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Reuse of Treated Wastewater for Crop Irrigation: Water Suitability, Fertilization Potential, and Impact on Selected Soil Physicochemical Properties

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Abstract: This study evaluates the suitability of treated wastewater (TWW: secondary effluent and membrane effluent) for crop irrigation and the resultant impact on crop growth and soil physicochemical characteristics. Carrot seeds (*Daucus carota* subsp. *sativus*) were grown on loam soil and irrigated with tap water (Tap), secondary effluent (SE), and membrane effluent (ME) until maturity. Bacteriological analyses showed four log counts of *E. coli* and thermotolerant coliforms for secondary effluent, making it unsafe for the irrigation of carrots. Tap water and membrane effluent fulfilled the microbial limit for water reuse and were suitable for irrigation. The sodium absorption ratio, Kelly index, and magnesium hazard assessments indicated that all three irrigation water streams were suitable for irrigation. The average mass of carrot fruits for Tap, SE, and ME was 2.14 g, 3.96 g, and 3.03 g, respectively. A similar trend was observed for the dry matter composition: Tap had 15.9%, SE had 18.3%, and ME had 16.6%. The soil pH increased from 7.08 to 7.26, 7.39, and 7.33 for tap water-, secondary effluent-, and membrane effluent-irrigated soils, respectively. Nitrate-nitrogen and potassium levels increased in the TWW-irrigated soil, while that of the tap water-irrigated soil decreased. Sodium levels in the TWW-irrigated soil increased significantly but did not induce soil sodicity. The application of the TWW enhanced the growth of the carrot plants and increased the soil nutrient levels. Hence, using TWW in agricultural irrigation could promote food production and also limit the overdependency on freshwater resources. However, TWW should be disinfected by using UV disinfection and ozonation to reduce the risk of microbial contamination. Such disinfection methods may not lead to the formation of toxic byproducts, and therefore secondary pollution to crops is not anticipated.

Keywords: wastewater treatment; water reuse; irrigation; sodium adsorption ratio; crop growth; biomass production

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1. Introduction

Water is considered an essential natural commodity that is required for the development of life on Earth [1]. Its (freshwater) availability has been acknowledged as a critical environmental control factor for driving anthropogenic activities to maintain socioeconomic strategies [2]. However, freshwater availability is becoming scarce, especially in arid regions of the globe [3]. Global warming, climate change, uneven water resource distribution, and high population growth have led to this water scarcity [2,4,5]. According to estimates, 2.4 billion people currently reside in water-scarce basins because of climate change, and predictions show that this deficit will grow due to the high sensitivity of water scarcity to climate change patterns [6–8]. The agriculture sector is most likely to be the sector that will be affected the most due to its high dependency on water resources. Water scarcity has been identified as the principal constraint to the sustainability of agriculture production [5].

The world's population is projected to reach approximately 10 billion in 2050, which will require an increase of more than 60% in agricultural production (from the 2005 production baseline) [9,10]. The increased demand for food will translate into higher agricultural water requirements, especially for the irrigation of food crops [4]. The continuous reliance on rainfed agriculture or/and the irrigation of crops by conventional water sources, such as rivers, streams, lakes, groundwater, springs, etc., may not be sustainable. An alternative nonconventional water source for crop irrigation is essential for the sustainability of agricultural production. Treated wastewater (TWW) or recycled water has emerged as a viable alternative nonconventional water source for crop irrigation, particularly in countries experiencing water stress [3]. TWW could supply important nutrients to crops, improve soil fertility, and improve soil physical and chemical properties to promote plant growth and crop production, as well as reduce farmers' dependency on commercial fertilizers [1,11]. Additionally, TWW has the potential to remediate and restore degraded and contaminated sandy soils [12–14]. The use of TWW for irrigation could come with some demerits, such as soil salinization, the risk of heavy metals, and the microbial contamination of food crops.

The irrigation of crops with TWW has been studied by several authors under different climatic conditions. Studies have involved crops like alfalfa, radish, wheat, maize, citrus, acacias, eucalyptus, cotton, and vegetable crops [1,15]. Al-Lahham et al. [16] studied the impact of TWW irrigation on the quality attributes of tomato fruits. The tomato fruits increased in diameter by 2 cm and in weight by up to 78.8 g. The authors concluded that TWW could be an alternative water source for irrigating tomatoes and care should be taken to avoid bacteriological contamination. In another study, the authors found that the eggplant yield under TWW irrigation was twice that of the yield under freshwater irrigation [17]. Other works involving barley and maize plants all recorded higher dry matter content and yields (respectively) under wastewater irrigation [18,19]. The increase in the fruit diameter, weight, length, and yield is due to the fertilization effect of TWW, since it is a reservoir of nutrients, such as nitrogen, phosphorus, and potassium [11].

One vegetable crop that has gained attention under TWW irrigation is carrot (*Daucus carota* subsp. *sativus*). Carrot is a root vegetable belonging to the *apiaceae* family and is the most important crop of the family [20]. Several studies involving the irrigation of carrot plants with TWW have been conducted. However, most of the studies focused on health and environmental risks, such as bacteriological contamination, pharmaceutical compound uptake and accumulation, and heavy metal bioaccumulation and bioaccessibility [15,21–25]. Few works have focused on the impact of the TWW on the physiological and morphological traits of the carrot, and the suitability assessment of TWW using water quality criteria [15,24,25]. The suitability of irrigation water is key to the effective growth of crops and the sustainability of arable lands. Higher levels of exchangeable sodium in irrigation water could increase the soil salinity and reduce the soil permeability, thereby affecting crops' nutrient uptake and osmotic activities. Elevated levels of magnesium, on the other hand, could cause the deterioration of the soil structure and quality, thereby affecting crop production [26]. Also, the suitability of irrigation water is key to protecting public health. Bacteriological characteristics or quality is one of the most important parameters in the evaluation of the suitability of TWW for crop irrigation. The presence of pathogenic microorganisms could pose serious health risks to farm employees and consumers through the inhalation and ingestion of these pathogenic organisms [27]. Several guidelines and legislations have been enacted globally to ensure public safety [28–30]. Notable among them are the World Health Organization Guidelines for the Safe Use of Wastewater, Excreta and Grey Water [29], the California Water Recycling Criteria [30], the United States Environmental Protection Agency Guidelines on Water Reuse [31], and the European Union Regulation 2020/741 on the minimum requirements for water reuse [32]. The latter mandates member countries across Europe to evaluate the microbial quality of TWW before application for reuse in agriculture.

