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Reuse of Treated Municipal Wastewater under Different Growing Seasons for the Spinach Production

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Abstract

A study was conducted in the Bekaa valley of Lebanon aiming to produce spinach leaves with treated effluent from Joub Janine plant under two growing seasons. Two experiments were laid out in a randomized complete block design. The effect of water quality on the qualitative, quantitative aspects and microbiological contamination of leaves was assessed. The results showed that the treated wastewater from Joub Janine plant was of category III. The highest mean marketable yields was recorded for T7 (4727 $g\cdot m^{-2}$) followed by T6 (3533 $g\cdot m^{-2}$) that were drip irrigated with treated wastewater. The uptake of K, Mg, Na and Cl was significantly 49.09%, 30.20%, 96.79% and 33.20%, respectively, higher in the spring than in autumn. The nitrate levels in all treatments and seasons were below the maximum level in foodstuffs as provided by the European Commission regulations. For the lipophilic fraction, there was no significant difference among treatments and also among treatments and seasons interacting together and the highest hydrophilic fraction and total phenols levels recorded for the autumn rather than the summer season. In general, pathogenic bacteria was absent on spinach leaves for all treatments and growing seasons.

Keywords

Treated Wastewater, Spinach Cultivation, Mineral Composition, Qualitative Parameters

1. Introduction

Water scarcity poses serious economic, social and even political concerns in all of its aspects. Many irrigated areas around the world are experiencing water shortages due to severe factors, such as climate change and ground water pollution [1].

Most of the water consumption in many countries is allocated to agricultural practices, which consume 66% of the available water [2].

Countries suffering from water shortages are forced to use non-conventional resources, mainly water harvesting and treated wastewater. Under these circumstances, treated wastewater use can help to mitigate the damaging effects of local water deficits but under controlled conditions to minimize hazards from pathogenic and toxic contaminants of the agricultural products, soils, surface, and ground water [3].

In Lebanon, like in many other Mediterranean countries, demands on water resources for households, commercial, industrial, and agricultural use are on the increase due to rapid population growth and demographic shifts [4] [5]. In this context, the lack of sufficient resources makes the use of wastewater a necessity particularly for agricultural purposes, and hence become an economically attractive proposition [6] [7]. However, there is still little knowledge about the harmful effects on human health and the environment.

Harmful effects are mainly due to associated pathogens and other undesirable constituents depending on the source [8] [9]. According to the proposed Lebanese guidelines, it is strictly forbidden to grow vegetables to be eaten raw; however, cooked vegetables are allowed to be grown with an effluent classified as "category I" [10].

Spinach leaves are currently cultivated and consumed as part of a main course or side dish in Lebanon, The Bekaa valley is one of the main farming areas of spinach currently being irrigated with non-conventional water resources due to the prevailing water scarcity in the plain. Harvested leaves are transported to markets in urban areas. According to the recent census conducted by the Ministry of Agriculture and the Food and Agriculture Organization of the United Nations, the cultivated area of spinach in Lebanon is around 342.1 hectares giving a total production of 3665.9 tons [11]. Spinach is available throughout the year, although its primary season runs from about early spring in March through May, then summer season and again in the fall from September through October.

The reuse of treated wastewater for crop production largely depends on adopting appropriate measures aiming at optimizing crop yields and quality, maintaining soil productivity and safeguarding the environment. Therefore, the aim of the current work was to assess the production of spinach leaves, in two growing seasons (summer and fall) with treated municipal wastewater in order to study the impact on yield, mineral composition, qualitative parameters and microbial contamination.

2. Materials and Methods

2.1. Experimental Site and Climate

The experiment was carried out during two consecutive growing seasons in 2016 at the experimental field of the Litani River Authority (LRA) agricultural extension center in Khirbet Kanafar village (Lebanon, 33.63˚N lat, 35.77˚E long, 859 m above sea level) (Figure 1). Spinach was cultivated in summer season from 20 August 2016 to 6 October 2016, and in autumn season from 14 October 2016 to 8 December 2016. The treated effluent of the Wastewater Treatment Plant (WWTP) that is serving the nearby village of Joub Janine and located very close to the LRA was used in the experiment. The WWTP provides a secondary treatment. It has a maximum capacity of 10,000 m^3 per day and currently receives 7000 - 8000 m^3 per day during the summer time. It is connected to the LRA and serves part of the irrigated land belonging to the station.

The climate of the area is typically Mediterranean, characterized by a hot and dry season from April to October. The main weather parameters were obtained from a standard agro-meteorological station located at the experimental station of the LRA that is very close to the field trial. The weather regimes, in terms of reference evapotranspiration (ETo), precipitation (P), maximum temperature (Tmax), minimum temperature (Tmin) and mean relative humidity (RHmean) during the season 2016 are given in Figure 2.

In general, the average maximum air temperature was 30.4˚C from 20 August 2016 to 6 October 2016 and 20.7˚C from 14 October 2016 to 8 December 2016, whereas the average minimum air temperature was 14.3˚C during August-October and 7.0˚C during Mid October-Mid December. Total rain was 0.6 mm and 201 mm during the summer and autumn growing season, respectively.

