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Assessing the Reliability of Treated Grey Water Irrigation on Soil and Tomatoes (*Solanum lycopersicum* L.)

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Abstract: Under a water scarcity situation, it is expected to manage water more efficiently. This study aims to evaluate the effect of treated grey water (from laundry and tableware), pre-treated through a wetland mini-reactor with a horizontal underground flow, on soil and tomatoes. The experiment included two tomato cultivars (Dart and Firenze), planted in completely randomized bloc design, and irrigated with treated grey water (TGW) vs. ground water (C) as control. Soil, tomato leaves and fruits were assessed for microbial contamination. Tomato yield, physico-chemical characteristics and antioxidant contents were studied. Results showed that TGW met the standards for irrigation water for most water quality variables. Irrigation with TGW increased the concentrations of phosphorus (P), iron (Fe) and copper (Cu) in the soil. Although, the soil ionic composition was still in the suitable range for agriculture. Opportunely, there was no contamination by fecal coliforms, *streptococcus* and *E. coli* in soil and tomatoes. Dart cultivar seems to be more responsive to TGW and had higher fruit number and weight. This response was accompanied by an effective antioxidant response, higher water and juice content. The findings of this study emphasize that TGW may provide a way to preserve water resources and to avoid soil contamination.

Keywords: grey water; tomato; soil; yield; microbes; fruit quality

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1. Introduction

Considerations comprising, the lack of freshwater resources and the increased demand especially in arid and semi-arid areas have directed water reuse concept. It is expected that 1.8 million people in developing countries will live in water scarce regions by 2025 due to the lack of suitable management strategies for the reuse of treated wastewater in crop production [1]. Grey water reuse may constitute an emerging water management tool, promoting water preservation and limiting environmental pollution. Oteng-Peprah et al. [2] reported that the treatment of grey water can help to reduce the dependency on freshwater resources and the pollution caused by discharge of untreated grey water. In this line, Lubbe et al. [3] declared that supplementing irrigation sources with grey water, in arid regions, may reduce water consumption by up to 50%. Studies focused on wastewater treatment technologies, suggest that there is a potential for grey water reuse in the developing world [1,3]. In these countries, household wastewater arising from bathing, washing clothes, dish washers and wash basins activities (grey water) is commonly thrown [4]. Non-treated grey water has been identified as a major cause of soil and ground water contamination, due to the infiltration of salts, oils and grease [5]. These impacts are exacerbated by poor effective methods of grey water treatment [4].

Nonetheless, grey water contains some nutrients that are beneficial to plant growth [1]. In this way, Al-Zu'bi and Al-Mohamad [6] found that tomato plants irrigated with grey water gave significant higher yield. Moreover, Hashem and Xuebin [7] reported that TGW provides nutriments and organic matter needed for maintaining the soil fertility and plant

productivity. According to Hussain and Qureshi [8], the treatment of grey water may constitute a valuable way to improve food security, and to contribute to environmental progress particularly in areas served with dry sanitation. In another work, Misra et al. [9] found higher biomass accumulation in tomato plants watered with grey water compared to tap water and explained this by an increased nutrient uptake. In fact, grey water contains some macronutrients (NPK) and micronutrients (Fe, Mn, Zn and Cu) necessary for plant growth and also a significant amount of organic matter [10]. Such composition of grey water constitutes the main cause of increased crops production [11]. In the interim, there are always worries about microbial pathogen in wastewater. According to Victor et al. [12] microbial growth in food crops, watered with wastewater, constitute a major limit of such approach which requires more gain of knowledge. Generally, growth of bacteria and fungi are linked to water and soil qualities [13]. From previous research, environmental effects of grey water on soils are jointly supportive and unsupportive depending on how it is managed. Qishlaqi et al. [14] indicated that irrigation with untreated wastewater elevated the soil pH level by 2 to 3 units and resulted in the accumulation of heavy metals to levels that were above the maximum permissible limits. As well, Pinto et al. [15] revealed an increase of soil electrical conductivity (EC) and pH due to grey-water irrigation compared to potable and diluted grey-water (1:1). However, in the study of Rusan et al. [16], grey water irrigation caused the accumulation of some pollutants, while the soil pH was not affected. Despite these reports, some findings suggest that grey water does not pose any risks to the appropriateness of soils [15,17]. Whereas Sharvelle et al. [18] considered the effect of long term (around 5 years) irrigation with grey water on landscapes and noticed accumulation of salts in the soil. These same authors recommended that with a sound grey water management system, the contamination of the water table could be minimized. In any case, water delivered for irrigation purposes should meet the high requirements of modern hygiene and basically be free from pathogenic organisms and toxic substances. Recently, increasing attention has been paid for monitoring and assessing the microbiological quality of treated wastewater all over the world. Therefore, the present study was conducted to explore the impacts of treated grey water, on soil physico-chemical and microbial flora, as well as the yield, fruit quality and composition field grown tomato plants. Microbial contamination of tomatoes leaves and fruits were also assessed.

2. Materials and Methods

2.1. Experimental Set-Up

The study was conducted in the spring season (February- June) of 2016–2017, under local climatic conditions of the regional center of agricultural research of Sidi Bouzid Tunisia (CRRA-Sidi Bouzid), Tunisia (9° 43′ E, 35° 01′ N; altitude 354 m). The growth period is characterized by mean precipitation of 32 mm, mean evapotranspiration of 99 mm and mean temperatures of 18 °C. The experimental layout precedes a completely randomized bloc design by means of two factors, with two levels each one, (i) tomato (*Solanum lycopersicum* L.) cultivars (Firenze and Dart) and (ii) irrigation treatments (C: ground water; TGW: treated grey water). Dart is characterized by an oval fruit shape and bright red fruit color whereas Firenze has a pear fruit shape and red fruit color, both cultivars are early or mid-season. Grey water was collected from the guest house of the CRRA, and it represents a mix of laundry and tableware activities. Ionic and microbial characteristics of irrigation water were indicated in Table 1. Drip irrigation lines were spaced by 80 cm with 40 cm spacing between plants. Drippers have a flow rate of 4 $L \cdot h^{-1}$. The planting depth was around 17 cm. The soil has sandy-clay texture (clay = 14%, silt = 1%, sand = 85%), 7.8 pH, 1.4% organic matter and 3.4 $Ds \cdot m^{-1}$ electrical conductivity (EC). Crop water requirement was calculated according to Penman formula: ETM = Kc × ETP, where ETP is the maximum evapotranspiration and Kc is a function of tomatoes phonological stages (0.2 at vegetative stage; 0.6 during flowering; 0.9 at mid-harvest and 0.7 for the rest of the developmental cycle).

Table 1. Ionic and microbial characteristics of irrigation water.

