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Sustainable Use of Treated Municipal Wastewater after Chlorination: Short-Term Effects on Crops and Soils

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Abstract: Due to the scarcity of fresh water for crop irrigation in semi-arid areas, sustainable use of treated municipal wastewater is essential. Chlorine for wastewater disinfection added in wastewater treatment plants may be toxic for crops and can degrade cultivated soils. This study evaluates the crop and soil response to irrigation with treated municipal wastewater (with or without chlorination) in comparison to clear water. Small plants of tomato and cabbage and young bergamot trees were irrigated in pots throughout two months. The use of chlorinated or non-chlorinated wastewater did not significantly change biomass growth, morphological parameters and the efficiency of energy transfer. Significant reductions (40–50%) in the stem diameter of tomato and bergamot plants and differences (−25% to 53%) in all physiological parameters were measured for tomato immediately after the irrigation start. A decrease (−55%) in stomatal conductance and transpiration rate together with an increase (+80%) in water use efficiency were also recorded in bergamot after 30 days of irrigation. This type of irrigation water did not induce significant changes in soil properties, except for a decrease in pH (−20%) in bergamot soils after the irrigation start and in electric conductivity (EC, −40%) at the end of the irrigation period for all species. Irrigation of plants with chlorinated wastewater increased the weight of the fresh biomass (+56%) of leaves and the stem diameter (−60%) of tomato and decreased water use efficiency (+67%) in bergamot after the irrigation start. After two months, decreases in stomatal conductance and transpiration rate in cabbage (over 50%) and increases in water use efficiency in cabbage and bergamot (by 40% and 70%, respectively) were evident. Among the studied soil properties, land application of chlorinated wastewater only reduced electrical conductivity (−47%). Overall, this study demonstrated that the use of treated municipal wastewater (with or without chlorination) does not have detrimental impacts on both plant growth (at least for tomato, cabbage and bergamot) and soil health in the short term.

Keywords: water scarcity; non-conventional water; crop irrigation; biomass growth; plant morphology; plant physiology; soil health

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1. Introduction

The sustainable reuse of treated or untreated wastewater in irrigated agriculture is quite common in many countries [1–5]. Globally, wastewater is used to irrigate approximately 20 million hectares of agricultural land, mostly in the form of untreated wastewater [6]. In the Mediterranean croplands, the reuse of wastewater for irrigation has been progressively adopted by almost all countries [7]. Nowadays, approximately 10% of the total irrigated surface worldwide is supplied with wastewater [8]. According to future projections [9,10], this share will increase, due to the forecasted climate changes as well as the intensification of agricultural activities [11,12].

Past research has explored the effects of municipal wastewater reused for irrigation on various crops [13,14], including, for example, tomatoes [15], citruses [7], chickpeas [16] and cotton [17]. The effects of wastewater application on crop growth and yield [18,19], as well as the physico-chemical properties of soils [16,20], were in general non-negative. However, many studies have shown that the reuse of municipal wastewater for crop irrigation can lead to negative impacts, such as soil salinization, damage to the most sensitive plants and possible contamination of both plants and soils with toxic compounds and pathogenic microorganisms (such as coliforms [15,21]). Since the impacts of reused wastewater depend on the irrigated species [22,23], it is essential to explore the response of specific plants by field experiments, evaluating both crop yield and growth as well as the changes in soil properties.

Some crops exhibit a low tolerance to non-negligible concentrations of some compounds or elements (e.g., chlorine, sodium, boron, heavy metals) even in fully treated municipal wastewater [24]. Some of these compounds are added in tertiary treatments (e.g., flocculation and disinfection), which are commonly used to limit microbial risks to human health [25,26] and are required by almost all national regulations [27,28]. Disinfection of wastewater is often carried out by adding chlorine or chlorinated compounds, such as sodium hypochlorite (NaClO) solution. This is the most widely used disinfection option, thanks to its highly bactericidal properties, simple operation and low cost [29]. Chlorine is generally supplied to the final effluent at a dose of about 5–10 mg/L and with a contact time of 30 min [30]. However, chlorine addition to municipal effluents used for irrigation could be toxic for some crops [31–33]. Vegetation can uptake and store chlorine, chlorides and chlorinated compounds, which is the main problem for plant growth together with sodium [31]. Chlorine and chlorinated compounds may also alter some chemical and biochemical properties of soils, with particular reference to microbial communities [7,34,35]. The possible accumulation of chloride may increase the mobility and bioavailability of heavy metals in the soil, with transfer to plant leaves and the food chain [30,36]. According to [37], less than 1 mg/L of residual chlorine should not be harmful to plants, but sensitive crops do not tolerate concentrations close to 0.05 mg/L [27].

Although the effects of chlorination on the environment have been widely investigated (e.g., [38,39]), little is known about the consequences of the use of chlorinated effluents on some important crops and soils [30]. As far as now, studies about the effects of wastewater disinfection on crops that are typical of the Mediterranean agriculture (e.g., citrus trees and vegetables) are scarce in the scientific literature. Moreover, to the authors' best knowledge, there are no specific investigations on tomato, cabbage and bergamot (a typical citrus tree growing on the Ionian Coast of Southern Italy, whose fruit essence and juice are widely used in perfumery and food industries). This is an important research gap, which requires more research on the topic.

This study evaluates the crop and soil response to irrigation with the treated municipal wastewater of cabbage, tomato and bergamot tree. To this aim, the most important soil properties (pH, electrical conductivity, organic carbon, nitrogen and phosphorous) and plant parameters (photosynthesis, stomatal conductance, transpiration and water use efficiency) were measured on small plants of the three species growing in pots and irrigated with non-chlorinated and chlorinated wastewater, as well as clear water, throughout two months. We hypothesize that, due to the disinfection treatment, irrigation with treated wastewater can potentially decrease plant growth and biomass yield and can significantly modify the main soil properties. The reply to this concern should provide farmers and agronomists with indications about the most sustainable wastewater type for the irrigation of these crops.

2. Materials and Methods

2.1. Experimental Site

The investigation was carried out in experimental farm of the "AGRARIA" Department at the Mediterranean University of Reggio Calabria (Southern Italy). The farm is located at an altitude of 136 m a.s.l. with South-East aspect. The climate of the area is semi-arid (Csa, "Hot-summer Mediterranean climate"), according to the classification proposed by [40], with mild and humid winters and hot and dry summers. The average annual rainfall and temperatures are 607 mm and 17.4 °C, respectively (data for the period of 2000–2020, weather station of Reggio Calabria-Villa Comunale, about 5 km from the farm).

2.2. Experimental Design

Three plant species were selected for this experiment: a fruit tree crop (bergamot, *Citrus bergamia* ssp. *Fantastica*) and two vegetable crops (tomato, *Solanum lycopersicum* ssp. *Cuore di Bue*, and cabbage, *Brassica oleracea* ssp. *Ramoso Calabrese*).

Following the guidelines by [32], the experiments were carried out in pots, in order to avoid uncontrolled water dispersion, as it may happen in field. Undoubtedly, the irrigation tests in pots are not representative of full-scale biotic and abiotic processes, but these tests allow full control of the water input to the complex plant soil, whose only escape is evapotranspiration. In this sense, the compounds supplied with wastewater (e.g., organic matter, nutrients and inhibiting compounds) are confined by the impervious surfaces of the plastic pot and therefore may impact plant growth and soil characteristics.

The bergamot seedlings grew in pots in a nursery until 18 months. On 15 July 2022, these seedlings and the seeds of cabbage and tomato plants were planted in pots (22 cm high and 24 cm in diameter) on the experimental farm. The soils in pots were loamy-textured, with 40% sand, 25% silt and 35% clay and a pH of 7.1. No optimization tests for plant, soil and water parameters were deliberately carried out.

Three treatments were carried out for each species: (i) irrigation with clear water, assumed as control (hereafter indicated by "CW"); (ii) wastewater collected upstream of the chlorination treatment (WW) in a treatment plant with nitrification and denitrification processing of municipal wastewater in Reggio Calabria city; and (iii) wastewater collected downstream of NaClO chlorination (Cl-WW). The NaClO solution (at a minimum chlorine concentration of 12%) is dosed by a probe working at a concentration range of free chlorine between 0.05 and 0.1 mg/L. Irrigation started immediately after planting (for tomato and cabbage) or transplanting (for bergamot).

Therefore, the experimental design consisted of three species (tomato, cabbage and bergamot) × three treatments (CW, WW and Cl-WW) × three replicates, totaling 27 experiments.

2.3. Irrigation Tests

Before planting the species in the pots, soil water content (SWC) at field capacity was determined using a common gravimetric method. This allowed the estimation of the crop irrigation requirement, which was set to keep the soil at 80% of field capacity. These requirements were 39.5 mm for bergamot and 29 mm for tomato and cabbage. SWC was measured at each watering using a HydroSense II probe (Campbell Scientific, Logan, UT, USA) as control. Simultaneously, the rainfall supply was measured with a simple rain gauge placed near the pots. Figure 1 reports the irrigation scheduling and volume as well as the rainfall depth input. Vegetables may take from 60 to 100 days (for tomato) and from four to six months (for cabbage) to be harvested after transplanting in the field and less in the greenhouse and only need irrigation for two to three months in the dry season. The studied fruit tree species is a young individual (about 1.5 years old) that is the transplanting age for most fruit crops, and this young age makes the tree (and the transplanting operation) very sensitive to external adverse conditions (such as irrigation with water of poor quality). Therefore, in the short time of the irrigation period adopted in this study, it is highly possible that the potentially toxic compounds in wastewater cause phytotoxic effects on plants, shown by negative impacts on growth and morphological and physiological traits. These are the reasons why only the short-term effects of plant irrigation (two months) with wastewater were analyzed.

