	-	4.21	0.77	4.30	0.00	-	-
	Jul/22	-	-	3.75	0.50	4.80	0.00	-	-
	Aug/22	-	-	4.64	1.28	4.00	0.00	-	-
	Sept/22	4.74	0.96	9.71	1.99	9.00	0.00	8.73	2.71
	Oct/22	6.76	0.66	9.91	1.66	-	-	9.67	2.59
	Nov/22	4.43	0.85	5.47	1.40	4.56	0.45	5.81	1.39
	Dec/22	4.70	0.00	4.33	0.61	4.85	0.25	-	-
	Jan/23	-	-	4.31	0.68	4.70	0.00	-	-
	Fev/23	-	-	5.65	1.51	6.90	0.00	-	-
Nitrites (mg/L NO_2)	Jan/22	-	-	-	-	-	-	0.90	0.00
	Feb/22	-	-	-	-	0.90	0.00	-	-
	Mar/22	-	-	-	-	0.90	0.00	-	-
	Apr/22	-	-	-	-	0.90	0.00	-	-
	May/22	-	-	-	-	0.90	0.00	-	-
	Jun/22	-	-	-	-	0.90	0.00	-	-
	Jul/22	-	-	-	-	0.90	0.00	-	-
	Aug/22	-	-	-	-	0.90	0.00	-	-
	Sept/22	-	-	-	-	0.90	0.00	0.90	0.00
	Oct/22	-	-	-	-	-	-	0.90	0.00

	Nov/22	-	-	-	-	-	-	0.90	0.00
	Dec/22	-	-	-	-	0.90	0.00	-	-
	Jan/23	-	-	-	-	0.90	0.00	-	-
	Fev/23	-	-	-	-	0.90	0.00	-	-
Oxidability (mg/L O_2)	Jan/22	1.40	0.00	-	-	-	-	1.40	0.00
	Feb/22	1.80	0.00	-	-	1.40	0.00	-	-
	Mar/22	2.00	0.00	-	-	1.40	0.00	-	-
	Apr/22	1.40	0.00	-	-	1.40	0.00	-	-
	May/22	1.50	0.00	-	-	1.40	0.00	-	-
	Jun/22	1.80	0.00	-	-	1.40	0.00	-	-
	Jul/22	1.50	0.00	-	-	1.40	0.00	-	-
	Aug/22	2.10	0.00	-	-	1.40	0.00	-	-
	Sept/22	2.89	0.23	-	-	1.40	0.00	1.41	0.03
	Oct/22	2.49	0.68	-	-	-	-	1.40	0.00
	Nov/22	1.78	0.30	-	-	1.40	0.00	1.44	0.11
	Dec/22	1.55	0.15	-	-	1.40	0.00	-	-
	Jan/23	2.70	0.00	-	-	1.40	0.00	-	-
	Fev/23	1.60	0.00	-	-	1.40	0.00	-	-
pH (Sorensen scale)	Jan/22	6.70	0.07	6.98	0.08	7.90	0.07	7.90	0.08
	Feb/22	6.80	0.06	6.86	0.08	7.84	0.10	-	-
	Mar/22	6.68	0.04	6.98	0.04	7.73	0.04	-	-