In this study, we hypothesize that TWW is suitable for the irrigation of crops (carrots) and will not adversely impact the physicochemical properties of the soil. The objectives are, therefore, (i) to assess the suitability of TWW (secondary effluent and membrane effluent) for crop irrigation using irrigation water quality assessment criteria, such as the sodium adsorption ratio (SAR), Kelly index (KI), and magnesium hazard (MH); (ii) to evaluate the bacteriological quality of TWW; (iii) to investigate the variation in the fertilization potential of TWW and tap water; (iv) to evaluate the impact of TWW on some selected physicochemical properties of the irrigated soil. To the best of our knowledge, studies incorporating SAR, KI, and MH in the evaluation of treated wastewater suitability for irrigation is scarce. These techniques are usually used in groundwater and surface water quality assessment. This study contributes to closing the knowledge gap on wastewater reuse for irrigation. The outcome of this study will be very useful for the promotion and implementation of treated wastewater reuse for irrigation.

2. Materials and Methods

2.1. Experimental Design

This study was conducted in a greenhouse facility at the Czech University of Life Sciences, Czech Republic. It involved irrigating carrot plants (*Daucus carota* subsp. *sativus*) with two different streams of TWW and tap water (control). The TWW irrigation streams consisted of secondary effluent and membrane effluent. Crop treatments were made up of secondary effluent-irrigated carrots (SE), membrane effluent-irrigated carrots (ME), and tap water-irrigated carrots (Tap). Tap water-irrigated carrots (Tap) represented the control group. Carrot seeds were purchased from a commercial supermarket and sown directly into soil contained in pots. Each pot had a volume of 0.0034 m^3 (3.4 L) and was filled with soil to about 85% of the volume. The soil was classified as loam, composed of clay (14.0%), silt (37.2%), and sand (48.7%) fractions. The pots were placed on a greenhouse growth bench and under a Growth Spectrum Advanced 600 W lamp (GIB Lighting, Berlin, Germany) with a 12 h daytime setting. After 66 days, the time setting was changed to a 9 h daytime setting. This was to reduce the high evaporation rate of water from the soil due to the heat from the lamp. The lamp has a nominal power of 600 W, a light intensity of 48,000 lm, a color temperature of 8000 K, and a photon flux (100 h) of 740 µmols/s. Each pot was adequately spaced from the other to ensure the effective circulation of air to the plants.

On average, a once-a-day irrigation regime was employed to ensure an adequate supply of water due to the high evaporation rate caused by the lamp. The mean temperature and relative humidity in the greenhouse were 26 °C and 35%, respectively. Sprinkling of water on the greenhouse floor was regularly performed to reduce the temperature and increase the humidity. Throughout the experiment, no fertilizer, manure, compost, or any other form of soil amendment was applied. The sowing of the seeds occurred in September 2021, and the matured carrots were harvested in February of the following year.

2.2. Irrigation Water Streams

Secondary effluent was obtained from a municipal wastewater treatment plant (WWTP) in Prague, which operates a conventional activated sludge system. The treatment tray consists of screening, grit removal, primary sedimentation, biological removal of nutrients and organics, and secondary sedimentation. Influent to the WWTP was initially treated using physical and mechanical processes. The partially treated wastewater was treated further using an activated sludge process to remove nutrients and organics. After this stage, the effluent was sent to the secondary clarifier, and afterwards discharged as secondary effluent. An amount of 0.02 m^3 of the secondary effluent was collected and stored in a reservoir for irrigation. Another 0.02 m^3 of secondary effluent was collected and post-treated with a laboratory-scale submersible hollow fiber ultrafiltration membrane (Figure 1). The membrane treatments were performed in batches using ZeeWeed-1, a polyvinylidene difluoride membrane from GE Water (Oakville, ON, Canada). The module had a nominal membrane surface area of 0.093 m^2 and a maximum transmembrane pressure of 62 kPa (manufacturer's manual). Effluent from the post-treatment was referred to as membrane effluent. Tap water was collected from the Biological Wastewater Treatment Laboratory (University of Chemistry and Technology-Prague) into a 0.01 m^3 reservoir and used for irrigation. All the irrigation water streams were frozen to minimize changes in the water quality and applied in batches to irrigate the carrot plants.

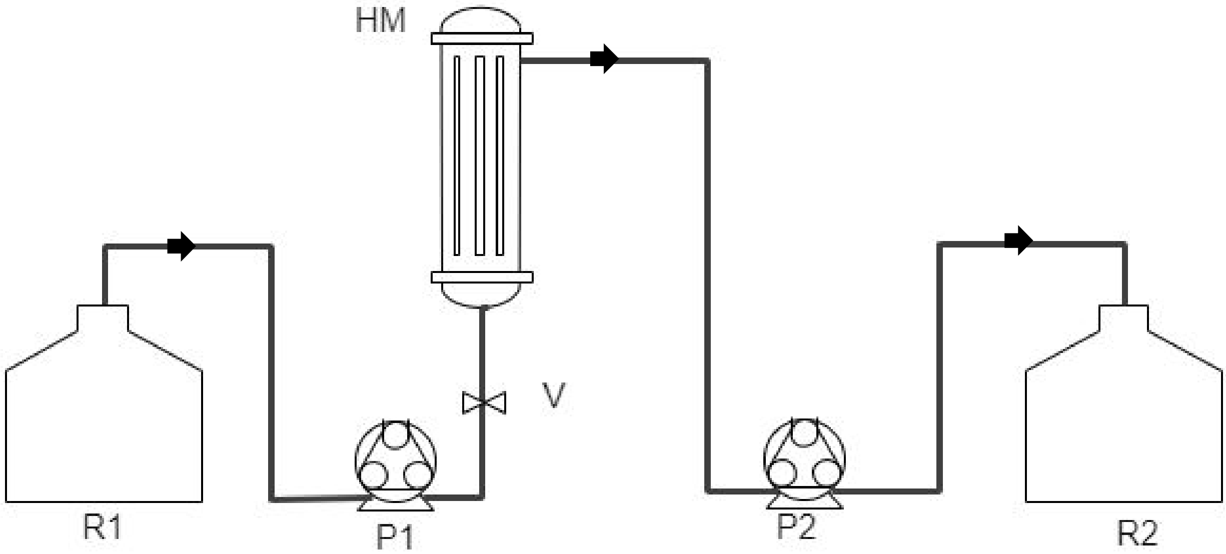

Figure 1. Schematic diagram of the ultrafiltration membrane laboratory set-up for the further treatment of the secondary effluent into the membrane effluent. R1 is the feed reservoir (secondary effluent); R2 is the permeate reservoir (membrane effluent); HM is the hollow tube ultrafiltration membrane; P1 and P2 are peristaltic pumps; V is a valve.

