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Effects of Irrigation with Treated Wastewater or Well Water on the Nutrient Contents of Two Alfalfa (*Medicago Sativa* L.) Cultivars in Riyadh, Saudi Arabia

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Abstract: Water scarcity has greatly increased the need for research into alternative irrigation methods. Irrigation with treated wastewater (TW) is considered an important alternative in terms of reducing our dependence upon groundwater and freshwater. In this study, we examined the effects of irrigation with TW on the nutrient contents of two alfalfa cultivars and compared them with the nutrient contents of plants irrigated with well water (WW). The two cultivars (Alhassawy—a local cultivar and CUV101) were cultivated from 2013 to 2015 and sampled twice. For both cultivars and in both sampling periods, irrigation with TW significantly affected the macronutrient and micronutrient contents of alfalfa tissues. Plants irrigated with TW had higher K and Ca contents, but lower N, P, and Mg contents. No significant variation in S content was noted between plants irrigated with WW and TW. Furthermore, cultivar and cutting time had significant effects on nutrient contents, and these variables interacted with the effect of the water type used for irrigation. However, before TW can be regularly used in irrigation processes, further long-term studies are needed that consider the variations in water treatment efficiency and differences between cultivated sites in addition to the crop being irrigated with TW.

Keywords: alfalfa; forage crops; water treatment

1. Introduction

Irrigation with treated wastewater (TW) is already employed in many countries, and the number of countries using TW in irrigation is increasing. In particular, less developed countries in Asia and Africa have been adopting the practice [1–4]. Treated wastewater can be used in many sites depending on water reliability, degree of treatment, and the environmental impact of TW application. Irrigation with TW can be used for industrial fields, large green areas, municipal plants in the streets, landscapes, industrial crops, or forage crops, depending on the degree of wastewater treatment [5,6]. Moreover, TW can be used to irrigate energy crops [7]. In Saudi Arabia, treated sewage is used to irrigate municipal plants in the streets, and this has now been expanded to include crops in some farms [8,9] under strict government supervision and control. In light of the scarcity of water sources in arid and semi-arid areas, and in order to conserve groundwater, it is important to look into other irrigation practices such as the use of TW to produce forage to feed livestock [10], such as alfalfa.

Several studies have examined the effects of irrigation with TW and have reported various effects ranging from positive to moderate and negative. The main positive effects of irrigation with TW include enhanced production and increases in the macronutrient and micronutrient contents of plant tissues [11]. Further, the positive environmental effects include improved soil fertility [12], reduced discharge of residual waters [13], and reduced groundwater uptake [14]. On the contrary, negative effects include the presence of contaminants that may enter the soil, groundwater, and plant tissues, as well as the presence of pathogens, heavy metals [14,15], pollutants (e.g., pharmaceuticals) [16], and pesticides in the water [17].

Alfalfa is considered to have been the first forage crop in the world, and the first in Saudi Arabia. Furthermore, it is the most cultivated forage crop in terms of cultivated area and quantity produced [18]. Alfalfa is classified as one of the most water-consuming crops during its growing season [19]. As a perennial crop, it is cultivated for over 5 years, and soil tillage is prevented during this period as the crop residues remain on the soil surface. Treated wastewater is a source of irrigation water and nutrients for plants [4,8]; however, it may contain bacteria and elements that are toxic to soils and plants [20–22]. In most cases, alfalfa is used in its dehydrated form to feed cattle, which inherently limits the risk of pathogen transfer via TW [23]. Furthermore, alfalfa can tolerate a moderately low-quality water supply [24].

Water scarcity is one of the most crucial problems currently being faced by the world, not only in arid and semi-arid regions but also in other parts of the world where water is more readily available. The main issues pertain to water quality and to sustainable water use that will not affect future generations. Therefore, methods of sustainable water use and the application of sustainability principles in water management have become some of the most important areas of research worldwide. Saudi Arabia has been dramatically affected by the global freshwater shortage, as many farms and agricultural projects have consumed huge amounts of deep non-renewable freshwater reserves and have experienced a gradual increase in well water salinity that has resulted in high levels of topsoil salination. Consequently, to stop the depletion of non-renewable freshwater resources, the Saudi cabinet has issued decrees (no. 66 dated 08/12/2015 and no. 39 dated 18/10/2016) to ban the cultivation of crops with low value and high water demand, such as wheat and forage crops including alfalfa [25]. Therefore, it has become crucial to find alternative irrigation methods and water resources.