	Apr/22	6.85	0.15	7.00	0.09	7.68	0.04	-	-
	May/22	6.82	0.07	6.94	0.10	7.70	0.07	7.60	0.00
	Jun/22	6.70	0.19	7.00	0.00	7.83	0.04	-	-
	Jul/22	6.65	0.15	7.00	0.07	7.85	0.05	-	-
	Aug/22	6.78	0.27	6.90	0.13	7.88	0.07	-	-
	Sept/22	6.61	0.16	6.88	0.13	7.64	0.08	8.01	2.10
	Oct/22	6.56	0.13	6.78	0.10	-	-	7.45	1.54
	Nov/22	6.70	0.13	6.93	0.11	7.88	0.14	7.83	0.11
	Dec/22	6.72	0.07	7.00	0.11	7.92	0.07	-	-
	Jan/23	6.88	0.13	6.98	0.11	7.94	0.10	-	-
	Fev/23	6.83	0.13	6.83	0.05	7.83	0.05	-	-
Scent (Dilution factor)	Jan/22	-	-	-	-	0.90	0.00	0.90	0.00
	Feb/22	-	-	-	-	0.90	0.00	-	-
	Mar/22	-	-	-	-	0.90	0.00	-	-
	Apr/22	-	-	-	-	0.90	0.00	-	-
	May/22	-	-	-	-	0.90	0.00	0.90	0.00
	Jun/22	-	-	-	-	0.90	0.00	-	-
	Jul/22	-	-	-	-	0.93	0.04	-	-
	Aug/22	-	-	-	-	0.90	0.00	-	-
	Sept/22	-	-	-	-	0.90	0.00	-	-
	Oct/22	-	-	-	-	-	-	0.90	0.00

	Nov/22	-	-	-	-	0.90	0.00	0.90	0.00
	Dec/22	-	-	-	-	0.90	0.00	-	-
	Jan/23	-	-	-	-	0.90	0.00	-	-
	Fev/23	-	-	-	-	0.90	0.00	-	-
Taste (Dilution factor)	Jan/22	-	-	-	-	0.90	0.00	0.90	0.00
	Feb/22	-	-	-	-	0.90	0.00	-	-
	Mar/22	-	-	-	-	0.90	0.00	-	-
	Apr/22	-	-	-	-	0.90	0.00	-	-
	May/22	-	-	-	-	0.90	0.00	0.90	0.00
	Jun/22	-	-	-	-	0.90	0.00	-	-
	Jul/22	-	-	-	-	0.93	0.04	-	-
	Aug/22	-	-	-	-	0.90	0.00	-	-
	Sept/22	-	-	-	-	0.90	0.00	0.90	0.00
	Oct/22	-	-	-	-	-	-	0.90	0.00
	Nov/22	-	-	-	-	0.90	0.00	0.90	0.00
	Dec/22	-	-	-	-	0.90	0.00	-	-
	Jan/23	-	-	-	-	0.90	0.00	-	-
	Fev/23	-	-	-	-	0.90	0.00	-	-
Temperature (˚C)	Jan/22	10.50	1.12	10.75	1.09	10.00	0.00	12.33	0.94
	Feb/22	11.20	1.60	11.20	1.17	11.40	0.80	-	-
	Mar/22	15.00	1.87	12.75	1.79	13.18	1.38	-	-

	Apr/22	15.50	2.06	15.00	1.41	15.40	1.02	-	-
	May/22	18.00	0.63	18.00	0.63	19.00	1.17	18.80	0.00
	Jun/22	19.75	1.48	20.50	1.80	19.40	3.01	-	-
	Jul/22	23.00	0.71	23.75	0.43	23.50	0.87	-	-
	Aug/22	23.60	0.80	24.00	0.71	24.60	0.80	-	-
	Sept/22	21.87	1.53	22.48	0.87	24.00	0.00	22.73	1.20
	Oct/22	18.90	0.93	-	-	20.02	0.94	20.60	1.58
	Nov/22	15.54	1.01	15.97	0.74	15.51	0.95	16.85	0.31
	Dec/22	13.46	0.65	13.52	0.54	13.78	0.44	-	-
	Jan/23	11.88	1.22	11.86	1.47	12.30	1.14	-	-
	Fev/23	11.60	0.91	11.20	0.57	11.46	0.66	-	-
Total Heterotrophs at 22 ˚C (N/mL)	Jan/22	-	-	-	-	-	-	0.00E+00	0.00E+00
	Feb/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Mar/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Apr/22	-	-	-	-	5.00E-01	5.00E-01	-	-
	May/22	-	-	-	-	3.00E+00	5.20E+00	-	-
	Jun/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Jul/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Aug/22	-	-	-	-	1.00E+00	0.00E+00	-	-
	Sept/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Oct/22	-	-	-	-	-	-	0.00E+00	0.00E+00