2.3. Determination of the Physicochemical Characteristics of the Different Irrigation Water Streams

The physicochemical quality of the irrigation streams was analyzed using Thermo Fischer's Gallery Analyzer (Thermo Fischer Scientific Inc., Vantaa, Finland), except for the total suspended solids (TSS), chemical oxygen demand (COD), and total nitrogen. Water samples were collected into new and clean cuvettes, inserted into the sample rack, and placed inside the Gallery analyzer for the analyses. The limit of detection for the methods of phosphate (PO_4), magnesium (Mg), chloride (Cl), and calcium (Ca) was 3.6 µg/L, 1.5 mg/L, 0.35 mg/L, and 1.16 mg/L, respectively. The TSS was determined by the gravimetric method at 105 °C and the COD was determined by the colorimetry method [33]. The COD was measured by using a photoLab 7100 Vis series spectrophotometer (WTW GmbH, Wellheim, Germany) at a limit detection of 10 mg/L. Total nitrogen was analyzed photometrically using a spectroquant nitrogen cell test kit (Merck, Darmstadt, Germany). The determination followed the manufacturer's protocol, which was analogous to ISO and DIN standards. The test kit had a measurement range of 0.5–15 mg/L.

Analyses of potentially toxic elements (lead (Pb), zinc (Zn), cadmium (Cd), essential metals (sodium (Na), potassium (K)), and boron (B)) were performed using atomic absorption spectroscopy (AAS). The limit of detection for all the analytes was 0.01 mg/L, except Pb, which was 0.05 mg/L. Water samples for such analyses were collected in clean plastic containers, preserved ($pH < 2$) with nitric acid, and refrigerated until the measurement. For each 0.02 m^3 of irrigation water (irrigation cycle), the water quality test was performed twice, before the start of the irrigation and in the latter part of the cycle, except for the boron and the metals. The physical and chemical quality characteristics of the different irrigation water streams are presented in Table 1.

2.4. Determination of the Irrigation Water Quality Characteristics according to Salt and Ion Toxicity

The evaluation of the suitability of the different irrigation water streams for irrigation was performed using the sodium absorption ratio (SAR), Kelly index (KI), and magnesium hazard (MH). These indicators provide vital information on the potential risk of sodium and magnesium hazards posed by irrigation water to soil and crops. Below are the equations of the indicators, which were adopted from [34]:

$$SAR = \frac{Na^{+}}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}} \quad (1)$$

$$KI = \frac{Na^{+}}{Ca^{2+} + Mg^{2+}} \quad (2)$$

$$MH = \frac{Mg^{2+} \times 100}{Ca^{2+} + Mg^{2+}} \quad (3)$$

The ions Na, Ca, and Mg are very essential for the evaluation of the suitability of water for irrigation. Sodium ions could induce plant toxicity and reduce soil permeability, while Ca and Mg could reduce the crop yield by making the soil alkaline [35]. The FAO guideline stipulates a limit of >3 meq/L for Na. For Ca and Mg, the guideline provides the usual range of these ions in irrigation water as $\leq$20 meq/L and $\leq$5 meq/L, respectively [28].

2.5. Determination of the Microbiological Quality of the Irrigation Water Streams

Water samples were collected into sterilized bottles for the determination of the presence of total coliforms, thermotolerant coliform, *Escherichia coliform* (*E. coli*), and *Clostridium perfringens*. The determination of total coliform bacteria was performed according to the Czech Standard. An amount of 100 cm^3 of the diluted water sample was filtered through a 0.45 µm membrane filter. The membrane filter was transferred to a selective culture agar medium containing lactose (Endo agar) in a Petri dish. The Petri dish containing the membrane filter was placed upside down in a thermostat and cultured at a temperature of 36 °C for about 18–24 h. The filter was then transferred from the Petri dish to a piece of filter paper saturated with cytochrome oxidase solution. After 2 min, the colonies of coliform bacteria formed were counted [36,37].

The detection of *Clostridium perfringens* in the irrigation water streams followed the Czech Decree 252/2004. An amount of 100 cm^3 of the diluted water sample was filtered through a membrane filter and the filter was transferred to a solid m-CP agar medium. Cultivation was then carried out in an anaerobic environment at 44 °C for about 21 h. After cultivation, the grown colonies, yellow in color, were counted. The colonies were exposed to ammonia vapor for 20–30 s. A change in the color from yellow to pink to red was observed, confirming the colonies as *Clostridium perfringens* [38,39].

In the evaluation of the presence of thermotolerant coliform and *E. coli* in the irrigation water, 100 cm^3 of the diluted water sample was filtered with a 0.45 µm membrane filter. The filter was then transferred to m-FC agar in a Petri dish. Cultivation of the bacteria occurred at a temperature of 44 °C for 18–24 h, after which the number of thermotolerant coliform bacteria colonies formed was counted. The membrane filter was later transferred to a culture mat saturated with liquid culture medium for 2–4 h in the dark at a temperature of 36 °C. After this step, the filter was visualized under a UV lamp at 360 nm, and the fluorescent colonies were counted. The method of detection was based on the Czech Standard ČSN 75 7835 [40,41].

Table 1. Physicochemical analyses of the three streams of irrigation water (tap water, secondary effluent, and membrane effluent) in comparison with the Food and Agriculture Organization (FAO) guidelines on irrigation water quality.

Irrigation Water Quality Parameters	Tap Water	Secondary Effluent	Membrane Effluent	FAO Irrigation Water Quality Guidelines [28]
pH	7.68 ± 0.69	7.90 ± 0.53	8.01± 0.43	6.5–8.4
TSS (mg/L)	0.14 ± 0.06	4.33 ± 0.67	0.29 ± 0.14	≤10.0 **
Conductivity (dS/m)	0.37 ± 0.14	0.74 ± 0.10	0.72 ± 0.13	<0.7
Alkalinity (mg/L $CaCO_3$)	77.84 ± 8.62	120.76 ± 24.44	118.12 ± 20.20	n.p
Total Nitrogen (mg/L)	4.12 ± 1.00	12.27 ± 0.50	12.57 ± 1.17	n.p
CODcr (mg/L)	<20	<20	<20	n.p
NO_3-N (mg/L)	3.36 ± 0.57	9.00 ± 2.01	8.96 ± 2.33	<5.0
PO_4 (mg/L)	0.08 ± 0.02	0.72 ± 0.12	0.77 ± 0.19	n.p
B (mg/L)	0.02 ± 0.003	0.07 ± 0.002	0.07 ± 0.001	<0.7
Ca (mg/L)	58.92 ± 4.64	79.89 ± 12.25	79.41±10.37	n.p
Mg (mg/L)	7.56 ± 0.40	14.77 ± 2.85	14.99 ± 2.59	n.p
Cl (meq/L)	0.74 ± 0.05	3.23 ± 0.72	3.24 ± 0.74	<4.0
Pb (mg/L)	<0.05	<0.05	<0.05	5.0
Zn (mg/L)	0.04 ± 0.01	0.13 ± 0.04	0.12 ± 0.05	2.0
Cd (mg/L)	<0.01	<0.01	<0.01	0.01
K (mg/L)	4.97 ± 0.34	26.76 ± 2.72	27.86 ± 6.01	n.p
Na (meq/L)	0.75 ± 0.01	3.77 ± 0.32	3.80 ± 0.17	<3.0

Notes: Values are mean plus standard deviation (±); "meq/L" is milliequivalent per liter; "n.p" means the limit is not provided by the FAO guidelines; ** limit from EU Regulation 2020/741 on the minimum requirement for water reuse. Part of the data in the table are presented in another manuscript which is under review [42].