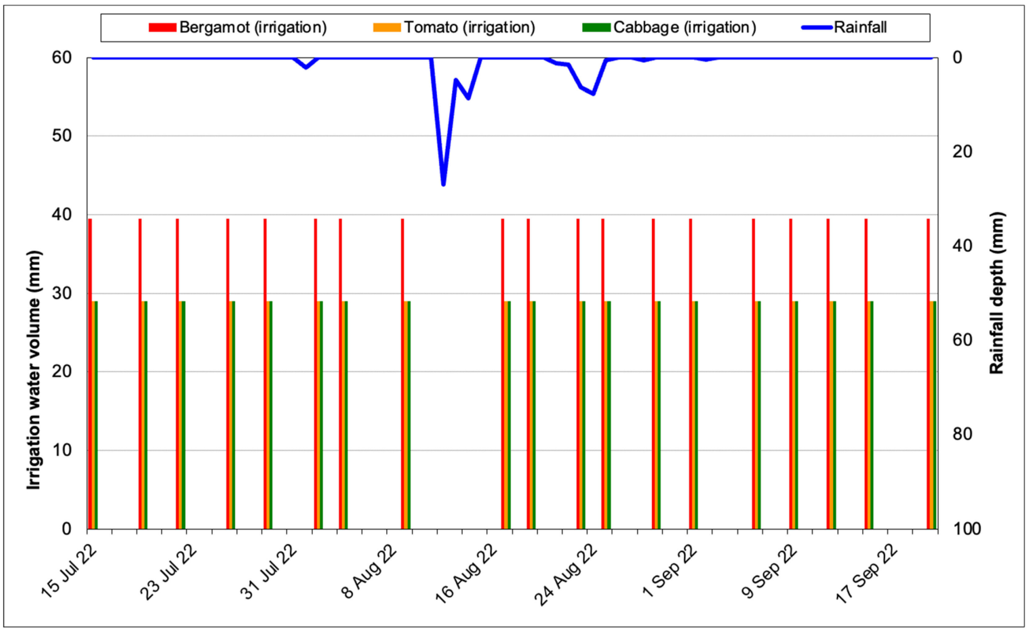


Figure 1. Crop irrigation requirement and rainfall supply throughout the experimental tests.

2.4. Measurement of Physico-Chemical Characteristics of Wastewater

The wastewater used was periodically characterized (chemical oxygen demand, COD, concentration of total nitrogen, phosphorus and chlorine) during periodic sampling at the treatment plant, using common analytical methods for water measurements [41] (Table 1).

Table 1. Main physico-chemical characteristics of municipal wastewater used for the irrigation tests (activated sludge plant of Ravagnese, Reggio Calabria, Italy).

Parameters	Wastewater Type	
	Before Chlorination	**After Chlorination**
	Sampling date: 14 July 2022	
COD (mg/L)	42.6	43.6
Phosphorous (mg/L)	2.37	1.58
Total nitrogen (mg/L)	14.8	15.2
Chlorine (mg/L)	-	0.02
	Sampling date: 28 July 2022	
COD (mg/L)	45.7	46.5
Phosphorous (mg/L)	1.05	2.18
Total nitrogen (mg/L)	14.8	11.2
Chlorine (mg/L)	-	0.03
	Sampling date: 14 September 2022	
COD (mg/L)	44.6	46.1
Phosphorous (mg/L)	2.02	1.98
Total nitrogen (mg/L)	20.5	22.6
Chlorine (mg/L)	-	0.02

2.5. Plant Surveys and Analysis

Immediately after irrigation start and in the short term (after 18, 26 and 42 days for cabbage, 6 and 17 days for tomato and 30 days for bergamot), the main growth, morphological and physiological parameters were measured using destructive and non-destructive methods. Fresh weight and dry weight were selected as growth parameters for tomato and cabbage, together with the number of new shoots for bergamot. Plant height and number of leaves (for tomato and cabbage), stem diameter (for tomato) and shoot length and diameter (for bergamot) were adopted as morphological parameters. Gas exchanges, namely photosynthesis, transpiration rate and stomatal conductance (for all three species), chlorophyll fluorescence (for bergamot), as well as the intrinsic water use efficiency (WUE, for all species), were adopted as physiological parameters. Finally, the efficiency of energy transfer from the antenna pigments to the PSII reaction center (hereafter "Fv/Fm") was measured. This parameter indicates the capacity of the leaf system to convey energy for photosynthetic activity.

Based on the morphological parameters, the relative growth rate (RGR, i.e., plant growth per unit of time) was calculated for each species. The following equations were applied for bergamot:

$$RGR(SL) = [\ln(SL_1) - \ln(SL_0)]/(t_1 - t_0) \quad (1)$$

$$RGR(SD) = [\ln(SD_1) - \ln(SD_0)]/(t_1 - t_0) \quad (2)$$

where RGR(SL) and RGR(SD) are the relative growth rate of stem length (SL) and diameter (SD), respectively; SL_1 and SL_0 and SD_1 and SD_0 are the length and diameter of shoots, respectively, at time t_0 (immediately after irrigation start) and t_1 (after 30 days). For the cabbage and tomato seedlings, the RGR of the morphological parameters at the intermediate dates was linearly interpolated based on the natural logarithms of the relative values at the time of the field surveys.

Gas exchange and chlorophyll fluorescence were determined with a portable photosynthesis meter (LICOR LI6400XT), assuming the following setup parameters: (i) flow rate = 500 cm^3 min^{-1}; (ii) leaf temperature = 26 °C; CO_2 concentration (monitored by cartridges) = 400 μmol(CO_2) mol(air)$^{-1}$; (iii) photosynthetic active radiation (provided by led) = 1200 μmol m^{-2} s^{-1}. Each measurement was carried out with a minimum and maximum waiting time of 120 and 200 s, respectively, using a "matching" operation of the infrared gas analyzer for differences in CO_2 concentrations between the "sample" and the "reference heat" of 50 μmol (CO_2) mol(air)$^{-1}$.

2.6. Soil Surveys and Analysis

Soil samples were taken in pots with each species and under each irrigation treatment immediately after irrigation start and after 60 days to measure pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (TN) and phosphorus (P) contents. In more detail, pH and electrical conductivity (EC) were determined in distilled water, at a soil:solution ratio of 1:2.5 with a multiparameter portable device (Hanna Instruments® model HI2040-02, Gipuzkoa, Spain). TOC was measured by the potassium dichromate oxidation method [42]. TN was determined using an elemental analyzer detector with a thermal conductivity sensor (Elementar Rapid N; Elementar Analysen systeme GmbH, Hanau, Germany). Available P was determined following the method described by [43]. According to [32], chloride and chlorine were not determined in the irrigated soils, since these are the most mobile ions and are easily lost by leaching and evaporation [44,45].

2.7. Statistical Processing

A one-way ANOVA was applied to the growth and morphological (for all species) and physiological (for bergamot) parameters, assuming the treatment as factor. Physiological parameters of cabbage and tomato seedlings were subjected to a one-way ANOVA with repeated measures at all survey dates with the same experimental factor. A two-way ANOVA was further applied to the soil parameters, assuming the treatment and survey dates (July and September) as factors. The equality of variance and normal distribution, which are assumptions of the statistical tests, were evaluated by normality tests, or the data were square-root-transformed when necessary. In all cases, Tukey's test was used to find statistical differences between pairs of means in the measured parameters ($p < 0.05$).

Then, two multivariate statistical techniques (principal component analysis, PCA, and agglomerative hierarchical cluster analysis, AHCA) were applied to observations of plant and soil variables measured at the last surveys. In more detail, PCA was applied to identify derivative variables (principal components, PCs) from the original dataset of observations. The original variables (expressed by different measuring units) were first standardized, and Pearson's coefficients were computed to build the correlation matrix. The latter was used to explore possible correlations between pairs of original variables. The first two PCs, explaining at least 70% of the variance of the original variables, were considered. Finally, the observations were grouped in clusters using AHCA, which allows one to group samples with similar characteristics by considering the scores on the first two PCs. Euclidean distance was used as the similarity–dissimilarity measure.

The statistical analysis was carried out using SPSS and XLSTAT software (release 2019).

3. Results

3.1. Plant Response to Irrigation

3.1.1. Growth Parameters

The two-way ANOVA showed that none of the evaluated growth plant parameters were significantly different among the treatments (p-level < 0.05) (Table 2).

In more detail, irrigation of tomato and cabbage seedlings with both types of wastewater (WW and Cl-WW) did not significantly change the dry and fresh biomass of the aerial part compared to CW (in the range of 7.85–11.3 g, for tomato, and 5.87–9.31 g, for cabbage). Only the fresh biomass of tomato leaves significantly increased from 12.7 $\pm$ 0.82 g (CW) to 19.9 $\pm$ 1.03 g when Cl-WW was used (Figure 2).

3.1.2. Morphological Parameters

According to the two-way ANOVA, among the morphological parameters, only the RGR diameter of tomato stems and bergamot sprouts was significantly different among the treatments (p-level < 0.05) (Table 2). More specifically, the stem diameter of tomato plants decreased from 0.022 $\pm$ 0.001 mm/day (CW) to 0.01 $\pm$ 0.004 mm/day (WW) and 0.009 $\pm$ 0.003 mm/day (Cl-WW) (Figure 3). The emission of new sprouts of bergamot seedlings irrigated with WW or Cl-WW was from one to ten during the irrigation period (Figure 3). The relative growth rate of the sprout diameter was lower in bergamots irrigated with WW (0.0018 $\pm$ 0.0004 mm/day) compared to seedlings irrigated with CW (0.0029 $\pm$ 0.0003 mm/day) and Cl-WW (0.0032 $\pm$ 0.0003 mm/day) (Figure 3).