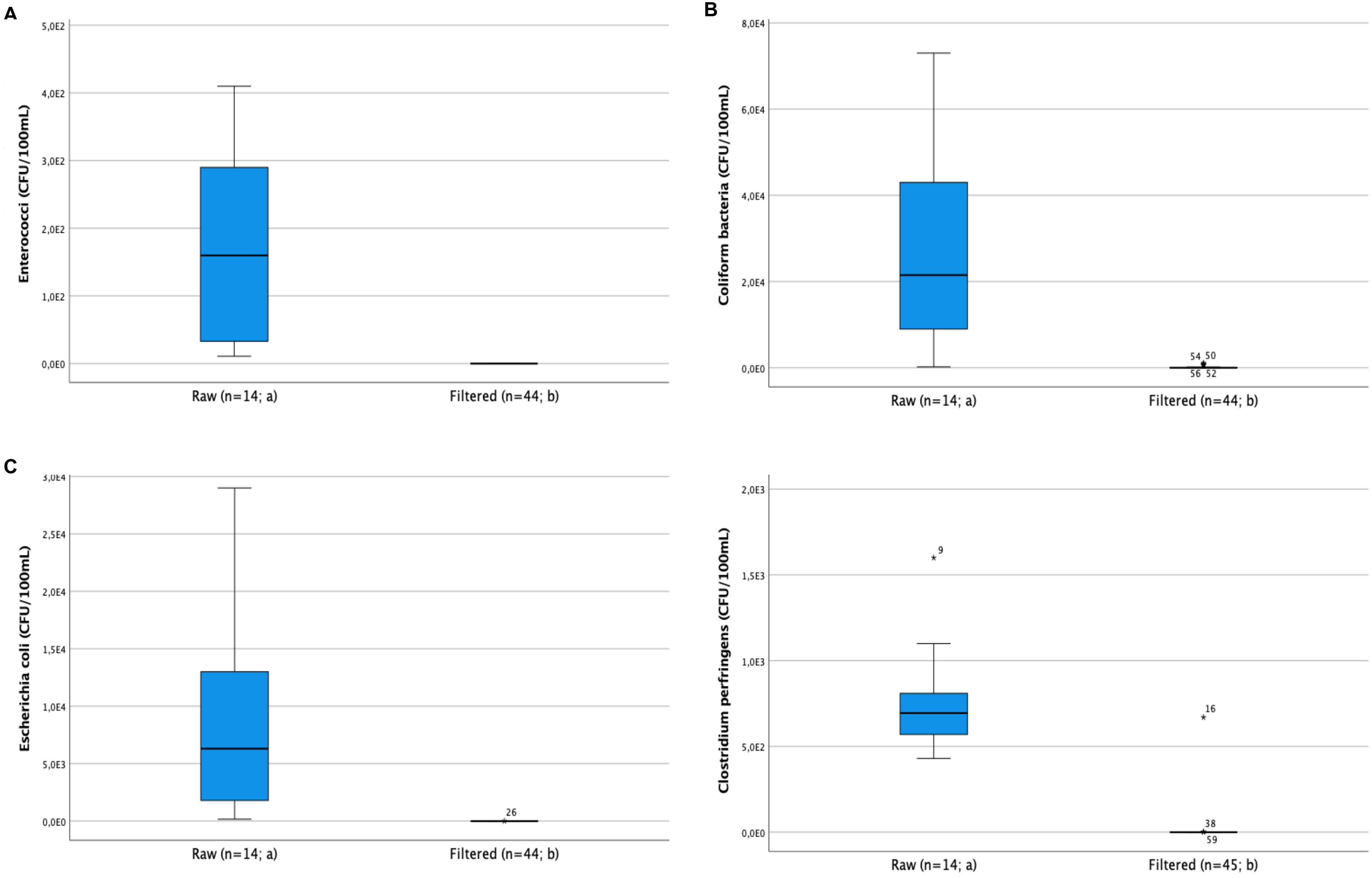
	Nov/22	-	-	-	-	-	-	0.00E+00	0.00E+00
	Dec/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Jan/23	-	-	-	-	1.00E+00	0.00E+00	-	-
	Fev/23	-	-	-	-	0.00E+00	0.00E+00	-	-
Total heterotrophs at 36 ˚C (N/mL)	Jan/22	-	-	-	-	-	-	0.00E+00	0.00E+00
	Feb/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Mar/22	-	-	-	-	1.00E+00	0.00E+00	-	-
	Apr/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	May/22	-	-	-	-	6.75E+01	6.83E+01	-	-
	Jun/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Jul/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Aug/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Sept/22	-	-	-	-	0.00E+00	0.00E+00	-	-
	Oct/22	-	-	-	-	-	-	0.00E+00	0.00E+00
	Nov/22	-	-	-	-	-	-	0.00E+00	0.00E+00
	Dec/22	-	-	-	-	1.00E+00	0.00E+00	-	-
	Jan/23	-	-	-	-	3.00E+00	0.00E+00	-	-
	Fev/23	-	-	-	-	0.00E+00	0.00E+00	-	-
Total organic carbon (mg/L C)	Jan/22	1.10	0.00	0.50	0.00	-	-	-	-
	Feb/22	1.40	0.00	0.70	0.00	-	-	-	-
	Mar/22	1.50	0.00	0.70	0.00	-	-	-	-