2.6. Determination of the Physical and Chemical Properties of Soil

Soil samples were air-dried and sieved with a 2 mm sieve. The pH was measured with a WTW Multi 9420 pH meter (WTW GmbH, Wellheim, Germany) in a 0.01 M calcium chloride solution ($CaCl_2$). A 2:1 (v/w) ratio of $CaCl_2$ solution to soil was used, following the procedure outlined by Motsara and Roy [43]. Soil extract for nitrate-nitrogen (NO_3-N), total nitrogen (Total-N), phosphate (PO_4), sulfate (SO_4), lead (Pb), zinc (Zn), cadmium (Cd), potassium (K), sodium (Na), and magnesium (Mg) analyses were obtained by using the same 0.01 M $CaCl_2$ extractant in a 10:1 (v/w) ratio. The soil samples were weighed, and a measured volume of the $CaCl_2$ solution was added. The mixture was shaken mechanically for about 2 h and then filtered with filter paper to obtain the extract. The extraction procedure was based on the protocol by Houba et al. [44] and Motsara and Roy [43]. The NO_3, PO_4, SO_4, and Mg were analyzed in the soil extract using Thermo Fischer's Gallery Analyzer (Thermo Fischer Scientific Inc., Vantaa, Finland), and the total nitrogen was measured by the spectroquant nitrogen cell test kit (Merck, Darmstadt, Germany). Soil extract analyses for Pb, Zn, Cd, K, and Na were preserved (pH < 2) with nitric acid and refrigerated below 4 °C. The measurements were performed using atomic absorption spectroscopy (Agilent 280FS AA, Agilent Technologies, Mulgrave, Australia) with the flame atomization technique. The flame type used was acetylene–air and the wavelengths were 217 nm, 213.9 nm, 228.8 nm, 766.5 nm, and 589 nm for Pb, Zn, Cd, K, and Na, respectively. The choice of extractant ($CaCl_2$) was to enable the extraction of the bioavailable fraction of the elements of interest.

The soil particle density was calculated from the ratio of the mass to the volume of the soil particles. The mass was determined by weighing the dry soil sample, whereas the volume was determined by calculating the mass and density of the water displaced by the soil sample in a 100 cm^3 pycnometer [45]. Four replicate tests were carried out and averaged as 2.44 g/cm^3 (standard deviation < 0.03).

The hydrometer method (ČSN EN ISO 17892-4) was used in determining the fraction of particles for fitting the particle size distribution curve. The density of the soil sample suspended in distilled water was measured with a calibrated hydrometer at predefined time intervals (30, 60, 120, 300, 900, 2700, 7200, 18,000, 97,200, and 172,800 s after the start of sedimentation). Temperature readings of the samples were simultaneously recorded with the hydrometer readings [46].

2.7. Measurement of Plant Height, Fresh Mass, and Dry Matter Content of Biomass

Plant height was measured against a white background using a measuring ruler. The measurement involved only the shoot (upper vegetative part), excluding the root or fruit. The determination of the fresh mass/weight and percentage dry matter content was based on the Organization for Economic Cooperation and Development guidelines (OECD) [47]. The edible part (fruit) was washed with distilled water several times to remove soil particles. It was then dried quickly with tissue paper and weighed to obtain the mass. Samples for the dry matter content determination were oven-dried at 70 °C for about 5 h after the initial mass was recorded. The difference in the mass of the dried sample and the fresh sample was used to compute the dry matter content using Equation (4).

$$\text{Percentage Dry Matter} = \frac{(C - A)}{(B - A)} \times 100\% \tag{4}$$

where A is the mass of the Petri dish; B is the mass of the fresh sample and Petri dish; C is the mass of the dried sample and Petri dish [47].

2.8. Statistical Analyses of Data

Statistical analyses and graphical representations of the obtained data were performed using Microsoft Excel 2019 and Statistica 13.5 by TIBCO Software Inc., Palo Alto, CA, USA. The test of significance of the means was performed by the Student *t*-test (paired) at a confidence interval of 95% ($p < 0.05$) for the investigated parameters, except for the fresh mass and dry matter composition. An ANOVA was used to analyze difference in the means of fresh mass at a 95% confidence level. Pearson correlation was used to evaluate the relationship between biomass mass and plant height for SE and Tap.

3. Results and Discussion

3.1. Evaluation of the Physicochemical Suitability of the Different Water Streams for Irrigation

The SAR results showed that all three irrigation water streams had values below the 15-value threshold limit (Table 2), classifying the TWW and tap water as suitable for irrigation. This implies that the use of these streams of water for irrigation may not pose a high risk of soil salinization. This was also confirmed by the KI, which classified all the irrigation water as acceptable. Water with a very high SAR (>26) and KI (≥1) may cause the deposition and accumulation of sodium ions in the soil, and therefore is considered unsuitable. The build-up of sodium in the soil could lead to the clogging of the soil pores and permeability problems. Such a phenomenon could adversely alter the uptake of nutrients and water by the crops [26]. The soil could become sodic due to the excess sodium ions, and negatively impact the physical, structural, and nutritional characteristics of the soil [48]. The SAR of the secondary effluent (2.34) and membrane effluent (2.36) were higher than the tap water (0.57) due to their relatively high sodium content (Table 1). The high sodium content is attributed to their source, which was municipal wastewater. Municipal wastewater is usually high in sodium, and the conventional biological treatment process may not be effective in removing sodium, thereby leading to the relatively high sodium content in the effluent. The works of Kalavrouziotis et al. [49] and Bedbabis et al. [50] reported sodium levels above 150 mg/L in the treated wastewater effluent. Notwithstanding, the use of the secondary effluent and the membrane effluent for crop irrigation may not have a significantly adverse effect on crop growth due to the low SAR value. This was evident during the experiment, where no negative impact was observed on the carrot plants, nor was the biomass production adversely affected.

Tap water had a very low KI value (0.26), indicating a lower sodium content and making it more suitable for irrigation. Irrigation water with a KI value of ≥1 is considered unsuitable because such waters contain excess sodium ions (salt) than magnesium and calcium, and therefore have the potential to transform alkaline soil into saline soil [26,34]. The KI values of the secondary effluent and membrane (0.92 and 0.93, respectively) were below the threshold limit of 1. This indicates that the TWW did not contain excess sodium ions and is suitable for irrigation. The level of sodium in the TWW (Table 1) was well within the usual range of 0–40 meq/L typical of irrigation water [28]. Both the SAR and KI analyses suggested that the TWW used in this study may not pose a high salinity or sodicity risk, and therefore could be used for agricultural irrigation. The pH of the tap water- and secondary and membrane effluent-irrigated soils were below 8.5, confirming no occurrence of soil sodicity [51].

