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Treatment of wastewater effluents using nanofiltration and low pressure UV photolysis to produce high quality water for food production

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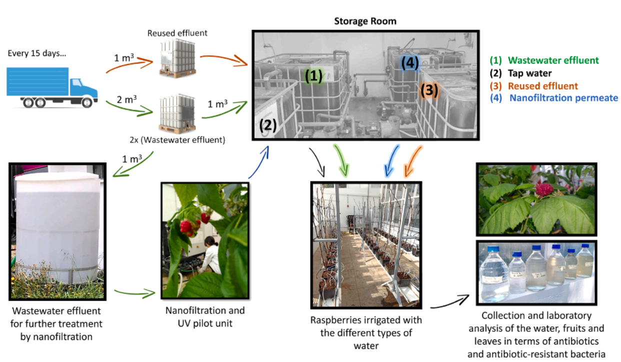
HIGHLIGHTS

- Wastewater effluents were tested as alternative water sources for crop irrigation.
- Antibiotics and bacteria were monitored during a 5-month irrigation.
- Nanofiltration produced high quality irrigation water while retaining nutrients.
- UV photolysis effectively inactivated bacteria in the retentate.
- No antibiotic uptake was detected in raspberry fruits or leaves above 10 µg/kg.

GRAPHICAL ABSTRACT



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ABSTRACT

Agriculture irrigation accounts for approximately 70 % of all water withdrawals. Climate change and the increase in human population will lead to a higher food and water demand. Hence, it is crucial to explore new water sources for agriculture irrigation, such as the reuse of wastewater effluents. Prior to irrigation with wastewater effluents, it is important to evaluate the potential uptake of contaminants by food crops.

In this case study, different water sources (three wastewater effluents with different qualities and tap water) were used for the irrigation of raspberries, selected due to their high water demand, rapid fruiting cycle, health benefits, and commercial value. Antibiotics (ciprofloxacin, levofloxacin, meropenem and ampicillin) were targeted due to their widespread clinical use, environmental persistence, and potential to promote antimicrobial resistance. Moreover, water quality indicators total coliforms, *Escherichia coli* and enterococci as well as

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Pseudomonas aeruginosa resistant to different antibiotics were monitored in the different irrigation waters over a five-month irrigation period.

The combination of nanofiltration and UV photolysis produced high-quality water for irrigation. UV photolysis was tested to further treat the permeate and retentate samples from a nanofiltration process. When ciprofloxacin was present in the permeate samples, after exposure to UV photolysis using a low-pressure mercury lamp, the antibiotic was not detected. In the highly concentrated retentate samples, 57 % of ciprofloxacin and 31 % of levofloxacin were degraded while all the target bacteria were inactivated successfully by UV photolysis, with logarithmic inactivation's higher than 4.

Analytical methods were also used for the detection of the target contaminants in the solid matrices and the potential uptake of the target contaminants by the fruits and leaves was evaluated. The target antibiotics were not detected in the fresh fruits and leaves at levels above the detection limit of 10 μg/kg.

This short-term research study encompasses wastewater treatment plant effluent characterization, advanced treatment through nanofiltration and UV photolysis, and the subsequent reuse of treated water for crop irrigation, providing a comprehensive evaluation of wastewater reuse as a sustainable solution to meet the growing demands for water in agriculture.

1. Introduction

Water scarcity is a worldwide critical environmental issue that raises major concerns on the sustainable future of humanity and the conservation of important ecosystem functions (Lorenzo et al., 2020). Recent data (from World Resources Institute's Aqueduct Water Risk Atlas) reveals that 25 countries, home to a quarter of the global population, experience extremely high water stress annually, consistently depleting nearly all of their available water resources. Over half of the world's population, approximately 4 billion people, endure highly water-stressed conditions for at least one month each year (Kuzma et al., 2023). This number is expected to increase as the world's population grows and climate change impacts water availability. In Europe, water scarcity is a particular concern in the southern areas, with around 30 % of the population in areas with permanent water stress and up to 70 % in areas with seasonal summer stress (EEA, 2025). Agriculture, public water supply, and tourism place significant pressure on freshwater resources. Agriculture accounts for a significant portion (approximately 70 %) of global freshwater use (UNESCO, 2024). Additionally, a quarter of the world's crops are grown in regions facing highly stressed or unreliable water supplies, or both. Increasing challenges such as climate change and growing competition for water are putting pressure on these resources, ultimately jeopardizing food security (Saccoccia and Kuzma, 2024).

As food demand and water scarcity continue to rise, sustainable irrigation is an essential strategy to ensure a reliable and resilient global food supply in the face of a changing climate (Lorenzo et al., 2020).

Wastewater reuse for agriculture can help to address water scarcity by reducing the demand for freshwater resources. For example, in Europe, there is potential to reuse six times more treated wastewater than current levels (European Commission, 2025). This can be a valuable way to conserve water resources, especially in areas where water is scarce. Additionally, wastewater can be a source of nutrients that can help to fertilize crops, and it can also help to reduce the need for chemical fertilizers. The use of treated wastewater for irrigation is projected to increase globally due to growing water scarcity, particularly in arid and semi-arid regions where it serves as a critical alternative water source (Al-Hazmi et al., 2023).

However, it is important to treat wastewater properly before it is used for agriculture, as it can contain harmful bacteria and chemical contaminants. Addressing these challenges requires a comprehensive understanding of the potential risks and benefits associated with wastewater reuse and the treatment technologies that may be used to produce safe water for irrigation.

Membrane filtration processes such as nanofiltration and reverse osmosis, are highly effective in removing suspended solids, pathogens, and chemical contaminants with low molecular mass from wastewater (e.g. Cristovão et al., 2021, 2022; Radjenović et al., 2008). Likewise, UV photolysis is extremely effective to inactivate pathogens and degrade photolabile chemical contaminants (e.g. Pereira et al., 2007; Rodrigues et al., 2014; Sanches et al., 2010; Zimmer et al., 2003).

Used in sequence these advanced treatment methods may enable the production of high-quality effluents that minimize the risks associated with the introduction of harmful microorganisms and chemical pollutants into irrigation systems, meet stringent regulatory standards and foster the sustainable reuse of wastewater effluents in agriculture.

In 2022, Portugal ranked 5th globally in exports of small berry fruits (fresh raspberries, blackberries, mulberries, and loganberries) according to data from the International Trade Centre compiled by the United Nations Statistics Division. Red raspberries (*Rubus idaeus*) contain essential nutrients and other bioactive components important to a healthy diet. They stand out among edible plants for their rich dietary fiber content per 100 kcal, making them a nutritious choice. Moreover, they contain ellagitannins and anthocyanins, both known for their health benefits. Research suggests a key role for red raspberries in reducing the risk for metabolically based chronic diseases, particularly cardiovascular disease, type 2 diabetes mellitus and Alzheimer disease (Burton-Freeman et al., 2016). Primocane fruiting red raspberries were chosen for this irrigation study due to their fast-growing habit with plants producing fruit in a short period. Furthermore, the long-cane system - a production method in which mature raspberry canes (1.6 to 2 m tall) with flower buds are grown in containers and stored in cold chambers at 0–4 °C to induce dormancy - allows cold-treated plants (those stored in the cold chamber) to begin fruiting within three months of placement in the glasshouse. Raspberries also have high water demands and a high transpiration rate, which can increase the uptake of undesirable contaminants.

In this study, four different water sources – tap water and three different wastewater effluents (one effluent discharged into the river, another effluent that undergoes additional filtration and chlorination before being reused for street cleaning, and an effluent generated after pilot-scale nanofiltration and UV treatment) – were used for the irrigation of raspberries. Antibiotics (ciprofloxacin, levofloxacin, meropenem, and ampicillin), as well as different bacteria (total coliforms, *Escherichia coli*, enterococci, and *Pseudomonas aeruginosa*) resistant to different antibiotics, were monitored in the various irrigation waters over a five-month period. Additionally, the study evaluated the treatment efficacy of nanofiltration and UV photolysis in removing these contaminants from wastewater effluents and investigated the potential uptake of the antibiotics by raspberries and their leaves.