Figure 1. Satellite imagery of study site in Joub Janine region.

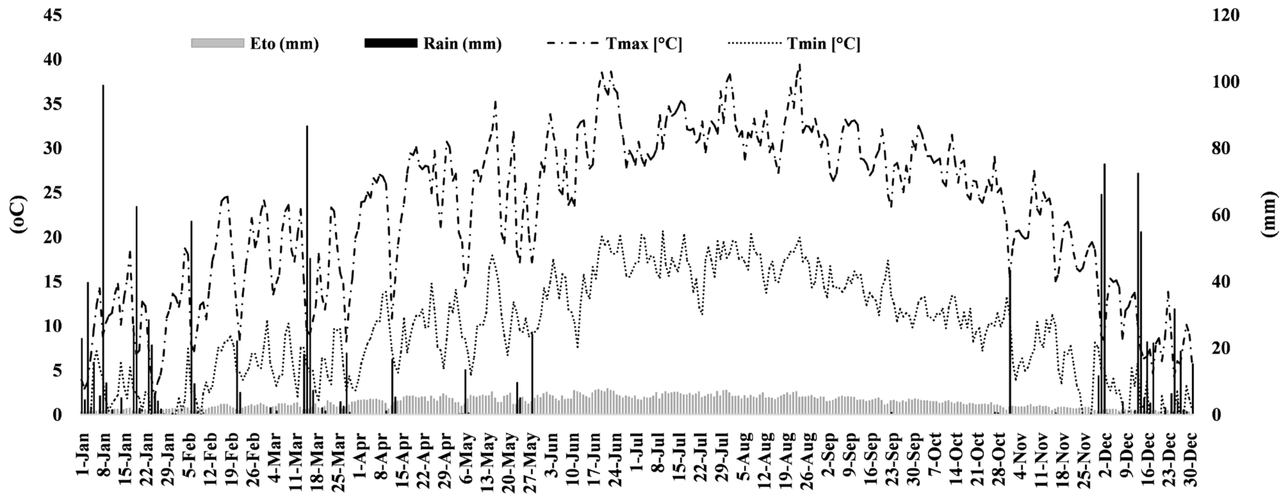


Figure 2. Daily climate data for the year 2016 as recorded for Joub Janine region.

The soil of the study area is sandy clay loam (USDA textural soil classification) with 53% Clay, 31% clay and 15% silt. Field slope was less than 0.1% and total available water holding capacity within the top 1 m of soil profile was 109 mm.

2.2. Experimental Design, Treatments and Agronomic Management

The experiment was laid out with eight treatments: T1: Faten variety sprinkler irrigated with fresh water (FW-S-V1); T2: Fayez variety sprinkler irrigated with fresh water (FW-S-V2); T3: Faten variety drip irrigated with fresh water (FW-D-V1); T4: Fayez variety drip irrigated with fresh water (FW-D-V2); T5: Faten variety sprinkler irrigated with treated water (TW-S-V1); T6: Fayez variety sprinkler irrigated with treated water (TW-S-V2); T7: Faten variety drip irrigated with treated water (TW-D-V1); T8: Fayez variety drip irrigated with treated water (TW-D-V2);. Treatments were organized in a randomized complete block design with three replicates. Each experimental unit consisted of a 9 m^2 plot. The plants in both seasons were placed with 25 cm between the rows and 15 cm within the row living a plant density of 27 plants m^{-2}.

The plots under drip irrigation were equipped with low polyethylene surface laterals. All the laterals were supplied with in-line drippers (theoretical discharge rate of 4 $L \cdot h^{-1}$); each lateral fed one row of plants. The plots under sprinkler irrigation were equipped with minisprayers having a discharge of 0.26 m^3/hr and placed on the middle of plot. The experiment was equipped with separate reservoirs and head units for the treatments irrigated with fresh water and those irrigated with treated effluent. Filters were manually cleaned.

2.3. Soil and Water Sampling and Analysis

Soil samples were taken at the beginning and at the end of each growing season at two different depths: 0 - 20 cm and 20 - 40 cm. The samples were analyzed for physical chemical properties, the presence of micronutrients and trace elements.

Concerning irrigation water, the main physic-chemical, microbial characteristics and trace metals content of both kinds of water (F and TW) were monitored. Standard methods were used in the laboratory to measure the biochemical oxygen demand (BOD5) [12], the total dissolved salts (TDS), electrical conductivity (EC), the pH, etc. The microbiological analysis of *Total coliforms* (TC), *Faecal coliforms* (FC), *Escherichia coli* (*E. coli*) and salmonella was done according to standard methods) [10].

2.4. Production and Microbial Contamination of Spinach

At the end of both experiments the marketable fresh yield expressing per square meter as well as the percentage of dry matter were recorded.