Table 2. Irrigation water quality classification according to sodium absorption ratio (SAR), Kelly index (KI), and magnesium hazard (MH). Description of the quality classification was adopted from Gorfie et al. [52] and Mukherjee et al. [26].

Quality Criteria	Tap Water	Secondary Effluent	Membrane Effluent
SAR	0.57	2.34	2.36
Quality Classification	Suitable	Suitable	Suitable
KI	0.26	0.92	0.93
Quality Classification	Acceptable	Acceptable	Acceptable
MH (%)	11.37	15.6	15.88
Quality Classification	Suitable	Suitable	Suitable

Notes: The computation was performed using the mean concentrations of sodium, calcium, and magnesium from Table 1. The concentrations of sodium, magnesium, and calcium were converted to milliequivalent per liter (meq/L) for the SAR computation. For a full description of the water quality classification of the SAR, KI, and MH, refer to Gorfie et al. [52] and Mukherjee et al. [26].

The magnesium hazard (MH) assessment, which is a measure of excess calcium and magnesium in water, showed that the tap water and the TWW were all suitable for agricultural application. None of the irrigation water exhibited higher MH values. The MH of the tap water, secondary effluent, and membrane effluent were 11.37%, 15.60%, and 15.66% respectively, far below the threshold limit of 50% [52]. This implies that none of the irrigation water poses a risk of clay particle dispersion or decreasing the hydraulic conductivity of the soil [26]. The order of quality or suitability according to the SAR, KI, and MH assessments was tap water > secondary effluent > membrane effluent in all the cases.

However, the order is different when the quality of the water is considered from the point of view of the classical nutrient supply. In Table 1, both the secondary effluent and membrane effluent exhibited significantly ($p < 0.05$) higher nitrate-nitrogen, phosphate, and potassium content than the tap water. Therefore, the TWW is of higher quality and has a higher potential to supply nutrients for crop growth than tap water. This is evident in the higher biomass production of carrots that were irrigated with the TWW. Also, the relative increase in the nitrate-nitrogen, potassium, and magnesium content of the secondary effluent-irrigated soil and membrane effluent-irrigated soil attests to the fertilization potential of the TWW. No significant difference was observed between the fertilization potential of the secondary effluent and the membrane effluent.

The pH of the irrigation water ranged between 7.68 and 8.01 for the tap water and TWW (Table 1). Irrigation water with extreme acidity or alkalinity affects the bioavailability of nutrients, such as phosphates. At a high pH, the precipitation of phosphates occurs, while fixation occurs at a low pH [52,53]. The tap water, secondary effluent, and membrane effluent were slightly alkaline and did not contain excessive bicarbonates. All three irrigation water streams do not pose a high risk of turning nonalkaline soil into alkaline soil. With the appropriate irrigation management strategy, the reuse of the TWW over a longer period may not significantly alter the acidic or alkaline status of the soil. The pH of the secondary effluent (7.90) and membrane effluent (8.01) were well within the acceptable range of 6.5–8.4 [28,54]. Potentially toxic elements (PTEs), such as lead, zinc, and cadmium, were all within the acceptable limit stipulated by the Food and Agricultural Organization

(FAO) guidelines on irrigation water quality [28]. Except for nitrate-nitrogen and sodium, all the physicochemical quality limits provided in the FAO guidelines were fulfilled by the two streams of the TWW used in the present study. Generally, the TWW used in this study could therefore be considered suitable for agricultural irrigation from the point of view of the potential risk of soil salinity or sodicity and the promotion of crop growth. However, irrigation management practices, such as the periodic dilution of the TWW by freshwater, are recommended to prevent groundwater salinization by nitrates and soil salinization by salts.

3.2. Evaluation of the Bacteriological Suitability of the Different Irrigation Water Streams

None of the selected indicator microorganisms were detected in the tap water, as expected (Table 3). The tap water had a high purity and its quality was consistent with typical drinking water quality. It fulfilled the criteria set out in the EU guideline, Regulation (EU) 2020/741, on water reuse for irrigation. On the other hand, the two streams of the TWW showed varied bacteriological quality characteristics. Secondary effluent had a relatively high microbial load in the order of four logs for the total coliform, thermotolerant coliform, and *E. coli*, and three logs for *Clostridium perfringens*. The *E. coli* count per 100 mL of water sample was higher than the $\leq$10 CFU/100 mL limit stipulated in the EU Regulation (EU) 2020/741 [32]. Thermotolerant coliform counts were also several thousand higher than the recommended 0–200 CFU/100 mL count [29]. Secondary effluent is therefore not considered suitable for irrigating carrots and other edible crops, and poses considerable risk to human health. The membrane effluent had different bacteriological quality characteristics when compared to the secondary effluent. Just like tap water, *Clostridium perfringens* was not detected in the membrane effluent. Thermotolerant coliform and *E. coli* counts were below 10 CFU/100 mL, fulfilling the limit stated in the EU Regulation (EU) 2020/741. The membrane effluent is therefore suitable for the irrigation of carrot plants and other edible crops, and poses little or no risk to human health.

The significant difference observed between the two streams of the TWW was due to the extra treatment or polishing by the ultrafiltration membrane module. Even though both streams came from the same source, further treatment by the membrane led to the removal or filtering of the microorganisms from the water. The outcome of the bacteriological analyses affirms the assertion that, for the safe use of TWW for crop irrigation, the disinfection or polishing of the water is required. The new EU Regulation 2020/741, which came into force in 2023, stipulates that TWW should be disinfected before being used for crop irrigation [32]. This is to ensure the safe use of TWW and to protect public health. It is evident from the microbiological results that the secondary effluent was not safe for crop irrigation and will require further treatment. Chlorination could be effective in significantly reducing the bacteriological or pathogenic loads of the secondary effluent to safe limits. Also, a combination of membrane technology coupled with UV disinfection could produce an effluent of high quality that meets the requirements for reuse [55]. Ozonation, an advanced treatment process, could also sanitize the TWW, making it safe for crop irrigation. The use of ozonation or UV disinfection may be preferred over chlorination due to the formation of toxic byproducts, such as trihalomethanes and haloacetic acids, by chlorination [56]. The effluent disinfected by these preferred processes may not pose a threat to plants, as well as soil, since no toxic byproducts will be formed.

Table 3. Microbiological water quality characteristics of the tap water, secondary effluent, and membrane effluent compared with the European Union (EU) Regulation 2020/741 on water reuse.