3.1.3. Physiological Parameters

After the two-way ANOVA, the following physiological parameters showed significant differences among the treatments (p-level < 0.05) (Table 2): (i) net photosynthesis, stomatal conductance, transpiration rate and intrinsic WUE (immediately after the irrigation start) for tomato; (ii) net photosynthesis (at the 18th day), stomatal conductance (at the 18th and 42nd days), transpiration rate (at the 18th day) and intrinsic WUE (at the 42nd day) for cabbage; and (iii) all parameters, except for net photosynthesis, immediately after the irrigation start for bergamot (Table 2).

In the period immediately after the irrigation start, the plants irrigated with Cl-WW showed an increase in net photosynthesis (7.5 $\pm$ 1.91 μmol m^{-2} s^{-1}), stomatal conductance (0.024 $\pm$ 0.005 μmol m^{-2} s^{-1}), transpiration rate (0.62 $\pm$ 0.13 μmol m^{-2} s^{-1}) and water use efficiency (310 $\pm$ 19.2 μmol CO_2/μmol H_2O) compared to the seedlings treated with WW (1.8 $\pm$ 0.55, 0.009 $\pm$ 0.002, 0.24 $\pm$ 0.05 μmol m^{-2} s^{-1} and 193 $\pm$ 26.4 μmol CO_2/μmol H_2O, respectively) (Figure 4). The intrinsic WUE showed a significant difference only between Cl-WW (266 $\pm$ 6.8 mol CO_2/μmol H_2O) and CW (169 $\pm$ 27.8 mol CO_2/μmol H_2O) at the end of the survey period (Figure 4a).

Table 2. Results of one-way ANOVA applied to the main growth, morphological and physiological parameters of tomato, cabbage and bergamot plants irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW).

Factor	Variable	Sampling Time (Days)	Degrees of Freedom	Sum of Squares	Mean Squares	F	Pr > F
			Tomato				
Treatment	Fresh weight (plant)	60	2	13.942	6.971	0.557	0.600
	Fresh weight (stem)	60		98.353	49.176	4.611	0.061
	Fresh weight (leaves)	60		77.428	38.714	4.267	0.070
	Dry weight (plant)	60		0.295	0.147	0.087	0.918
	Dry weight (stem)	60		0.096	0.048	0.116	0.893
	Dry weight (leaves)	60		0.106	0.053	0.112	0.896
	RGR plant height	60		0.000	0.000	2.385	0.173
	RGR number of leaves	60		0.000	0.000	1.036	0.411
	RGR stem diameter	60		0.000	0.000	6.020	**0.037**
	Net photosynthesis	0		50.180	25.090	5.868	**0.039**
	Net photosynthesis	6		23.619	11.809	1.346	0.329
	Net photosynthesis	17		18.705	9.352	0.743	0.515
	Stomatal conductance	0		0.000	0.000	4.957	**0.044**
	Stomatal conductance	6		0.000	0.000	1.754	0.251
	Stomatal conductance	17		0.000	0.000	0.054	0.948
	Transpiration rate	0		0.215	0.108	5.029	**0.042**
	Transpiration rate	6		0.230	0.115	1.276	0.345
	Transpiration rate	17		0.009	0.005	0.064	0.939
	Intrinsic WUE	0		20,727.186	10,363.593	7.407	**0.024**
	Intrinsic WUE	6		2520.163	1260.082	0.655	0.553
	Intrinsic WUE	17		14,207.267	7103.633	6.304	**0.034**
	Fv/Fm	0		0.001	0.000	0.128	0.882
	Fv/Fm	6		0.004	0.002	0.213	0.814
	Fv/Fm	17		0.009	0.005	0.632	0.564
			Cabbage				
Treatment	Plant weight (fresh)	60	2	276.781	138.391	2.667	0.148
	Plant weight (dry)	60		5.722	2.861	2.330	0.178
	RGR plant height	60		0.000	0.000	0.452	0.657
	RGR number of leaves	60		0.000	0.000	2.216	0.190
	Net photosynthesis	0		27.334	13.667	0.330	0.731
	Net photosynthesis	18		230.149	115.074	8.561	**0.018**
	Net photosynthesis	26		13.556	6.778	0.489	0.636
	Net photosynthesis	42		66.072	33.036	2.159	0.197
	Stomatal conductance	0		0.001	0.001	0.599	0.579
	Stomatal conductance	18		0.005	0.003	5.569	**0.043**
	Stomatal conductance	26		0.000	0.000	0.196	0.827
	Stomatal conductance	42		0.002	0.001	5.317	0.047
	Transpiration rate	0		0.386	0.193	0.500	0.630
	Transpiration rate	18		3.000	1.500	6.098	**0.036**
	Transpiration rate	26		0.160	0.080	0.450	0.658
	Transpiration rate	42		1.145	0.572	5.305	**0.047**
	Intrinsic WUE	0		3502.908	1751.454	2.476	0.165
	Intrinsic WUE	18		2359.618	1179.809	0.437	0.665
	Intrinsic WUE	26		151.802	75.901	0.191	0.831
	Intrinsic WUE	42		19,237.228	9618.614	12.116	**0.008**
	Fv/Fm	0		0.053	0.027	4.181	0.073
	Fv/Fm	18		0.043	0.021	2.521	0.160
	Fv/Fm	26		0.003	0.002	0.581	0.588
	Fv/Fm	42		0.006	0.003	5.792	**0.040**
			Bergamot				
Treatment	New sprouts	60	2	20.667	10.333	0.846	0.475
	RGR sprout diameter	60		0.000	0.000	5.719	**0.041**
	RGR sprout length	60		0.000	0.000	1.865	0.235
	Net photosynthesis	0		4.747	2.374	1.721	0.195
	Net photosynthesis	30		15.519	7.759	4.356	**0.021**
	Stomatal conductance	0		0.000	0.000	13.729	**<0.0001**
	Stomatal conductance	30		0.000	0.000	15.546	**<0.0001**
	Transpiration rate	0		0.187	0.094	12.067	**0.000**
	Transpiration rate	30		0.165	0.083	16.352	**<0.0001**
	Intrinsic WUE	0		397,197.650	198,598.825	1657.266	**<0.0001**
	Intrinsic WUE	30		224,959.271	112,479.635	28.285	**<0.0001**

Note: bold characters indicate significant differences after Tukey's test ($p < 0.05$).

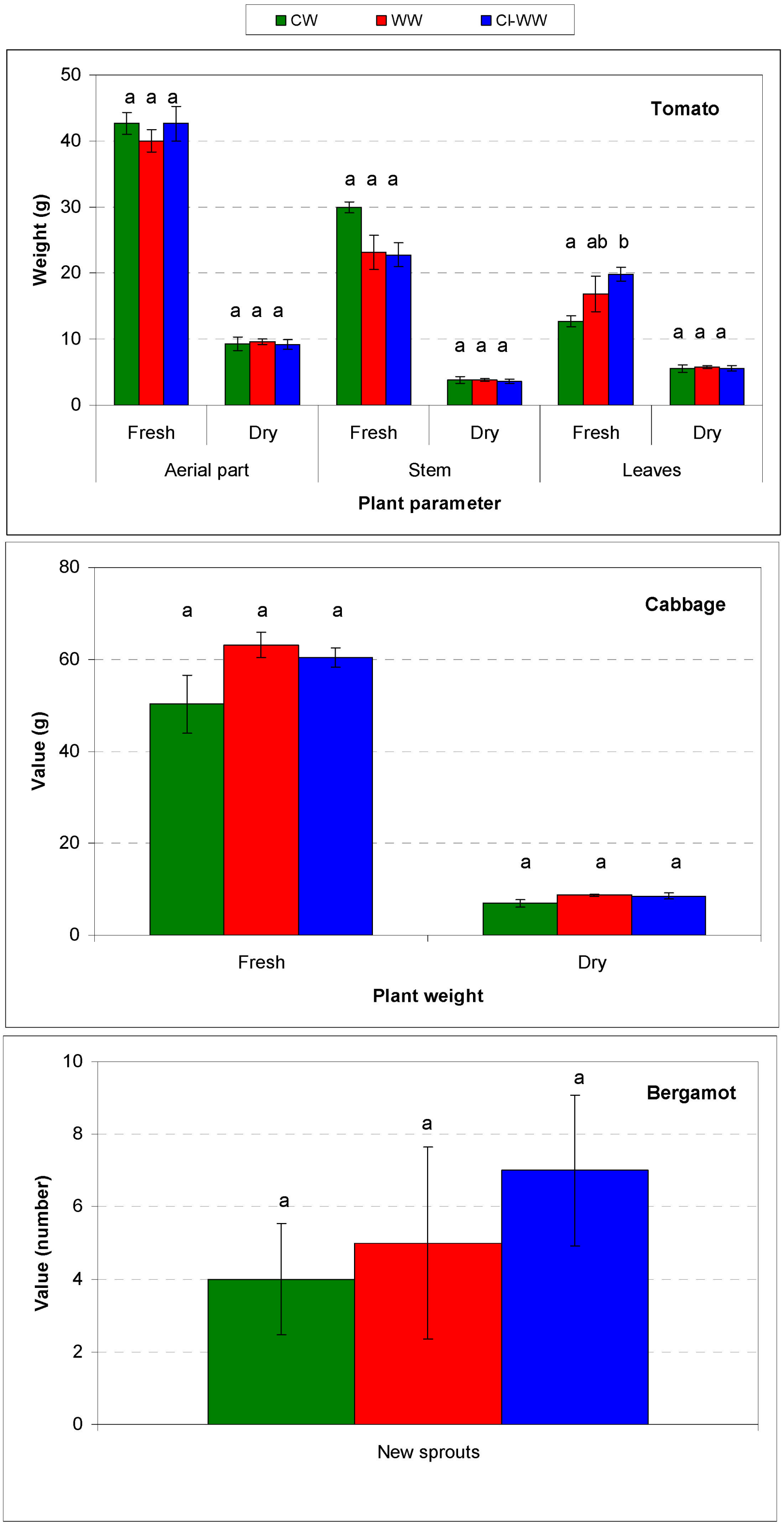


Figure 2. Mean ± standard error (n = 3) of fresh and dry weight of tomato, cabbage and bergamot plants irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW). Different letters indicate significant differences after Tukey's test ($p < 0.05$).