	GW	TGW
pH	7.34 ± 0.01 [a]	7.42 ± 0.02 [a]
EC (mS cm^{-1})	3.23 ± 0.20 [a]	3.63 ± 0.04 [a]
SS (mg L^{-1})	13.66±1.08 [b]	50.33 ± 1.08 [a]
Turbidity (NTU)	0.47 ± 0.00 [b]	104.33 ± 0.40 [a]
CDO (mg O_2 g^{-1})	27.33 ± 1.77 [b]	86.33 ± 1.08 [a]
BDO (mg O_2 g^{-1})	0.33 ± 0.40 [b]	46.33 ± 3.34 [a]
HCO_3^- (meq L^{-1})	6.00 ± 1.23 [b]	12.00 ± 0.00 [a]
NO_3^- (mg L^{-1})	63.33 ± 0.40 [b]	96.00 ± 3.74 [a]
SO_4^{2-} (meq L^{-1})	22.83 ± 1.24 [a]	16.83 ± 1.81 [a]
K^+ (mg L^{-1})	0.06 ± 0.01 [b]	0.12 ± 0.00 [a]
Na^+ (meq L^{-1})	4.13 ± 0.16 [a]	5.90 ± 0.00 [a]
Ca^{2+} (meq L^{-1})	8.53 ± 0.16 [b]	10.40 ± 0.74 [a]
Mg^{2+} (meq L^{-1})	12.93 ± 0.58 [a]	12.26 ± 1.14 [a]
Cl^- (meq L^{-1})	16.00 ± 0.00 [a]	16.00 ± 0.00 [a]
Ni (mg L^{-1})	0.20 ± 0.00 [b]	0.50 ± 0.01 [a]
Fe^{2+} (mg L^{-1})	-	0.10 ± 0.00 [a]
Pb (mg L^{-1})	0.30 ± 0.00 [b]	1.10 ± 0.01 [a]
Cu^{2+} (mg L^{-1})	-	-
Zn^{2+} (mg L^{-1})	-	-
Cd (mg L^{-1})	-	-
SAR (mg L^{-1})	1.26 ± 0.07 [b]	1.75 ± 0.02 [a]
Total coliforms (UFC 100/mL)	-	$1.2.10^2 \pm 1.4.10^3$
Fecal coliforms (UFC 100/mL)	-	-
Escherichia coli (UFC 100/mL)	-	$2.1.10^1 \pm 1.1.10^2$
Streptococcus (UFC 100/mL)	-	-
Salmonella (UFC 100/mL)	-	-
Vibrio cholera (UFC 100/mL)	-	-

Means are presented ± SE (n = 3). Different letters within each line indicate significant differences between treatments according to LSD's test at the significance level (p <0.05). BDO, biochemical oxygen demand; CDO, chemical oxygen demand; EC, electrical conductivity; GW, ground water; SS, soluble solids; TGW, treated grey water.

The experimental unit for grey water treatment is composed of a horizontal sub-surface flow constructed mini-wetland reactor (HSSFCW). The constructed wetland operates in a subsurface-flow mode, in which grey water was conveyed to flow through the constructed wetland beds. Firstly, water was collected in a polyethylene barrel (50 L) equipped with sieve filter to remove large particles. Then, water was evacuated to second barrel of filtration and dispersion (50 l capacity), this barrel is secured inside a pit filled with gravel (30 mm size, 50% porosity and 3% bottom slope) and it is holed over its entire surface to achieve uniform distribution of water. The treated grey water was collected in a high-density polyethylene (HDPE) tank, placed downstream of the filter bed and drilled at its base to facilitate the entry of water. This tank contained also a submersible pump and a float switch to evacuate treated grey water to the experimental area after passing through a screen filter [19].

2.2. Soil Analysis

Soil samples were taken using a soil auger from the depths 0–20 cm and 20–40 cm, oven dried (105 °C) and sieved. The pH and the electrical conductivity (EC) were, respectively, measured using pH-meter (MP 22, Mettler Toledo, Switzerland) and conductivity-meter (Hanna HI8424, Canada) in a (1/2.5) soil/water suspension. Ions of bicarbonate (HCO_3^-), calcium (Ca^{2+}), magnesium (Mg^{2+}) and chloride (Cl^-) were measured using titration method. Sodium (Na^+) and potassium (K^+) contents were measured using flame photometer [20]. An atomic absorption photometer (HITACHI model Z-6100, Germany) was used to detect heavy metals (Cu, Cd, Fe, Ni, Pb and Zn). For that, samples of soil solutions (1g of soil + 10 mL hydrogen fluoride + 5 mL $HCLO_4$) were digested in a microwave. Then, 70 mL of perchloric acid ($HClO_4$) was added to the mixture, filtered and brought to 100 mL with distilled water. Finally, the obtained filtrate was analyzed [21].

2.3. Microbiological Examination

Microorganism enumeration in soil and tomatoes leaves and fruits was realized, according to the method of inoculation in liquid medium and enumeration by most probable number (MPN). Soil samples were grounded, mixed with sterilized distilled water (10 g/100 mL) and shaken vigorously (30–60 s) to dislodge bacteria. The resulting suspension was used to prepare serial dilutions, which were then spread onto proper medium. Microbiological analysis concerned total coliforms, fecal coliforms, *Streptococcus*, *Salmonella*, *Vibrio cholera* and *Escherichia coli* (*E.coli*). Culture media and incubation procedures used for each microorganism were indicated in Table 2. Further, microorganisms in fresh leaves and fruits were enumerated and isolated using the same selective media as soil samples. Despite plant samples (20 g) were suspended in 80 mL sterile peptone water (0.1%), homogenized for 2 min. Thereafter, serial dilutions were prepared and plated into each specific medium [22,23].

Table 2. Methods of microbiological analysis.

Microorganism	Microbiological Analysis
Total and fecal coliforms	Total coliforms were determined using multiple tube fermentation (MTF) procedure. Fecal coliforms were enumerated by inoculation of tubes containing a lactose broth with diluted samples. After incubation at 37 °C for 24–48 h, we looked for gas production and positive tubes were subcultured on a selective medium (lactose broth, Brilliant Green Bile). The confirmatory test consists of verifying the production of indole by adding kovacs reagent.
Escherichia coli	*E. Coli* were detected on the presemptive tubes (indole+) when a dark red ring appeared.
Streptococci	Incubation was done in a Roth medium at 35–37 °C for 48 h. Positive tubes are those which present a cloudiness often accompanied by sediment. The confirmatory test consists of subculturing the presumptive positive tubes into tubes of Litsky medium at 35–37 °C for 24–48 h, and streptococci presence were confirmed by a cloudiness often accompanied by blue–purple sediment.
Salmonellae	*Salmonellae* was detected on salmonella–shegella media and colonies are indicated by transparent black colonies.
Vibrio cholerae	*Vibrio cholerae* was detected on thiosulfate-citrate-bile salts-sucrose (T.C.B.S) medium and colonies were indicated by yellow color and flat appearance.