The findings of this work provide important insights for water reuse policies, the development of sustainable irrigation strategies, and the design of effective treatment processes that the water industry can implement to address various pollutants and comply with increasingly stringent future regulations.

2. Materials and methods

2.1. Irrigation study

Every 15 days, over a 5 month irrigation period (from January to May), four 1000 L tanks were filled with different water sources (two wastewater effluents collected at a tertiary treatment facility, a wastewater effluent after nanofiltration treatment and tap water) that were used for the irrigation of raspberries (Fig. 1). Two wastewater effluents were collected at a wastewater treatment plant: the wastewater effluent discharged in the river (identified in this study as wastewater effluent) and a wastewater effluent that the wastewater utility further treats by filtration and chlorination and reuses for street cleaning (identified in this study as reused effluent).

Each tank/water quality was used to irrigate raspberry plants grown in 4.7 L pots with a commercial substrate mixture. 12 pots per repetition were used. Each pot was drip irrigated with a 2 L/h regime. No broken emitters were observed throughout the entire cropping season. Fertigation was applied equally for all water sources according to the growth and development stages of the raspberry plants (Bushway et al., 2008).

Water samples were collected during the study period (five months) and characterized, as detailed below, in terms of: (i) physico-chemical properties (total solids, suspended solids, dissolved solids, chemical oxygen demand and inorganic constituents that influence plant growth: total nitrogen, ammonium, nitrate, total phosphorous and chloride); (ii) antibiotics (ciprofloxacin, levofloxacin, meropenem and ampicillin); (iii) total coliforms, *E. coli*, enterococci, and *P. aeruginosa* (as well as detection of antibiotic resistances in the same samples).

2.1.1. Physico-chemical characterization of the different irrigation waters

Total solids (TS), total suspended solids (TSS), and total dissolved solids (TDS) were determined in the water samples following Standard Method 2540 (Lipps et al., 2023).

The total solids content, amount of residue left in a crucible after evaporation and drying in an oven, was determined by adding 30 mL of the samples to a previously weighted ceramic crucible. The mass of total solids was calculated by subtracting the original weight of the crucible from its weight after the evaporation and drying procedure. The crucible was cooled to room temperature in a desiccator before being weighted.

The total suspended solids content, portion of total solids retained by filtration, was determined by vacuum filtration of 250 mL of the sample through a 1.2 μm pore diameter glass microfiber filter (Whatman GF/C, CAT No 1822–047). The filter was then dried at approximately 103 °C, cooled to room temperature in a desiccator and weighted. The mass of total suspended solids was calculated by subtracting the weight after filtration from the weight of the original filter.

The total dissolved solids content, portion of total solids not retained by filtration, was calculated by subtracting the calculated value of total suspended solids from the value of total solids.

Chemical oxygen demand and inorganic constituents that can influence plant growth: total nitrogen, ammonium, nitrate, total phosphorous and chloride were measured using a HACH DR3900 spectrophotometer, a thermostat HT200S (used for the determination of chemical oxygen demand, total nitrogen and total phosphorous) and different test methods: LCI 500 for chemical oxygen demand, LCK 238 LATON for total nitrogen, LCK 303 for ammonium, LCK 340 for nitrate, LCK 348 for total phosphorous and LCK311 for chloride.

2.1.2. Occurrence of antibiotics in the different irrigation waters, produced raspberries and leaves

2.1.2.1. Analysis of antibiotics in the water samples. An analytical method, previously described by Rodriguez-Mozaz et al. (2020), was adjusted to test the concentration of the target antibiotics (ciprofloxacin, levofloxacin, meropenem and ampicillin) in the different water matrices (wastewater effluent, reused effluent, nanofiltration effluent, tap water) used for the irrigation of the raspberries from January to May. The target antibiotics were selected due to their widespread clinical use, environmental persistence, and potential to promote antimicrobial resistance.

Wastewater samples were first filtered using 1.2 μm glass microfiber filters (Whatman, Maidstone, UK) and 0.45 μm polyamide membrane filters (nylon) (Filter-Lab, Barcelona, Spain). Subsequently, 0.1 M Na_2EDTA solution was added to achieve a final concentration of 0.1 % (g solute/g solution), and the sample pH was adjusted to 2.5 with 0.1 M hydrochloric acid. Afterwards, the method involved the solid phase extraction of the antibiotics from the different matrices using 200 mg Oasis HLB cartridges (Waters, Milford, MA, USA). Depending on the water matrix, different volumes of samples passed through the cartridges (500 mL of the wastewater effluents and 1000 mL of the tap water and nanofiltration samples) at a flow rate of 5 mL min^{-1} under

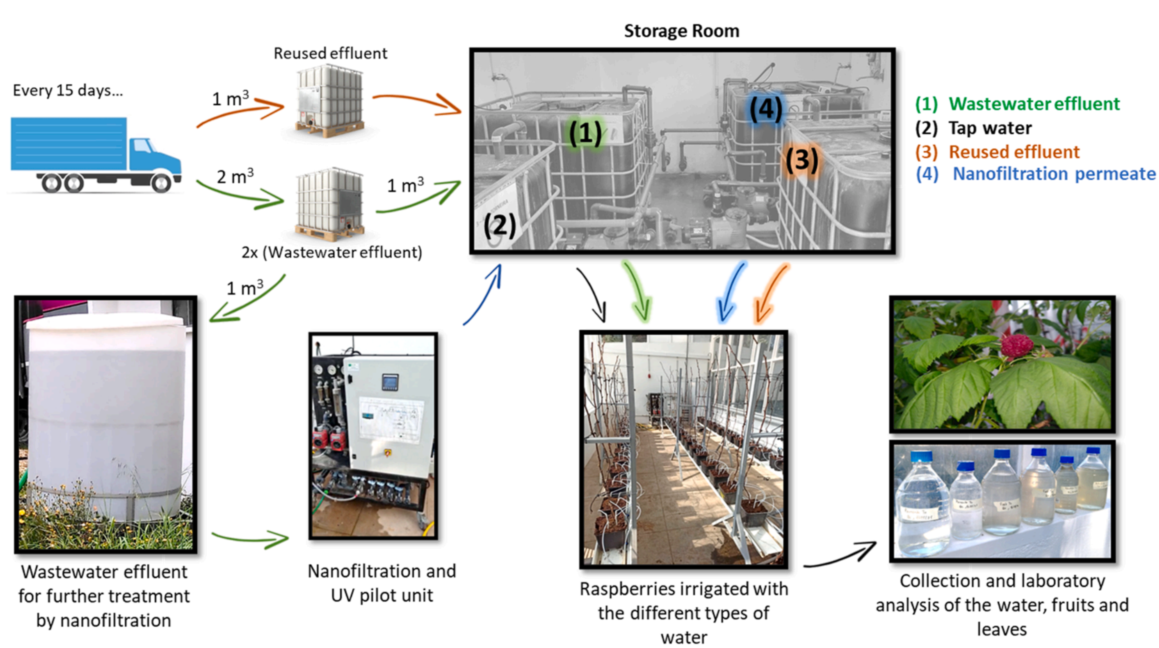

Fig. 1. Diagram of the irrigation study.

vacuum, after which the cartridge was rinsed with 3 mL of laboratory grade water, dried for approximately one hour, eluted with methanol, evaporated by a nitrogen stream near dryness, and reconstituted with 500 μL of a mixture of methanol and Milli-Q water (50:50, v/v) and stored at −20 °C. The samples were then filtered with 0.2 μm filters (GE Healthcare, UK Limited) prior to UPLC-MS/MS analysis (Waters® Acquity™ ultra-high-performance LC) using a Mediterranean Sea 18 analytical column (2.2 μm; 100 × 2.1 mm) kept at 35 °C during the analysis. The mobile phases consisted of 0.1 % (v/v) formic acid in Milli-Q water (A) and 0.1 % (v/v) formic acid in acetonitrile (B) with a flow rate of 0.3 mL min^{-1}. Initially, the column was kept at 100 % A for 1 min, followed by a decrease to 80 % A from 1 to 7 min. The column was then kept at 80 % A for 1 min and at 10 % A for 2 min, finally returning to the initial conditions for 10 min. Tandem mass spectrometry (MS/MS) detection of the target antibiotics, ionized in positive ion mode (ESI+), was performed on an Acquity™ triple quadrupole (Waters®, Dublin, Ireland) using an electrospray ionization source (temperature: 130 °C; capillary voltage: 2.2 kV). MS/MS conditions were optimized, and the analysis was performed in multiple reaction monitoring (MRM) mode, in order to achieve a higher selectivity and sensitivity. Two transitions were used to identify the compounds in samples, with a maximum deviation of 15 % between MRM1/MRM2 ratio, compared to the stock solutions. Table S1 (in the supporting documentation section) describes the analytical conditions optimized for each compound.