	Apr/22	1.50	0.00	0.70	0.00	-	-	-	-
	May/22	1.60	0.00	1.00	0.00	-	-	-	-
	Jun/22	1.60	0.00	0.60	0.00	-	-	-	-
	Jul/22	1.90	0.00	0.80	0.00	-	-	-	-
	Aug/22	1.70	0.00	0.60	0.00	-	-	-	-
	Sept/22	2.58	0.16	0.94	0.07	-	-	0.90	0.07
	Oct/22	2.53	0.13	0.96	0.11	-	-	0.93	0.12
	Nov/22	1.47	0.31	0.47	0.10	0.40	0.00	0.46	0.06
	Dec/22	1.35	0.05	0.45	0.05	0.40	0.00	-	-
	Jan/23	-	-	0.40	0.00	-	-	-	-
	Fev/23	-	-	-	-	-	-	-	-
Trihalomethanes (μg/L)	Jan/22	-	-	-	-	-	-	4.23	0.00
	Feb/22	-	-	-	-	7.05	0.00	-	-
	Mar/22	-	-	-	-	5.12	0.00	-	-
	Apr/22	-	-	-	-	5.56	0.00	-	-
	May/22	-	-	-	-	5.60	0.00	-	-
	Jun/22	-	-	-	-	6.15	0.00	-	-
	Jul/22	-	-	-	-	6.30	0.00	-	-
	Aug/22	-	-	-	-	6.80	0.00	-	-
	Sept/22	-	-	-	-	10.98	1.73	12.01	1.27
	Oct/22	-	-	-	-	-	-	10.88	2.68