In addition, spinach leaves (500) were harvested from each experimental unit in order to measure microbial contamination, mainly the *Enterobacteriacae*, *Staphylococcus aureus*, *Clostridium perfringens*, *L. monocytogenes*, *Faecal coliforms*, *E. coli* and Salmonella. In the laboratory, 100 g of leaves were homogenized with 900 mL of sterile water by a stomacher. Then, ten-fold dilutions were made within the same medium. Faecal coliform and *E. coli* were measured using membrane filtration techniques [12]. The Salmonella detection was done acording to the method of [13].

2.5. Mineral Analysis

Dried spinach leaf tissues were ground in a Wiley Mill to pass through an 841 microns screen, and then portions of the dried tissues were used for mineral analysis. Total N concentration in fruit tissue was determined by Kjeldahl method following mineralization with sulphuric acid in the presence of potassium sulfate and low concentration of copper catalyst [14].

For the NO_3, P, S, K, Ca, Mg and Na analysis, 250 mg of finely ground dried plant tissues (leaf and fruit) were suspended in 50 ml of ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany) and subjected to four freeze-thaw cycles in liquid nitrogen followed by shaking water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80˚C for 10 min. The mixture was centrifuged at 6000 rpm for 10 min (R-10M, Remi Elektrotechnik Limited, India), then filtered through a 0.20 µm filter paper (Whatman International Ltd., Maidstone, U.K.), as described previously by [15], Potassium, Ca, Mg and Na were separated by ion chromatography (ICS-3000, Dionex, and Sunnyvale, CA, USA) and quantified through an electrical conductivity detector. Chromatographic separation was achieved in isocratic mode on an IonPac CS12A analytical column (4 × 250 mm, Dionex, Corporation) equipped with an IonPac CG12A precolumn (4 × 250 mm, Dionex, Corporation) and a self-regenerating suppressor CERS500 (4 mm, Dionex, Corporation). The nitrate, P and S contents were also measured through ion chromatography coupled to a conductivity detector. A IonPac ATC-HC anion trap (9 × 75 mm), and a AS11-HC analytical column (4 × 250 mm) equipped with an AG11-HC precolumn (4 × 50 mm) and a self-regenerating suppressor AERS500 (4 mm) were used for separation.

2.6. Leaf Quality Assessment

The hydrophilic fraction (HAA) from freeze-dried spinach leaves (200 mg) was extracted with distilled water and its antioxidant activity was measured with the N,N-dimethyl-p-phenylenediamine (DMPD) method [16]. The lipophilic fraction (LAA) was also extracted from freeze-dried fruits (200 mg) with methanol, and antioxidant activity of this extract was measured with the 2,2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ABTS method [17]. The hydrophilic and lipophilic antioxidant activities were determined by UV–Vis spectrophotometry. The absorbance of the solutions was measured at 505 and 734 nm, respectively. HAA and LAA were expressed as mmol ascorbic acid and as mmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per 100 g of dry weight, respectively.

The total phenolic content in methanolic extracts was determined using the Folin-Ciocalteau procedure [18] with gallic acid as a standard. A 100 µl aliquot of the supernatant was combined with 500 µl of Folin-Ciocalteau's reagent (Sigma Aldrich Inc, St Louis, MO, USA) and 400 µl of 7.5% sodium carbonate/water (w/v). Absorption was measured after 30 min at 765 nm using a UV-Vis spectrophotometer, and the result was expressed as mg gallic acid (Sigma Aldrich Inc, St Louis, MO, USA) per 100 g dry weight.

2.7. Statistical Analysis

Analysis of variance (ANOVA) of the experimental data was performed using SPSS 10 for Windows, 2001 (SPSS Inc., USA). To separate treatment means within each measured parameter, Duncan's multiple-range test was performed at $P \le 0.05$. Combined analysis of variance over two growing seasons was performed for crop growth parameters, mineral composition, leaf quality parameters, heavy metals and bacterial contamination.

3. Results and Discussions

3.1. Water Quality

Table 1 reports the physico-chemical parameters, microbial characteristics and trace metals of the fresh and the treated water analysed during the trial.

The level of TSS (52 $mg \cdot L^{-1}$) in wastewater is within the level proposed by the environmental limit values decision of MOE for surface water [19] as well as for the proposed Lebanese guidelines for wastewater reuse in irrigation [20]. The levels of COD (210 $mg \cdot L^{-1}$) and BOD5 (45 $mg \cdot L^{-1}$) in treated wastewater were higher than the admissible limits of water category I, however, those parameters in freshwater were within the admissible limits. The levels of nitrates and phosphates were low in both fresh and wastewater. The *Total coliforms* were highly present in both fresh and wastewater while the Feacal coliforms (3.5 × 10^3

CFU/100 mL) were present in treated water and they were exceeding the proposed limit value. Furthermore, the Salmonella was detected in treated water however in fresh water it was completely absent. [21] observed that pathogens can enter plants and become internalized. Other studies showed that *E. coli* could be transported into the edible part of lettuce from soil through root system, and Salmonella could be transported from contaminated roots to the aerial parts of lettuce seedlings [22].