2.1.2.2. Analysis of antibiotics in the raspberries and leaf samples. From each irrigation line, 1 kg of raspberries was collected once a week during the two months of harvesting whereas the leaves were collected in the last harvesting event.

The potential uptake of the target contaminants by the fruits and leaves collected was assessed following a procedure proposed by Danezis et al. (2016).

For the analysis of fresh fruit and leaves, approximately 10 g of fresh raspberries and 5 g of fresh leaves were weighed and blended. Afterwards, 10 mL of extraction solvent (acidified methanol with 1 % formic acid v/v) was added. The samples were then shaken by hand for 2 min and centrifuged at 4000 rpm for 5 min. The extract was transferred into a vial and stored in the freezer (at −20 °C) until analysis. Before the UPLC-MS/MS analysis described above, samples were filtered with 0.2 μm filters (GE Healthcare, UK Limited).

2.1.3. Microbiological characterization of the different irrigation waters

A microbiological characterization of the different irrigation waters was performed in terms of total coliforms, *E. coli*, and enterococci, that are frequently monitored as microbiological indicators of water quality, as well as *P. aeruginosa*, an ubiquitous environmental bacterium.

Total coliforms and *E. coli* present in the irrigation waters were detected using the Colilert-18 assay (IDEXX, Maine, USA; ISO 9308–2:2012). Enterococci, such as *Enterococcus faecium* and *E. faecalis* were detected in the irrigation waters using the Enterolert-E test (IDEXX, Maine, USA; ISO 7899–1). *Pseudomonas aeruginosa* was detected using the Pseudalert test (IDEXX, Maine, USA; ISO: 16266–2:2018). The most probable number of the bacteria in the different samples was estimated by placing the samples in Quanti-Tray/2000 (IDEXX, Maine, USA). The Quanti-Trays were incubated at 35±0.5 °C during 18 h for Colilert-18, at 41±0.5 °C during 24 h for Enterolert-E, and 38±0.5 °C during 24 to 28 h for Pseudalert. After the incubation period, the positive wells were counted and converted into most probable number per 100 mL of sample.

The rapid bacterial enzyme detection methods described above were also used for the detection of antibiotic-resistant bacteria in the different irrigation samples. Galvin et al. (2010) adapted the protocol of the Colilert-18 assay for the enumeration of antimicrobial-resistant *E. coli* in different wastewater effluents. The proposed procedure follows the same main steps, however, before mixing the substrate with the samples, a specific concentration of different antibiotics was added to each sample.

Stock solutions of ciprofloxacin (Acros Organics), levofloxacin (Sigma), meropenem (Sigma Aldrich) and ampicillin (nzytech) were prepared. The target antibiotics were selected based on surveillance of antimicrobial resistance in Europe (ECDC, 2023). The concentrations of the antibiotics added to the IDEXX tests (detailed in Table S2 of the supporting information section) were defined based on the minimal inhibitory concentration break-points levels for Enterobacterales, *Enterococcus* spp. and *P. aeruginosa* (CLSI, 2021). Using the adapted IDEXX procedures, the presence of resistant bacteria by the end of incubation is indicated by a color change to yellow or fluorescence in the Quanti-Tray wells.

2.2. Treatment of wastewater effluents using nanofiltration and low pressure UV photolysis

Every 15 days during the 5-month irrigation period (from January to May), 1000 L of wastewater effluent was further treated by nanofiltration and the 1000 L of nanofiltration permeate (treated water) was placed in a tank and used for the irrigation of raspberries (Fig. 1). A pilot treatment unit equipped with nanofiltration and a low-pressure mercury ultraviolet lamp Puro-1S (PURO SRL, Italy; Lamp power: 40 W) was used in this study. The nanofiltration conditions that maximized the rejection of different pharmaceutically active compounds (anticancer drugs and antibiotics) and minimized fouling formation when tested at pilot scale with real wastewater effluents were optimized with this pilot scale unit and described in previous studies (Cristovão et al., 2021, 2022).

The nanofiltration membrane used was a spiral wound Desal 5DK module (model DK4040F30, Suez membranes, Lenntech, Delfgauw, Netherlands), with a molecular weight cut-off between 150 and 300 Da and a filtration area of 7.9 m^2. The system was operated at a controlled transmembrane pressure of 6 bar and recovery rate of 73 % to minimize fouling (Cristovão et al., 2021, 2022).

Samples of feed (wastewater effluent), permeate and retentate were collected and analysed in terms of the presence of the physico-chemical compounds, inorganic constituents that influence plant growth, antibiotics as well as microorganisms as described above. The pilot scale treatment unit allows further treatment of the permeate or retentate streams using the low-pressure mercury lamp. When the permeate or retentate streams were subject to UV treatment using the low-pressure mercury lamp, a flow rate of 40 L/h was set, which corresponded to a hydraulic retention time of 6.5 min.

2.3. Spectral characterisation of raspberry leaves and fruits

To gain further insight into the physiological and biochemical status of the plants under the tested irrigation treatments, spectral reflectance of raspberry leaves and fruits was measured with a FieldSpec4 portable full-range spectroradiometer (ASD Inc. PANalytical Company, Boulder, USA). At fruit maturity stage (BBCH stage 87), reflectance spectra of a minimum of six leaves and fruits irrigated with the different water sources were recorded. Leaves and fruits were analysed with an ASD leaf clip accessory assembled on a standard ASD plant contact probe provided with a halogen bulb and coupled to the FieldSpec4 spectrometer with an optical fibre. Reflectance spectra and derivative-transformed reflectance spectra were obtained and used for further data analysis. Spectral reflectance indices were calculated according to Vergara-Diaz, et al. (2018). R 3.5.1 (R core team) was used for conducting and drawing the Principal Component analyses.

3. Results and discussion

3.1. Characterization of water sources used for the irrigation of raspberries

The four water sources used for the irrigation of raspberries were characterized in terms of different parameters: pH (ranging from 7.1 to 7.8), total solids, dissolved solids, suspended solids, chemical oxygen demand as well as inorganic constituents that influence plant growth: total nitrogen, ammonium, nitrate, total phosphorous and chloride (Figs. 2–5).

High levels of solids can clog irrigation devices, and, in extreme cases, the irrigated plant roots may lose water rather than gain it. In the four water sources used for irrigation, the total suspended solids (plotted in the secondary y-axis) constituted an extremely low percentage of the total solids (between 0.03 and 2 %). Most of the solids were dissolved solids, organic and inorganic molecules as well as ions present in the different matrices. As expected, greater variability in solids concentrations was observed in the wastewater effluent and reused effluent compared to the permeate and tap water sampled over the 5-month irrigation period, due to the lower level of treatment applied to these samples. The tap water samples presented a level of dissolved solids below 500 mg/L, which is considered desirable for drinking water since waters with high dissolved solids are generally of inferior palatability (Standard methods 2540). The average concentration of total suspended solids measured in the wastewater effluent (22±11 mg/L) and reused effluent (16±6 mg/L) were higher than the total suspended solids limit of 10 mg/L required for agriculture irrigation with reclaimed water of class A, but lower than the total suspended solids limit of 35 mg/L required for agriculture irrigation with reclaimed water of class C (EU 2020/741). Crops belonging to a given category should be irrigated with different qualities of reclaimed water. Class A should be used in all food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw, whereas class C should be used in food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals (EU 2020/741). In the case of the raspberry crops planted in this study, class C water quality limits should be considered, so the total suspended solids measured in the wastewater effluents comply with the minimum requirements for water reuse. However, for irrigation of lettuce for example, the wastewater effluent and reused effluent did not comply with the total suspended solids limit set for agriculture irrigation with reclaimed water of class A and require further treatment.