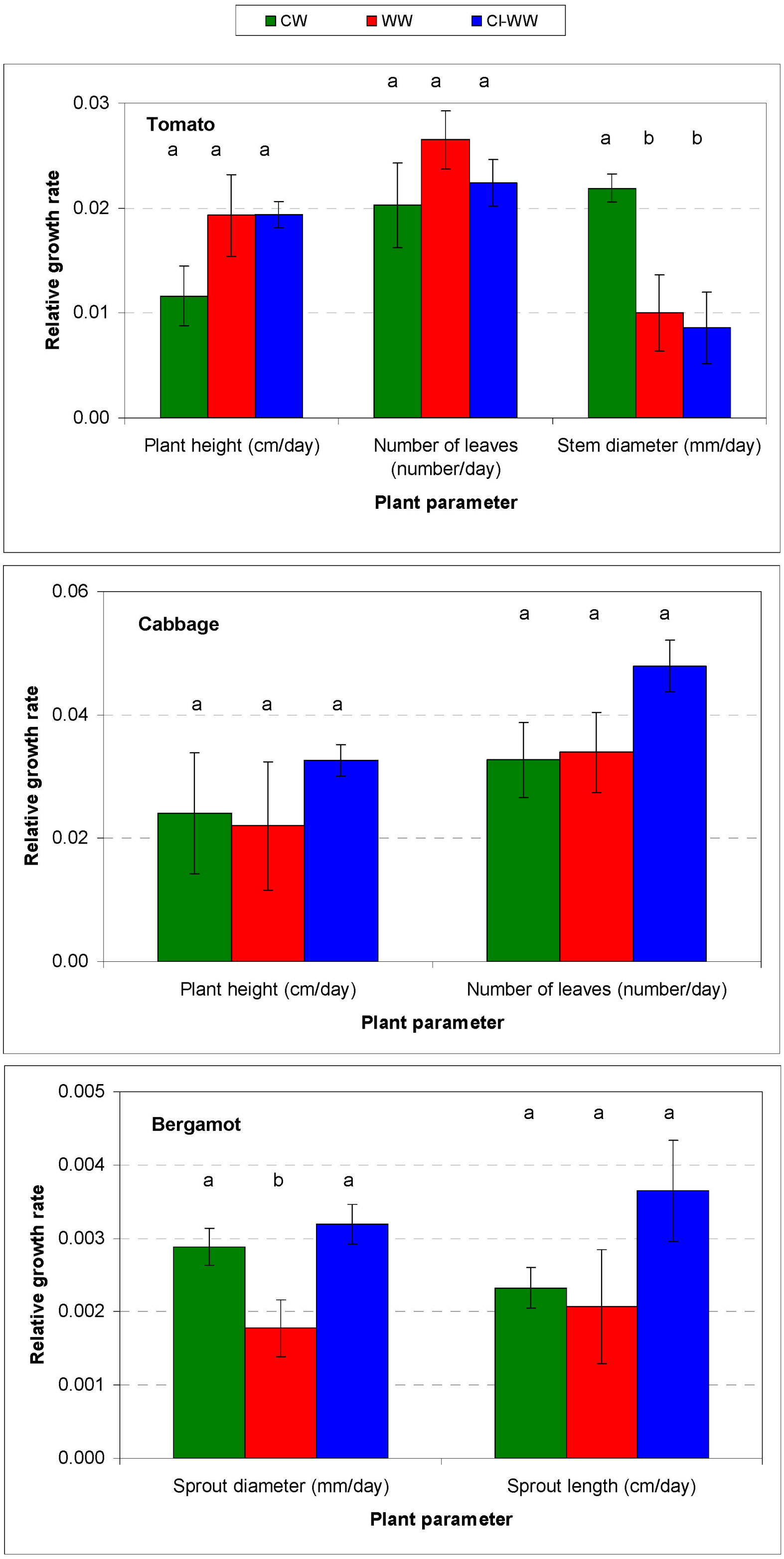
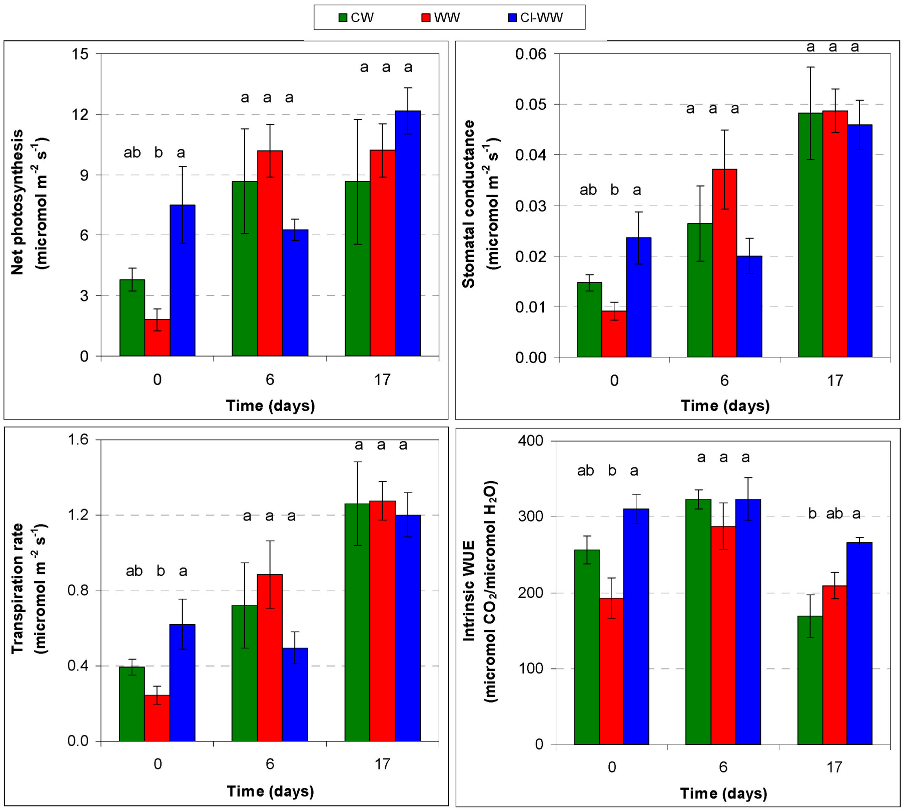
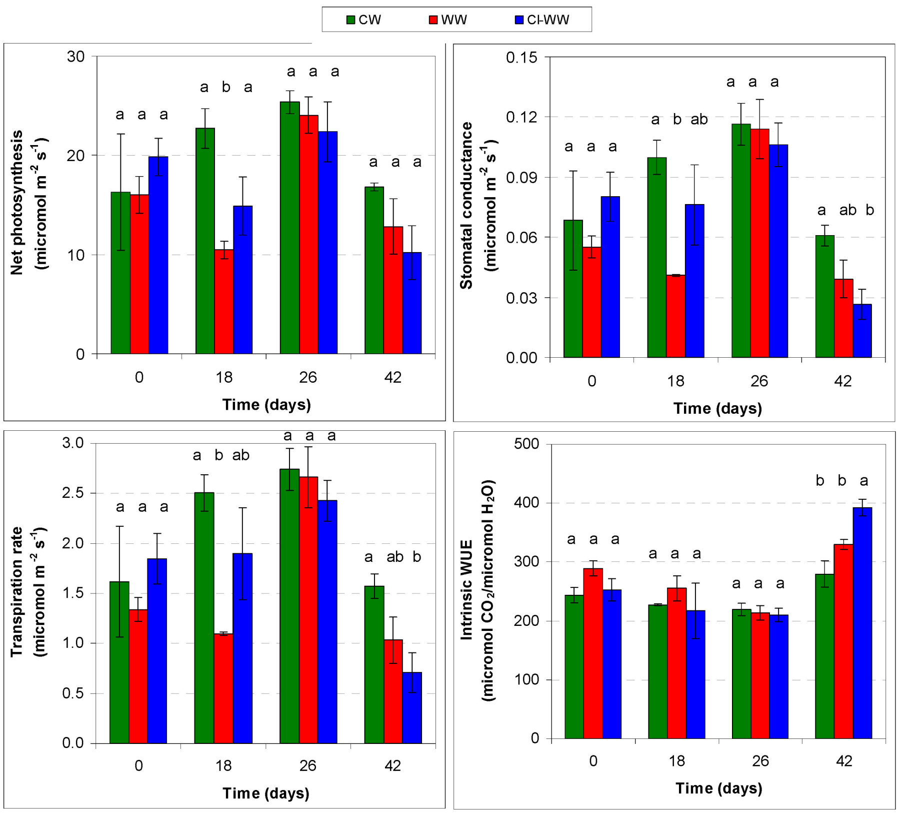


Figure 3. Mean ± standard error (n = 3) of morphological parameters of tomato, cabbage and bergamot plants irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW). Different letters indicate significant differences after Tukey's test ($p < 0.05$).