Indicator Microorganism	Tap Water	Secondary Effluent	Membrane Effluent	EU Water Reuse Guidelines [32]
Coliform [CFU/100 mL]	nd	$9.85 \pm 6.52 \times 10^4$	12.75 ± 7.93	≤1000 **
Thermotolerant Coliform [CFU/100 mL]	nd	$5.80 \pm 4.15 \times 10^4$	<10	≤10 *
Escherichia coli [CFU/100 mL]	nd	$2.76 \pm 2.33 \times 10^4$	<10	≤10
Clostridium perfringens [CFU/100 mL]	nd	$1.22 \pm 0.87 \times 10^3$	nd	>4log

Notes: "CFU" means colony-forming unit; "nd" implies not detected; * ISO guidelines cited from Drechsel et al. [57]; ** FAO guidelines [58].

3.3. Impact on Plant Growth and Biomass Production

The TWW-irrigated plants (SE and ME) showed better growth and had a higher biomass production than carrot plants irrigated with tap water (Tap). The fresh mass (mean) of the carrot fruits produced under tap water, secondary effluent, and membrane effluent irrigation were 2.14 g, 3.96 g, and 3.03 g, respectively (Figure 2). A similar pattern was observed for the percentage dry mass composition. The SE had the highest dry matter composition (18.3%), followed by the ME (16.6%), and then the Tap (15.9%), as can be seen in Figure 2. In Figure 3, the growth trend was not different from the aforementioned; the SE and ME exhibited better growth characteristics in terms of the height than the Tap. However, the difference in the growth indicators among the different irrigation treatments was not significant. A very strong correlation existed between the plant height and the corresponding fresh mass of the fruit produced (Table 4); the carrot plants with longer shoots produced heavier fruits. The observed trend in the growth characteristics and biomass production could be attributed to the higher fertilization potential of the secondary effluent and membrane effluent. The high levels of NPK in these two streams of the TWW promoted the supply of nutrients to the carrot plants and boosted their growth and biomass production.

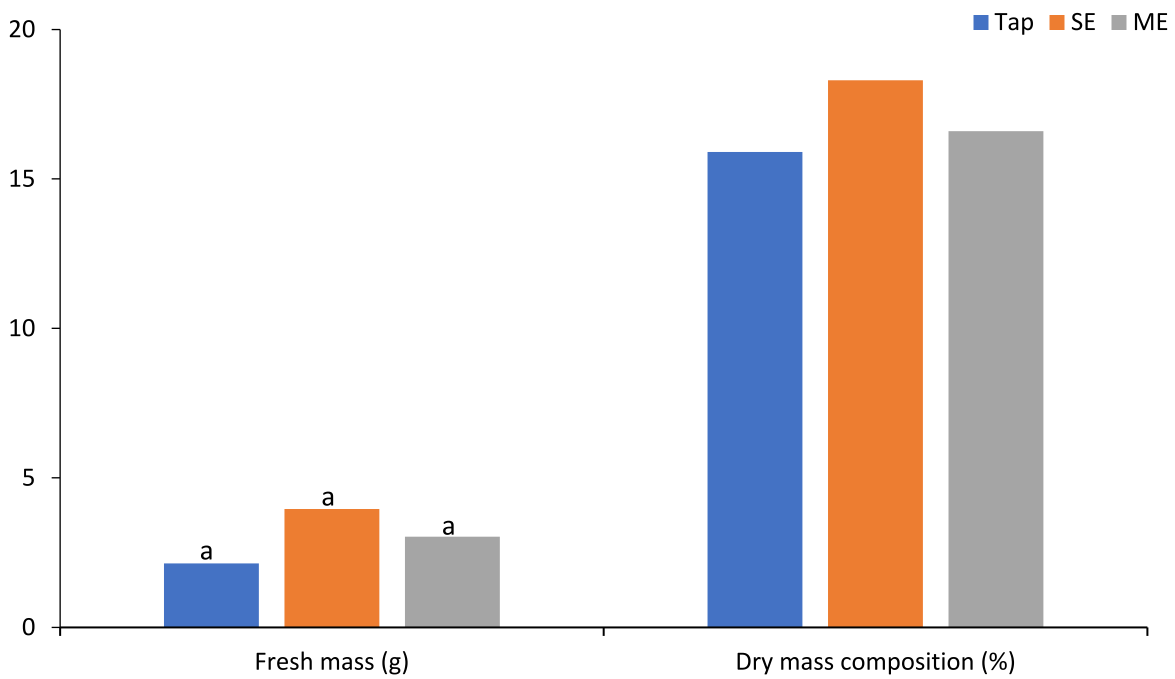

Figure 2. Effect of the TWW and tap water irrigation on the fresh mass (mean) and percentage dry matter composition/content of the carrot plants. The same letter indicates no significant difference ($p > 0.05$) was observed among the different treatments. Fresh mass is expressed in grams and dry matter content is in percentages.