It remains unclear whether TW is safe to be used in the irrigation of food and forage crops, mainly because of the risk of contamination with heavy metals that might transfer to the food chain. However, arid and semi-arid areas need alternative ways of irrigation to cope with water scarcity prevalence in these areas. One of the major barriers preventing the usage of TW in irrigation is the lack of social acceptance and belief, especially among farmers, in its beneficial effects [26–28]. Increasing farmers' awareness about the benefits as well as risks resulting from irrigation with wastewater could help in overcoming this problem. However, such a task needs more accurate and robust analysis of the risks and hazards or benefits associated with irrigation using TW. Therefore, further studies are needed to assess and clarify the risks associated with using TW in irrigation of food and forage crops and, thus, provide the required information for farmers and decision makers to help in increasing the adoption of TW in irrigation of food and forage crops. Especially, the presence of heavy metals is considered as the main limitation factor of using TW in irrigation. Translocation of heavy metals in alfalfa plants irrigated with TW needs more investigation considering different kinds of heavy metals. In the current study, we examined the concentrations of different heavy metals in alfalfa tissues over two seasons, aiming to analyze the effects of irrigation with TW on the nutrient contents of two alfalfa cultivars and measure the accumulation of harmful or toxic elements in the plants at the start and end of growing. The results of this study will help in providing the required information regarding the benefits and risks associated with irrigating alfalfa crops using TW for farmers and decision makers.

2. Materials and Methods

2.1. Experimental Design

In this study, we examined the effects of irrigation with TW on alfalfa nutrient contents and compared them with plants irrigated with well water (WW). The experiment was conducted at the Dirab Agricultural Research and Experiment Station (24°25'34.43'' N, 46°39'10.86'' E, 571 m a.s.l.), College of Food and Agriculture Science, King Saud University (KSU), Riyadh, Saudi Arabia, from 2013 to 2015. Seeds of two alfalfa cultivars (Alhassawy—a local cultivar and CUV101) were obtained from the College of Food and Agriculture Science, KSU. Well water was obtained from a well that is used for irrigation in the Dirab Agricultural Research and Experiment Station, while the TW was obtained from a sewage treatment plant in Riyadh. This treatment plant applies tertiary treatment procedures to wastewater. The treated water is transferred to the farms surrounding the treatment plant via a pipeline network starting from the plant and ending in the farms. Therefore, our experiment was receiving the water daily from the treatment plant via such a pipeline network without any special means of transportation or storage.

The experiment was carried out in a split-plot design, with the cultivar as the main plot and the irrigation type as the split plot factor, with three replicates for each treatment. Seeds of the two studied cultivars were planted in plots (3 × 4 m) on 10 November 2013, with 15 cm spacing between lines. The seeding rate was 40 kg seeds ha⁻¹. All irrigation and fertilization processes were carried out according to the standards followed by the farmers in the study area as recommended by the Ministry of Environment, Water and Agriculture of Saudi Arabia (<https://www.mewa.gov.sa/en/>). In brief, TW reached our experimental site via the pipeline network already installed by the sewage treatment in Riyadh. All the plots were irrigated using surface irrigation methods once a week in winter and twice a week in the summer season until reaching field capacity. Prior to cultivation, plots were fertilized with 120 kg ha⁻¹ diammonium phosphate (DAP; 18 N, 46 P₂O₅). After sowing, fertilizers were applied three times a year, after each harvest, with 120 kg ha⁻¹ DAP, 50 kg ha⁻¹ urea (50 N) and kg ha⁻¹ potassium sulfate (K₂SO₄; 50 K₂O, 18 S). Weeds were removed manually as needed.

2.2. Soil and Water Analysis

Water samples were analyzed in the laboratories of KSU and the Ministry of Environment, Water and Agriculture in Saudi Arabia before starting the experiment. Physical parameters including pH, electrical conductivity (EC), and total dissolved solids (TDS) were examined, in addition to the contents of several nutrients and heavy metals (N, P, K, Ca, Mg, Na, Fe, Cu, Zn, Mn, Cd, Pb, Ba, and Cr) for both WW and TW. Table 1 shows the physical and chemical properties of the two water types used in this study.

Table 1. Chemical and physical properties of the water types used for irrigation.

