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Nutrient Content of Vineyard Leaves after Prolonged Treated Wastewater Irrigation

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Abstract: Water is essential for agricultural productivity and is a vital component of food security. In areas with limited water supplies, new water resources must be identified. Given these challenges, we attempted to determine whether the use of treated wastewater for vineyard irrigation is compatible with sensible agricultural methods within the context of a circular economy, where resource sustainability is a key tenet of foodtech. The main purpose of this study was to determine whether using treated wastewater for vineyard irrigation influences foliar nutrient content identifying differences according to irrigation water. A field experiment was designed to compare vineyards that had been irrigated with treated wastewater for years to those that had been irrigated with conventional well water. For characterization, water and soil were analyzed. Furthermore, the macro and micronutrient contents of vine leaves, as well as chlorophyll (SPAD units) measured directly in the field, were tracked over several seasons to determine the relationship between them. We found no nutritional imbalances in the crop at the end of the study, although there were improvements in the concentrations of some nutrients (Mg, Mn and Zn). It was also noticed that plots irrigated with treated wastewater run the risk of increasing soil saline concentrations.

Keywords: vineyard; nutrients; reclaimed wastewater; soil

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1. Introduction

New water resources must be identified in areas with restricted water supplies. As one of the Sustainable Development Goals (SDGs) in the 2030 Agenda to end hunger and poverty, safeguard the global ecosystem, and promote prosperity for all people, the United Nations (UN) is promoting the use of treated wastewater in agriculture ("Transforming our world: the 2030 Agenda for Sustainable Development | Department of Economic and Social Affairs", n.d.) [1]. One of the consequences is that it is becoming increasingly common to use treated wastewater from agricultural operations all over the world [2].

Among all reuse alternatives, reusing treated wastewater as irrigation water has the highest rate of use in the world [3]. While 44% of the recovered water in southern Europe is utilized for irrigation, 51% of it is used for environmental purposes in northern Europe [4]. The EC issued a regulation requiring a minimum level of reclaimed water quality for the European Union, but each country has its own regulatory structure, and strict regulations discourage the use of recycled water. Pioneers in the use of recovered water in agriculture include Spain, Italy, Cyprus and Greece. Overall, 10% of wastewater in Spain is recovered and 61% of it is used in agriculture [4].

The widespread use of wastewater for irrigation is, however, constrained not just by quality standards but also by institutional and social factors [3]. In general, there is a rejection of the use of reclaimed water due to a general lack of technical knowledge about its properties, depending on the intended use (environmental, agricultural, industrial, etc.). The reluctance of reclaimed water use in agriculture is connected with social barriers and a high sense of pathogen revulsion [4]. Moreover, politicians and decision-makers are sometimes reluctant to take action to promote reclaimed water because they do not have a thorough understanding of the reuse of wastewater in agriculture [5]. Only through effective interdisciplinarity, social learning and adaptive management can water concerns be effectively addressed [6].

In this sense, several studies have been conducted to evaluate the long-term impacts of wastewater irrigation on crops [7] and, in most cases, it is advised that adequately treated wastewater can be utilized safely for agricultural production amid the Mediterranean region's water shortage [2]. It would free up freshwater for other uses, such as domestic and urban supplies, if more of the available treated, partially treated or untreated wastewater was used to replace even a small amount of the freshwater currently required for irrigation [8].

However, since some instances have indicated somewhat increased soil salinity and electrical conductivity values in wastewater treated irrigated areas, the long-term effects are not universally agreed upon [9].

Because of the worldwide popularity of wine and related products, vineyard agriculture is very important on a global scale [10]. Grape and wine production provide numerous economic, social, and environmental benefits, including trade, rural income, employment and tourism [8]. The use of treated wastewater in vineyards has also been increasing in various countries such as the U.S.A, Spain and France [7], and Mexico and South Africa [8]. It must be considered that local government wastewater standards vary greatly; though, they are becoming more stringent as tertiary treatments are implemented in conventional wastewater treatment plants [11].

Researchers have trouble determining the long-term impact of irrigation with treated wastewater because farmers who have been using this type of water on their crops for years, particularly woody crops such as olive groves or vineyards, have not had their soil and plant material monitored in the beginning years of use. This information should have been available from the beginning, but that is not the situation. Resuming monitoring at a specific time and comparing it to a similar crop patch irrigated with conventional water will help compensate for the lack of data.

In many instances, this is challenging, since we are starting from actual field scenarios that are influenced by variables that are difficult to control rather than a controlled laboratory experiment [12].

Considering these challenges, we have attempted to determine if the use of treated wastewater for vineyard irrigation is compatible with sound agricultural methods within the context of a circular economy, where the sustainable use of resources is a core tenet of foodtech.

Water is a key factor in food security and is necessary for agricultural productivity, which should not be overlooked. The understanding of how the use of treated residual water can impact agricultural crops nutritionally is essential [11]. The study of nutrients in any crop of agricultural interest is of particular concern because it is directly related to product quality [10]. Although soil and water testing have traditionally been used to assess the potential for nutrient stress in crops, tissue analysis is thought to be a more reliable diagnostic tool for determining nutritional status [13].

Plant nutrition research is required to understand plant growth and development. Roots absorb approximately 60 elements from the soil, but not all of them are required for plant growth. Nutrients are essential elements that primarily serve structural functions, act as enzyme activators, and act as osmotic regulators in plants. There are 17 plant nutrients that are required [14]. In contrast, functional elements are those that promote growth but are not required for survival.