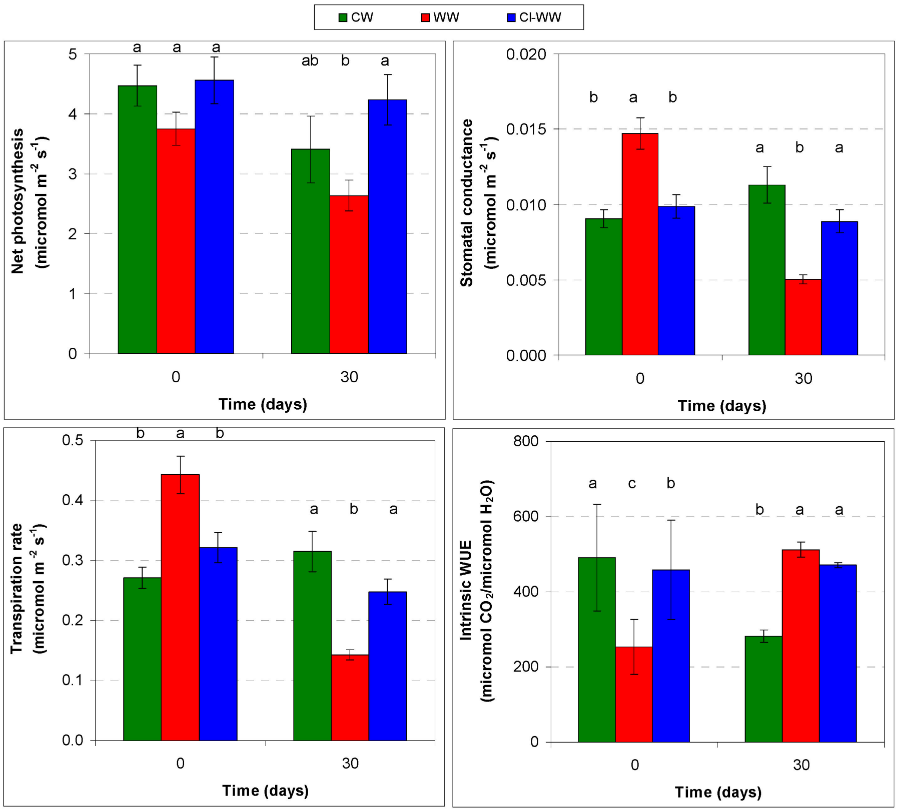


(**a**)



(**b**)

Figure 4. *Cont.*



(**c**)

Figure 4. Mean ± standard error (n = 3) of physiological parameters of tomato (**a**), cabbage (**b**) and bergamot (**c**) plants irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW). Different letters indicate significant differences after Tukey's test ($p < 0.05$); WUE stands for water use efficiency.

For the cabbage plants, only at the end of the observation period, the stomatal conductance and transpiration rate of plants treated with Cl-WW were lower compared to the same species irrigated with CW (0.027 ± 0.008 vs. 0.061 ± 0.005 µmol m^{-2} s^{-1} and 0.71 ± 0.2 vs. 1.57 ± 0.12 µmol m^{-2} s^{-1}, respectively). After 42 days, chlorination influenced the intrinsic WUE, and this parameter was higher (393 ± 14.6 µmol CO_2/µmol H_2O) compared to irrigation with CW and WW (280 ± 22.5 and 330 ± 8.6 µmol CO_2/µmol H_2O, respectively) (Figure 4b).

For bergamot plants, after 30 days from the irrigation start, net photosynthesis was higher in the plants irrigated with Cl-WW (4.24 ± 0.42 µmol m^{-2} s^{-1}) only in comparison with the treatment with WW (2.63 ± 0.26 µmol m^{-2} s^{-1}). Both stomatal conductance and respiration rate increased in the bergamot seedlings irrigated with WW (0.015 ± 0.001 and 0.44 ± 0.03 µmol m^{-2} s^{-1}, respectively) compared to plants irrigated with both CW (0.009 ± 0.001 and 0.27 ± 0.02 µmol m^{-2} s^{-1}) and Cl-WW (0.01 ± 0.001 and 0.32 ± 0.03 µmol m^{-2} s^{-1}). A significant decrease in the stomatal conductance and respiration rate was instead noticed for the plants irrigated with WW (0.005 ± 0.0003 µmol m^{-2} s^{-1}, stomatal conductance, and 0.14 ± 0.01 µmol m^{-2} s^{-1}, respiration rate), while the seedlings irrigated with CW (0.011 ± 0.001 and 0.32 ± 0.03 µmol m^{-2} s^{-1}) and Cl-WW (0.009 ± 0.001 and 0.25 ± 0.02 µmol m^{-2} s^{-1}) showed much higher values. Intrinsic WUE was different at the start of the monitoring period (491 ± 142 µmol CO_2/µmol H_2O for CW, 254 ± 73.2 µmol CO_2/µmol H_2O for WW and 459 ± 132 µmol CO_2/µmol H_2O for Cl-WW) and, after 30 days of irrigation, was higher in the treatments with wastewater (512 ± 19.9 µmol CO_2/µmol H_2O, WW, and 471 ± 6.4 µmol CO_2/µmol H_2O, Cl-WW) compared to irrigation with CW (282 ± 16.9 µmol CO_2/µmol H_2O) (Figure 4c).

3.1.4. Efficiency of Energy Transfer

The capacity of the leaf system to convey energy for photosynthetic activity (measured by the Fv/Fm parameter) was lower only at the 42nd day of the irrigation period for the cabbage plants treated with WW (0.557 ± 0.008) compared to irrigation with Cl-WW (0.617 ± 0.02) (Table 2 and Figure 5).

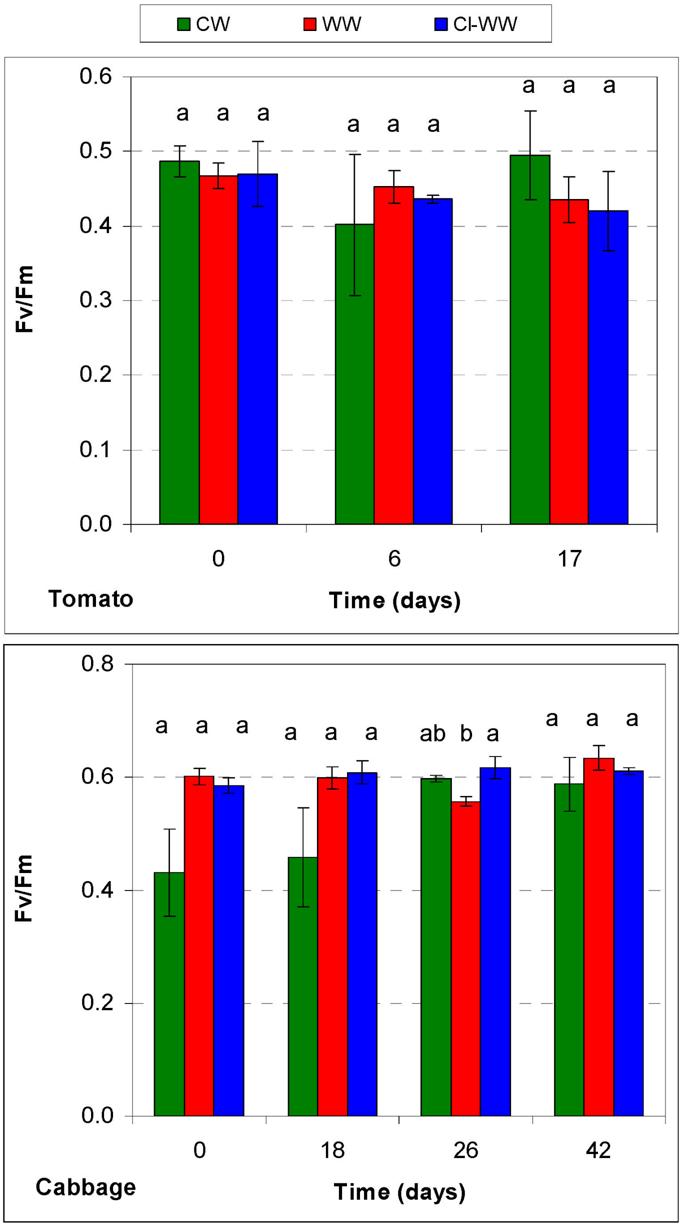


Figure 5. Mean ± standard error (*n* = 3) of efficiency of energy transfer (Fv/Fm) of tomato and cabbage plants irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW). Different letters indicate significant differences after Tukey's test ($p < 0.05$).

3.2. Effects of Irrigation on Soils

The two-way ANOVA applied to the soil parameters showed that the treatments determined significant differences in P, pH and EC, while only P and TN changed over time. The interaction between the two factors (treatment × sampling date) was never significant (Table 3).