Parameters	Well Water (WW)	Treated Wastewater (TW)
pH	7.15	7.82
EC (Electrical Conductivity) ($\mu\text{S}/\text{cm}$)	3560	2480
TDS ppm (Total dissolved solids)	1773	1253
Total N (mg/L)	5.8	9.2
P	2.2%	1.7%
K	4.4%	1.5%
Ca	2.9%	1.1%
Mg	3.2%	1.6%
Na	1.1%	1.8%
F	3.7%	2.0%
Cu	4.0%	2.6%
Zn	3.2%	1.9%
Mn	1.1%	3.4%
Cd	1.8%	4.0%
Pb	2.1%	1.8%
Ba	2.2%	1.5%
Cr	2.4%	1.6%

Soil samples were taken from each plot at the beginning and the end of the experiment at four different depths ranging from 0 to 70 cm. All collected samples were pooled together according to type of water used for irrigation and were sent for analysis. Physical (pH, EC, soil texture) and chemical (P, K, Ca, Mg, Na, Fe, Cu, Zn, Mn, Cd, Pb and Cr) properties were examined, and samples were analyzed in the laboratories of KSU and the Ministry of Environment, Water and Agriculture in Saudi Arabia using Inductively Coupled Plasma-Mass Spectrometer (NexION 300D, Perkin Elmer, USA). Table 2 shows the physical and chemical properties of the soil samples at the beginning and end of the experiment.

Table 2. Chemical and physical properties of soils at the beginning and end of the experiment.

Parameters (%)	Beginning of the Experiment	End of the Experiment	
		WW	TW
pH	8.28	7.98	8.07
EC	0.25	0.49	0.22
Sand	62	60	54
Silt	28	30	34
Clay	10	10	12
P	8.2	4.1	4.8
K	12.7	7.8	8.3
Ca	11.9	5.7	8
Mg	s *	3.7	6.1
Na	s *	s *	s *
Fe	11.3	6.5	7.7
Cu	11.7	7.1	4.8
Zn	83.8	2.7	8
Mn	7.6	6.8	2.7
Cd	53	18.3	25.8
Pb	7	13.8	3.7
Cr	8.7	7.1	6

* s indicates that reading was above upper sample concentration.

2.3. Plant Sampling and Analysis

Each replicate of the four treatments (two cultivars $\times$ two water types) was harvested twice; first in the 2013/2014 season and second in the 2014/2015 season. Alfalfa plants were harvested at a height of 5 cm at the start of the flowering stage, and the collected aerial parts were immediately transferred to laboratories in the College of Food and Agriculture Science, KSU. The fresh green leaves were weighed, before being dried in an oven at 60 °C for 48 h. Dried samples were weighed, and these weights were recorded. Dried samples were ground using a grinder (IKA®, Staufen, Breisgau, Germany, MF 10.1 cutting-grinding head) with a speed range of 5500 rpm and a grinding sieve with a diameter of 1 mm.

Quantities of around 500 g of the powdered (dried and grinded) plant samples were packed in sealed plastic bags and shipped by air to Germany (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA) e. V. Germany and Landesbetrieb Hessisches Landeslabor, Germany) for nutrient contents analysis. Analysis of nutrient contents was performed using a near-infrared spectroscopy (NIRS) instrument (Technicon 500, Technicon Industrial Systems, NY, USA).

2.4. Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) on IBM SPSS Statistics 20 with three factors, cultivar, cutting time, and water type. Means are reported as averages of three replicates and were separated by Duncan's Multiple Range Test ($P \leq 0.05$). Correlation analyses between nutrient contents in alfalfa samples were performed using Pearson's correlation analysis on IBM SPSS Statistics 20.