Overall, the water treated in Joub Janine plant is of category III as proposed by the Lebanese guidelines and it could easily reach category I if well treated.

Table 1. Fresh water and treated effluent average quality and limit values for the reuse of TWW in Lebanon.

			Environmental limit values for surface water based on MoE Decision 8/1 (MoE, 2001)	Effluent specifications for wastewater reuse in irrigation based on proposed Lebanese guidelines (FAO, 2011)		
	FW	TW		Water Category I	Water Category II	Water Category III
Physico-chemical parameters (mg·L^{-1})						
pH	7.85	7.82	6 - 9	6 - 9	6 - 9	6 - 9
COD	90.50	210.00	125	125	250	250
BOD5	14.00	45.00	25	25	100	100
Total Suspended Solids	5.32	52.00	60	60	200	200
Nitrates	9.30	3.00	90	30	30	30
Phosphates	0.06	0.50	5	_	_	_
Potassium	4.50	9.60	_	_	_	_
Pathogens in water						
Total Coliform (CFU/100mL)	2.6 × 10^2	6.16 × 10^4	_	_	_	_
Fecal Coliform (CFU/100mL)		3.5 × 10^3	<2000	<200	<1000	_
E. coli (CFU/100mL)		7.5 × 10^2	<2000	<200	<1000	_
Salmonella	Absent	Present	Absent	Absent	Absent	Absent
Trace metals (mg·L^{-1})						
Zn	789.47	789.47	5			
Cu	<0.001	<0.001	0.5			
Pb	444.44	444.44	0.5			
Mn	0.23	0.52	1			
Ni	372.09	3651.16	0.5			
Hg	0.0100	0.0003	0.05			
As	<0.001	<0.001	0.1			
Cd	111.1100	<0.001	0.2			
Cr	<0.001	<0.001	2			

3.2. Marketable Yield and Dry Matter Percentage

The analysis of variance and mean comparisons for marketable yield and dry matter percentage of spinach plants grown under different water quality regimes, irrigation methods and growing seasons were reported in Figure 3 and Figure 4.

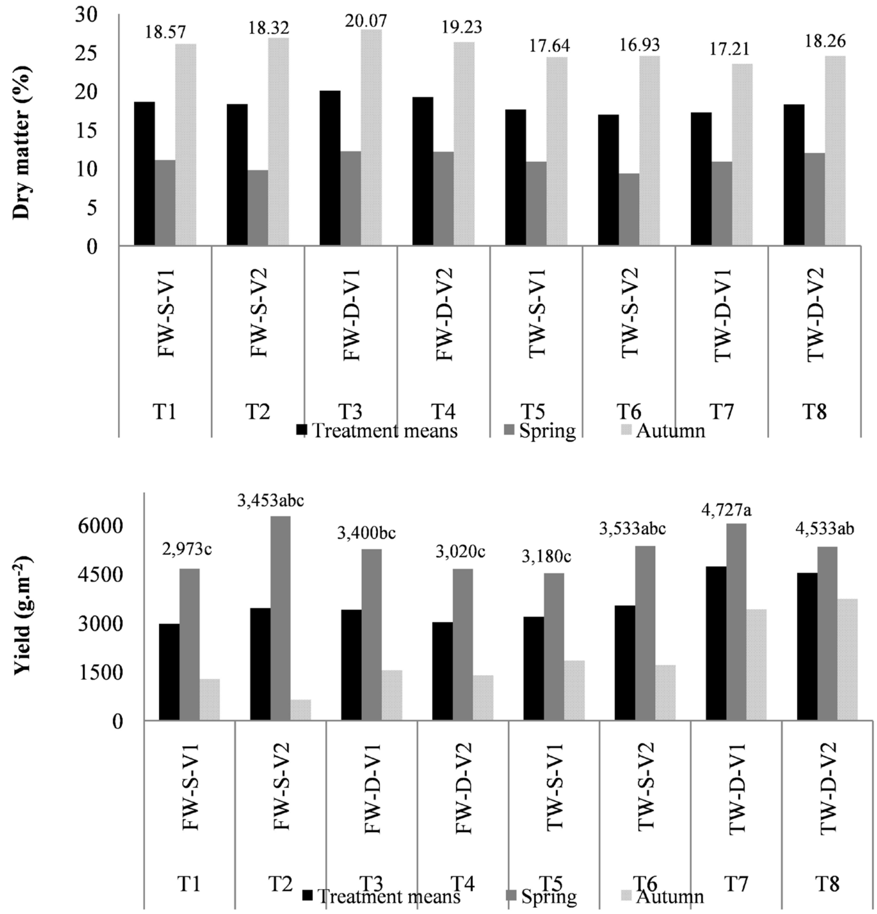
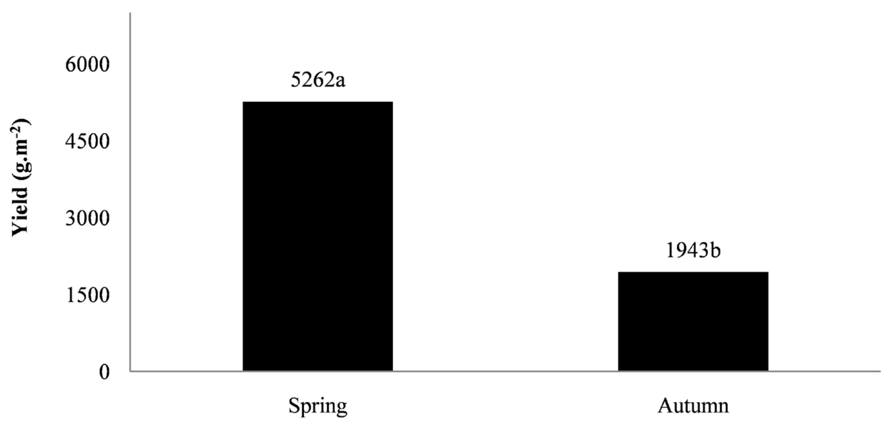