As is well known, there are two categories of nutrients that can be distinguished: macronutrients, which are those that are consumed in greater amounts and make up 0.2–4.0% of the dry matter weight; and micronutrients, which are found in plant tissues at concentrations between 5 and 200 ppm, or less than 0.02% dry weight [14]. Most micronutrients have a very small appropriate range, and even a small variation in concentration can cause symptoms.

Mobility of nutrients within the soil is related to properties of the soil, such as cation exchange capacity, moisture or pH. Any nutrient's absence or shortage results in the emergence of symptoms. Therefore, when evaluating the nutritional value of plants, any factor that modifies the soil's conditions, such as the type of irrigation water, must be taken into consideration.

In comparison to other studies where reclaimed water is used to irrigate vineyards, our study intends to determine whether any specific nutrient is absorbed differently when compared to a control irrigated with conventional well water. Simultaneously, we intend to investigate whether it is possible to determine the nutritional status of the vineyard more quickly than by laboratory analysis.

Hence, the main goal of this study is to find out whether the use of treated wastewater for the continued irrigation of vineyards has a consequence on the foliar nutrient content.

To achieve this objective, (i) two vineyard plots irrigated with reclaimed water for years were compared with a control plot in the same area and with the same type of crop; (ii) soil and irrigation water in the studied areas were characterized; (iii) macro and micronutrients in vine leaves were analyzed; (iv) a correlation between the amount of nutrients found in leaves that have undergone laboratory analysis and the amount of chlorophyll that has been non-destructively measured in the field was sought.

2. Materials and Methods

2.1. Study Area: Demo Sites Description

The field trial was carried out in 3 different study areas distributed in agricultural parcels of Albacete province (SE of Spain). Two vineyard plantations (V1 and V2) irrigated with treated wastewater (TW) for more than ten years were compared with other plantation (VC) irrigated with conventional well water used as the control plot. The VC plot had 7.7 ha and was located in Mahora (39°11′8.18″ N; 1°39′32.69″ W) and V1 plot was located in Fuenteálamo (38°41′34.54″ N; 1°28′26.53″ W) and had 4.8 ha; finally, V2 plot was in Valdeganga (39°7′23.71″ N; 1°40′23.41″ W) and had 4.3 ha. In all cases, the monitored area was 300 m^2.

During the study period (July 2016–September 2018), soil and irrigation water were characterized.

Treated wastewater came from two different wastewater treatment plants (WWTPs) with biological secondary treatment without disinfection and different numbers of Inhabitants Equivalent (IE) located in Valdeganga (3000 IE) and Fuenteálamo (5600 IE). Both WWTPs are placed in an agrarian and rural area without industrial activity. As a result, there is no concern of heavy metal accumulation because the pollutant load is domestic in origin and comes from nearby communities. Table 1 shows the irrigation water composition.

Table 1. Irrigation water composition. WC: control water; W1 and W2: treated wastewater. EC: Electrical Conductivity; TSS: Total Suspended Solids. Different lowercase letters indicate different groups with significant differences at $p < 0.05$ among treatments according to the Tukey test. (*) Significant differences. Values are average ± standard deviation.

PARAMETER	UNIT	VALUE			METHODOLOGY
		WC (n = 6)	W1 (n = 6)	W2 (n = 6)	
EC	mS cm^{-1}	1.22 ± 0.04	2.14 ± 0.09	1.67 ± 0.10	Conductimety (1:5)
pH		7.61 ± 0.14	7.98 ± 0.27	7.92 ± 0.19	Potentiometry

Table 1. *Cont.*

PARAMETER	UNIT	VALUE			METHODOLOGY
		WC (n = 6)	W1 (n = 6)	W2 (n = 6)	
NO_3^-	mg L^{-1}	31.34 ± 2.71	2.50 ± 2.52	12.78 ± 12.28	Spectrophotometry UV/VIS
CO_3^{2-}	mg L^{-1}	4.00 ± 9.80	17.00 ± 25.33	16.00 ± 19.60	Volumetry
HCO_3^-	mg L^{-1}	288.67 ± 28.51	422.92 ± 93.39	427.00 ± 57.14	Volumetry
SO_4^{2-}	mg L^{-1}	260.17 ± 66.01	426.42 ± 117.29	279.50 ± 66.11	Spectrophotometry UV/VIS
Cl^-	mg L^{-1}	109.83 ± 4.45	274.75 ± 33.75	206.00 ± 32.86	Volumetry
K	mg L^{-1}	2.49 ± 0.85	20.42 ± 3.83	21.40 ± 9.21	Atomic absorption spectroph.
Na	mg L^{-1}	32.83 ± 13.80	120.40 ± 39.72	95.50 ± 29.67	Atomic absorption spectroph.
Mg	mg L^{-1}	55.50 ± 6.98	119.62 ± 23.90	84.50 ± 16.32	Complexometry
Ca	mg L^{-1}	151.50 ± 26.68	160.83 ± 54.70	127.33 ± 29.97	Complexometry
NH_4^+	mg L^{-1}	Not detectable	16.43 ± 15.86	5.85 ± 7.83	Volumetry
PO_4^{3-}	mg L^{-1}	0.05 ± 0.12	7.34 ± 5.81	8.87 ± 11.94	Spectrophotometry UV/VIS
B	mg L^{-1}	0.10 ± 0.06	0.31 ± 0.10	0.29 ± 0.07	Spectrophotometry UV/VIS
Organic matter	mg L^{-1}	1.54 ± 0.74	7.55 ± 4.39	6.01 ± 1.66	Permanganometry
TSS	mg L^{-1}	85.20 ± 99.46	88.82 ± 21.77	67.20 ± 28.76	Gravimetry

This way, three different water sources were used for the study, namely (1) WC: Control water (to irrigate control plot: VC); (2) W1: TW (to irrigate V1 and collected from Fuenteálamo WWTP); (3) W2: TW (to irrigate V2 and collected from Valdeganga WWTP).