3. Results and Discussion

In the current study, the effect of using TW on the metal contents of two alfalfa cultivars was examined. Concentrations of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (Na, Cl, Cu, Zn, Fe, Mn, Pb, Ni, and Co) were also estimated. The obtained results showed that all of the studied independent variables (water type, cultivar, and cutting time) had a significant ($P \leq 0.05$) effect on macronutrient and micronutrient contents in alfalfa plants. Furthermore, the interactions between each pair of independent variables, and the overall interactions between the studied parameters, produced significant ($P \leq 0.05$) differences in nutrient contents in the plants. Although K and Ca contents were higher in WW as compared to TW (Table 1), irrigation with TW produced plants with slightly higher K and Ca contents, while no differences in S content were observed between plants irrigated with WW and plants irrigated with TW. This agrees with the results obtained by Chávez, et al. [11], who found that irrigation with untreated wastewater led to higher S levels in alfalfa tissues than that with partially TW. Enhancement of nutrient uptake was reported as one of beneficial gains of irrigation with TW [29,30]. Nevertheless, plants irrigated with TW had lower N, P, and Mg contents than those irrigated with WW, regardless of cutting time and cultivar (Table 3). Furthermore, irrigation with TW led to slight decreases in the contents of all studied micronutrients apart from Cl, which increased by an average of 3.2% compared to plants irrigated with WW. Surprisingly, the contents of Mn and Cd in plants irrigated with TW were lower than in those irrigated with WW; however, TW was characterized by higher Mn and Cd contents as compared to WW. Hussain et al. [31] showed that levels of different heavy metals (i.e., Cd, Co, Cu, Mn, Zn, Pb, Ni and Cr) in radish, spinach and carrot plants irrigated with TW were below toxic limits, but plants irrigated with 100% TW had lower contents of heavy metals as compared to others irrigated with 25%, 50% and 75% TW. In general, metals uptake could be affected by several factors including plant cultivar (genotype), external concentrations, and soil pH. Although soils irrigated with TW showed a roughly 50% decrease in Pb content (Table 2), no differences in Pb content were observed between treatments (Table 4). Such a result could be partially explained by the ability of Pb to move in the soil solution and leach to the underground water [32], in addition to its high dependability on the solubility and pH of soil [33]. Chávez, et al. [11] studied

alfalfa tissues and found that Cu and Zn were the most sensitive micronutrients to irrigation with wastewater; however, in the present study, significant decreases in N, P, and K contents were observed after irrigation with TW. Variations were also detected in the macronutrient and micronutrient contents of the different cultivars, regardless of cutting time and the type of water used for irrigation. Plants of the Alhassawy cultivar contained higher N, P, and K contents but lower Ca, Mg, and S contents than plants of the CUV101 cultivar (Table 3). In general, cultivar variation affects several alfalfa yield traits, including morphology, forage quality, overall yield [34], and elemental composition of the plant shoots [35]. It is perhaps unsurprising, then, that plants of the Alhassawy cultivar had higher contents of all micronutrients apart from Cu, Zn, and Co than plants of the CUV101 cultivar (Table 4). James et al. [36] examined five different cultivars of alfalfa and found significant variations in P, Ca, Na, Mn, Sr, and Zn contents. The plant material obtained in the first cutting period (2013/2014) had higher contents of the majority of macronutrients and micronutrients than plant materials obtained during the second cutting period (2014/2015). This could be correlated with the reduction observed in contents of macronutrients in the soils irrigated with TW or WW at the end of the experiment. It was previously reported that long-term application of TW led to significant reductions in plants' contents of heavy metals [37]. Another study that sampled alfalfa cultivated lands that had been irrigated with TW since 80 years found that levels of Fe, Cu, Zn and Mn were higher if compared with their levels in soils irrigated with potable water [38]. The exceptions to this were S, Na, Ni, and Co, which were present in higher contents in plants obtained during the second cutting period than the first cutting period (Tables 3 and 4). Other studies have also shown that cutting time significantly affects the yield, nutritive value, and elemental composition of alfalfa plants [39].

Table 3. Effects of cutting time, cultivar, and water type used for irrigation on alfalfa macronutrient contents.

Factor		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Cutting time	1	3.60 a	0.26 a	2.87 a	3.27 a	0.38 a	0.37 b
	2	2.96 b	0.21 b	2.24 b	1.82 b	0.31 b	0.39 a
LSD ($P \leq 0.05$)		0.02	0.01	0.01	0.02	0.01	0.01
Cultivar	Alhassawy	3.12 b	0.21 b	2.07 b	3.07 a	0.35 a	0.38 a
	CUV101	3.44 a	0.26 a	3.04 a	2.02 b	0.34 b	0.37 a
LSD ($P \leq 0.05$)		0.02	0.01	0.01	0.021	0.01	0.01
Water type	WW	3.30 a	0.24 a	2.51 b	2.42 b	0.35 a	0.38 a
	TW	3.25 b	0.23 b	2.60 a	2.68 a	0.33 b	0.38 a
LSD ($P \leq 0.05$)		0.02	0.01	0.01	0.02	0.01	0.01

Means followed by the same letter are not significantly different ($P \leq 0.05$). LSD: Least Significant Difference.

Table 4. Effects of cutting time, cultivar, and water type used for irrigation on alfalfa micronutrient contents.