Figure 3. Analysis of variance and mean comparisons for marketable yield and dry matter percentage of spinach plants grown under different water quality regimes, irrigation methods and growing seasons.



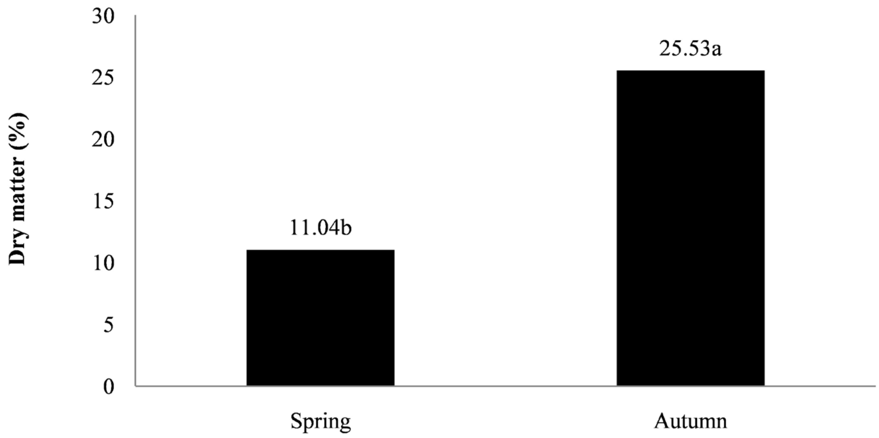


Figure 4. Seasonal means for marketable yield and dry matter percentage of spinach plants grown under different water quality regimes, irrigation methods and growing seasons.

For the marketable yield, considering the treatment as the source of variance, there was a significant difference among the different treatments (**Figure 3**), with the highest mean marketable yields recorded for T7 (4727 g·m^{-2}) followed by T6 (3533 g·m^{-2}) that were drip irrigated with treated wastewater. The treatments that were drip irrigated with fresh water gave respectively lower yields with 3400 g·m^{-2} for T3 and 3020 g·m^{-2} for T4. In addition, under drip irrigation, spinach variety Faten (V1) showed 12.5% and 4% higher yield than variety Fayez (V2) under fresh water and wastewater, respectively. On the contrary, under sprinkler irrigation, spinach V2 showed a better performance than V1 with 16% and 11% higher yields under fresh water and wastewater, respectively. However, the treatments means showed close values under sprinkler irrigation independently of the quality of irrigation water.

Considering the season as the source of variance, there was a significant difference, among both growing seasons with the highest seasonal mean for the summer (5262 g·m^{-2}) while the mean in autumn was 1943 g·m^{-2} (**Figure 4**). The lowest marketable yield recorded in autumn than in summer growing season could be attributed to the reduced evaporative demand of the environment such as lower global radiation and air temperature. In addition, the uptake of available nutrients existing in the soil and in irrigation waters of different qualities could also explain the fluctuations between seasons and among treatments. The present results are in agreement with the findings of [23] who found variation in spinach yield among different growing seasons. Moreover, in a similar study [24] indicated higher crop production under treated wastewater. Probably, treated wastewater provides part of the nutrient amount necessary during the crop cycle, as emphasized by [25] and [26].

For the percentage dry matter, considering the treatment as the source of variance, there was no significant difference among the different treatments (**Figure 4**). Considering the season as the source of variance, there was a significant difference, among the seasons with the highest seasonal mean for the autumn (25.5%) while the mean in the summer was 11.0% (Figure 4).

3.3. Leaf Mineral Composition of Spinach Plants

The leaf mineral composition (N, PO_4, K, Ca, Mg, Na and Cl) were significantly influenced by the treatment (T), season (S) and the interaction of T × S for most nutrients (Table 2).

Table 2. Analysis of variance and mean comparisons for leaf mineral composition of spinach plants grown under water quality regimes, irrigation methods and growing seasons.