2.4. Plant Analysis

2.4.1. Yield Attributes

The fruit set of each plant was counted and used to calculate the percentage of setting rate (SR%). Fruit weight (FW) and fruit number (FN) per plant were determined at maturity stage. The number and the weight of fruits free from rotting, deterioration, foreign smell and/or taste, greenbacks and other defects were determined and considered as marketable yield. Transversal (TD) and longitudinal diameter (LD) of fruits were measured with digital caliper and were expressed in millimeters. Fruit setting rate (SR), number and weight of fruits per plant, number and weight of marketable fruits, TD and LD were taken in 15 plants per treatment for each cultivar.

2.4.2. Fruit Quality Measures

The fruit water content (WC) was calculated as follows: $WC(\%) = [(FWfruit - DWfruit)/FWfruit] \times 100$, where FW fruit and DW fruit are the fruit fresh weight and fruit dry weight, respectively. The DW fruit was obtained by drying tomatoes in a Hotcold-UM, Selecta type drying oven at 100 °C for 24 h. For firmness determination, tomatoes were marked in a cross shape from their stem, around 3 cm to each side. Perforations were done using a Portable penetrometer FT 327, Italy. Color of ripe tomatoes was performed by the analysis of three parameters defined by the CIEL*a*b* system. Parameters L*, a* and b* were measured by CR-10 Konica Minolta Chromameter (Chuo-Ku, Osaka, Japan), where L* defines light (L* = 0 black and L* = 100 white) and a* and b* define chromaticity (+a* = red and –a* = green, +b* = yellow and –b* = blue), a*/b*. The value of chroma (C*) consisting of color saturation was obtained by the following Equation: $c* = \sqrt{a*^2} + b*^2$. The measurements were made in six points per fruit in a total of ten tomato fruits. The fruit WC, firmness and color measurements were made in a sample of ten fruits per treatment, for each cultivar.

2.4.3. Physico-Chemical Parameters

Tomato juice was extracted after homogenizing for a minute using a stainless-steel blender, and juice content (JC) was calculated according to the following equation: $JC(\%) = (Juiceweight/Fruitweight) \times 100$. The pH, electrical conductivity (EC), total salt content (TSC), soluble solids (SS) and titratable acidity (TA) were determined in tomato juice filtered with nylon-type cotton cheesecloth. Total salt content (TSC) was determined according to the formula: $TSC\ (gl^{-1}) = 0.7 \times EC\ (mScm^{-1})$, where EC is the electrical conductivity of juice [24]. Soluble solids were measured with digital refractometer (ATAGO PAL-1) by adding 1 to 2 drops of juice on the prism surface, results were expressed in ºBrix. Titrable acidity was measured according to the method adopted by [25]. In fact, ten ml of juice sample was titrated with NaOH (0.1 N) by adding 2 to 3 drops of indicator (phenolphthalein) and the results were calculated as percentage.

Membrane stability index (MSI) was determined with the following formula: $MSI = (\frac{EC1}{EC2}) \times 100$, where EC1 and EC2 are the electrical conductivity measured after incubation of tomato fruits portions in a 32 °C water bath for 2 h, and the electrical conductivity after autoclaving at 121 °C for 20 min, respectively [26].

2.4.4. Biochemical Traits

Vitamin C quantity was quantified by the potassium iodate method [27].

For antioxidant compounds (phenolics, flavonoids and anthocyanin) determinations, 5 g of frozen fruits were extracted with 10 mL HCl (0.5 N) in methanol (80% v/v). The mixture was then centrifuged at 4.000× g for 15 min at 4 °C. Total phenols, were determined using the method of Folin-Ciocalteau reagent, the absorbance was measured at 725 nm and results were expressed in milligrams of Gallic acid equivalents (GAE) per 100 g of FW. Flavonoids were determined at the absorbance 510 nm and the results were expressed as mg of catechin equivalents (CE) per 100 g of FW. Anthocyanins were calculated with values of absorbance at 535 and 700 nm and the molar extinction absorptivity coefficient

ε = 25.965/cm M and expressed in mg of cyanidin 3-glucoside equivalents (C3GE) per kg of fresh weight.

Lycopene content was determined according to the method of reduced volumes of organic solvents [28]. About 0.6 g of unfiltered tomato puree was added to a 5 mL of acetone with 0.05% butylated hydroxytoluene, 5 mL of ethanol, and 10 mL of hexane. The mixture was shaken for 15 min at 180 rpm. Three milliliters of water was then added, prior to an additional 5 min of stirring. Finally, the absorbance of the upper layer was measured at 503 nm. The following relationship was then used for the estimation of lycopene content: Lycopene $= (\text{A503} \times 31.2)/\text{W}_{\text{Sample}}$, were A503 is the absorbance at 503 nm and Wsample is the weight of used sample.

2.5. Data Analysis

Treatments were arranged under completely randomized bloc design under two factorials. Data is the mean of four replicates. Statistical analysis was performed using SPSS Statistics Version 20.0 and data was subjected to two-way analysis of variance (ANOVA) using treatment (T) and cultivar (C) as main factors and LSD's test at a 5% level of significance.

3. Results

3.1. Physico-Chemical Proprieties of the Soil

From Table 3, irrigation with treated grey water (TGW) does not show any significant effect on soil electrical conductivity (EC), pH and Ni contents. Conversely, the concentration of Ca^{2+} + Mg^{2+} was 70% ($p < 0.05$) and 40% ($p < 0.05$) greater in the soil irrigated with TGW compared to soil irrigated with ground water, in the 0–20 cm and 20–40 cm depths, respectively. As well, nitrate ($NO_3{}^-$) concentration was significantly higher in the soil irrigated with TGW, in both depths (0–20 cm; 20–40 cm). Additionally, we found a significant increase in phosphorous (P) (0–20 cm: 25%, 20–40 cm: 60%) after irrigation with TGW. Likewise, the increase registered for Cu and Zn, under TGW, was significant only in the 0–20 cm depth (33% and 22%, respectively). As for irrigation water, cadmium (Cd) ion was not identified in the soil. However, potassium (K^+) content was decreased by TGW irrigation (50% and 33% in the 1st and the 2nd depth, respectively).

3.2. Microbiological Examination of Soil

Data in Table 4 revealed a significant difference in total coliform content between soils irrigated with ground water and treated grey water. In fact, TGW increased the amount of total coliforms. Although, the level of *Streptococcus* and *E. coli* was not affected by irrigation treatments, as they preserve similar contents under C and TGW treatments. Results also suggest that *Streptococcus* exist in the soil from the establishment of the experiment, as it was not identified in both irrigation treatments (Table 2). Likewise, *Salmonella* and *Vibrio cholera* were not identified similarly in soils irrigated with ground water and treated grey water.

3.3. Microbial Examination of Tomato Leaves and Fruits

Table 5 shows the means of germs counts in tomatoes leaves and fruits, under the two water quality treatments (C and TGW). Total coliform content was increased in leaves of both cultivars, grown under TGW. The increase in total coliform count was more pronounced in Firenze leaves. Meanwhile, in fruit tissues the level of total coliform was increased only in Dart. Indeed, Dart cultivar had lesser initial (under C treatment) total coliform content than Firenze. However, there is no clear impact of irrigation treatments in the other bacterial categories (fecal coliform and *Salmonella*), as they were not identified equally in plants watered with C and TGW.