Factor		Na (%)	Cl (%)	Cu	Zn	Fe	Mn	Pb	Ni	Co
		(mg/kg)								
Cutting time	1	0.17 b	1.41 a	7.80 a	32.37 a	312.75 a	41.79 a	0.51 a	1.19 b	0.16 b
	2	0.32 a	1.13 b	6.48 b	21.20 b	185.17 b	40.15 b	0.50 a	1.70 a	0.17 a
LSD ($P \leq 0.05$)		0.01	0.03	0.16	0.35	2.18	0.53	0.01	0.01	0.01
Cultivar	Alhassawy	0.33 a	1.37 a	6.13 b	25.40 b	282.08 a	41.91 a	0.51 a	1.31 a	0.16 b
	CUV101	0.16 b	1.17 b	8.15 a	28.17 a	218.83 b	40.03 b	0.50 a	1.57 b	0.18 a
LSD ($P \leq 0.05$)		0.01	0.03	0.16	0.35	2.18	0.53	0.01	0.01	0.01
Water type	WW	0.28 a	1.25 b	8.03 a	27.28 a	275.00 a	41.78 a	0.51 a	1.58 a	0.19 a
	TW	0.21 b	1.29 a	6.25 b	26.28 b	222.92 b	40.47 b	0.51 a	1.30 b	0.15 b
LSD ($P \leq 0.05$)		0.01	0.03	0.16	0.35	2.18	0.53	0.01	0.01	0.01

Means followed by the same letter are not significantly different ($P \leq 0.05$). LSD: Least Significant Difference.

The interaction between each pair of independent variables significantly ($P \leq 0.05$) affected the nutrient content of alfalfa plants. Tables 5 and 6 show the interactive effects between each pair of independent variables on alfalfa macronutrient and micronutrient contents, respectively. Alhassawy cultivar plants irrigated with WW showed the highest contents of all macronutrients (apart from S, as no significant differences in S content were observed in any of the studied plants), and most micronutrients (except Na and Ni) compared to Alhassawy plants irrigated with TW, CUV101 plants irrigated with TW, and CUV101 plants irrigated with WW. CUV101 plants irrigated with WW contained the highest Na and Ni contents out of any of the treatments. The interaction between cutting time and water type used for irrigation did not produce significant variations in micronutrient contents among the different treatments; however, plants obtained in the second cutting period had lower nutrient contents than those obtained in the first cutting period. Plants from the first cutting period irrigated with WW contained the highest micronutrient contents apart from Na and Ni, which were highest in plants from the second cutting period irrigated with WW. There was no significant variation in Pb content among any of the treatments. CUV101 plants obtained in the first cutting period had the highest contents of all macronutrients except S and Ca, which were highest in CUV101 plants of the second cutting period and in Alhassawy plants obtained during the first cutting period, respectively. The contents of several micronutrients (Cl, Fe, Mn, and Co) were highest in Alhassawy plants from the first cutting period. However, the contents of other micronutrients (Cu, Zn) were higher in CUV101 plants of the first cutting period. No significant changes in Pb content were observed among the treatments, and the highest Co content was found in CUV101 plants obtained during the second period. The results obtained in this study indicate that the effect of irrigation water quality significantly depends on other factors, mainly cultivar and cutting time. For example, irrigation with saline water has been shown to affect total forage production and iron relations (including iron uptake and translocation inside the plant tissues) in alfalfa plants, and this effect varies from one cultivar to another [40]. Nevertheless, usage of untreated wastewater increases yield as well as N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, Pb, Ni, and Cd contents in cauliflower (*Brassica oleracea* L. var. botrytis) and red cabbage (*Brassica oleracea* L. var. rubra), without contaminating the soil or plant tissues with heavy metals [41].

Table 5. Effects of the interactions between each pair of independent variables on alfalfa macronutrient contents.