2.2. Abiotic Conditions

Study plots were in the local steppe climate. There is little rainfall throughout the year. This climate is considered BSk according to the Köppen–Geiger climate classification. According to the weather stations of the area, the meteorological conditions monitored were mean average temperature (15.25 °C), and minimum absolute and maximum absolute temperatures which were −8.4 °C and 40.3 °C, respectively. Total precipitation was 809.2 mm and mean daily sunshine was 9.79 h.

2.3. Crops Description

All the vineyards studied were red grape varieties for wine. The plantation was supported by trellises and framework which were 1.5 m (row distance) × 3 m (tree spacing along each row). The V1 plot was irrigated with TW since 2000 and V2 since 2009.

Every year, the farmers of all the vineyards fertilize the crop, primarily in the winter, with the help of agricultural machinery and complex fertilizers. They also treat for fungus to guard off oidium and mildew, and every three years, they used to spread sheep manure on alternate streets.

2.4. Irrigation System

All study plots were watered by dripping, and 16 mm diameter, polyethylene pipelines were used and the height of pipelines to soil was 50 cm. Emitter flow was 2 L h^{-1} and irrigation was 100 mm per year.

2.5. Sampling and Measurement

2.5.1. Water

Six times water samples were collected during the study and parameters analyzed in water were (Table 1) electrical conductivity, pH, NO_3^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, Cl^-, K, Na, Mg, Ca, NH_4^+, PO_4^{3-}, B, organic matter and total suspended solids (TSS).

2.5.2. Soil

From the start of the study, monthly soil samples were collected in the three monitoring plots. Following a zigzag distribution, ten soil samples were randomly selected from each plot, and they were then homogenized and subjected to a soil characterization study using the parameters provided in Table 2.

Table 2. Characterization and differences between vineyard soil simples (VC, V1, V2). Period August 2016–September 2018. VC: control vineyard; V1 and V2: vineyards irrigated with treated wastewater. Different letters indicate different groups with statistically significant differences for $p < 0.05$ between treatments according to Tukey's test. (*) Significant differences. Values are means ± standard deviation.

PARAMETER	UNIT	VALUE (n = 17)				METHODOLOGY
		VC	V2	V1	p-Value	
Sandy	%	51.32 ± 7.94	56.13 ± 5.58	42.85 ± 4.09		Bouyoucos Densimeter
Loam	%	29.71 ± 6.70	24.64 ± 4.27	36.03 ± 2.70		Bouyoucos Densimeter
Clay	%	18.97 ± 1.70	19.23 ± 1.78	21.11 ± 2.34		Bouyoucos Densimeter
Texture		Sandy clay loam	Loam	Clay loam		USDA
pH		8.62 ± 0.10 a	8.63 ± 0.16 a	8.68 ± 0.22 a	0.8264	Potentiometry in 1:2.5 extract
Electrical conductivity(1:5)	mmhos cm^{-1}	0.24 ± 0.06 a	0.44 ± 0.53 a	0.47 ± 0.60 a	0.3259	Conductimetry on 1:5 extract
Cl^-	ppm	14.62 ± 4.23 a	14.48 ± 4.01 a	16.72 ± 1.74 a	0.5466	Argentometry in 1:5 extract
SO_4^{2-}	meq 100 g^{-1}	34.60 ± 19.60 a	39.01 ± 19.62 a	29.02 ± 5.56 a	0.6361	Turbidimetry on extract 1:5
Total organic matter	%	1.14 ± 0.29 a	1.37 ± 0.22 b	1.88 ± 0.33 c	<0.0001 *	Walkley Black Method
Total-N	%	0.05 ± 0.01 a	0.08 ± 0.02 a	0.10 ± 0.03 c	<0.0001 *	Kjeldahl Method
C:N		12.81 ± 3.80 a	10.69 ± 3.44 a	11.71 ± 3.53 a	0.2364	Arithmetical operation
Nitric-N	ppm	6.22 ± 4.49 a	18.29 ± 21.57 a	15.03 ± 22.46 a	0.1451	Spectrophotometry UV/VIS
Assimilable P	ppm	21.43 ± 5.20 a	40.98 ± 8.65 a	41.40 ± 11.45 b	<0.0001 *	Olsen Method
Total carbonates	%	30.06 ± 9.01 a	27.35 ± 1.66 a	23.67 ± 1.06 a	0.2045	Bernard Method
Active limestone	%	13.50 ± 5.32 a	14.18 ± 4.83 a	12.25 ± 1.32 a	0.7681	Gasometry
Assimilable K	meq 100 g^{-1}	0.83 ± 0.16 a	1.17 ± 0.20 a	1.22 ± 0.59 b	<0.0001 *	Atomic emission spectrophotometry
Assimilable Na	meq 100 g^{-1}	0.38 ± 0.19 a	0.52 ± 0.25 a	0.59 ± 0.34 a	0.0707	Atomic emission spectrophotometry
Assimilable Ca	meq 100 g^{-1}	37.91 ± 5.28 a	37.01 ± 4.48 a	38.76 ± 4.97 a	0.5851	Spectrophotometry Atomic absorption
Assimilable Mg	meq 100 g^{-1}	1.47 ± 0.65 a	2.10 ± 0.74 a	3.88 ± 1.42 b	<0.0001 *	Spectrophotometry Atomic absorption
K:Mg		0.75 ± 0.55 b	0.62 ± 0.26 ab	0.34 ± 0.08 a	0.0062 *	Arithmetical operation
Ca:Mg		34.35 ± 0.65 b	20.60 ± 10.45 a	11.03 ± 3.63 a	0.0005 *	Arithmetical operation
Cation Exchange Capacity	meq 100 g^{-1}	9.37 ± 2.31 a	8.98 ± 1.70 a	12.08 ± 2.54 b	0.0002 *	Atomic emission spectrophotometry
Assimilable Fe	ppm	1.20 ± 0.73 a	0.97 ± 0.31 a	0.97 ± 1.43 a	0.7222	Spectrophotometry Atomic absorption
Assimilable Zn	ppm	0.41 ± 0.13 a	1.20 ± 0.39 c	0.76 ± 0.30 b	<0.0001 *	Spectrophotometry Atomic absorption
Assimilable Cu	ppm	0.62 ± 0.08 a	0.80 ± 0.11 b	1.43 ± 0.31 c	<0.0001 *	Spectrophotometry Atomic absorption
Assimilable Mn	ppm	2.63 ± 0.92 a	4.28 ± 1.26 b	4.67 ± 2.69 b	0.0039 *	Spectrophotometry Atomic absorption
Assimilable B	ppm	0.19 ± 0.19 a	0.34 ± 0.29 ab	0.44 ± 0.15 b	0.0055 *	UV/VIS Spectrophotometry