3.4. Yield Attributes

From Table 6 and ANOVA results summarized in Table 7, the setting rate (SR) of tomato plants was not influenced by water treatments and by cultivars. In the interim, the number of fruits per plant was significantly increased with TGW in Dart cultivar. The

weight of fruits per plant was increased under TGW in both cultivars. The ANOVA results revealed that FN and FW were significantly affected by cultivar factor, as they were more elevated in Dart. In the other hand, proportions of number and weight of marketable fruits were generally higher in TGW watered plants compared to plants grown under ground water treatment. However, the number of non-marketable fruits (NNMF) was not affected by water treatments.

Table 3. Effect of irrigation treatments (C: ground water and TGW: treated grey water) on soil physico-chemical proprieties in the 0–20 cm and 20–40 cm depths.

	C		TGW	
	0–20 cm	**20–40 cm**	**0–20 cm**	**20–40 cm**
pH	8.04 ± 0.39 ^{Aa}	8.05 ± 0.11 ^{aA}	7.87 ± 0.02 ^{aA}	8.04 ± 0.10 ^{aA}
EC (mS cm^{-1})	1.36 ± 0.38 ^{aA}	1.30 ± 0.08 ^{aA}	1.38 ± 0.24 ^{aA}	1.39 ± 0.15 ^{aA}
$NO_3{}^-$ (ppm)	1466.66 ± 28.6 ^{aB}	1566.00 ± 20.16 ^{aB}	2550.00 ± 50.00 ^{aA}	2433.33 ± 50.30 ^{aA}
Na^+ (meq L^{-1})	0.93 ± 0.15 ^{aA}	0.71 ± 0.05 ^{aA}	1.08 ± 0.10 ^{aA}	0.98 ± 0.03 ^{aA}
K^+ (meq L^{-1})	0.08 ± 0.05 ^{aA}	0.06 ± 0.00 ^{aA}	0.04 ± 0.01 ^{aB}	0.04 ± 0.00 ^{aB}
P (meq L^{-1})	64.66 ± 13.79 ^{aB}	24.33 ± 4.16 ^{bB}	86.00 ± 3.00 ^{aA}	60.00 ± 1.73 ^{bA}
Ca^{2+} + Mg^{2+} (meq L^{-1})	25.33 ± 5.03 ^{aB}	16.00 ± 3.46 ^{bB}	87.00 ± 6.08 ^{aA}	27.00 ± 6.0 ^{bA}
Cl^- (meq L^{-1})	17.24 ± 2.51 ^{aA}	16.80 ± 4.56 ^{aA}	18.00 ± 3.05 ^{aA}	17.51 ± 2.44 ^{aA}
Pb (meq L^{-1})	4.31 ± 3.61 ^{aA}	4.00 ± 0.00 ^{aA}	5.33 ± 2.52 ^{aA}	4.33 ± 2.30 ^{aA}
Fe (meq L^{-1})	1895 ± 12.85 ^{aA}	1227.33 ± 34.88 ^{aA}	2894.33 ± 29.60 ^{aA}	1821.00 ± 21.35 ^{aA}
Cu (meq L^{-1})	0.80 ± 0.00 ^{aB}	0.70 ± 0.50 ^{aA}	1.20 ± 0.60 ^{aA}	0.60 ± 0.60 ^{aA}
Zn (meq L^{-1})	2.33 ± 1.15 ^{aB}	4.33 ± 1.51 ^{aA}	3.00 ± 3.98 ^{aA}	3.33 ± 0.80 ^{aA}
Cd (meq L^{-1})	NI	NI	NI	NI
Ni (meq L^{-1})	5.66 ± 1.51 ^{Aa}	3.33 ± 0.57 ^{bA}	5.90 ± 1.43 ^{aA}	3.33 ± 0.31 ^{bA}

Means are ± SE of n = 4, different lowercase letters within the same line indicate significant difference between depths, different uppercase letters within the same line indicate significant difference between treatments according to LSD test. Abbreviation: NI: Not identified.

Table 4. Effect of treated grey water irrigation on soil microorganism's content.

Bacterial Count	**C**	**TGW**
Total coliform (CFU mL^{-1})	1100	>1900
Fecal coliform (CFU mL^{-1})	NI	NI
Streptococcus (NPP mL^{-1})	<3	<3
Escherichia coli (NPP mL^{-1})	>2400	>2400
Salmonellae	NI	NI
Vibrio cholera	NI	NI

C, ground water; NI, Not identified; TGW, treated grey water.

3.5. Transverse (TD) and longitudinal (LD) Diameters of Tomato Fruits

Significant distinction between cultivars was observed for transversal diameter (TD) and longitudinal diameter (DL) of tomato fruits (Table 8). Fruits from Dart cultivar presented higher LD although, fruits from Firenze cultivar exhibited higher TD, demonstrating that Dart had longer oblong fruits then Firenze. Both cultivars had relatively higher TD and LD under TGW compared to C treatment, suggesting that tomato fruits fruit diameter was enhanced with TGW. Additionally, the oblong shape of the two tomato cultivars belong to small class (TD between 40 mm and 50 mm) [29].

Table 5. Bacterial counts in tomato leaves and fruits under ground water (C) and treated grey water (TGW) irrigation treatments.

Bacterial Count 100 g^{-1}	Plant Organ	Firenze		Dart	
		C	TGW	C	TGW
Total coliform	Leaves	200	500	240	500
	Fruits	200	200	100	200
Fecal coliform	Leaves	NI	NI	NI	NI
	Fruits	NI	NI	NI	NI
E. coli	Leaves	NI	NI	NI	NI
	Fruits	NI	NI	NI	NI
Streptococcus	Leaves	NI	NI	NI	NI
	Fruits	NI	NI	NI	NI

NI: Not identified.

Table 6. Changes in the setting rate (SR), fruit number (FN), number of marketable fruits (NMF%), number of non-marketable fruits (NNMF%), fruit weight (FW), weight of marketable fruits (WMF %), weight of non-marketable fruits (WNMF%) of two tomato cultivars irrigated with ground water (C) and treated grey water (TGW).

	C		TGW	
	Dart	Firenze	Dart	Firenze
SR(%)	55.71 ± 16.64^{aA}	58.69 ± 19.15^{aA}	68.08 ± 24.05^{aA}	65.36 ± 26.17^{aA}
FN (plant^{-1})	36.00 ± 1.52^{bB}	34.80 ± 1.20^{aB}	42.93 ± 1.14^{aA}	35.40 ± 1.00^{aB}
NMF (%)	82^{aB}	83^{aA}	85^{aA}	85^{aA}
NNMF (%)	18^{aA}	17^{aA}	15^{aA}	15^{aA}
FW(g plant^{-1})	1160.71 ± 58.39^{aB}	944.16 ± 17.93^{aB}	1526.22 ± 78.38^{aA}	1419.55 ± 58.65^{aA}
WMF(%)	87^{aA}	85^{aA}	87^{aA}	80^{bB}
WNMF (%)	13^{aA}	14^{aA}	13^{bA}	20^{aA}

Means are ± SE, different lowercase letter within the same line indicate significant ($p \leq 0.05$) difference between cultivars, different uppercase letter within the same line indicate significant difference between treatments according to LSD test.