Source of variance	N	PO4		K		Ca		Mg		Na		Cl	
	($g·kg^{-1}$ d.wt.)	($g·kg^{-1}$ d.wt.)		($g·kg^{-1}$ d.wt.)		($g·kg^{-1}$ d.wt.)		($g·kg^{-1}$ d.wt.)		($g·kg^{-1}$ d.wt.)		($g·kg^{-1}$ d.wt.)	
Treatment (T)	ns	*		*		***		***		***		ns	
Season (S)	***	*		***		ns		***		***		***	
T x S	ns	***		***		**		*		*		**	
Treatment													
T1	39.60	6.21	a	52.41	a	0.72	a	9.39	ab	9.15	b	8.64	
T2	40.71	6.80	a	51.63	a	0.34	bc	10.84	a	15.03	a	10.58	
T3	36.90	6.46	a	52.23	a	0.40	bc	9.87	ab	9.92	b	9.77	
T4	37.19	6.60	a	49.15	a	0.55	ab	10.44	a	6.38	c	9.38	
T5	39.89	5.80	ab	49.56	a	0.28	c	9.85	ab	9.51	b	8.51	
T6	40.10	4.17	b	39.80	b	0.24	c	8.65	b	10.98	b	8.00	
T7	41.06	5.30	ab	43.39	ab	0.34	bc	6.48	c	6.28	c	10.95	
T8	41.56	6.47	a	46.50	ab	0.26	c	6.89	c	6.06	c	9.59	
Season													
Autumn	44.40a	6.37	a	37.99	b	0.43		7.76	b	5.92	b	8.01	B
Summer	35.50b	5.65	b	56.94	a	0.36		10.09	a	11.65	a	10.67	A
T x S													
T1 Autumn	44.33	7.90	abc	50.02	abcd	1.04	a	9.10	ab	6.39	efgh	8.77	Bc
T1 Summer	34.88	4.52	ef	54.80	abc	0.40	bc	9.68	ab	11.92	b	8.50	Bc
T2 Autumn	49.74	9.87	a	49.84	abcd	0.29	c	10.11	ab	10.32	bcde	10.09	Bc
T2 Summer	34.69	4.75	def	52.83	abc	0.37	bc	11.33	a	18.16	a	10.91	Bc
T3 Autumn	45.03	6.68	bcde	43.55	cde	0.29	c	8.34	b	7.65	bcdefgh	8.32	Bc
T3 Summer	31.48	6.31	bcde	58.01	ab	0.47	bc	10.89	a	11.44	bcd	10.74	Bc
T4 Autumn	40.95	8.61	ab	46.34	bcd	0.42	bc	9.80	ab	5.58	fgh	10.75	Bc
T4 Summer	33.42	4.58	ef	51.95	abc	0.69	b	11.08	a	7.18	defgh	8.02	Bc
T5 Autumn	43.40	5.62	cdef	37.30	def	0.35	bc	9.70	ab	7.44	cdefgh	7.40	Cd

Continued

T5 Summer	36.38	5.99	bcde	61.83	a	0.22	c	10.00	ab	11.59	bc	9.63	bc
T6 Autumn	48.46	2.95	f	21.03	g	0.28	c	4.98	c	3.57	h	3.85	D
T6 Spring	34.52	4.99	def	52.31	abc	0.20	c	11.10	a	15.93	a	10.77	Bc
T7 Autumn	43.75	4.00	ef	24.79	fg	0.35	bc	4.61	c	3.76	h	6.94	Cd
T7 Spring	38.38	6.60	bcde	61.99	a	0.33	c	8.35	b	8.79	bcdef	14.96	A
T8 Autumn	42.89	5.47	cdef	31.19	efg	0.29	c	5.53	c	3.91	gh	7.38	Cd
T8 Spring	40.23	7.48	abcd	61.81	a	0.22	c	8.25	b	8.22	bcdefg	11.80	Ab

ns, *, **, *** Non-significant or significant at $P \le 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05).

Considering the treatment as the source of variance, there was no significant difference among treatments for N and Cl. However, for PO_4 and K, a significant difference, at $P \le 0.05$ was found among treatments with the highest levels of PO_4 and K recorded for the treatments under fresh water, T1, T2, T3 and T4. Similarly, for Ca, Mg and Na, a significant difference, at $P \le 0.001$ was found among treatments with the highest levels recorded for the treatments under fresh water, T1, T2, T3 and T4.

Considering the season as the source of variance, there was a significant difference with the highest N and PO_4 levels, 25% and 12% respectively, recorded for the autumn rather than the spring. The uptake of K, Mg, Na and Cl was significantly 49.09%, 30.20%, 96.79% and 33.20%, respectively, higher in the summer than in autumn.

Vegetables contribute normally by 11%, 35%, 7%, and 24% to the human dietary intake of total P, K, Ca and Mg, respectively [27]. In the context of the current study, K was the predominant macronutrient present (**Table 2**). The results are in agreement with [15] who found that the highest P and K contents were recorded in rocket, spinach and green lettuce. Data on the mineral content of spinach have also been reported in the USDA database. Our results on mineral contents of spinach were proximate to those reported by the National Nutrient Database for Standard References (USDA). In addition, obtained results were below the Recommended Dietary Allowances (RDA) or Recommended Nutrient Intake of Spinach leaves for adults and children aged four years and older [28].