Table 3. Results of two-way ANOVA applied to the main growth, morphological and physiological parameters of tomato, cabbage and bergamot plants irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW).

Factor	Degrees of Freedom	Sum of Squares	Mean Squares	F	Pr > F
		TOC			
Treatment	2	0.053	0.027	1.072	0.353
Sampling date	1	0.002	0.002	0.097	0.758
Treatment × sampling date	2	0.003	0.001	0.052	0.949
		P			
Treatment	2	0.000	0.000	4.154	**0.024**
Sampling date	1	0.000	0.000	17.382	**0.000**
Treatment × sampling date	2	0.000	0.000	1.476	0.242
		TN			
Treatment	2	0.000	0.000	0.026	0.974
Sampling date	1	0.005	0.005	26.827	**<0.0001**
Treatment × sampling date	2	0.000	0.000	0.337	0.716
		pH			
Treatment	2	1.219	0.609	3.574	**0.038**
Sampling date	1	0.068	0.068	0.400	0.531
Treatment × sampling date	2	0.155	0.078	0.455	0.638
		EC			
Treatment	2	491,911.000	245,955.500	72.732	**<0.0001**
Sampling date	1	7656.463	7656.463	2.264	0.141
Treatment × sampling date	2	7578.037	3789.019	1.120	0.337

Note: bold characters indicate significant differences after Tukey's test ($p < 0.05$). In more detail, for soils with bergamot, P content decreased over time only in the plants irrigated with CW (0.06 ± 0.01%) or Cl-WW (0.07 ± 0.01%) compared to the value measured for irrigation with WW at the start of the irrigation period (0.06 ± 0.01%) (Figure 6). The only difference in pH was detected between soils with bergamot plants irrigated with CW (8 ± 0.99) and WW (6.4 ± 0.12) at the start of the experiment. EC showed the highest variability in the analyzed parameters for all species and irrigation treatments and over time. In general, a gradient Cl-WW < WW < CW was found in soils supporting all species, and, only for tomato, the EC values were higher at the start of the experiment compared to the final survey. The highest and lowest values for this parameter were measured for cabbage irrigated with Cl-WW (205 ± 18 µS/cm) at the start of the irrigation period and with CW at its end (600 ± 55 µS/cm), respectively (Figure 7).

3.3. Analysis of Combined Effects of Irrigation on Plants and Soils Using Multivariate Statistical Techniques

Pearson's matrix shows significant linear correlations between several pairs of plant and soil variables. In more detail, among the plant parameters, PS was noticeably correlated to SC and TR (r > 0.89), and the latter parameters were strongly associated with each other (r = 0.99). Also, WUE was inversely correlated to SC and TR (r > |−0.72|). Among the soil properties, a strong correlation was found between TOC and P (r = 0.78), and the latter soil property was negatively correlated to TN (r = −0.68). The associations between plant parameters and soil properties were strong for the pairs of P vs. SC (r = −0.75), P vs. TR (r = −0.76) and P vs. PS (r = −0.66) (Table 4).

The PCA provided two PCs, which explain together 72.7% of the variance of the original variables, the first PC (PC1) explaining 59% of this variance. This PC had high loadings (>0.689) on all plant parameters. In more detail, these loadings were positive for PS, PC, TR and RGR among the plant parameters and for TN and pH among the soil properties, while WUE, TOC and PC had negative loadings on PC1. The second PC (PC2) was strongly associated only with EC (loading of 0.866) (Figure 8a and Table 5).

The PCA and AHCA grouped the observations of plant parameters and soil properties in three clusters, showing high overlapping. The first cluster groups three observations related to irrigation with CW and one with WW. The second cluster consists of plant soil irrigated with Cl-WW (six samples), WW (five samples) and CW (three samples). The third cluster also contains observations made on samples irrigated with CW, WW and Cl-WW (three samples for each water type) (Figure 8b,c).

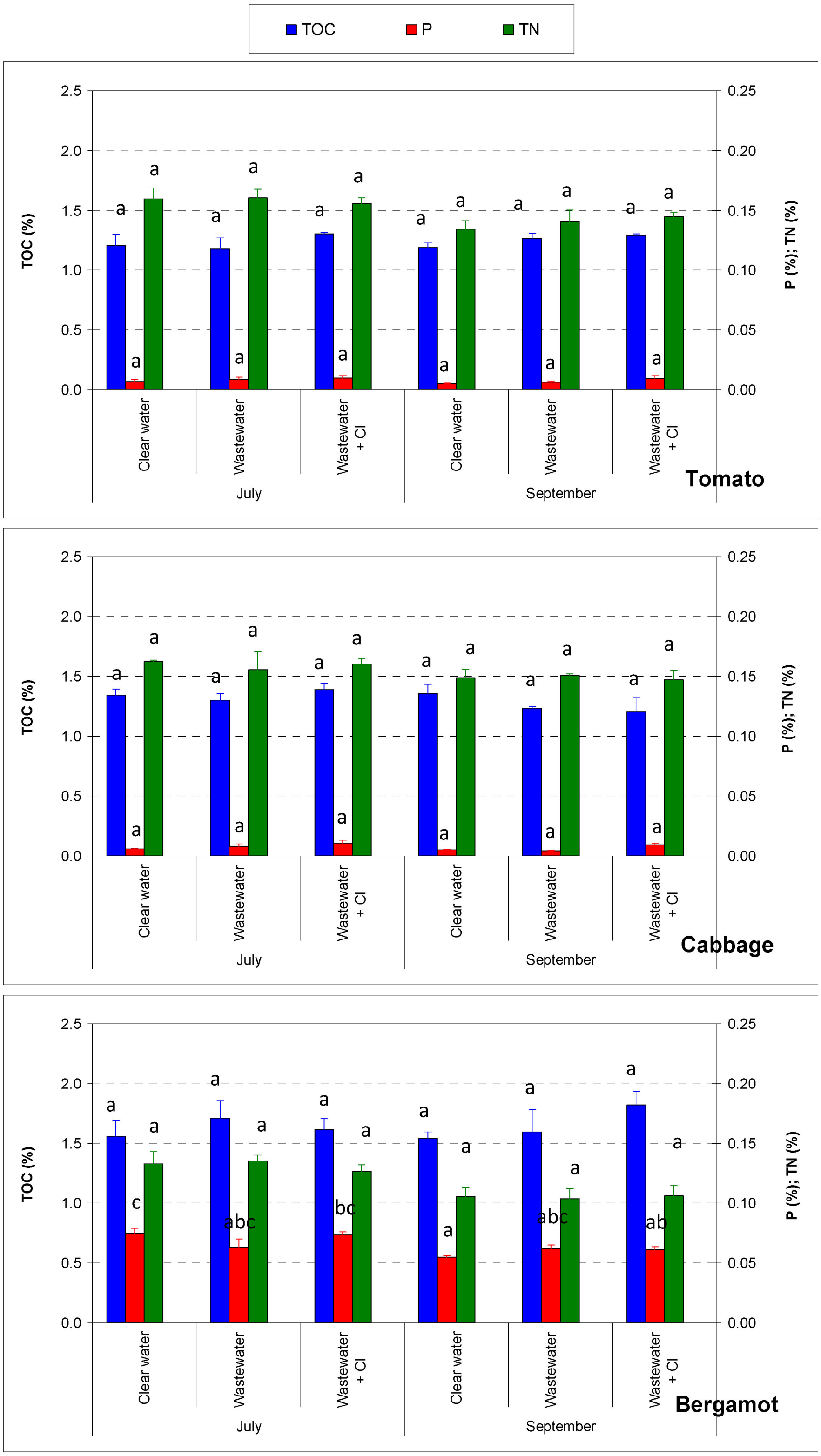


Figure 6. Mean ± standard error (n = 3) of soil total organic carbon (TOC), nitrogen (TN) and phosphorous (P) for tomato, cabbage and bergamot plants irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW). Different letters indicate significant differences after Tukey's test ($p < 0.05$).