Table 7. Analysis of variance for the effect of cultivar (C), treatment (T) and their interactions on tomato yield attributes.

	SR	FN	NMF	NNMF	FW	WMF	WNMF
T	0.57^{ns}	8.37 *	12.91 **	0.40^{ns}	49.17 ***	46.72 **	18.85 **
C	0.1^{ns}	11.30 **	1.69^{ns}	8.60 **	7.42 *	5.65 *	12.50 **
T×C	0.05^{ns}	5.87 *	7.11 **	0.16^{ns}	0.80^{ns}	1.44^{ns}	2.98^{ns}

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ^{ns}, non significant.

Table 8. Changes of transversal diameter (TD) and longitudinal diameter (DL) of two tomato cultivars irrigated with ground water (C) and treated grey water (TGW).

Cultivar	Treatment	TD	LD
Dart	C	42.40 ± 0.76^{Bb}	66.40 ± 1.11^{Aa}
	TGW	44.40 ± 1.08^{Ab}	67.06 ± 1.29^{Aa}

Table 8. *Cont.*

Cultivar	Treatment	TD	LD
Firenze	C	46.73 ± 1.02 ^{Ba}	56.73 ± 1.02 ^{Bb}
	TGW	49.53 ± 0.98 ^{Aa}	61.53 ± 1.39 ^{Aa}
ANOVA	T	12.64 *	5.53 *
	C	20.13 **	23.64 ***
	T×C	0.10 ^{ns}	0.14 ^{ns}

Means are ± SE, different lowercase letters within the same column indicates significant difference between cultivars, and different uppercase letters within the same column indicate significant difference between treatments. T: Treatment; C: Cultivars. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ^{ns}, non-significant.

3.6. Fruit Quality Traits

From Figure 1, the water content of Dart fruits was significantly higher in plants watered with TGW compared to those irrigated with ground water (C). However, values of WC of Firenze fruits were not statistically significant among water treatments. Concomitantly to the less WC in Firenze fruits, this cultivar had higher flesh firmness and less juice content (JC) values, as compared to Dart. Meanwhile, °Brix values were not statistically different among cultivars and in response to irrigation treatments, though registered values were above 4 °Brix, suggesting that harvested fruits can be commercialized. The same response was registered for total soluble salts (TSS), titratable acidity (TA), pH and EC as they were not influenced by irrigation treatments or by cultivars. Regarding vitamin C content, values were significantly higher in Dart compared to Firenze. Membrane stability index of tomato fruits were enhanced significantly with TGW treatment in both cultivars.

3.7. Fruit Color Measurement

We measured the impact of irrigation with treated grey water and ground water on the color of tomatoes. From Figure 2, irrigation treatments had no significant effect on color components (L*, a*, b*, a*/b* and C*). Meanwhile, Firenze had higher b* values and Dart had relatively higher a* values which is a cultivar characteristic. It is also remarked that the redness (+a*) degree of Dart fruits was increased by TGW treatment.

3.8. Biochemical Analysis

Analysis of the antioxydants contained in tomato fruits are shown in Figure 3. Flavonoids content was not influenced by water quality treatment and by cultivar. Conversely, total phenols concentrations were significantly higher in Dart fruits watered with TGW as compared to C treatment. In the other hand, the highest lycopene and anthocyanin content were recorded for plants watered with TGW in both cultivars. It is also remarked that Dart fruits had comparatively higher lycopene and anthocyanin concentrations than Firenze.