2.5.3. Crop

During two consecutive wine seasons from May through October, a random leaf of ten different trees per row (three rows in total) was taken monthly in the 2016/17 growing season (five sampling dates), and three sampling dates (June, July and September) in the 2017/18 growing season (thirty different leaves in total in each sampling event).

Foliar nutrients were tested in the laboratory. To quantify chlorophyll, an optical sensor was used. The soil plant analysis development (SPAD) meter (SPAD-502, Konica Minolta, Tokyo, Japan) is a lightweight hand-held chlorophyll meter for use directly in the field. This small tool is frequently used in studies when it is necessary to determine the content of chlorophyll quickly and non-destructively [15,16]. The SPAD-502 measures the light transmissions at 650 nm, where light is absorbed by the chlorophyll molecule, and at 940 nm, when no absorption is present, to quantitatively assess the intensity of leafy green [17]. The meter calculates a numerical SPAD value based on these two absorbances, which is proportional to the amount of chlorophyll in the leaf [18]. Chlorophyll in SPAD units were determined in leaves.

2.6. Experimental Design and Statistical Analysis

Soil and irrigation water analysis was conducted to describe the agricultural growing environment.

The experimental design compared the nutrient concentrations in vine leaves (6 macronutrients, 5 micronutrients, and chlorophyll content) as a function of two irrigation treatments: WC (control water) and TW (treated water) (treated wastewater: W1 and W2).

ANOVA was used to analyze the data, and Fisher's least significant differences (LSD) and the Tukey test for $p < 0.05$ were used to distinguish between the means. Statgraphic Centurion XV (Statgraphics Technologies, Inc. P.O. Box 134, The Plains, Virginia 20198) was used to perform all the statistical calculations.

3. Results

3.1. Analytical Results

3.1.1. Water Quality

Overall, six water samples were analyzed. Average values of water results analysis are reported in Table 1. There were significant differences between the three types of irrigation water (WC, W1, and W2). Comparing control (WC) with reclaimed water (W1 and W2),W1 had higher concentration values in several values than W2 and especially when compared to the WC.

Commenting on the Table 1 values, no differences in pH were observed between the reclaimed water and the control water, as was also observed for calcium and total suspended solids. However, electrical conductivity was higher in TW, especially W1, and the concentrations of carbonates, bicarbonates, sulphates, chlorides, potassium, sodium, magnesium, ammonium, phosphate, boron and organic matter were always higher in the reclaimed water samples than in the control water samples. Nevertheless, it is noteworthy that in both cases of treated wastewater, the concentration of nitrate decreases with respect to control.

3.1.2. Soil

When the data in the soil characterization Table 2 are analyzed, the differences in irrigation water input in the three plots studied are not apparent in the case of some parameters analyzed, such as texture (it must be noted that they are soils in the same geographic area and have not undergone changes), pH, Cl^-, SO_4^{2-}, C/N ratio, active limestone and Ca. The higher content of organic matter, N, P, cation exchange capacity and assimilable compounds such as Zn, Cu, Mn and B are notable when compared to the control soil, which has never been irrigated with reclaimed water. In terms of soil fertility, all these parameters are positive. This is not the case for Na concentration, which can be related to a higher value of electrical conductivity and is not thought to be beneficial to crop productivity in some cases.

3.1.3. Crop

Table 3 compares the average nutrient concentrations. In most of the parameters analyzed (N, P, K, Ca, Na, Fe, B and chlorophyll), no significant differences were observed between vine leaves irrigated with reclaimed water for years and those irrigated with conventional well water, which served as a control.

Table 3. Macro and micronutrient composition of leaves. VC: control vineyard; V1 and V2: vineyards irrigated with treated wastewater. Different letters indicate differences between groups at the significance level of $p < 0.05$ between treatments according to the LSD test. (*) Significant differences at 95% confidence level. Values are means ± standard deviation. Period: May 2017–September 2018.