Interacting Variables		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Cutting time × cultivar	1 × Alhassawy	3.46 b	0.23 b	2.28 c	4.05 a	0.38 a	0.35 c
	1 × CUV101	3.74 a	0.29 a	3.45 a	2.49 b	0.37 a	0.39 b
	2 × Alhassawy	2.78 d	0.20 c	1.85 d	2.09 c	0.31 b	0.42 a
	2 × CUV101	3.14 a	0.23 b	2.63 b	1.55 d	0.31 b	0.36 bc
LSD ($P \leq 0.05$)		0.11	0.02	0.14	0.29	0.02	0.03
Cutting time × water type	1 × WW	3.68 a	0.27 a	2.91 a	3.10 a	0.40 a	0.38 a
	1 × TW	3.52 a	0.25 ab	2.83 a	3.44 a	0.36 b	0.36 a
	2 × WW	2.93 b	0.21 b	2.11 b	1.72 b	0.30 c	0.38 a
	2 × TW	2.98 b	0.22 b	2.37 ab	1.91 b	0.31 c	0.40 a
LSD ($P \leq 0.05$)		0.23	0.04	0.66	0.80	0.02	0.05
Cultivar × water type	Alhassawy × WW	3.68 a	0.27 a	2.91 a	3.10 a	0.40 a	0.38 a
	Alhassawy × TW	3.52 a	0.25 ab	2.83 a	3.44 a	0.36 b	0.36 a
	CUV101 × WW	2.93 b	0.21 b	2.11 b	1.72 b	0.30 c	0.38 a
	CUV101 × TW	2.98 b	0.22 b	2.37 ab	1.91 b	0.31 c	0.40 a
LSD ($P \leq 0.05$)		0.23	0.04	0.66	0.80	0.02	0.05

Means followed by the same letter are not significantly different ($P \leq 0.05$). LSD: Least Significant Difference.

Table 6. Effects of the interactions between each pair of independent variables on alfalfa macronutrient contents.

Interacting Variables		Na (%)	Cl (%)	Cu	Zn	Fe	Mn	Pb	Ni	Co
		(mg/kg)								
Cutting time × cultivar	1 × Alhassawy	0.20 b	1.49 a	5.88 b	28.63 b	359.50 a	43.58 a	0.52 a	1.23 b	0.18 ab
	1 × CUV101	0.14 b	1.33 b	9.72 a	36.10 a	266.00 b	40.00 b	0.51 a	1.16 b	0.15 b
	2 × Alhassawy	0.47 a	1.25 b	6.38 b	22.17 c	204.67 c	40.23 b	0.51 a	1.40 b	0.15 b
	2 × CUV101	0.18 b	1.01 c	6.58 b	20.24 c	165.67 c	40.06 b	0.50 a	1.99 a	0.20 a
LSD ($P \leq 0.05$)		0.07	0.11	1.79	3.30	40.27	2.00	0.02	0.24	0.03
Cutting time × water type	1 × WW	0.19 b	1.45 a	9.85 a	35.27 a	353.17 a	43.03 a	0.52 a	1.23 b	0.19 ab
	1 × TW	0.15 b	1.36 a	6.02 b	29.47 b	272.33 b	40.55 b	0.51 a	1.15 b	0.14 c
	2 × WW	0.37 a	1.05 c	6.48 b	19.30 d	196.83 c	39.92 b	0.51 a	1.93 a	0.20 a
	2 × TW	0.28 ab	1.21 b	6.48 b	23.10 c	173.50 c	40.38 b	0.50 a	1.45 b	0.16 bc
LSD ($P \leq 0.05$)		0.15	0.15	1.91	3.64	48.11	2.32	0.02	0.29	0.03
Cultivar × water type	Alhassawy × WW	0.19 b	1.45 a	9.85 a	35.27 a	353.17 a	43.03 a	0.52 a	1.23 b	0.19 ab
	Alhassawy × TW	0.15 b	1.36 a	6.02 b	29.47 b	272.33 b	40.55 b	0.51 a	1.15 b	0.14 c
	CUV101 × WW	0.37 a	1.05 c	6.48 b	19.30 d	196.83 c	39.92 b	0.51 a	1.93 a	0.20 a
	CUV101 × TW	0.28 ab	1.21 b	6.48 b	23.10 c	173.50 c	40.38 b	0.50 a	1.45 b	0.16 bc
LSD ($P \leq 0.05$)		0.15	0.15	1.91	3.64	48.11	2.32	0.02	0.29	0.03

Means followed by the same letter are not significantly different ($P \leq 0.05$). LSD: Least Significant Difference.