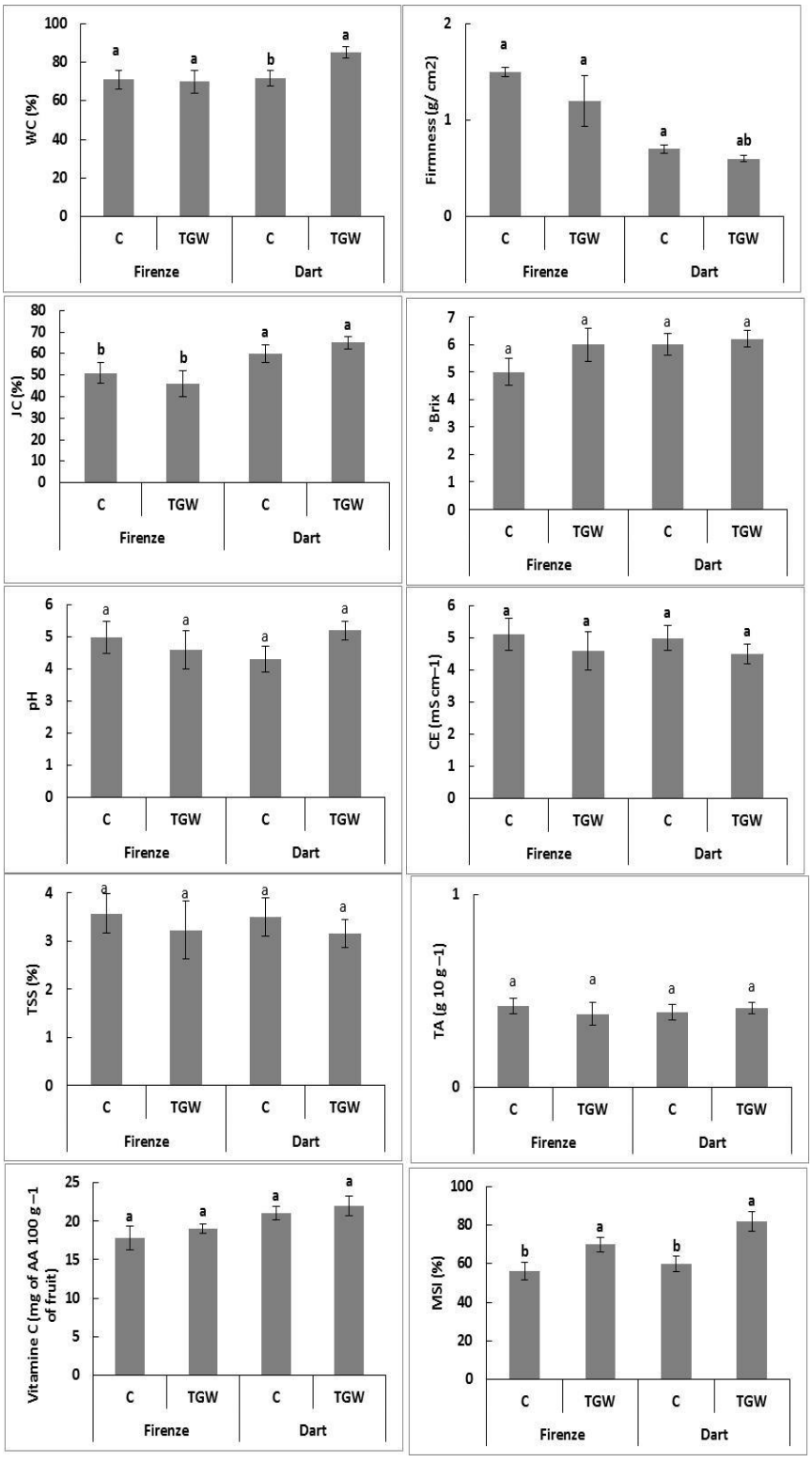

Figure 1. Changes in fruit water content (WC), fruit firmness, fruit juice content (JC), ºBrix (%), pH, electrical conductivity (EC), total soluble salts (TSS), titratable acidity (TA), vitamin C, membrane stability index (MSI) of two tomato cultivars (Firenze and Dart) under two irrigation treatments (C: ground water and TGW: treated grey water). Values are means ± SE of $n = 4$, different lowercase letters indicate significant difference among treatments and different uppercase letters indicate significant difference between cultivars according to LSD test at $p \leq 0.05$.

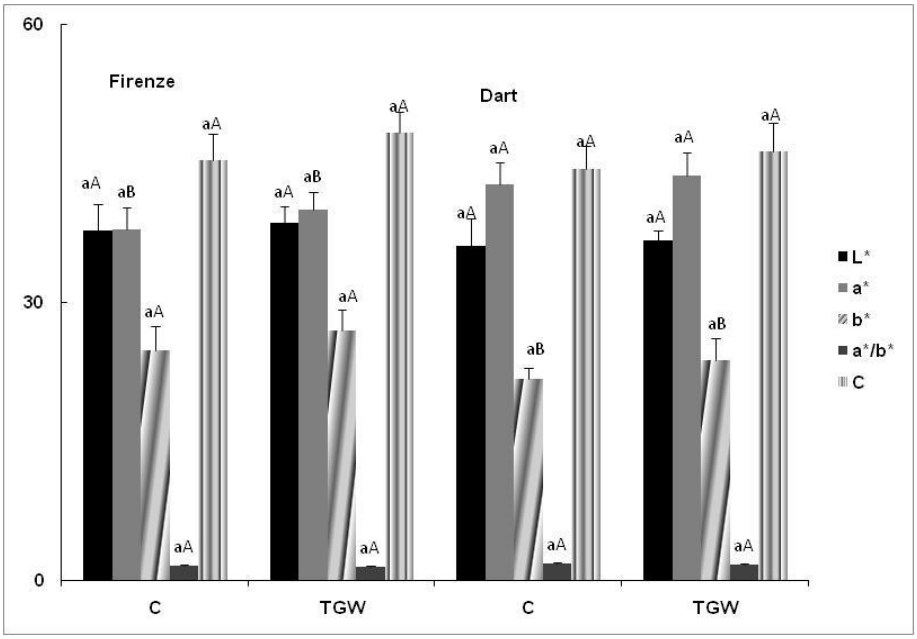

Figure 2. Effect of treated grey water on tomato flesh color. L* (lightness), a* (−a* = greenness, +a* = redness), b* (−b* = blueness, +b* = yellowness), a*/b* and C* (chroma). Values are means ± SE n = 4, different lowercase letters indicate significant difference among treatments and different uppercase letters indicate significant difference between cultivars according to LSD test at $p \leq 0.05$.

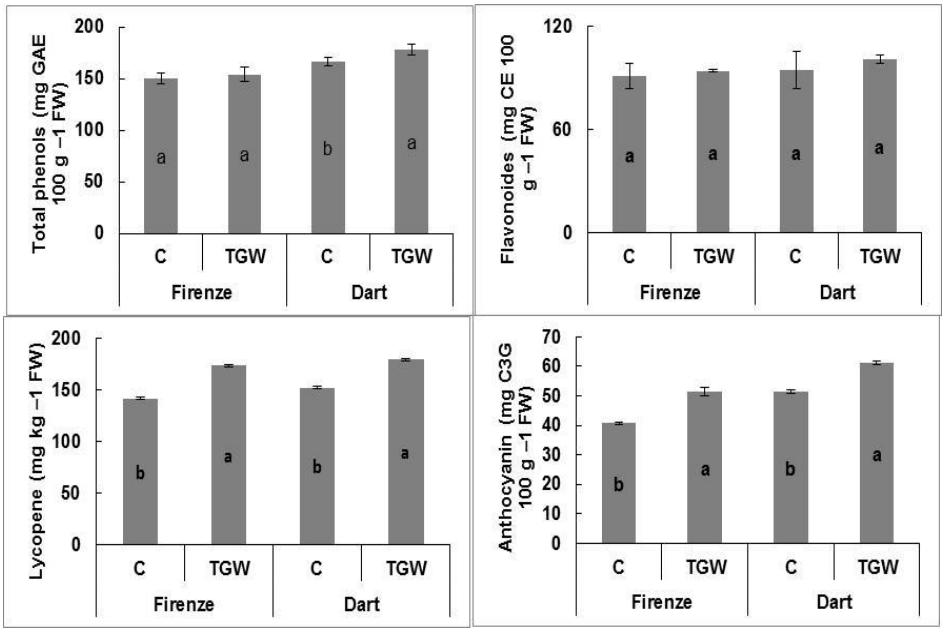

Figure 3. Changes in total phenolics, flavonoids, lycopene and anthocyanin concentrations of two tomato cultivars (Firenze and Dart) under two irrigation treatments (C: ground water and TGW: treated grey water). Values are means ± SE of n = 4, different lowercase letters indicate significant difference among treatments and different uppercase letters indicate significant difference between cultivars according to LSD test at $p \leq 0.05$.

4. Discussion

In several arid and semi-arid regions, usage of grey water for irrigation purposes becomes decisive for water management programs. Meanwhile, the changes in soil physico-chemical and microbial characteristics, due to grey water and/wastewater irrigation, influence greatly the plant growth and yield. In this experiment grey water (from laundry

and tableware) treated with a wetland mini reactor was used to irrigate tomatoes. The results showed an increase in soil P and NO_3^- content, probably enhancing the plant yield. Likewise, Chen et al. [30] registered an increase in soil phosphorus concentration due to wastewater irrigation. Pereira et al. [31] declared that P is an essential element for the plant, that favored crop development and yield. Additionally, the improved nitrate content in TGW irrigated soil (Table 3) had an important role in stimulating plant growth [32]. However, the marked increase in soil salinity registered by Chen et al. [30] differ from our results, as changes in water and soil EC owing to TGW irrigation was not significant. This result may support the efficiency of the grey water treatment unit used in this study [19]. Our further results about heavy metals accumulation in the soil put forward that the increase in Cu and Zn, under TGW, was more pronounced in the 1st depth (33% and 22%, respectively). This suggest that the choice of an alternative irrigation management involves not only an effective technique of water treatment but also an adequate soil texture, to overcome the excessive accumulation of toxic elements in the soil with poor infiltration rate. Fortunately, in this study Na^+, Cl^-, Fe and Ni accumulation in the soil was not changed by TGW irrigation. On the other hand, the decrease of potassium content in soil irrigated with TGW, may indicate better potassium uptake by plants. Considering that, K^+ concentration was in the order TGW > C in water treatments.