LEAVES ANALYSIS						
PARAMETER	**UNIT**	**VALUE**			***p*-Value (n = 9)**	**METHODOLOGY**
		VC	**V2**	**V1**		
MACROELEMENTS						
N	%	2.26 ± 1.05 a	2.51 ± 0.79 a	2.53 ± 0.79 a	0.7757	Kjeldahl method
P	%	0.15 ± 0.08 a	0.14 ± 0.08 a	0.16 ± 0.09 a	0.8138	UV/VIS Spectrophotometry
K	%	1.16 ± 0.87 a	1.00 ± 0.51 a	0.78 ± 0.41 a	0.4499	Atomic emission spectrophotometry
Mg	%	0.22 ± 0.06 a	0.36 ± 0.07 b	0.31 ± 1.70 b	0.0081 *	Atomic absorption spectrophotometry
Ca	%	2.00 ± 0.73 a	1.94 ± 0.66 a	1.70 ± 0.65 a	0.6209	Atomic absorption spectrophotometry
Na	%	0.04 ± 0.04 a	0.01 ± 0.03 a	0.05 ± 0.06 a	0.1812	Atomic emission Spectrophotometry
MICROELEMENTS AND CHLOROPHYLL						
Fe	ppm	84.68 ± 17.72 a	89.44 ± 23.31 a	75.54 ± 19.01 a	0.3475	Atomic absorption spectrophotometry
Mn	ppm	48.74 ± 20.43 a	124.82 ± 59.14 b	67.28 ± 18.18 a	0.0007 *	Atomic absorption spectrophotometry
Cu	ppm	1.38 ± 0.50 a	2.31 ± 0.91 ab	5.48 ± 6.77 b	0.0907	Atomic absorption spectrophotometry
Zn	ppm	10.89 ± 2.98 a	19.98 ± 5.93 b	12.22 ± 3.19 a	0.0002 *	Atomic absorption spectrophotometry
B	ppm	45.12 ± 22.30 a	48.24 ± 35.11 a	57.90 ± 23.93 a	0.6005	UV/VIS Spectrophotometry
Chlorophyll	SPAD units	42.01 ± 6.22 a	40.93 ± 7.64 a	38.90 ± 8.60 a	0.7393	Direct measurement: SPAD-502 Plus

In contrast, Mg concentration was significantly higher in the plots irrigated with reclaimed water (V1: 0.31%; V2: 0.36%) than in the leaves from the control plot (VC: 0.22%).

Regarding microelements, the significant differences between treatments were observed in the concentrations of Mn, Cu and Zn. In these three cases, the lowest values occurred in the control leaves. The differences were observed at different levels. Thus, the amount of Mn in leaves from plot V2 was significantly higher (124.82 ppm) than those from the other plot irrigated with reclaimed water (V1: 67.28 ppm) and the control (VC: 48.74 ppm).

A similar situation was observed for Zn concentrations, where leaves from V2 had higher values (19.98 ppm) than those obtained in V1 (12.22 ppm) and VC (10.89 ppm) for this micronutrient.

Finally, it is remarkable that the Cu concentration in leaves from V1 (5.48 ppm) was significantly higher than those from V2 (2.31 ppm) and VC (1.38 ppm).

As shown in Table 3, the mean amounts of chlorophyll in leaves did not differ between irrigation treatments. Figure 1 shows that the evolution curves in the three study plots were similar. The graph curves increased in all cases until they reached a peak in the two summer months (July and August), then fell. This decrease in chlorophyll content is normal at this point in the crop's growing season and is associated with an increase in carotenoid concentration. This causes the grapevine leaves to turn yellow or pale green [16].

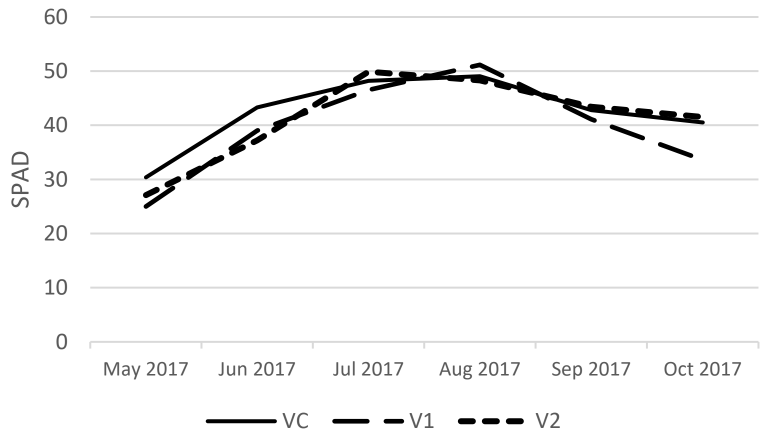

Figure 1. Chlorophyll content (SPAD units) evolution from May to October 2017.

Chlorophyll concentration has been related to the amount of nutrients in Figure 2.

The phosphorus curve, Figure 2b, showed the best correlation (R2 = 0.8191; P(%) = 0.0008 SPAD2 − 0.0676 SPAD + 1.5785), followed by the curves with K (Figure 2c: R2 = 0.684; K(%) = 0.0031 SPAD2 − 0.2555 SPAD + 5.7124), Ca (Figure 2d: R2 = 0.6126; Ca(%) = −0.0024 SPAD2 + 0.2538 SPAD − 4.2641) and N (Figure 2a: R2 = 0.5047; N(%) = 0.0061 SPAD2 − 0.524 SPAD + 13.333). The graphics for the remaining nutrients showed lower correlation values.

In addition, to compare between irrigation treatments, an ANOVA was performed considering the type of irrigation water factor (control water: WC or treated wastewater: TW). The differences for the parameters Mg, Mn, Cu and Zn are still evident in Table 4 and they are noticeably higher in the vine irrigated with TW.

Table 4. Differences between nutrients content, and chlorophyll in vine foliar tissues for plants irrigated with treated wastewater (TW) and control (C). For the same parameter, different lowercase letters indicate different groups with significant differences at $p < 0.05$ among treatments according to the Tukey test. (*) Significant differences. Values are average ± standard deviation. Period: May 2017–September 2018.