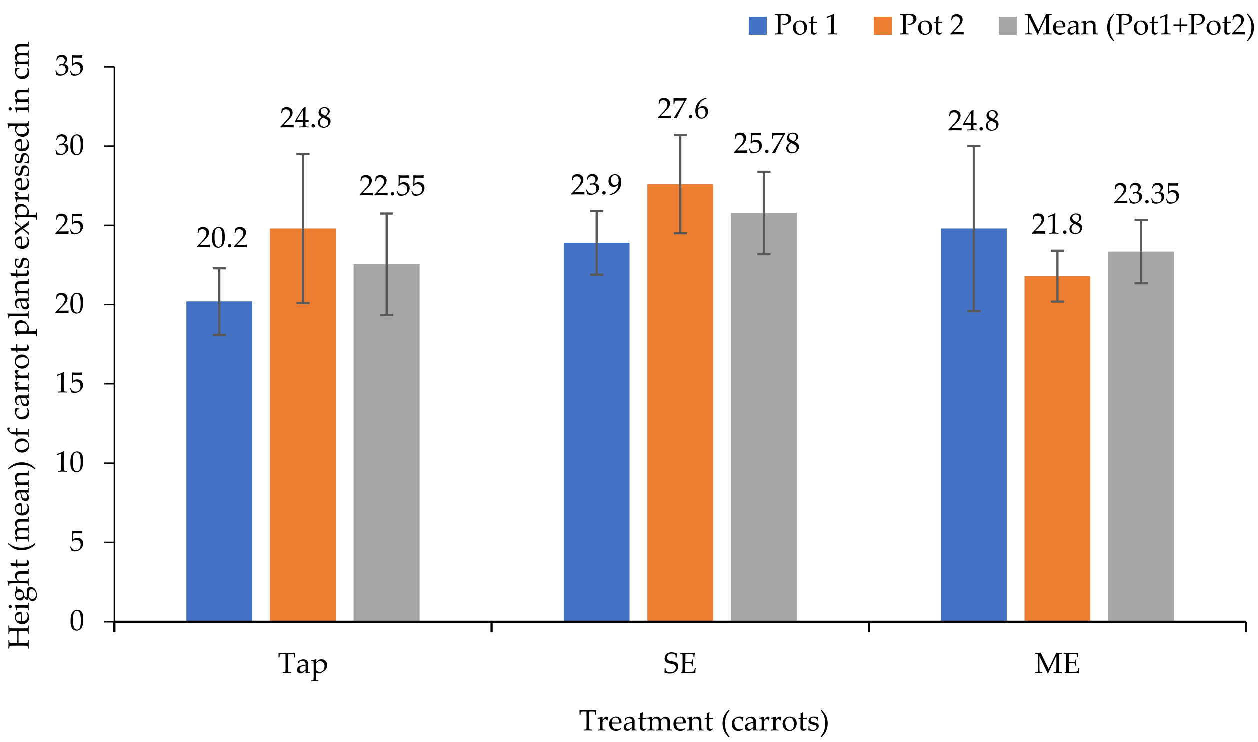

Figure 3. Effect of the TWW and tap water on the height (mean) of the carrot plant. Only the vegetative part of the carrot plant was used in the measurement. The blue and brown bar graphs represent the mean height of the individual pot treatments, and the gray bar graph represents the mean for six plants per treatment. Error bars are the standard deviation.

Table 4. Pearson correlation shows a strong relationship between the plant height and the fresh mass of the carrot plants under tap water and secondary effluent irrigation. Tap 1 and SE 2 are the fresh mass and Tap 3 and SE 4 are the height, respectively.

	Tap 1	SE 2	Tap 3	SE 4
Tap 1	1			
SE 2	0.478449	1		
Tap 3	0.814232	0.818247	1	
SE 4	0.522865	0.972042	0.798666	1

The findings of the study are not in isolation from similar studies that have been conducted. Alkhamisi et al. [3] reported a significant increase in the height of wheat and cowpeas irrigated with the TWW compared to groundwater-irrigated plants. The yield of cowpeas was significantly higher for the TWW-irrigated cowpeas. Another study also found that TWW-irrigated vegetables exhibited better growth and had higher biomass production [22].

3.4. Impact of Irrigation Water on Selected Physical and Chemical Properties of Soil

3.4.1. pH

Tap water- and TWW-irrigated soils changed from neutral (7.08) to moderately alkaline after irrigation. Tap water-irrigated soil increased to 7.26, while secondary and membrane effluent-irrigated soils increased to 7.39 and 7.33, respectively (Figure 4). This corresponds to an increase of 0.18, 0.31, and 0.25 units, accordingly. The irrigation water of the present study may have altered the pH status of the soil, changing it to moderately alkaline. The magnitude of the change in the soil pH corroborates with the alkaline status of the different irrigation water streams (Table 1). The pH of the TWW-irrigated soil was higher than tap water-irrigated soil. The increase may have been caused by the release of exchangeable cations during organic matter mineralization and the relatively high cation content of the TWW [52,59,60]. Also, the relatively high level of bicarbonates in the TWW might be responsible for the higher pH elevation of the TWW-irrigated soil, since bicarbonates have a pH-increasing effect on soil. This may have precipitated cations, such as calcium and magnesium, out of the soil solution and formed calcium bicarbonates and magnesium bicarbonates [61]. These formed products tend to increase the pH of soils.

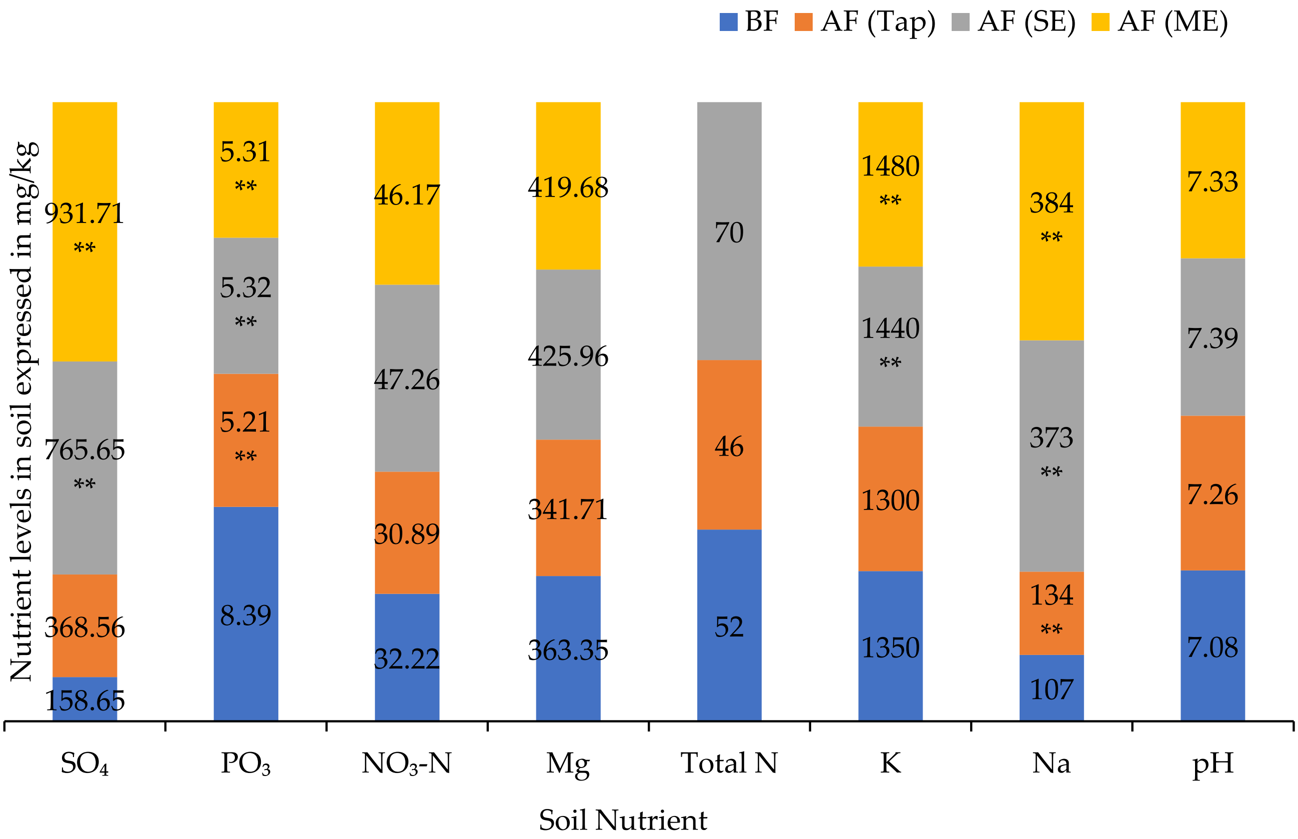

Figure 4. Soil pH and nutrient levels of the soil before and after the irrigation. Values indicated are nutrient contents in mg/kg, except pH. ** indicate a significant difference ($p < 0.05$) in nutrient levels before and after irrigation. BF refers to the level of nutrients in the soil before irrigation; AF refers to the level of nutrients after irrigation.