The results obtained in the current study demonstrate that the overall interaction between the studied variables, namely, water type used for irrigation, cultivar, and cutting time, significantly ($P \leq 0.05$) affected alfalfa macronutrient and micronutrient contents. CUV101 plants obtained in the first cutting period that were irrigated with WW showed the highest N, P, and Mg contents. There were no differences in P content for first cutting period CUV101 plants irrigated with TW (Table 7). Furthermore, first cutting period CUV101 plants irrigated with TW contained the highest K content among all the studied treatments. Moreover, Alhassawy plants irrigated with TW obtained in the first and the second cutting periods showed the highest Ca and S contents, respectively. N, K, Mg, and Na contents in alfalfa plants irrigated with TW were higher than in plants irrigated with tap water [42]. Regarding the present study, the effects of the three independent variables on alfalfa micronutrient contents are shown in Table 8. Alhassawy plants obtained in the first cutting period that were irrigated with WW had the highest Fe, Mn, and Pb contents. In contrast, Al-Karaki [42] found that Pb and Ni contents were higher in plants irrigated with TW than in plants irrigated with tap water. Irrigation with TW did not increase Cl content in the tissues of Alhassawy plants obtained in the first cutting period. The highest Cu and Zn contents were found in tissues of CUV101 plants irrigated with WW and harvested in the first cutting period. Higher Zn content in alfalfa tissues is considered an undesirable trait because it can lead to problems in crop yield. This is mainly due to the antagonistic effect of Zn against N in different parts of the plant [43], which might lead to N deficiency and, consequently, affect yield. In contrast, our results showed a significantly strong positive association between Zn content and N content (Table 9). The highest Ni and Co contents were found in Alhassawy plants irrigated with WW that were harvested in the second cutting period. The highest Na content was observed in Alhassawy plants irrigated with WW that were harvested in the second cutting period. The crucial nutrients for the health of alfalfa plants are N, P, K, Ca, Mg, and Na [44]. Our results demonstrate that irrigation with TW does not lead to dramatic decreases in the contents of these nutrients within plants. Nevertheless, irrigation with TW does lead to significant increases in K and Ca contents, without any adverse effects on P content. These results agree with those found in a study by Chávez et al. [11], who studied alfalfa irrigated with untreated or partially TW. Kiziloglu et al. [41] concluded that untreated wastewater can be used confidently, on a short-term basis, in agricultural land, while primary TW is suitable for long-term use in sustainable agriculture. Irrigation with TW has been shown to greatly enhance the physiological status, enzymatic activity, and nutritional composition of alfalfa plants, compared to irrigation with WW [45]. Changes in the contents of macro and micronutrients in plants

irrigated with TW could be attributed to several factors including the accumulation of heavy metals inside the plant tissues, which hamper the uptake of other essential ions. In the current study, a negative correlation between Ni and Mg contents was observed, evidence for the effect of heavy metals leading to decreased uptake of essential nutrients. Another factor is the presence of contaminants (e.g., pharmaceutical) that might stimulate or hinder the uptake of specific nutrients [46]. Such contaminants might bind to a plant cell's membrane fraction and thus facilitate or obscure the flux of several ions, e.g., Ca^{2+} [47,48]. Moreover, some nutrients play critical roles in a plant's tolerance to heavy metals, which might explain the vast increase in their levels in plant tissues. In the current study, increased levels of certain heavy metals (i.e., Fe, Cu, Zn and Mn) were associated with an increase in magnesium levels. Mg was shown to have a potential role in heavy metal stress tolerance in mangrove [49] and soybean [50] plants.

Table 7. Effect of the overall interaction between cutting time, cultivar, and water type on alfalfa macronutrient contents.

Cutting Time	Cultivar	Water Type	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
1	Alhassawy	WW	3.54 c	0.24 b	2.38 e	3.76 b	0.40 a	0.37 bc
1	Alhassawy	TW	3.39 d	0.21 c	2.18 f	4.35 a	0.36 b	0.32 d
1	CUV101	WW	3.82 a	0.30 a	3.43 b	2.45 d	0.39 a	0.38 b
1	CUV101	TW	3.65 b	0.28 a	3.48 a	2.54 c	0.35 b	0.39 b
2	Alhassawy	WW	2.83 g	0.18 d	1.73 h	1.79 f	0.31 c	0.39 b
2	Alhassawy	TW	2.72 h	0.21 c	1.97 g	2.38 e	0.31 c	0.45 a
2	CUV101	WW	3.03 f	0.24 b	2.49 d	1.65 g	0.30 c	0.38 b
2	CUV101	TW	3.25 d	0.22 c	2.77 c	1.44 h	0.31 c	0.35 c
LSD ($P \leq 0.05$)			0.04	0.02	0.03	0.04	0.03	0.02

Means followed by the same letter are not significantly different ($P \leq 0.05$). LSD: Least Significant Difference.