	(n)	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Na (%)**
TW	18	2.52 ± 0.77 a	0.15 ± 0.09 a	0.89 ± 0.46 a	1.82 ± 0.65 a	0.34 ± 0.09 b	0.03 ± 0.05 a
C	9	2.26 ± 1.04 a	0.15 ± 0.08 a	1.16 ± 0.086 a	2.00 ± 0.73 a	0.22 ± 0.06 a	0.04 ± 0.04 a
p-value		0.4732	0.9110	0.2931	0.5101	0.0030 *	0.7461
		Fe (ppm)	**Mn (ppm)**	**Cu (ppm)**	**Zn (ppm)**	**B (ppm)**	**Chloropyll (SPAD units)**
TW	18	82.5 ± 21.8 a	96.04 ± 51.7 b	3.89 ± 4.9 a	16.1 ± 6.1 b	53.07± 29.6 a	39.91 ± 7.8 a
C	9	84.7 ± 17.7 a	48.7 ± 20.42 a	1.37 ± 0.49 a	10.88 ± 29.0 a	45.11 ± 22.29 a	42.01 ± 6.2 a
p-value		0.7971	0.0147 *	0.0453 *	0.0238 *	0.4843	0.5479

The evolution graphs in the sampled campaigns are shown for these four parameters (Figure 3) where differences have been observed (Mg, Mn, Cu and Zn). Understanding how these parameters have changed will help us to determine whether there is a relationship between the type of water received and the health of the crop.

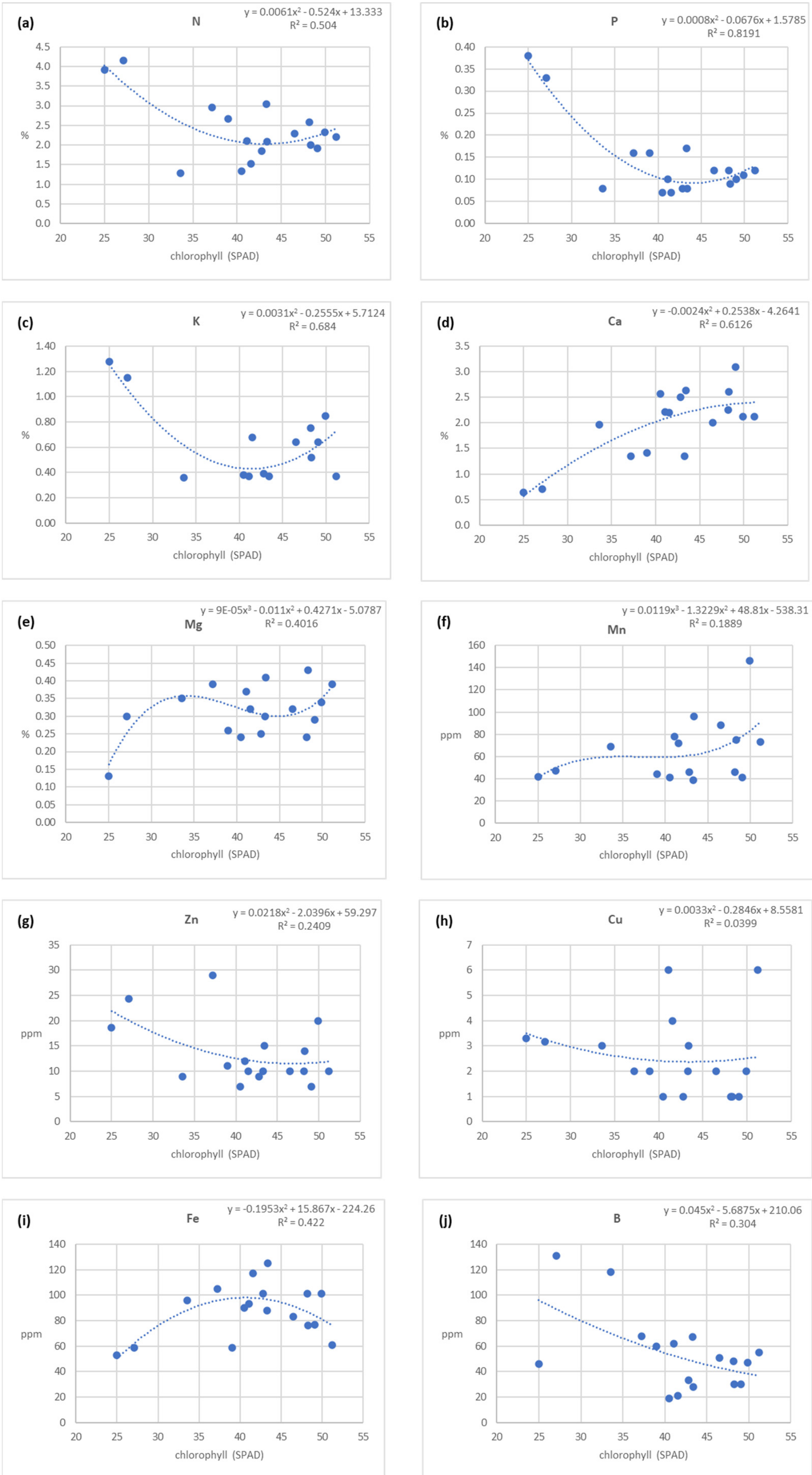

Figure 2. Relationship between chlorophyll concentration (in SPAD units) and nutrients (nitrogen (**a**), phosphorus (**b**), potassium (**c**), calcium (**d**), magnesium (**e**), manganese (**f**), zinc (**g**), copper (**h**), iron (**i**), boron (**j**)) in vine leaves.