Differences with the mineral composition reported in the published literature could be attributed to different farming practices, environmental conditions and also cultivars. Importantly, the average contents of N and PO4 were significantly higher in autumn.

3.4. Quality of Spinach Leaves

The results of nitrate, lipohilic (LAA) and hydrophilic (HA) antioxidant activities and total phenol contents of spinach as related to the different water quality regimes, irrigation methods and growing seasons are given in **Table 3**. In general, nitrate levels in all treatments and seasons were below the maximum level in foodstuffs as provided by the European Comission regulations (No 1258/2011) of 2500 - 3500 mg·kg^{-1} [29]. Obtained results present mean nitrate values higher than those given by [30] who found that spinach contains 24.0 - 457.0 mg NO3. kg^{-1}. However, according to the [31] and the results of two eminent surveys on leafy vegetables [32], [33], the concentration of nitrates in spinach ranged between 64 - 3048 mg·kg^{-1} (fresh weight). Thus, the nitrate contents evaluated in this experiment were in the range reported by the former studies. Considering the treatment as the source of variance, there was a significant difference at $P \leq 0.001$ among treatments with the highest levels of nitrates recorded for the treatments T1 and T2 sprinkler irrigated with fresh water, followed by T3 and T4 that were drip irrigated with fresh water. The lowest nitrates levels were recorded for the treatments irrigated with treated wastewater independently of the irrigation method. Considering the season as the source of variance, there was a significant difference at $P \leq 0.05$ with the highest nitrate levels recorded for the autumn rather than the spring.

For LAA, there was no significant difference among treatments and also among treatments and seasons interacting together. For ascorbic acid, treatments were significantly different at $P \leq 0.05$ with the highest values recorded under sprinkler irrigation independently of water quality. The lowest values were recorded under drip irrigation. There was a significant difference at $P \leq 0.001$ with the highest HAA levels recorded for the autumn rather than the spring. [34] found vitamin C concentration of spinach to be in the range of 25.0 - 71.0 mg 100 g^{-1}; however, [35] reported that spinach can contain up to 120.0 mg 100 g^{-1} vitamin C and also stated that vitamin C concentration increases during the winter season. Those results are in agreement with the findings of this experiment.

For the total phenols, there was significant difference among treatments at $P \leq 0.001$. However, there was not a significant difference among seasons with 39.51% higher total phenol level recorded for the autumn rather than the spring.

3.5. Bacterial Contamination of Spinach Leaves

The bacterial contamination of the two studied spinach varieties grown under different water quality regimes, irrigation methods and growing seasons is provided in **Table 4**.

In general, pathogenic bacteria such as salmonella and *Listeria monocytogenes* were absent on spinach leaves for all treatments and growing seasons. In addition, Staphylococcus aureus and Clostridium perfringens were not detected. However, spinach leaves were mostly contaminated with microorganisms consisting of Enterobacteriacae particularly during the spring season while no contamination was recorded for the autumn season. The contamination by the Enterobacteriacae that was found on spinach leaves could not be attributed to the use of treated wastewater in T5, T6 and T7 because the same order of magnitude contamination was determined on the samples irrigated with fresh water in T1,

Table 3. Analysis of variance and mean comparisons for nitrate, lipohilic (LAA) and hydrophilic (HA) antioxidant.

Source of variance	NO3		LAA	HAA		Total phenols	
	(mg·kg^{-1} f.wt.)		(mmol Trolox/100g d.wt.)	(mmol Ascorbic acid/100g d.wt.)		(mg Gallic acid/100g d.wt.)	
Treatment (T)	***		NS	*		***	
Season (S)	*		***	***		***	
T × S	*		NS	NS		NS	
Treatment							
T1	1978.65	a	7.51	2.30	ab	68.15	A
T2	1777.42	ab	6.51	2.22	bc	63.54	B
T3	1021.53	bc	6.72	2.13	c	64.91	Ab
T4	866.53	c	6.90	2.14	bc	63.01	Ab
T5	938.12	c	7.00	2.42	a	57.62	B
T6	737.36	c	7.45	2.27	bc	60.72	b
T7	537.11	c	7.61	2.04	c	68.57	a
T8	626.07	c	6.44	2.06	c	62.62	ab
Season							
Autumn	1251.67		7.81	2.58		74.89	
Summer	878.13		6.35	1.88		53.68	
T × S							
T1 Autumn	2891.62	a	8.17	2.63		82.79	
T1 Summer	1065.68	cd	6.85	1.98		53.51	
T2 Autumn	2327.78	ab	7.21	2.66		74.92	
T2 Summer	1410.52	bcd	5.80	1.79		52.16	
T3 Autumn	1674.72	bc	6.90	2.51		71.58	
T3 Summer	586.07	d	6.53	1.75		58.23	
T4 Autumn	880.58	cd	7.57	2.46		73.16	
T4 Summer	852.47	cd	6.23	1.82		52.86	
T5 Autumn	872.04	cd	8.46	2.68		67.38	
T5 Summer	1004.19	cd	5.53	2.15		47.86	
T6 Autumn	542.61	d	8.28	2.59		71.86	
T6 Spring	867.19	cd	6.62	1.95		49.59	
T7 Autumn	550.11	d	8.48	2.58		84.32	
T7 Spring	524.11	d	7.02	1.68		58.07	
T8 Autumn	537.30	d	7.18	2.50		78.93	
T8 Spring	714.84	cd	6.20	1.91		57.18	

ns, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05).