Table 8. Effect of the overall interaction between cutting time, cultivar, and water type on alfalfa micronutrient contents.

Cutting Time	Cultivar	Water Type	Na (%)	Cl (%)	Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Pb (mg/kg)	Ni (mg/kg)	Co (mg/kg)
1	Alhassawy	WW	0.22 c	1.50 a	7.40 bc	31.57 c	409.00 a	46.40 a	0.53 a	1.28 d	0.21 b
1	Alhassawy	TW	0.19 d	1.47 a	4.37 f	25.70 d	310.00 b	40.77 b	0.50 b	1.17 e	0.15 de
1	CUV101	WW	0.17 d	1.40 b	11.77 a	38.97 a	297.33 c	39.67 b	0.50 b	1.18 e	0.16 cd
1	CUV101	TW	0.11 e	1.25 c	7.67 b	33.23 b	234.67 d	40.33 b	0.51 b	1.13 f	0.14 e
2	Alhassawy	WW	0.57 a	1.13 d	7.00 d	20.60 g	214.67 e	40.00 b	0.50 b	1.73 c	0.16 cd
2	Alhassawy	TW	0.36 b	1.37 b	5.77 e	23.73 e	194.67 f	40.47 b	0.51 b	1.06 g	0.14 e
2	CUV101	WW	0.17 d	0.97 f	5.97 e	18.00 h	179.00 g	39.83 b	0.50 b	2.13 a	0.23 a
2	CUV101	TW	0.19 d	1.05 e	7.20 cd	22.47 f	152.33 h	40.29 b	0.50 b	1.84 b	0.17 c
LSD ($P \leq 0.05$)			0.02	0.07	0.31	0.70	4.36	1.07	0.02	0.02	0.01

Means followed by the same letter are not significantly different ($P \leq 0.05$). LSD: Least Significant Difference.

Table 9. Pearson's correlation coefficients between the contents of different nutrients in alfalfa plants.

	N	P	K	Ca	Mg	S	Na	Cl	Cu	Zn	Fe	Mn	Pb	Ni	Co
N	1														
P	0.761 **	1													
K	0.813 **	0.879 **	1												
Ca	0.404	0.032	−0.096	1											
Mg	0.779 **	0.484 *	0.401	0.674 **	1										
S	−0.433 *	0.078	−0.118	−0.354	−0.251	1									
Na	−0.718 **	−0.685 **	−0.761 **	−0.249	−0.400	0.375	1								
Cl	0.413 *	0.158	0.013	0.851 **	0.781 **	0.016	−0.127	1							
Cu	0.599 **	0.685 **	0.673 **	−0.245	0.444 *	0.119	−0.175	0.094	1						
Zn	0.856 **	0.763 **	0.709 **	0.399	0.819 **	0.006	−0.450 *	0.643 **	0.728 **	1					
Fe	0.577 **	0.257	0.057	0.829 **	0.855 **	−0.265	−0.191	0.808 **	0.190	0.614 **	1				
Mn	0.230	−0.010	−0.157	0.543 **	0.515 **	−0.164	−0.083	0.491 *	−0.055	0.228	0.742 **	1			
Pb	0.199	0.093	−0.068	0.339	0.299	−0.082	−0.056	0.284	0.035	0.205	0.539 **	0.706 **	1		
Ni	−0.388	−0.300	−0.203	−0.650 **	−0.589 **	−0.236	0.142	−0.869 **	−0.157	−0.701 **	−0.532 **	−0.220	−0.180	1	
Co	−0.028	0.018	−0.081	−0.152	−0.021	−0.236	−0.172	−0.340	−0.027	−0.292	0.109	0.378	0.194	0.667 **	1

** Correlation is significant at the 0.01 level (two-tailed). * Correlation is significant at the 0.05 level (two-tailed).

4. Conclusions

The results obtained in this study show that irrigation with TW is more advantageous than irrigation with (high saline) WW. Plant nutrient content was not adversely affected by irrigation with TW compared to irrigation with WW. Similarly, soil analysis showed no significant deterioration in soil physiochemical characteristics as a result of irrigation with TW. Based on these results, the use of irrigation with TW in the production of alfalfa can be recommended. However, further research is needed to approve the safety of using TW to irrigate other forage crops, considering the possible variations between sites in terms of TW quality and soil status. Even for alfalfa, research on the long-term effects needs to be performed as the current study is based only on results from 2 years.

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