	Nov/22	-	-	-	-	4.58	0.45	5.70	1.64
	Dec/22	-	-	-	-	5.17	0.64	-	-
	Jan/23	-	-	-	-	2.92	0.00	-	-
	Fev/23	-	-	-	-	-	-	-	-
Turbidity (NTU)	Jan/22	2.40	0.39	0.19	0.00	0.19	0.00	0.19	0.00
	Feb/22	2.20	0.92	0.19	0.00	0.19	0.00	-	-
	Mar/22	2.38	0.73	0.19	0.00	0.19	0.05	-	-
	Apr/22	2.65	0.27	0.19	0.00	0.19	0.00	-	-
	May/22	3.02	0.67	0.19	0.00	0.19	0.00	0.19	0.00
	Jun/22	1.98	0.79	0.19	0.04	0.19	0.00	-	-
	Jul/22	1.97	0.89	0.19	0.00	0.19	0.04	-	-
	Aug/22	1.38	0.40	0.19	0.00	0.19	0.00	-	-
	Sept/22	2.30	0.68	0.19	0.00	0.19	0.00	0.19	0.00
	Oct/22	3.45	2.00	0.19	0.00	-	-	0.20	0.04
	Nov/22	3.41	1.46	0.19	0.04	0.19	0.00	0.19	0.00
	Dec/22	1.91	0.65	1.90	0.00	1.90	0.00	-	-
	Jan/23	3.20	1.71	0.20	0.07	0.20	0.03	-	-
	Fev/23	1.50	0.57	0.20	0.05	0.20	0.04	-	-
UV (/cm)	Jan/22	0.04	0.00	0.01	0.00	0.01	0.00	0.01	0.00
	Feb/22	0.04	0.00	0.01	0.00	0.01	0.00	-	-
	Mar/22	0.04	0.00	0.01	0.00	0.01	0.00	-	-

	Apr/22	0.04	0.00	0.01	0.00	0.01	0.00	-	-
	May/22	0.04	0.00	0.01	0.00	0.01	0.00	0.013	0.01
	Jun/22	0.04	0.00	0.01	0.00	0.01	0.00	-	-
	Jul/22	0.04	0.00	0.01	0.00	0.01	0.00	-	-
	Aug/22	0.05	0.00	0.01	0.00	0.01	0.00	-	-
	Sept/2	0.07	0.01	0.02	0.00	0.02	0.00	0.01	0.00
	Oct/22	0.07	0.01	0.02	0.00	0.02	0.00	0.01	0.00
	Nov/22	0.06	0.08	0.01	0.00	0.01	0.00	0.01	0.00
	Dec/22	0.04	0.00	0.01	0.00	0.01	0.00	-	-
	Jan/23	0.03	0.01	0.01	0.00	0.01	0.00	-	-
	Fev/23	0.04	0.01	0.01	0.00	0.01	0.00	-	-

Appendix 5. A) Enterococci, B) Coliform bacteria, C) *Escherichia coli* and D) *Clostridium perfringens* boxplot representation of the distribution of determinations for each type of water- for raw and filtered water between the period of January/22 and February/23.



Appendix 9. Conditions used for quantitative PCR (qPCR) determinations in the study (retrieved from Teixeira *et al.,* 2023).

Target Gene	Primers	Primers Reference	qPCR conditions	Amplicon Length (bp)
aph (3'')-ib	FW- CCGACTTCTTACCGGACGAG	Teixeira et al., 2023	95 ºC 10 min (1 cycle), 95 ºC 15 s – 60 ºC 1 min (40 cycles) Other: SYBR® Select Master Mix (Applied); 500 nM of primers; BSA (20 mg/ml).	103
	R- ATATCGGTGCGCTCTTGGTC			
tetX	FW- AAATTTGTTACCGACACGGAAGTT	Pärnänen et al., 2019	95 ºC 10 min (1 cycle), 95 ºC 30 s – 60 ºC 30 s (40 cycles) Other: Fast SYBR Green® Master Mix (Applied); 200 nM of primers.	101
	R- CATAGCTGAAAAAATCCAGGACAGTT			
qacEΔ1	FW – CCCCTTCCGCCGTTGT	Pärnänen et al., 2019	95 ºC 10 min (1 cycle), 95 ºC 15 s - 60 ºC 1 min (40 cycles) Other: SYBR® Select Master Mix (Applied); 400 nM of primers; 2.5% DMSO.	159
	R- CGACCAGACTGCATAAGCAACA			
ermB	FW- TGAATCGAGACTTGAGTGTGCAA	Alexander et al., 2015	95 ºC 10 min (1 cycle), 95 ºC 15 s – 60 ºC 1 min (40 cycles) Other: Power SYBR Green® Master Mix (Applied); 200 nM of primers; BSA (20 mg/ml).	71
	R- GGATTCTACAAGCGTACCTT			
ermF	FW- TTTCTGGGAGGTTCCATTGTCC	Teixeira et al., 2023	95 ºC 10 min (1 cycle), 95 ºC 15 s - 60 ºC 1 min (40 cycles) Other: SYBR® Select Master Mix (Applied); 400 nM of primers; BSA (20 mg/ml).	163
	R- CTGATTTGACAGTTGGCGGTG			