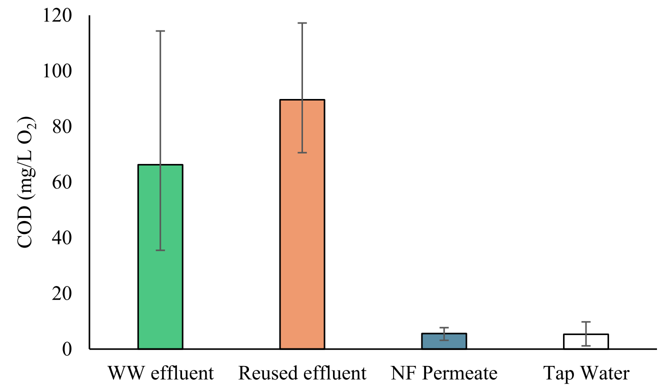

Fig. 3. Chemical oxygen demand (COD) in the water sources used for the irrigation of raspberries. The error bars represent the results from eight sampling events conducted during the 5-month irrigation period.

Additionally, the average levels of total suspended solids measured in the permeate (0.2 ± 0.1 mg/L) and tap water (0.5 ± 0.1 mg/L) samples were well below the reclaimed water class A quality requirement for agricultural irrigation (below 10 mg/L).

Fig. 3 presents the values of chemical oxygen demand in the water sources used for the irrigation of raspberries. This parameter is often used to characterize the organic matter content of wastewater and natural waters since it measures the oxygen equivalent of the organic matter that can be oxidized (Tchobanoglous and Burton, 1991). Organic matter can influence both soil and plant health, as well as affect the efficiency of the treatment processes evaluated in this study. In nanofiltration, it may cause membrane fouling, reducing performance. In UV photolysis, organic matter can have dual effects: it may either scavenge UV light, reducing treatment efficiency, or react with the light to produce highly reactive hydroxyl (•OH) radicals, potentially enhancing the degradation of contaminants.

As expected, the permeate and tap water samples contained much lower levels of chemically oxidized compounds due to their higher level of treatment. On the other hand, the higher levels of chemical oxygen demand in the reused effluent compared to the wastewater treatment can be explained since chlorine is a strong oxidizing agent (Abdullah and Rehaili, 1995). All of the water sources used for irrigation presented chemical oxygen demand values below 150 mg/L, that has been reported to pose no restriction to irrigation use (Alberta Environment, 2000).

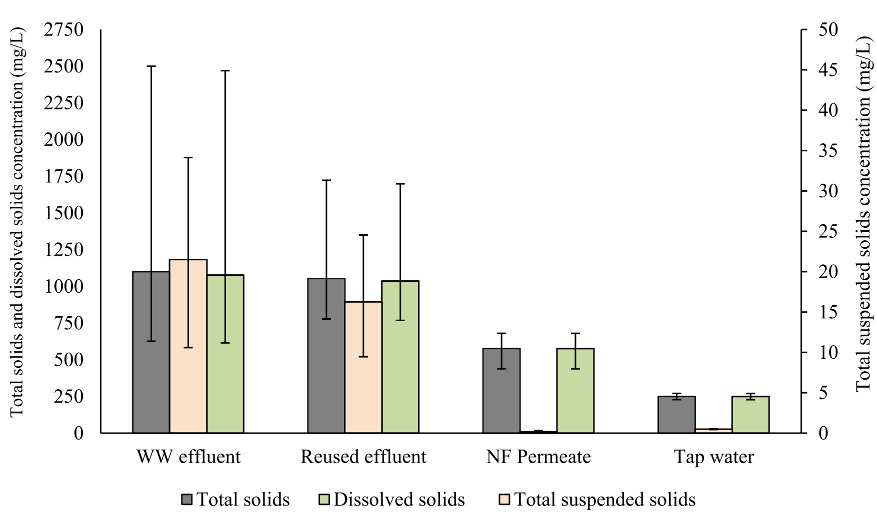

Fig. 2. Concentration of total solids, dissolved solids and suspended solids present in the water sources used for the irrigation of raspberries. The error bars represent the results from eight sampling events conducted during the 5-month irrigation period.

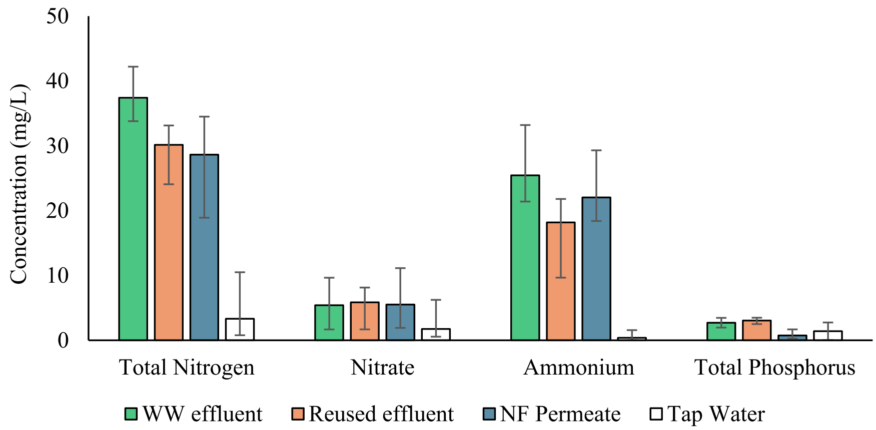

Fig. 4. Concentration of total nitrogen, nitrates, ammonium and total phosphorus in the water sources used for the irrigation of raspberries. The error bars represent the results from eight sampling events conducted during the 5-month irrigation period.

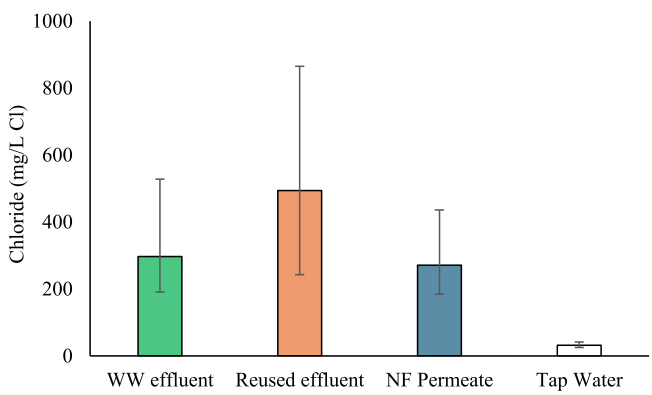

Fig. 5. Concentration of chloride in the water sources used for the irrigation of raspberries. The error bars represent the results from eight sampling events conducted during the 5-month irrigation period.

Elements such as nitrogen and phosphorus are essential to plant growth and productivity. Their presence in the water sources used for irrigation may decrease the need for fertilizer application. Fig. 4 presents the concentration of total nitrogen, nitrates, ammonium and total phosphorus measured in the different water sources used for the irrigation of raspberries.

The concentrations in Fig. 4 were determined as a function of nitrogen (mg/L as N) and used to calculate the percent of the different forms of nitrogen (Fig. S1 in supporting information). In the wastewater matrices used for irrigation (wastewater, reused and permeate effluents), ammonium accounted for 60 % to 72 % of the total nitrogen, nitrate for 14 % to 20 %, and the remaining was categorized as "other", which includes organic nitrogen and nitrite. In the tap water samples, nitrate made up 53 % of the total nitrogen, ammonium constituted 12 %, and the remaining 35 % was classified as "other".