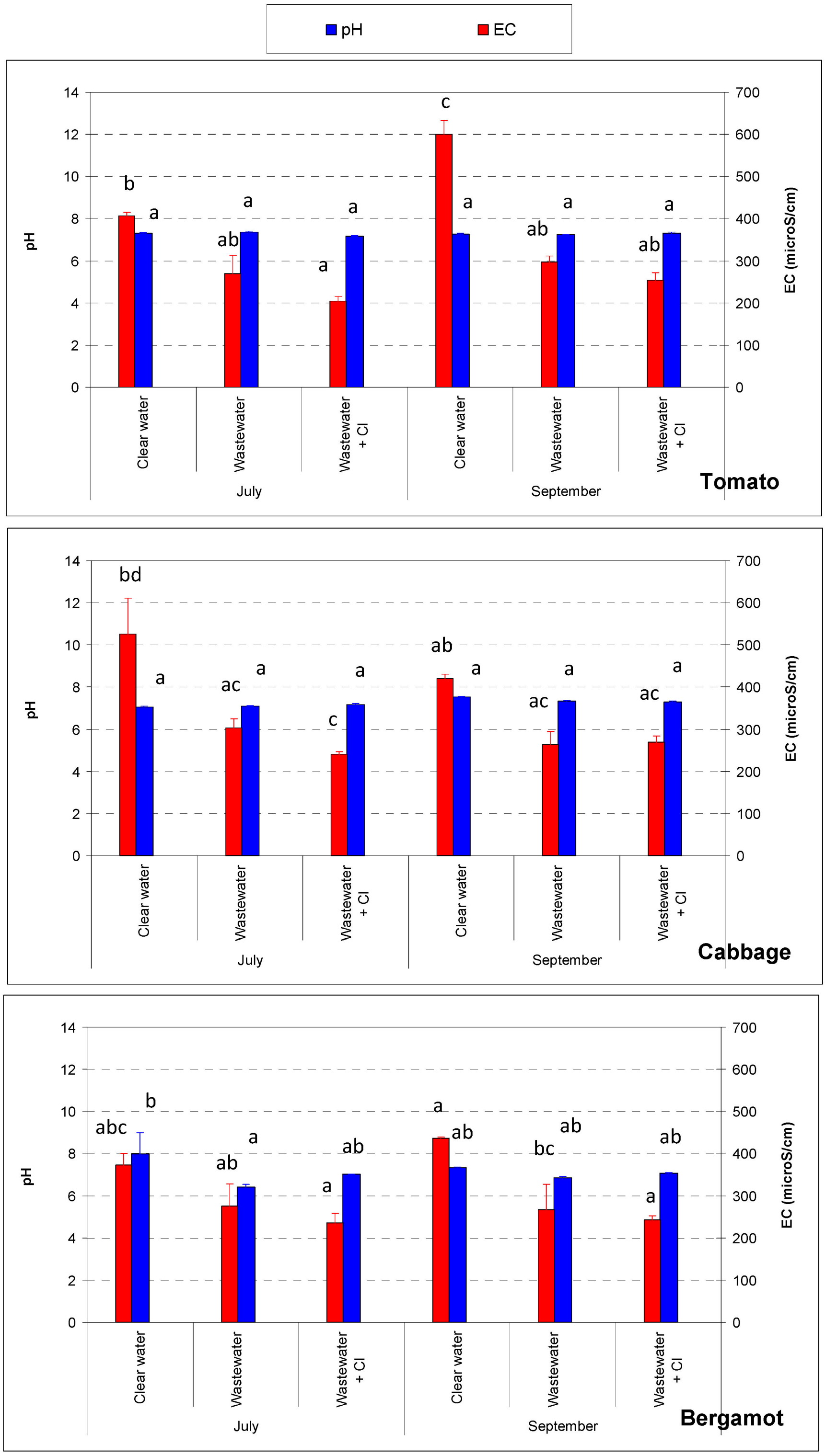
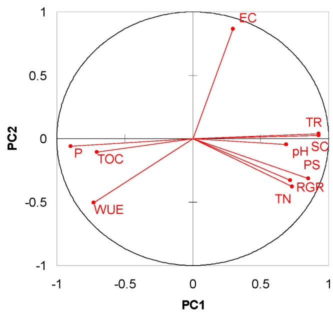


Figure 7. Mean ± standard error ($n = 3$) of soil pH and electrical conductivity (EC) for tomato, cabbage and bergamot plants irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW). Different letters indicate significant differences after Tukey's test ($p < 0.05$).

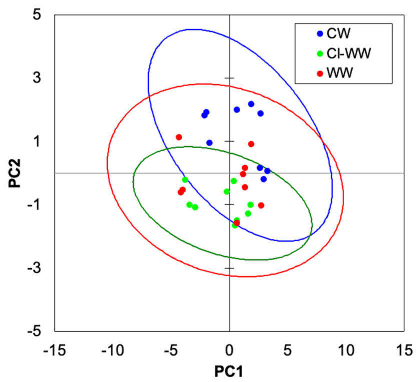
Table 4. Pearson's correlation matrix between pairs of plant and soil measurements of tomato, cabbage and bergamot irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW).

Variables	PS	SC	TR	WUE	RGR	TOC	P	TN	pH	EC
PS	1	0.90	0.89	−0.39	0.58	−0.39	−0.66	0.63	0.69	0.01
SC		1	0.99	−0.72	0.55	−0.50	−0.75	0.56	0.61	0.25
TR			1	−0.73	0.54	−0.50	−0.76	0.56	0.61	0.26
WUE				1	−0.37	0.55	0.65	−0.36	−0.36	−0.51
RGR					1	−0.59	−0.64	0.62	0.41	−0.01
TOC						1	0.78	−0.54	−0.28	−0.25
P							1	−0.68	−0.54	−0.31
TN								1	0.45	−0.04
pH									1	0.26
EC										1

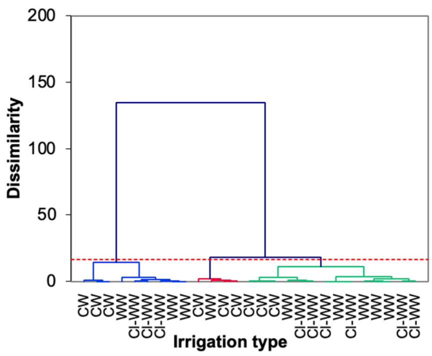
Notes: plant parameters: PS = photosynthesis; SC = stomatal conductance; TR = transpiration; WUE = water use efficiency; RGR = relative growth rate; soil properties: TOC = total organic carbon; P = available phosphorous; TN = total nitrogen; EC = electrical conductivity.



(**a**)



(**b**)



(**c**)

Figure 8. *Cont.*

Clusters		
1	**2**	**3**
CW	WW	CW
CW	WW	CW
CW	Cl-WW	CW
WW	Cl-WW	WW
	Cl-WW	WW
	CW	WW
	CW	Cl-WW
	CW	Cl-WW
	WW	Cl-WW
	WW	
	WW	
	Cl-WW	
	Cl-WW	
	Cl-WW	

(**d**)

Figure 8. Loadings of the original variables (**a**), plant parameters and soil properties, scores with relevant clusters (**b**) on the first two principal components (PC1 and PC2) provided by the principal component analysis, and dendrogram (**c**) with cluster composition (**d**) using analytical hierarchical cluster analysis applied to tomato, cabbage and bergamot irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW). Legend: PS = photosynthesis; SC = stomatal conductance; TR = transpiration; WUE = water use efficiency; RGR = relative growth rate; TOC = total organic carbon; P = available phosphorous; TN = total nitrogen; EC = electrical conductivity; values in bold are significant at $p < 0.05$; different colors in lines and characters refer to different clusters.

Table 5. Factor loadings of the original variables (plant parameters and soil properties) on the first two principal components (PC1 and PC2) provided by PCA applied to tomato, cabbage and bergamot irrigated with clear water (CW), depurated and non-chlorinated wastewater (WW) and depurated and chlorinated wastewater (Cl-WW).

Variables	Principal Components (PCs)	
	PC1	**PC2**
PS	**0.852**	−0.315
SC	**0.929**	0.023
TR	**0.929**	0.037
WUE	**−0.728**	−0.506
RGR	**0.717**	−0.327
TOC	**−0.704**	−0.105
P	**−0.899**	−0.061
TN	**0.731**	−0.377
pH	**0.689**	−0.045
EC	0.297	**0.866**

Notes: plant parameters: PS = photosynthesis; SC = stomatal conductance; TR = transpiration; WUE = water use efficiency; RGR = relative growth rate; soil properties: TOC = total organic carbon; P = available phosphorous; TN = total nitrogen; EC = electrical conductivity; values in bold are significant at $p < 0.05$.

4. Discussion

4.1. Effects of Non-Chlorinated Wastewater on Plants

Compared to irrigation with clear water, the plant treatment with non-chlorinated wastewater did not induce significant changes in the biomass growth, morphological parameters and efficiency of energy transfer, while the sensitivity of the physiological parameters of plants to the irrigation treatments was different among the tested species and over time. Only a significant decrease in the diameter of tomato stems (−55%) and bergamot sprouts (−38%) was noticed for morphological parameters. Moreover, this treatment resulted in a significant reduction in all physiological parameters (variations between −25% and 53%) but only immediately after the irrigation start and even for tomato. These changes decreased over time for water use efficiency, whose value was higher compared to CW (+24%). The stomatal conductance and transpiration rate significantly decreased (by 55%) after 30 days of irrigation in bergamot plants. Water use efficiency was also sensitive to this wastewater, but the short-term reduction (−48%) fully recovered after one month from the irrigation start, and even WUE increased (+82%) compared to irrigation with clear water. An analysis of the results by other authors reveals that [35] also found that wastewater application does not have any significant effects on alfalfa, radish and tomato plants growing on a silty loam soil. According to [46], higher production (total marketable heads as number and weight per hectare) of artichokes was achieved using secondary and tertiary wastewater in comparison with clear water. Also, [34] reported that the growth dynamics of crops irrigated with treated municipal wastewater were not noticeably different from plants irrigated with clear water, and even the yields were even higher (Table 6).

Table 6. Main results of studies about irrigation with municipal wastewater.