Research has shown that wastewater high in bicarbonates has the potential to increase the soil pH [62,63]. In a study, Tarchouna et al. [60] observed an increase in the soil pH after irrigation with slightly alkaline TWW. The soil changed from slightly acidic (6.85) to moderately alkaline (8.35). The pH increases in the present study did not have a significantly adverse impact on the availability of nutrients.

3.4.2. Nutrients

The nitrate-nitrogen increased from 32.22 mg/kg to 47.26 mg/kg and 46.17 mg/kg for the secondary effluent and membrane effluent-irrigated soil, respectively, but decreased to 30.89 mg/kg for the tap water-irrigated soil. A similar trend was observed for potassium and magnesium. This affirms the assertion that the TWW has a higher fertilization potential than tap water. Nitrate-nitrogen and potassium levels in the tap water were below the crop nutrient requirement, leading to a depletion of the already-existing levels in the soil. Phosphate decreased from 8.39 mg/kg to 5.21 mg/kg, 5.32 mg/kg, and 5.31 mg/kg for the tap water-, secondary effluent-, and membrane effluent-irrigated soils, respectively. Phosphate levels in all the irrigation water might have been below the crop requirement and were insufficient. Since no additional supply of phosphate in the form of fertilizer or manure was made, the carrot crops made use of the phosphate present in the soil, leading to the reduction in the initial amount. Crop nutrient requirement assessment is therefore necessary before the application of TWW irrigation to ensure an adequate supply of nutrients. Sulfate, a readily available form of sulfur for plant uptake, was significantly higher in the soil irrigated with the TWW than the tap water. Tabatabai [64] suggested that irrigation water influences the concentration of water-soluble sulfate in soil.

Tap water-irrigated soil increased from 158 mg/kg to 368 mg/kg, while secondary and membrane effluent-irrigated soil increased to 765 mg/kg and 931 mg/kg, respectively (Figure 4). The significant increase ($p < 0.05$) in sulfate in the TWW soil is attributed mainly to the fertilization potential of the TWW. Several studies have reported similar results of improvement in soil nutrients after TWW irrigation [51,63,65].

3.4.3. Sodium Accumulation in Irrigated Soil

Sodium levels in the soil increased from 107 mg/kg to 134 mg/kg, 373 mg/kg, and 383 mg/kg for tap water-, secondary effluent-, and membrane effluent-irrigated soil, respectively (Figure 4). The accumulation of sodium in the TWW-irrigated soil was significantly higher than in the tap water-irrigated soil. This increase corroborated with the quantity of sodium present in the respective irrigation water (Table 1) and is in line with the existing literature [63,65]. The increase was caused by the deposition of sodium by the irrigation water. The relatively high calcium content of the TWW may have enhanced the selectivity of the potassium uptake and transport over sodium, leading to the deposition. Also, the antagonistic activity of potassium may have caused a reduction in the sodium adsorption capacity [63].

Even though the accumulation of sodium in the TWW-irrigated soil was significantly higher, the carrot plants did not show signs of toxicity, nor was the soil sodic (pH < 8.5) [51]. The use of the TWW for long-term irrigation would require salinity control measures, such as the dilution of TWW with less-saline water and the leaching of salts beyond the root zone, to maintain the integrity of the soil.

3.4.4. Distribution of Potentially Toxic Elements (PTEs) in Irrigated Soil

Potentially toxic elements (Cd and Pb) were below the limit of detection for both tap water- and the TWW-irrigated soils (Table 5). The concentration of Cd was below 0.01 mg/kg, and that of Pb was below 0.04 mg/kg. This result is consistent with what has been reported by Bedbabis et al. [63]. After 5 years of irrigation with TWW, the authors found that the Cd and Pb contents in the soil were below the detection limit. In this present study, there was no increase in the Zn, Cd, and Pb contents of the soil after irrigation with secondary effluent and membrane effluent. The concentration of the PTEs remained the same for all the treatments, except for Zn, which increased to 0.02 mg/kg from the initial level of 0.01 mg/kg for the tap water-irrigated soil. The application of the TWW to the soil did not lead to the deposition and accumulation of Zn, Cd, and Pb in the soil. Hence, the TWW may not pose a risk of PTE dissemination in soil, and therefore its use for crop irrigation will not lead to the uptake of the PTEs by the crops. A comparison of the results to the WHO guidelines on the maximum tolerable soil concentrations of toxic chemicals based on human health protection revealed that Cd and Pb concentrations were far below the guideline limits of 4 mg/kg for Cd and 7 mg/kg for Pb. Therefore, both the secondary effluent- and membrane effluent-irrigated soils pose little or no risk to humans and are safe for food cultivation.

Table 5. Distribution of selected potentially toxic elements (PTEs) in the soil before and after irrigation compared to the maximum tolerable soil concentrations of toxic chemicals based on human health protection.

Potentially Toxic Elements	PTE Levels in Soil before Irrigation (mg/kg)	PTE Levels in Soil after Irrigation (mg/kg)			Maximum Tolerable Soil Concentration (mg/kg) [29]
		Tap	SE	ME	
Pb	<0.04	<0.04	<0.04	<0.04	7.00
Zn	0.1	0.02	0.01	0.01	n.p
Cd	<0.01	<0.01	<0.01	<0.01	4.00

Notes: The bioavailable fraction of the PTEs was considered for the study and not the total fraction. This was based on the consideration that, under normal environmental conditions, the bioavailable fraction is the fraction that could be available for crop uptake (expert opinion).

4. Conclusions

This study shed light on the suitability of TWW for crop irrigation and the resultant impact on crop growth and soil characteristics. Of the two streams of TWW that were used for the irrigation, secondary effluent did not meet the bacteriological quality standard for irrigating carrot plants. The use of the TWW for irrigation led to better plant growth in terms of height and biomass production in carrots, and significantly improved the nutrient levels in the soil. These improvements were due to the high fertilization potential of the TWW. The accumulation of potentially toxic elements in the soil and the deterioration of the soil quality, such as salinization or sodicity, were not observed after the TWW irrigation. This study concludes that TWW is suitable for crop irrigation, but must be disinfected to reduce the bacteriological load to an acceptable limit. The use of ozonation or UV disinfection methods may be preferred over chlorination due to the formation of toxic byproducts by chlorination. Also, salinization or sodicity minimization measures, such as leaching and the periodic dilution of the irrigation water, should be incorporated into the irrigation plan when TWW is used for long-term irrigation.

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