Assuming that all the water used to fill the tanks was used for irrigation, the total mass of the nutrients that was added to the irrigation pots using the different water sources during the 5-month irrigation period can be determined and is presented in Fig. S2 (supporting information section). The reuse of wastewater effluents, even after being treated with extremely efficient treatment processes such as nanofiltration, can provide a source of nutrients important for plant growth and productivity, help reduce the need for chemical fertilizers, and enhance water use efficiency.

One nutrient of special concern is ammonia, due to its toxicity to aquatic organisms at high concentrations, with unionized ammonia being the most toxic form. The balance between unionized and ionized ammonia in total ammonia concentrations is largely determined by water quality factors, particularly pH. As pH increases, the proportion of ammonia in its unionized form also rises. Other stressors, such as ionic strength and temperature, can also impact ammonia toxicity by affecting the tolerance and overall toxicity of ammonia to aquatic life (U.S. EPA, 2023). In this sense, ammonia uptake by plants can be an effective method for wastewater treatment, as they remove part of the ammonia by acting as water detoxifiers, making it safer for aquatic organisms, while benefiting their growth and production potential.

As expected, a much lower chloride concentration was measured in the tap water samples compared to the different wastewater effluents (Fig. 5). Since chloride is a monovalent ion extremely similar concentrations of this anion were expected in the feed and the nanofiltration permeate samples. In the reused samples, the increased level of chloride can be explained since the utility disinfects the water with chlorine prior to the reuse of this effluent for street cleaning.

3.1.1. Occurrence of antibiotics in the different irrigation waters

The occurrence of the target antibiotics (ciprofloxacin, levofloxacin, meropenem and ampicillin) was monitored in the different water sources in eight sampling events (Fig. 6 and Fig. S3 in supporting information). Ampicillin was not detected in any of the samples collected. As expected, due to the treatment processes applied to produce the different water sources, different concentrations of the other antibiotics were detected in the wastewater, reused, and nanofiltration permeate effluents, as reflected by the different y-axis scales in Fig. 6.

As expected, in the wastewater effluent (Fig. 6a), higher concentrations of antibiotics were detected. Wastewater treatment has played a vital role in advancing public health and safeguarding the environment. However, with the growing diversity and volume of chemical use, conventional methods for treating wastewater and drinking water became increasingly inadequate in effectively removing a wide range of emerging contaminants (Kümmerer et al., 2018). The occurrence of antibiotics in wastewater effluents has been reported worldwide (e.g. Zhang and Li, 2011).

The concentration of ciprofloxacin ranged from 466 ng/L to 1679 ng/L, levofloxacin was detected at levels ranging from 181 ng/L to 330 ng/L, while the concentrations of meropenem measured in the wastewater effluent samples were much lower, ranging from not detected in six of the sampling events to 56 ng/L. The higher levels of ciprofloxacin and levofloxacin detected in the wastewater effluents can be attributed to their more frequent use and excretion into wastewater, while the lower detection of meropenem aligns with its reserved clinical application for serious infections in healthcare environments.

In the reused effluent (Fig. 6b), a lower concentration of antibiotics was detected compared to the wastewater effluent that can be explained since this effluent is further treated (by filtration and chlorination) prior to being reused for street cleaning. Ciprofloxacin levels measured in this

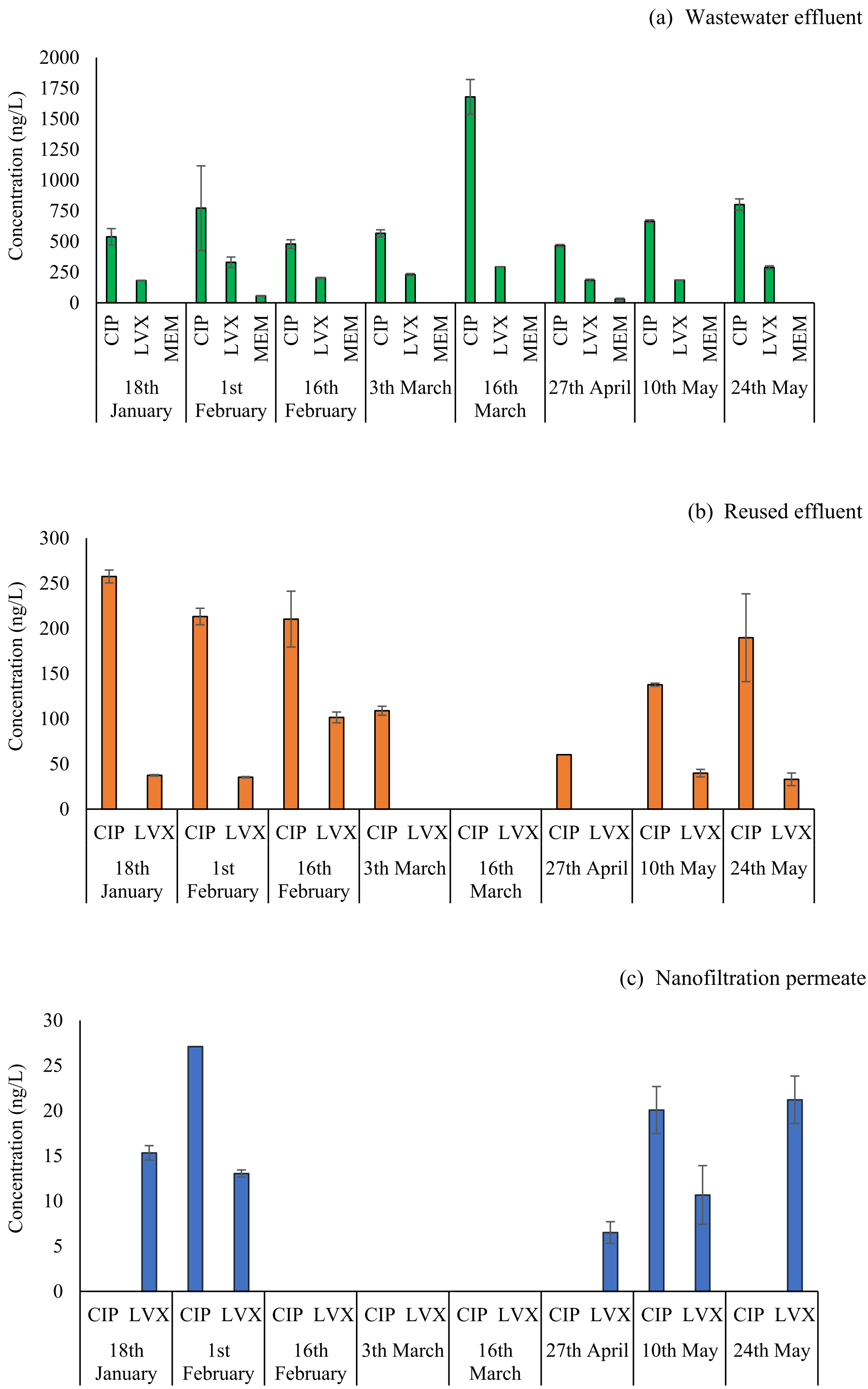

Fig. 6. Occurrence levels of antibiotics measured in the wastewater effluent (a), reused effluent (b) and nanofiltration permeate (c) samples. The error bars represent the results obtained from duplicate samples. CIP - ciprofloxacin, LVX - levofloxacin, MEM – meropenem.

water source ranged from not detected to 258 ng/L, levofloxacin concentrations ranged from not detected to 102 ng/L, and meropenem was not detected. Chlorine is commonly used in effluent treatment to eliminate pathogens, but it also reacts with chemical compounds (e.g. Dodd and Huang, 2007; Najjar et al., 2013), leading to their partial or complete degradation, which would explain the reduced concentrations of both ciprofloxacin and levofloxacin in the reused water samples.