In this experiment, the increase of total coliforms proportion in soil watered with TGW, may be related to the augment in P content which favored the consumption of carbohydrates by microbes [30]. Evidence provided in this study indicated that total coliform contained in TGW was not of fecal origin. Data showed that coliform count does not exceed 10^6 FC/100 mL and the *E.coli* microbes were in the range $2.1.10^1$UFC 100/mL, these values were slightly lower than that recommended for irrigation purposes [33,34]. In addition, the level of fecal coliforms and *E. coli* does not show a significant difference between soil irrigated with two water quality treatments (C and TGW).

Instead, the absence of fecal coliforms, *Streptococcus* and *E. coli* in tomato tissues (leaves and fruits) point toward more healthy fruits. Such observations corroborated Al Hamaiedeh and Bino [35] found, who noticed that irrigation of olive trees and vegetable crops with treated grey water does not show any adverse effect on chemical properties of the fruits and leaves. Meanwhile, Bino and Al-Beiruti [36] reported that some fruits of okra and bean plants grown under grey water irrigation had high concentrations of total and fecal coliform bacteria. In this context, Chen et al. [30] clarified that changes in soil pH due to wastewater irrigation are caused by the decomposition of organic matter and production of organic acids by microbes. This is different from our data about (i) soil pH stability and (ii) reduced amount of microbe content in TGW. In this way, Balkhair [1] reported that soil and plant microbial content is depended on grey water composition.

The results also showed that TGW irrigation improved tomato yield, fruit number (FN), fruit weight (FW), marketable fruits weight (WMF). This found supports the study of Lubbe et al. [3] who indicated that grey water can be used to irrigate freshly sown seeds of *A. dubius* without reducing the production. It was also reported that the yield of corn, potato, lettuce, olive trees and alfalfa was increased after irrigation with wastewater compared to plants irrigated with natural water resources which may be due to the presence of plant nutrients in treated wastewater (especially nitrogen and phosphorus) [37,38]. Moreover, it seems that Dart cultivar was more productive and more receptive to TGW comparing to Firenze. Additionally, the longer oblong fruits and red coloration prominence of Dart are generally preferred by costumers [39]. This may be attributed to more effective antioxidative response as indicated by the higher total phenols, lycopene and anthocyanin content in Dart fruits. Even so, Dart had higher fruit water content and juice content. This recommended that Dart plants were more efficient in water usage, but the obtained fruits had short shelf life so that they are not very favored for conservation dedications [40]. Taken as a whole, the wetland mini-reactor with a horizontal underground flow used in this study was successful in reducing microbial contamination of soil and tomato plants. Additionally, all bacteria categories except total coliform were not transmitted to both assessed cultivars.

This study also supports the fact that the tomato cultivar had an effective oxidative response it may be more productive under TGW. In the meantime, the need of more studies about contamination of soil and crops still compulsory, especially at long term irrigation.

5. Conclusions

The influence of treated grey water (TGW) irrigation in soil and tomatoes have been investigated. The wetland mini-reactor with a horizontal underground flow was used to treat the grey water resulting from laundry and tableware activities of a guest house, located next to the experimental area. According to the results, usage of TGW for three months irrigation period does not affect the soil salinity, pH, and Ni contents. Conversely, the concentrations of NO_3^-, Ca^{2+} + Mg^{2+} were slightly increased. The heavy metals, P, Cu and Fe were increased. Microbial assessments revealed that only total coliforms were increased by TGW in soil and leaves. Based on cultivars response, it was concluded that Dart was more reactive to TGW treatment as it had less microbial count, higher yield and antioxidants (Phenols, lycopene, anthocyanin). Accordingly, treatment of grey water may provide an efficient solution to preserve scarce water resources and to avoid soil contamination. Treated grey water seems to be suitable for field grown tomatoes especially in cultivars with effective antioxidative reaction.

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Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

1. Balkhair, K.S. Microbial contamination of vegetable crop and soil profile in arid regions under controlled application of domestic wastewater. *Saudi J. Biol. Sci.* **2015**, *23*, S83–S92. [CrossRef]
2. Oteng-Peprah, M.; Acheampong, M.A.; Devries, N.K. Greywater Characteristics, Treatment Systems, Reuse Strategies and User Perception—A Review. *Water Air Soil Pollut.* **2018**, *229*, 1–16. [CrossRef]
3. Lubbe, E.; Rodda, N.; Sershen, N. Effects of grey water irrigation on germination, growth and photosynthetic characteristics in selected African leafy vegetables. *Water SA* **2016**, *42*, 0378-4738. [CrossRef]
4. De Gisi, S.; Casella, P.; Notarnicola, M.; Farina, R. Grey water in buildings: A mini-review of guidelines, technologies and case studies. *Civ. Eng. Environ. Syst.* **2015**, *33*, 35–54. [CrossRef]
5. Voulvoulis, N. Water reuse from a circular economy perspective and potential risks from an unregulated approach. *Curr. Opin. Environ. Sci. Health* **2018**, *2*, 32–45. [CrossRef]
6. Al-Zu'bi, Y.; Al-Mohamad, I.F. Effect of grey water on soil chemical composition and yield of tomato plant. *J. Food Agric. Environ.* **2008**, *6*, 408–410.
7. Hashem, M.; Qi, X. Treated Wastewater Irrigation—A Review. *Water* **2021**, *13*, 1527. [CrossRef]
8. Hussain, M.I.; Qureshi, A.S. Health risks of heavy metal exposure and microbial contamination through consumption of vegetables irrigated with treated wastewater at Dubai, UAE. *Environ. Sci. Pollut. Res.* **2020**, *27*, 11213–11226. [CrossRef]
9. Misra, R.K.; Patel, J.H.; Baxi, V.R. Removal of pollutants by tomato plants during reuse of laundry greywater for irrigation. In Proceedings of the International Conference on Food Security and Environmental Sustainability (FSES 2009), Kharagpur, India, 17–19 December 2009; pp. 17–19.
10. Sánchez–González, A.; Chapela–Lara, M.; Germán–Venegas, E.; Fuentes-García, R.; del Río-Portilla, F.; Siebe, C. Changes in quality and quantity of soil organic matter stocks resulting from wastewater irrigation in formerly forested land. *Geoderma* **2017**, *306*, 99–107. [CrossRef]