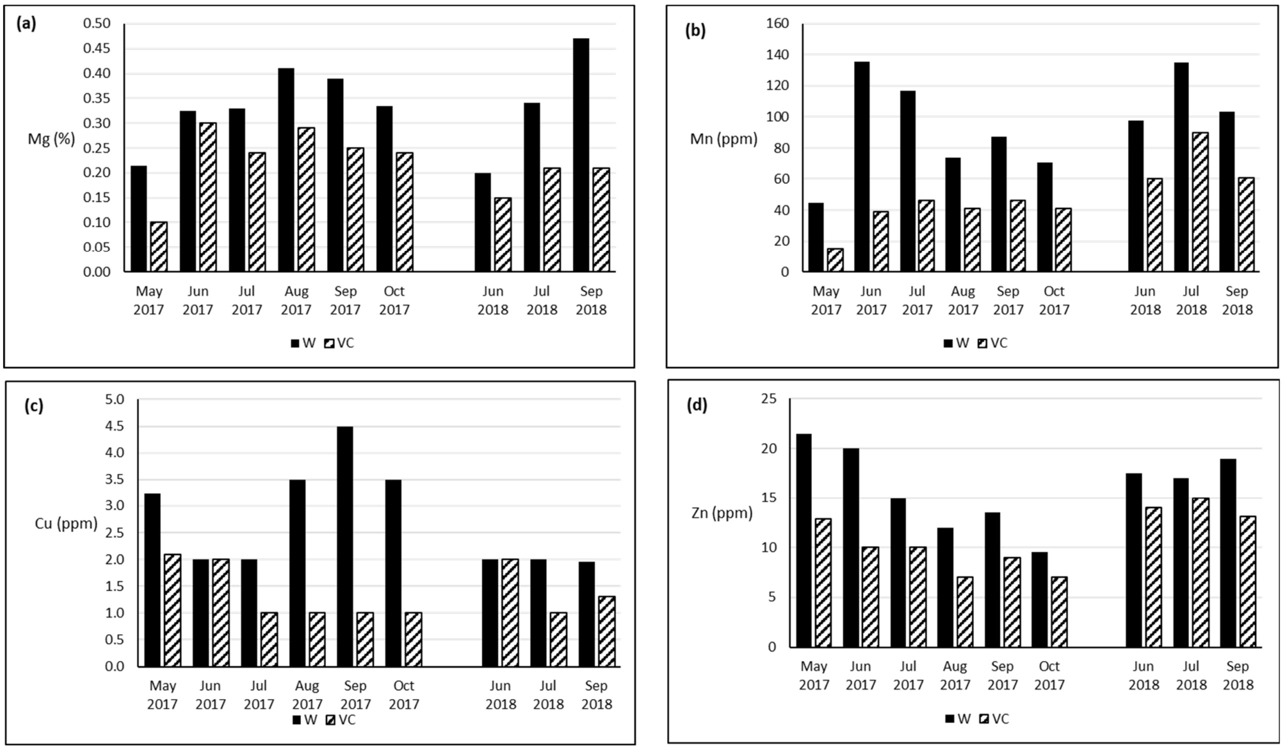

Figure 3. Magnesium (**a**), manganese (**b**), copper (**c**) and zinc (**d**) content in grapevine leaves.

After carefully examining Figure 3 as a whole, we can state that the evolution of nutrients is a multidimensional process that depends on numerous variables and fluctuates from season to season.

It is also evident that vine leaves from the wastewater irrigation plot had higher concentrations of any one of these four parameters than vine leaves from the control plot.

4. Discussion

Many previous studies were conducted in laboratories. In contrast, this was one of the few research projects in which reclaimed water was used for vineyard irrigation in the field. The limitations and drawbacks of this research are obvious. The fact that environmental variables linked to climatology could not be set at will, or that the initial situation of the crop was enforced because the vines were already planted before the monitoring process were the main challenges when designing the experiment.

However, these difficulties in having absolute control of the variables that affect the report of the research turn into an advantage over a laboratory trial, since the results obtained are an image of what happens in the reality of a vineyard field.

The fundamental precondition for water reuse is that its applications will not cause unacceptable public health risks. For this purpose, it is crucial to evaluate the quality of the TW used for irrigation and compare it with the quality of the conventional water sources adopted by local farmers.

Research is still being carried out to compare how agricultural crops respond to irrigation from treated wastewater and traditional irrigation sources [19].

There have been studies that show a link between increased sodium concentration and increased electrical conductivity of the soil, which can cause vine damage and, in some cases, plant death [7]. The grapevine response to salinity involved two mechanisms: (a) a reduction in transpiration and growth, which began as soon as salinity was experienced; and (b) vine mortality, which was correlated with salinity level, a sharp increase in sodium and chlorine content of leaves. At lower salinities, the onset of mortality occurred later and death rates increased as the duration of exposure to salinity increased [20]. Sodium can harm vines by creating osmotic stress in the short term or by causing direct toxicity in the long term. As osmotic potential decreases in saline soils, turgor pressure decreases, causing plants to dehydrate and slow photosynthesis, resulting in growth retardation, biomass loss, and eventually death ([20] in [7]).

This is in line with our results and the outcomes of other researchers [20–22]. Based on our findings, the higher saline concentration of irrigation water from WWTPs is directly related to the plots' soils having higher electrical conductivity and, thus, a higher risk of salinization. However, thus far, no obvious crop damage has been seen.

We can see from Figure 1 that the evolution of chlorophyll concentration has been unaffected by the type of water used for irrigation. As a result, if we can find a chlorophyll–nutrient ratio in the leaf, we will have a very useful tool for quickly determining the nutritional status of the crop—especially if the chlorophyll measurement can be performed in the field with a manual instrument, saving time waiting for laboratory analysis.