As expected, due to the higher level of treatment, in the nanofiltration permeate samples, antibiotics were only detected in five of the sampling events in the permeate samples at much lower concentrations (Fig. 6c). In the permeate samples, that consisted of 1000 L of wastewater effluent further treated by nanofiltration, ciprofloxacin was detected at levels lower than 27 ng/L, and levofloxacin at levels lower than 21 ng/L. These results suggest that the Desal 5DK nanofiltration membrane is extremely effective at removing these antibiotics from water. To the best of our knowledge, ciprofloxacin and levofloxacin are not currently included in any binding legislation for water quality standards and, while ciprofloxacin was previously part of the EU Watch List for emerging pollutants, neither antibiotic is presently monitored under mandatory regulatory frameworks.

3.1.2. *Microbiological characterization of the different irrigation waters*

Fig. 7 presents the concentration of the target microorganisms (total coliforms, *E. coli*, enterococci, and *P. aeruginosa*) detected in the wastewater effluent samples.

The wastewater effluent samples did not meet the minimum requirements set for water quality in terms of *E. coli* to produce class A (≤10 number/100 mL) nor class C (≤1 000 number/100 mL) reclaimed water (EU 2020/741). Moreover, resistance to different antibiotics was detected in the wastewater effluent samples. The wastewater effluent sample contained total coliforms and *E. coli* strains resistant to one or more of the following antibiotics - ciprofloxacin, levofloxacin, streptomycin and ampicillin; enterococci resistant to ciprofloxacin, levofloxacin, and ampicillin; and *P. aeruginosa* resistant to ciprofloxacin and levofloxacin (Table S3 of the supporting information section). Further treatment would be required to reuse this effluent for irrigation.

Antibiotic-resistant bacteria and resistance genes have been detected in humans, animals, and the environment, with wastewater treatment plants being considered major recipients of these contaminants (Manaia, 2022; Teixeira et al., 2023). Despite the partial elimination during treatment, final effluents have been reported to contain bacteria and antibiotic-resistant bacteria, even after disinfection (Manaia, 2022). As an example, levels between 10^4 and 10^6 CFU/mL of heterotrophic bacteria and between 10^3 and 10^5 CFU/mL of presumptive coliforms have been reported in treated effluents after tertiary treatment (Guardabassi et al., 2002).

None of the target microorganisms were detected in the further treated reused effluent, permeate and tap water samples (<1 MPN/100 mL). The reused effluent and the tap water samples were disinfected using chlorination, which is known for its broad-spectrum antimicrobial activity, making it a widely adopted method for ensuring the

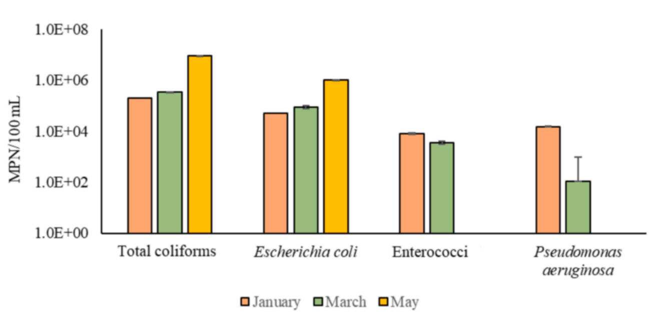

Fig. 7. Occurrence of the target bacteria in the wastewater effluents used for irrigation. The error bars represent the results obtained from duplicate samples.

microbiological safety of water. Even though there is limited information on the long-term effects of using chlorinated water for irrigation, chlorine has been linked to toxic effects on plant growth (Banach and Van Der Fels-Klerx, 2020). Lettuce plants grown in a deep-water culture hydroponic system with concentrations of chlorine higher than 0.5 mg/L exhibited different degrees of phytotoxic symptoms (McGehee and Raudales, 2023). Discoloration, necrosis, and reduced growth of leaves increased in lettuce plants treated with chlorine doses higher than 1 mg/L. Additionally, when irrigation water contains high levels of organic matter, as is typical of wastewater effluents, chlorination should be avoided due to the potential formation of hazardous disinfection by-products. Nanofiltration, on the other hand, is a physical process that improves the wastewater effluent quality without the addition of chemical reagents that may negatively influence plant growth and lead to the production of disinfection by-products that have been linked to adverse health effects. Besides the total elimination of the target bacteria in this study, nanofiltration using the same Desal-5DK membrane has been previously reported to ensure high removals of antibiotic resistance genes and viral genomes from wastewater effluents (Cristóvão et al., 2021).

3.2. *Treatment of wastewater effluents using nanofiltration and low pressure UV photolysis*

Fig. 8 presents the average rejection values obtained in eight sampling events of the permeate samples. These samples were obtained every 15 days, during the 5 month irrigation period, 1000 L of the collected wastewater effluents were further treated by nanofiltration using a spiral wound Desal 5DK module operated at a controlled transmembrane pressure of 6 bar and recovery rate of 73 % (Cristovão et al., 2021, 2022).

The rejection results obtained - defined as the percentage of a compound retained by the membrane - were expected to be small for low molecular mass and monovalent ions and high for total suspended solids, total phosphorus, chemical oxygen demand as well as of all the target microorganisms (none was detected in the permeate samples at levels above 1 MPN/100 mL). Additionally, high rejections were obtained for the antibiotics detected in the wastewater effluent (ciprofloxacin and levofloxacin). Rejections higher than 94 % were obtained for the target antibiotics. When the same membrane (Desal 5DK) and pilot scale installation was tested at a wastewater treatment facility, high rejections of ciprofloxacin and levofloxacin (the antibiotics were not detected in the permeate samples), carbapenem and fluoroquinolone resistance genes (rejections higher than 99.6 %), as well as virus (rejections higher than 41 %) were reported (Cristovão et al., 2022). Hence, this treatment was considered to be a promising treatment approach to cope with chemical and biological contaminants present in wastewater effluents and tested in this study to produce a high quality water used for irrigation. Similar high rejections were also reported by Dolar et al. (2011) for ciprofloxacin using other nanofiltration membranes, NF270 and NF90.

Besides the nanofiltration membrane, the pilot treatment unit was also equipped with a low pressure mercury lamp and valves that allowed testing the direct photolysis of the permeate or retentate streams, using an hydraulic retention time of 6.5 min.

None of the target microorganisms was detected in the permeate samples due to the high rejection of the microorganisms by the membranes so further treatment of the permeate samples could not be evaluated in terms of these parameters. In the sampling events in which ciprofloxacin and levofloxacin were detected in the permeate samples at extremely low concentrations (lower than 30 ng/L), after exposure to the UV photolysis, the antibiotics were not detected.

When UV photolysis treatment of a highly challenging matrix, the retentate stream, was also tested, the results exceeded expectations. The retentate samples, as expected, contained high levels of microorganisms, natural organic matter and solids. Particles and natural organic matter