Table 4. Bacterial contamination of two spinach varieties grown under different water quality regimes, irrigation methods and growing seasons.

Source of variance	Enterobacteriacae 37˚C (cfu·g^{-1})		*E. coli* 44˚C (cfu·g^{-1})	S. aureus 37˚C (cfu·g^{-1})	Cl. perfringens 37˚C (cfu·g^{-1})	Salmonella sp./25g (+ or -)	*L. monocytogenes*/25g (+ or -)
Treatment (T)	*		ns	-	-	-	-
Season (S)	**		ns	-	-	-	-
T × S	*		ns	-	-	-	-
Treatment							
T1	53	b	0	nd	nd	-	-
T2	387	b	0	nd	nd	-	-
T3	3183	a	967	nd	nd	-	-
T4	0	b	0	nd	nd	-	-
T5	115	b	0	nd	nd	-	-
T6	198	b	35	nd	nd	-	-
T7	980	b	0	nd	nd	-	-
T8	0	b	0	nd	nd	-	-
Season							
Autumn	0		0	nd	nd	-	-
Summer	1229		250	nd	nd	-	-
T × S							
T1 Autumn	0	b	0	nd	nd	-	-
T1 Summer	107	b	0	nd	nd	-	-
T2 Autumn	0	b	0	nd	nd	-	-
T2 Summer	773	b	0	nd	nd	-	-
T3 Autumn	0	b	0	nd	nd	-	-
T3 Summer	6367	a	1933	nd	nd	-	-
T4 Autumn	0	b	0	nd	nd	-	-
T4 Summer	0	b	0	nd	nd	-	-
T5 Autumn	0	b	0	nd	nd	-	-
T5 Summer	230	b	0	nd	nd	-	-
T6 Autumn	0	b	0	nd	nd	-	-
T6 Spring	397	b	70	nd	nd	-	-
T7 Autumn	0	b	0	nd	nd	-	-
T7 Spring	1960	b	0	nd	nd	-	-
T8 Autumn	0	b	0	nd	nd	-	-
T8 Spring	0	b	0	nd	nd	-	-

ns, *, **, *** Non-significant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P = 0.05$). nd, not detected.

T2 and T3. In fact, the T3 treatment irrigated with fresh water was significantly different, with the highest value than all other treatments. *E. coli* was not detected in autumn season, however, it was only found on the T3 irrigated with fresh water and T6 irrigated with treated effluent in the spring season. The microbiological characteristics of the irrigation water could be only one of the routes for crop contamination. Another route for contamination could be probably boosted by favorable climatic conditions for bacterial development in the spring time rather than in the autumn time.

Similar results were obtained by [36] that studied the effect of wastewater irrigation on lettuce and found that bacterial contamination was present on treatments irrigated with treated water as well as on treatments irrigated with fresh water. Moreover, [37] highlighted that even though lettuce contamination by coliforms could be caused by water quality, the planting interval, harvest hygiene, and postharvest washing must also be taken into account. In addition, the crop handling, packaging, and transportation conditions are contamination routes. Finally, according to [38] low oxygen atmospheres could be used to store harvested spinach leaves and to control spoilage micro-organisms such as the Enterobacteriacae for at least 7 days, so long as the storage does not exceed 5˚C.

4. Conclusions

For the foregoing, it can be concluded that the treated waste water of Joubjnaine is not suitable for cultivation of vegetable crops and plants in terms of water quality but the approach of using treated waste water for raising crops is towards minimising cost of fertilizers and conservation of water resources. And the drip irrigation method in irrigated agriculture with treated waste water shall be adopted in order to reduce the direct contact between the plant and the water, and to limit the possible contamination of the crops products. However in terms of quantitative aspects, the findings results in this paper noted that the highest mean marketable yields recorded for the treatments that were used drip irrigated with treated waste water and there is no significant difference between the treatments in terms of qualitative aspects.

The knowledge on the reuse of treated waste water in irrigation is still scarce and more studies are needed to evaluate the quality of the treated effluent under different conditions and strict protection measures, stringent guidelines and good management of recycling of waste water are needed to minimize the negative impact of waste water irrigation in plants, soil and human health.

Conflicts of Interest

We certify that there are no affiliations with or involvement in any organization or entity with any financial interest and i am not placed in a situation which could give rise to conflict of interests.

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