Author(s)	Year	Geographical Area	Climate	Municipal Wastewater Treatment Type	Irrigated Crop	Soil Type	Monitoring Period	Effects on Plants *	Effects on Soils *
[47]	1985	Southern Australia	Semi-arid	Chlorination	Orange	Sand	5 years	Yield decrease	No effects on soil salinity
[48]	1995	California (USA)	Semi-arid	Chlorination	Hydrangea, Nandina, Lace fern, Rhaphiolepis, hedge rose, Pittosporum, jasmine, Japanese boxwood and azalea	Fine textured	6 months	Significant differences in chloride tolerance among the species	Not evaluated
[49]	2000	Southern Spain	Semi-arid	Not specified	Orange	Clay	3 years	Same growth and fruit quality, no toxicity	Not evaluated
[32]	2005	Northern Greece	Semi-arid	Chlorination	Tobacco	Clay loam	3 months	Lower plant height and number of leaves, symptoms of toxicity	Not evaluated
[35]	2007	Jordan	Semi-arid	Rotating biological contactors	Alfalfa, radish and tomato	Silty loam	3 months	Non-significant	Slight changes in porosity and salinity
[34]	2007	North-western China	Semi-arid	High load biological adsorption and chlorination	Celery, wheat, maize, millet, rapeseed, yellow beans and apples	Not specified	14 months	Higher production in weight, no effect on quality	Non-significant
[33]	2009	Southern Spain	Semi-arid	Secondary and tertiary treatments	Lemon	Silty loam	12 months	Lower vegetative growth and leaf gas exchange, no toxicity	Higher salinity and B accumulation
[7]	2012	Southern Spain	Semi-arid	Secondary	Citrus	Clay loam	2 years	Lower growth, no toxicity	Higher salinity, Cl and B concentrations

Table 6. *Cont.*

Author(s)	Year	Geographical Area	Climate	Municipal Wastewater Treatment Type	Irrigated Crop	Soil Type	Monitoring Period	Effects on Plants *	Effects on Soils *
[39]	2014	Cyprus	Semi-arid	Secondary and tertiary treatments	Tomato	Sandy clay loam	150 days	No effect on crop yield	Noticeable variation in EC, no effect on pH and organic matter
[46]	2016	Southern Italy	Semi-arid	Secondary and tertiary	Artichokes	Loam	2 years	Higher yield	No effects on microbial population
[30]	2017	Southern Italy	Semi-arid	Chlorination	Lettuce	Sandy	2 months	Chlorosis, leaf necrosis and reduced crop yield	Accumulation of extractable organo-halogenated compounds (EOX)
This study	2023	Southern Italy	Semi-arid	Secondary treatment Chlorination	Tomato, cabbage, bergamot tree	Loam	2 months	No effects on crop growth, morphology and physiology	Non-significant effects on pH, OC, N and P, decrease in EC for irrigation with chlorinated wastewater

Note: * compared to irrigation with clear water.

4.2. Effects of Chlorinated Wastewater on Plants

Also, the irrigation of plants with chlorinated wastewater did not cause significant changes in the biomass growth, morphological parameters and efficiency of energy transfer. The application of this wastewater even increased the fresh biomass of tomato leaves by 56%, although a significant reduction in the stem diameter of the same species (about 60%) was measured. Since the dry biomass was not affected by significant changes compared to clear water (only −5.3%), the increase in fresh biomass means that tomato retained more water after irrigation with chlorinated wastewater but without any detrimental effect on biomass yield. Regarding other experiences of crop irrigation with chlorinated wastewater, refs. [7,33] found that the growth of citrus trees irrigated with wastewater receiving a secondary treatment was lower compared to the irrigation with clear water, but no toxicity effects for plants were observed. Therefore, according to these authors, the possible yield decrease in citrus between irrigation with wastewater from tertiary and secondary treatments may be ascribed to osmotic stress rather than toxicity. Again for citrus, [47] estimated a yield decrease of about 20% for each increase of 1 meq/L in chloride concentration (equal to 35.4 mg/L) in the irrigation water. In another study by [49], 3-year-long irrigation of orange trees with chlorinated wastewater did not affect the growth or fruit quality parameters, and no toxicity due to chlorine was observed [37]. In contrast, [32] reported that the adverse effects of chloride in irrigation water on plant height and the number of leaves are already substantial above 40 mg/L and visible within 30 days after the irrigation start (Table 6).

After the irrigation, the physiological parameters were subjected to variable changes among the tested species and over time. Immediately after the irrigation start, the chlorinated wastewater application to tomato plants increased all parameters by 21% to 98% compared to the irrigation with clear water, but these increases vanished over time. In contrast to tomato, the stomatal conductance and transpiration rate of cabbage plants significantly decreased in the plants treated with chlorinated wastewater (by 56% and 55%, respectively) at the end of the irrigation period, while water use efficiency significantly increased (by 40%). Moreover, the application of chlorinated wastewater significantly increased water use efficiency in bergamot plants (+67%), while the other physiological parameters did not undergo any significant changes. In contrast to our results, the supply of chlorine to plants may result in reduced vegetative growth and leaf gas exchange in citrus trees [33,50] (Table 6). In line with [51], a possible explanation for the good tolerance of the studied species to chlorine is the low uptake by plants and the minor changes in chlorine concentration in the root zone, the latter being due to leaching thanks to watering and rains.

According to [30], plants irrigated with chlorinated water and growing on sandy soil are commonly affected by symptoms of stress (i.e., chlorosis, leaf necrosis and reduced crop yield) from the first watering, especially at concentrations between 10 and 40 mg/L of free chlorine. In contrast, plants growing on finer soils (e.g., with silty clayey texture), such as in this study, show better growth and later symptoms. In any case, the intensity of these symptoms is positively correlated to the free chlorine concentration in the irrigation water. This statement indicates that a more suitable soil texture (i.e., a finer grain size) may help to contrast the negative effects of chlorine in wastewater. Moreover, the greater the amounts of accumulated chlorine, the higher the reduction in growth [48]. According to [52], this tolerance to chlorine should be ascribed to the high content of calcium in plant tissues [31] (Table 6).

4.3. Effects of Wastewater Application on Soils

Almost all the studied soil properties did not change after the application of chlorinated and non-chlorinated wastewater at both survey dates with a few exceptions. The soils with bergamot treated with non-chlorinated wastewater underwent a significant but not severe decrease in pH (−20%) after the irrigation start and in the electrical conductivity (−43%) after the irrigation period. Also, land application of chlorinated wastewater reduced the electrical conductivity of soils (−47%), which was the lowest among the irrigation treatments. This is an important result since a high electrical conductivity is proof of high saline concentrations in soil, which may increase the osmotic potential for vegetation and therefore can result in damage to plants. As reported by [7,33], high salinity in soils can be considered the main problem for irrigation with treated wastewater in semi-arid areas. Also, ref. [53] reported that irrigation with tertiary or secondary effluents induces noticeable variations in soil EC, but the treatment does not significantly affect soil pH, organic carbon or crop productivity (Table 6).

4.4. Analysis of Relationships among Plant and Soil Parameters among the Irrigation Conditions

The correlation analysis revealed close associations among the physiological parameters of plants and their fair correlations with growth rates. In contrast, the linkages between plant parameters and soil properties are much lower and generally non-significant, except for P content, which is inversely correlated to all physiological parameters. Moreover, the correlation between the TOC and P is positive and negative between TOC and TN. A combined analysis of the results given by PCA and AHCA did not evidence clear discrimination among plant-soil complexes treated with different types of irrigation water, since the observations were not grouped into separate clusters among the irrigation sources. Only a slight gradient between observations made after irrigation with clear water and wastewater (chlorinated or not) was evident. This gradient seems to be mainly controlled by the second PC, which is closely and negatively associated with the soil electrical conductivity, rather than PC1, which was instead noticeably influenced by all other plant parameters and soil properties. This result confirms the beneficial effects of reused municipal wastewater on this important soil property, since depuration allows a slight decrease in soil salinity compared to the soils irrigated with clean water.

5. Conclusions

Irrigation of tomato, cabbage and bergamot plants with treated municipal wastewater (with or without chlorination) in comparison to treatment with clear water indicated that the application of both chlorinated and non-chlorinated wastewater did not significantly modify the biomass growth, morphological parameters and efficiency of energy transfer. However, the impacts of irrigation water on the physiological parameters of plants were variable among the species and over time. In the case of treatments with non-chlorinated wastewater, a significant reduction in the stem diameter of tomato and bergamot plants and variability in all physiological parameters (between −25% and 53%) immediately after the irrigation start for tomato were measured. A decrease in the stomatal conductance and transpiration rate, as well as an increase in water use efficiency, after 30 days of irrigation in bergamot was also found. This irrigation water did not induce significant changes in soil properties, except for a significant decrease in pH in bergamot soils after the irrigation start and in EC at the end of the irrigation period.

Irrigation of plants with chlorinated wastewater increased the weight of the fresh biomass of leaves and the stem diameter of tomato and decreased the water use efficiency of bergamot immediately after the irrigation start. After two months, decreases in the stomatal conductance and transpiration rate were noticed in cabbage, and increases in water use efficiency in cabbage and bergamot were evident. Land application of chlorinated wastewater only reduced the electrical conductivity of soils among the studied soil properties.

Overall, the study demonstrated that the sustainable use of treated municipal wastewater (with or without chlorination) did not exert detrimental impacts on both the growth of tomato, cabbage and bergamot plants and soil health in the short term. As such, the working hypothesis that the disinfection treatment decreases plant growth and biomass yield and significantly modifies the main soil properties should be rejected, at least for the experimental crops and soils. Therefore, these water resources may be safely reused for crop cultivation, promoting water use efficiency in semi-arid areas affected by a chronic shortage of clear water and rainwater. However, the main characteristics of plants and soils must be properly monitored for environmentally sound reuse of treated wastewater.

Author Contributions: This study was conceived by D.A.Z. and P.S.C. The first draft was written by D.A.Z. and B.G.C. and was revised and commented on by all authors. A.S. and A.Z. measured and processed the plant parameters and discussed the relevant aspects. R.O., I.M. and R.S. carried out the measurements and processing of soil properties and commented on the related aspects. M.E.L.-B. performed the statistical analysis. D.A.Z., P.S.C. and S.M.Z. supervised the scientific and financial aspects of the investigation. All authors have read and agreed to the published version of the manuscript.

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