11. De Carlo, L.; Battilani, A.; Solimando, D.; Caputo, M.C. Application of time-lapse ERT to determine the impact of using brackish waste water for maize irrigation. *J. Hydrol.* **2020**, *582*, 124465. [CrossRef]
12. Akpan, V.E.; Omole, D.O.; Bassey, D.E. Assessing the public perceptions of treated wastewater reuse: Opportunities and implications for urban communities in developing countries. *Heliyon* **2020**, *6*, e05246. [CrossRef] [PubMed]
13. Ntuli, V.; Chatanga, P.; Kwiri, R.; Gadaga, H.T.; Gere, J.; Matsepo, T.; Potloane, R.P. Microbiological quality of selected dried fruits and vegetables in Maseru, Lesotho. *Afr. J. Microbiol. Res.* **2017**, *11*, 185–193. [CrossRef]
14. Qishlaqi, A.; Moore, F.; Forghani, G. Impact of untreated wastewater on soils and crops in Shiraz suburban area, SW. *Iran. Environ. Monit. Assess.* **2008**, *141*, 257–273. [CrossRef]
15. Pinto, U.; Maheshwari, B.; Grewal, H. Effects of greywater irrigation on plant growth, water use and soil properties. *Resour. Conserv. Recycl.* **2010**, *54*, 429–435. [CrossRef]
16. Rusan, M.J.M.; Hinnawi, S.; Rousan, L. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination* **2007**, *215*, 143–152. [CrossRef]
17. Faruqui, N.; Al-Jayyousi, O. Greywater Reuse in Urban Agriculture for Poverty Alleviation: A Case Study in Jordan. *Water Int.* **2002**, *27*, 387–394. [CrossRef]
18. Sharvelle, S.; Roesner, L.; Qian, Y.; Stomberger, M.; Azar, M.N. Long-Term Study on Landscape Irrigation Using Household Gray Water—Experimental Study. The Urban Water Centre, Colorado State University. 2012. Available online: http://www.aciscience.org/docs/graywater%20report%202012.pdf (accessed on 6 June 2014).
19. Hajlaoui, H.; Akrimi, R.; Sayahi, S.; Hachicha, M. Usage of treated greywater as an alternative irrigation source for tomatoes cultivation. *Water Environ. J.* **2022**, *36*, 484–493. [CrossRef]
20. Akrimi, R.; Hajlaoui, H.; Batelli, G.; Ruggiero, A.; Badri, M.; Grillo, S.; Mhamdi, M. Electromagnetic water enhanced metabolism and agro-physiological responses of potato (*Solanum tuberosum* L) under saline conditions. *J. Agron. Crop Sci.* **2020**, *207*, 44–58. [CrossRef]
21. Khaskhoussy, K.; Kahlaoui, B.; Nefzi, B.M.; Jozdan, O.; Dakheel, A.; Hachicha, M. Effect of Treated Waste water Irrigation on Heavy Metals Distribution in a Tunisian Soil. *ETASR* **2015**, *5*, 805–810.
22. APHA. *Compendium of Methods for Microbiological Examination of Foods*, 4th ed.; American Public Health Association: Washington, DC, USA, 2001; 676p.
23. APHA. *Standard Methods for the Examination of Water and Waste Water*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
24. Fransolet, E.; Crine, M.; L'Homme, G.; Toye, D.; Marchot, P. Analysis of electrical resistance tomography measurements obtained on a bubble column. *Meas. Sci. Technol.* **2001**, *12*, 1055–1060. [CrossRef]
25. Dashti, N.H.; Montasser, M.S.; Ali, N.Y.; Cherian, V.M. Influence of plant growth promoting rhizobacteria on fruit yield, pomological characteristics and chemical contents in cucumber mosaic virus-infected tomato plants. *Kuwait J. Sci.* **2014**, *41*, 205–220.
26. Daneshmand, F.A.; Javad, M.K.; Manouchehri, K. Physiological responses to NaCl stress in three wild species of potato in vitro. *Acta Physiol. Plant.* **2010**, *32*, 91–101. [CrossRef]
27. Instituto Adolfo Lutz—IAL. *Métodos físico-químicos para análise de alimentos*; IAL: São Paulo, Brazil, 2008.
28. Fish, W.W.; Perkins-Veazie, P.; Collins, J.K. A Quantitative Assay for Lycopene That Utilizes Reduced Volumes of Organic Solvents. *J. Food Compos. Anal.* **2002**, *15*, 309–317. [CrossRef]
29. Toledo, D.S.; Costa, C.A.; Bacci, L.; Fernandes, L.A.; Souza, M.F. Production and quality of tomato fruits under organic management. *Hort. Brasil.* **2011**, *29*, 253–257. [CrossRef]
30. Chen, L.; Feng, Q.; Li, C.; Wei, Y.; Zhao, Y.; Feng, Y.; Zheng, H.; Li, F.; Li, H. Impacts of aquaculture wastewater irrigation on soil microbial functional diversity and community structure in arid regions. *Sci. Rep.* **2017**, *7*, 1–10. [CrossRef]
31. Pereira, B.; He, Z.; Silva, M.; Herpin, U.; Nogueira, S.; Montes, C.; Melfi, A. Reclaimed waste water: Impact on soil-plant system under tropical conditions. *J. Hazard. Mater.* **2011**, *192*, 54–61.
32. Magesan, G.N. Changes in soil physical properties after irrigation of two forested soils with municipal waste water. *N. Z. J. For. Sci.* **2001**, *31*, 188–195.
33. WHO. *Health Guidelines for the Use of Waste Water in Agriculture and Aquaculture*; Report of a WHO Scientific Group; (Technical Report Series 1989, No. 778); WHO: Geneva, Switzerland, 1989.
34. WHO. *Water Quality: Guidelines, Standards and Health*; Fewtrell, L., Bartram, J., Eds.; IWA Publishing: London, UK, 2001; ISBN 1900222280.
35. Al-Hamaiedeh, H.; Bino, M. Effect of treated grey water reuse in irrigation on soil and plants. *Desalination* **2010**, *256*, 115–119. [CrossRef]
36. Bino, M.J.; Al-Beiruti, S.N. Studies of IDRC SupportedResearch on Greywater in JordanConducted by INWRDAM. In *Compilation of Greywater Studies and Reports on Policy, Economic Feasibility, Health Impacts and Reuse Quality Guidelines and the Aqaba Declaration on Greywater Use*; 2007; pp. 1–76.
37. Munir, M.; Mohammad, A. Forage yield and nutrient uptake as influenced by secondary treated sewage water. *J. Plant Nutr.* **2004**, *27*, 351–365.
38. Lopez, A.; Pollice, A.; Lonigro, A.; Masi, S.; Palese, A.M.; Cirelli, G.L.; Toscano, A.; Passino, R. Agricultural sewage water reuse in southern Italy. *Desalination* **2006**, *187*, 323–334. [CrossRef]

39. Nascimento, A.D.R.; Junior, M.S.S.; Caliari, M.; Fernandes, P.M.; Rodrigues, J.P.; De Carvalho, W.T. Qualidade de tomates de mesa cultivados em sistema orgânico e convencional no estado de Goiás. *Hortic. Bras.* **2013**, *31*, 628–635. [CrossRef]
40. Yadav, A.K.; Singh, S.V. Osmotic dehydration of fruits and vegetables: A review. *J. Food Sci. Technol.* **2012**, *51*, 1654–1673. [CrossRef]