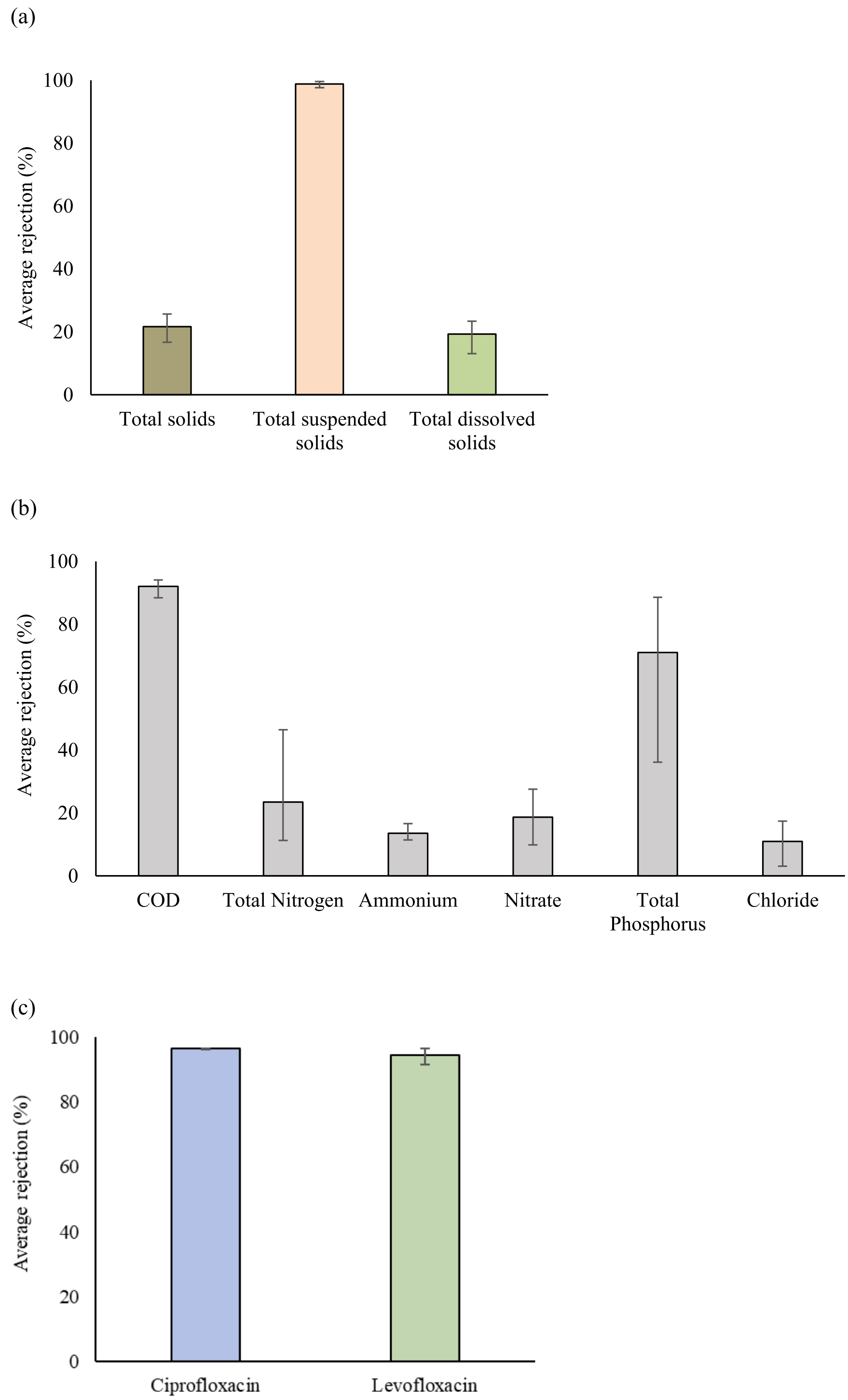

Fig. 8. Average rejection of solids (a), chemical oxygen demand (COD), total nitrogen, ammonium, nitrate, total phosphorus and chloride (b), and antibiotics (c) detected in the feed source using the pilot scale nanofiltration treatment process used to produce permeate water for irrigation. The error bars represent the results from eight sampling events conducted during the 5-month irrigation period.

significantly affect UV photolysis, as they can create a shielding effect or absorb UV light, preventing it from reaching its intended target and reducing the overall effectiveness of the process.

Fig. 9 shows some level of degradation of ciprofloxacin (57 %±0.4 %) and levofloxacin (22 %±9 %) despite the challenging retentate matrix tested (with high levels of natural organic matter and solids), the low exposure time and the low absorbance of ciprofloxacin and levofloxacin at 254 nm (Czyrski, 2022). The maximum absorbance wavelengths range from 271 nm to 277 nm for ciprofloxacin and from 287 nm to 295 nm for levofloxacin. To increase the removal of these compounds in the retentate matrices, advanced oxidation processes could be tested in future studies that could address the removal of a wide range of chemical compounds rejected by the membranes. However, if advanced oxidation processes are considered, the possibility of toxic by-product formation should also be evaluated due to the high levels of organic matter in the retentate matrices.

Despite the highly challenging matrix tested, extremely high inactivation of the target microorganisms and antibiotic-resistant bacteria was

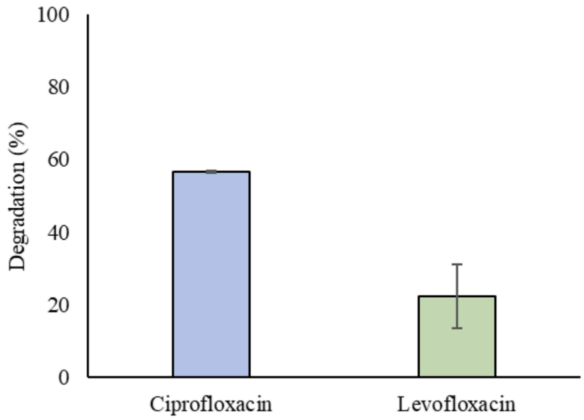

Fig. 9. Degradation by low pressure UV direct photolysis of ciprofloxacin and levofloxacin in the retentate samples. The error bars represent the results obtained from duplicate samples.

obtained when low pressure UV photolysis was tested to inactivate the retentate samples (Fig. 10). The lower inactivation percentage observed for *P. aeruginosa* compared to other bacteria may be due to its ability to form aggregates and biofilms as well as to produce extracellular polymeric substances (Wang et al., 2012; Xue et al., 2013), which offer protection from disinfection. In the retentate matrix, the high levels of natural organic matter and particles may provide additional support for the aggregates compared to cleaner matrices.

Low-pressure UV photolysis proved to be highly effective in treating the target bacteria and antibiotic-resistant bacteria present in the concentrated retentate, providing a solution to address one of the key challenges of membrane processes—the production of a highly concentrated retentate.

3.3. Analysis of antibiotics in raspberries and leaf samples

During the two-month harvesting period, approximately 1 kg of raspberries was collected from each irrigation line once a week to monitor the potential contaminant uptake in the fruits over time.

In addition to the fruits, leaves were collected during the final harvest to assess the potential presence of contaminants in the foliage, providing insight into possible accumulation in edible plants irrigated under similar conditions.

The potential uptake of the target contaminants by the raspberries was evaluated by analyzing samples collected on different days throughout the harvest. None of the target antibiotics were detected in either the fresh fruits or the leaves at levels exceeding 10 μg/kg. This suggests that the raspberries and leaves did not accumulate the target antibiotics to a significant extent during the irrigation period.

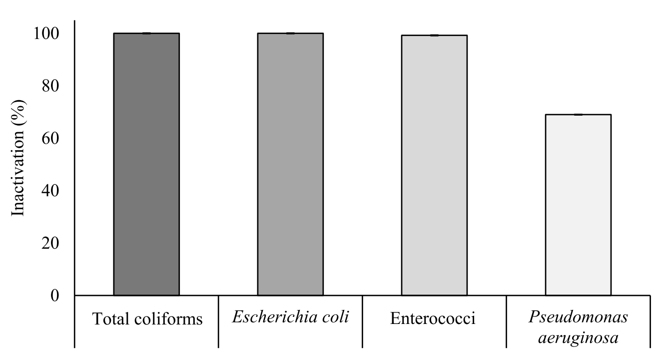

Fig. 10. Low pressure UV inactivation of the target microorganisms in the retentate samples. The error bars represent the results obtained from duplicate samples.

The method of irrigation, where water was applied directly to the substrate rather than to the top of the plant, may have minimized the exposure of the plant to these potential contaminants. It is important to note that different results could be obtained with crops that are irrigated on top, as direct exposure to irrigation water on the leaves or fruits could lead to higher levels of contaminant uptake. For example, Ferro et al. (2015) reported that after 5 weeks of irrigating lettuce crops with treated wastewater, carbamazepine and thiabendazole accumulated in the soil at concentrations of up to 472 ng/g and 256 ng/g, respectively, and were detected in lettuce at up to 109 ng/g and 18 ng/g, respectively. Further studies focusing on different types of irrigation systems would help clarify how different watering methods affect the accumulation of contaminants in various crops.

Moreover, the internalization of other possible contaminants that may be present in wastewater effluents such as heavy metals should also be considered in future studies.