crAssphage	FW- TGTATAGATGCTGCTGCAACTGTACTC	Karkman et al., 2019	95 ºC 10 min (1 cycle), 95 ºC 15 s – 60 ºC 1 min (40 cycles) Other: SYBR® Select Master Mix (Applied); 400 nM of primers; 2.5% DMSO.	148
	R- CGTTGTTTTCATCTTTATCTTGTCCAT			
mefC	FW- GCTTACAAGTTATGCTGTTCAG	Milaković et al., 2019	95 ºC 15 min (1 cycle), 95 ºC 15 s – 60 ºC 30 s – 72 ºC 30 s (40 cycles) Other: SYBR® Select Master Mix (Applied); 400 nM of primers; 2.5% DMSO.	195
	R- CAGAGAGCTATAAAAGCATCC			
uidA	FW- CAACGAACTGAACTGGCAGA	Chern et al., 2009	95 ºC 10 min (1 cycle), 95 ºC 15 s – 60 ºC 1 min (40 cycles) Other: SYBR® Select Master Mix (Applied); 200 nM of primers; 5% DMSO.	121
	R- CATTACGCTGCGATGGAT			
sul1	FW- CGCACCGGAAACATCGCTGCAC	Pei et al., 2006	95 ºC 5 min (1 cycle), 95 ºC 10 s – 60 ºC 30 s (35 cycles) Other: Fast SYBR Green® Master Mix (Applied); 300 nM of primers.	163
	R- TGAAGTTCCGCCGCAAGGCTCG			
intI1	FW- GATCGGTCGAATGCGTGT	Barraud et al., 2010	95 ºC 10 min (1 cycle); 95 ºC 15 s - 60 ºC 1 min (40 cycles) Other: Power SYBR Green® Master Mix (Applied); 200 nM of primers; 2.5% DMSO.	196
	R- GCCTTGATGTTACCCGAGAG			
16S rRNA	FW- CGGCAACGAGCGCAACCC	Denman and McSweeney, 2006	95 °C 10 min (1 cycle); 95 °C 15 s, 55 °C 20 s, and 72 °C 10 s (35 cycles) Other: KAPA SYBR® FAST ABI Prism®; 200 nM of primers; BSA (20 mg/ml).	130
	R- CCATTGTAGCACGTGTGTAGCC			

phoE	FW- TGCCCAGACCGATAACTTTA	Sun et al., 2010	95 °C 10 min (1 cycle); 95 °C 15 s, 55 °C 30 s, and 72 °C 30 s (40 cycles) Other: KAPA SYBR® FAST ABI Prism®; 200 nM of primers; BSA (20 mg/ml).	142
	R- CTGTTTCTTCGCTTCACGG			
bla$_{\mathbf{KPC}}$	FW- CAGCTCATTCAAGGGCTTTC	Subirats et al., 2017	95 °C 10 min (1 cycle); 95 °C 15 s, 60 °C 20 s, and 72 °C 10 s (40 cycles) Other: KAPA SYBR® FAST ABI Prism®; 200 nM of primers; BSA (20 mg/ml).	196
	R- GGCGGCGTTATCACTGTATT			
bla$_{\mathbf{OXA-1}}$	FW- TATCTACAGCAGCGCCAGTG	Rocha et al., 2019	95 °C 10 min (1 cycle); 95 °C 15 s and 60 °C 60 s (40 cycles) Other: KAPA SYBR® FAST ABI Prism®; 200 nM of primers; BSA (20 mg/ml).	79
	R- CGCATCAAATGCCATAAGTG			