3.4. In-vivo plant spectral assessments

The spectral characteristics of leaves and fruits were analyzed using principal component analysis (PCA) on a set of spectral indices, which describe the composition and overall condition of the plant organs (Fig. 11). The PCA of leaf data explained up to 66 % of the variability and grouped together plants irrigated with reused and nanofiltered water, while those irrigated with tap water and wastewater effluent showed slightly different responses, with their centroids, representing the average spectral performance of the tested treatments, exhibiting distinct spatial separation. In general, plants irrigated with reused and nanofiltered water showed higher signal for pigment content (chlorophylls, anthocyanins and carotenoids) and nitrogen, whereas the plants irrigated with tap water and wastewater effluent appeared to show higher signal for water and biomass spectral indices. For the fruits, PCA accounted for approximately 77 % of the spectral data variability, showing substantial overlap among the treatment distributions. Plants irrigated with reused and tap water exhibited intermediate spectral values, whereas wastewater effluent irrigation was associated with increased anthocyanin and carotenoid signals. In contrast, fruits from plants irrigated with nanofiltered water showed higher moisture content and stronger flavonol signals.

Although based on a limited number of measurements, these results provide clear evidence that both the origin and treatment of irrigation water influence the biochemical composition of plants. Notably, the findings suggest a potential positive impact on plant health, growth potential, and fruit nutritional quality under the use of permeate effluent irrigation, as inferred from increased plant spectral signal in biomass, pigment and antioxidant indices. These aspects should be examined in more detail in subsequent studies.

4. Conclusions

In this study, four different water sources—three different wastewater effluents (one effluent discharged into the river, another that undergoes additional filtration and chlorination before being reused for street cleaning, and a third permeate from a nanofiltration-treated wastewater effluent), as well as tap water—were used to irrigate raspberries and characterized. Among the four sources tested, only the untreated wastewater effluent did not meet the minimum water quality requirements for producing reclaimed water, as outlined in EU 2020/741.

The wastewater effluent was further treated by nanofiltration under optimized conditions, which minimized fouling while maximizing water production and the rejection of contaminants. This treatment resulted in a rejection higher than 94% of both the target antibiotics and antibiotic-resistant bacteria, demonstrating that nanofiltration can effectively reduce these contaminants from wastewater effluents and produce water suitable for agricultural irrigation while leaving in the wastewater

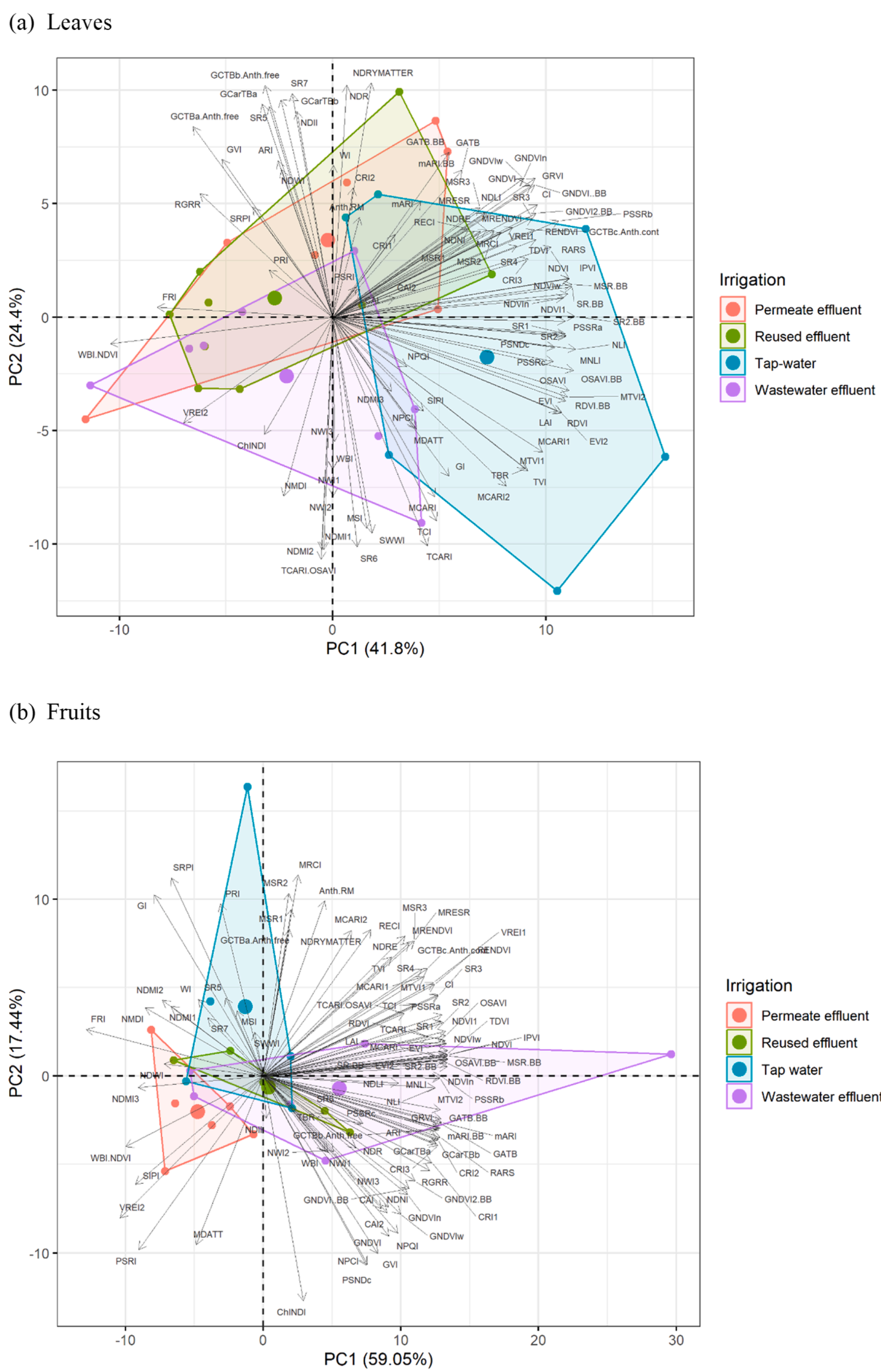

Fig. 11. Principal component analysis (PCA) of spectral reflectance indices from raspberry leaves (a) and fruits (b). Data points are grouped by irrigation treatment, as indicated in the legend.

effluent nutrients important for plant growth and productivity.

Additionally, low-pressure UV photolysis was highly effective in treating the target bacteria in the concentrated retentate, further enhancing the overall treatment process. The integration of nanofiltration for effluent treatment and UV photolysis for retentate management presents a promising and sustainable approach, ensuring high-quality permeate suitable for agricultural reuse while enabling safe and effective disposal of the concentrated waste stream.

The fresh raspberries and leaves irrigated with the different waters (including the wastewater effluent) showed no detectable levels of the target antibiotics above 10 μg/kg, indicating that the antibiotics did not accumulate to significant levels in the plants. The irrigation method used likely limited plant exposure to contaminants, highlighting the need for further research on irrigation methods as well as the potential uptake of other pollutants not monitored in this work such as heavy metals.

These findings support the use of nanofiltration and UV treatment as promising methods for treating wastewater effluents, ensuring the removal of harmful contaminants and the production of high quality wastewater that can be reused for agricultural irrigation.

CRediT authorship contribution statement

Maria Beatriz Cristóvão: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **João Sério:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Ana Paula Marques:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Andreia Bento-Silva:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Maria Gossard:** Methodology, Investigation, Formal analysis. **Omar Vergara-Diaz:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Ana Filipa Silva:** Funding acquisition. **Mónica Nunes:** Investigation, Project administration. **Maria Rosário Bronze:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Pedro Brás de Oliveira:** Writing – review & editing, Writing – original draft, Investigation. **Maria Teresa Barreto Crespo:** Writing – review & editing, Validation, Resources, Methodology, Investigation. **João Goulão Crespo:** Writing – review & editing, Validation, Resources, Methodology. **Vanessa Jorge Pereira:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2025.124782.

Data availability

Data will be made available on request.

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