In our research, we aimed to link the laboratory analysis of leaf nutrients with the direct field measurement of chlorophyll in SPAD units. Knowing this relationship is important for planning effective and more agile fertilization programs for vineyards, particularly in the case of nitrogen fertilization where the destructive sampling of leaf lamina or petiole sap for elemental tissue analysis is the usual method used in vineyards to determine the nitrogen status of the vines [23]. Nitrogen is an essential nutrient in grapevines, where it is responsible for important metabolic functions, adequate shoot and bunch formation, optimal grape juice fermentation, and the formation of aromatic wine compounds [24]. Measuring vine nutrient status during the growing season can help inform N management strategies and mitigate the negative effects of insufficient or excessive N fertilization, such as poor yields, undesirable grape, must, and wine composition, and negative environmental effects such as contamination of ground and surface waters. A simple and real-time method for estimating vine N status in the field would be beneficial to the viticulture industry [15].

Like nitrogen, phosphorus is essential for the process of photosynthesis [14], as well as K^+ is also easily redistributed from mature to younger organs, so its deficiency symptoms first appear in older leaves.

In this sense, when planning fertilization, it is possible to directly determine the crop's P, K, Ca and N contents by measuring the amount of chlorophyll present in the field. However, it should be noted that the outcome of these measurements is an indirect calculation of the nutritional needs of this crop, and in doubtful situations, the nutrient analysis in the laboratory is advisable.

Micronutrient deficiencies are corrected in viticulture by adding trace elements necessary for plant growth, such as Cu, Zn, Fe, Mn and B [25]. However, if irrigation water contains these micronutrients, as in the case of reclaimed water, it is necessary to know this in order to properly plan crop fertilization.

In addition, the potential phytotoxicity of several nutrients is an important factor to consider. High quantities of trace elements accumulate in plant tissues, which leads to micronutrient toxicity. Regarding the nutrients where we found differences in the foliar analysis (Mg, Mn, Cu and Zn), it is documented [14] that copper and zinc have critical toxicity levels of 20 and 200 g g^{-1} dry weight, respectively, and manganese's critical toxicity level can range from 200 g g^{-1} dry weight to 5300 g g^{-1} depending on the crop. According to the results of our analysis in Figure 3 and Table 3, these toxicity values were not reached by any of the treatments used in our study.

It has been thoroughly documented [26] that because an overabundance of one nutrient may result in a deficit of another component, the symptoms of toxicity are difficult to anticipate. For instance, an excess of manganese also induces deficiency of iron, magnesium and calcium. Manganese competes with iron and magnesium for their uptake [14]. It also competes with magnesium for binding sites in some enzymes.

Magnesium is a mobile element that is an essential component of chlorophyll. It acts as an enzyme cofactor in the ATP production process. It is also required for ribosome subunit binding. It also activates ribulose bisphosphate carboxylase (Rubisco) and phosphoenolpyruvate carboxylase (PEP carboxylase), two important enzymes involved in photosynthesis's dark reaction [27]. Additionally, it is necessary for the action of several respiration and nucleic acid biosynthesis enzymes.

A lack of magnesium causes premature leaf abscission. It enters the cell as a divalent cation (Mg^{2+}) and travels through the xylem vessels. It is never limited in soil, but plants growing in acidic and sandy soils do not have access to it due to soil pH [28].

In our study, the concentration of magnesium in vine leaves was always higher in those irrigated with reclaimed water. This is a positive aspect since, as we have said, this element is of vital importance in the main physiological phenomena of the grapevine as it is part of the chlorophyll molecule. From this perspective, reusing reclaimed water for irrigation would preserve photosynthetic efficiency.

Furthermore, due to the study area's alkaline pH and loamy soil texture, there is no risk of magnesium insufficiency, and this risk is even lower in areas that use treated wastewater for irrigation.

Manganese toxicity, which causes the "sudden crash," is more likely to occur in acidic, moist soils. Manganese function in cell metabolism acts as a growth catalyst; an oxygen evolving constituent and at the plant level accelerates seed germination and maturity; and increases the availability of phosphate and calcium complexes [27].

Manganese is absorbed primarily as Mn^{2+}. Manganese ions move very quickly from roots to shoots, making them less toxic to roots when compared to other metals in the soil. Mn^{2+} is required for chloroplast development as well as the activation of many photosynthesis, respiration and nitrogen metabolism enzymes. It donates electrons to chlorophyll b and participates in the decarboxylation reaction during respiration.

Mn^{2+} deficiency causes interveinal chlorosis and grey spots on leaves, and the absence of manganese ions also causes thylakoid membrane disorganization.

Manganese may be able to compete with magnesium. However, vines irrigated with treated wastewater had leaves with higher concentrations of these two elements than the control, suggesting that this competition has been at least partially eliminated.

This is another reason for recommending the use of treated wastewater as a water resource since it represents an increase in this essential micronutrient for plants.

Copper activates photosynthesis and respiration enzymes, is a component of cytochrome oxidase and polyphenol oxidase, and is found in the ethylene signal receptor. It is well known that the excessive application of fungicides containing copper and the subsequent soil contamination causes copper toxicity in crops such as vineyards [25].

According to Figure 1, there is a significant increase in copper concentration in the vineyard irrigated with reclaimed water in the August–September 2017 period compared to the control and even compared to the same dates the following year. We cannot be certain that this increase is due to irrigation because it coincides with the addition of copper-containing fungicides to the crop this year. It should not be forgotten that the trial plots belonged to farmers who did their field work in the traditional way, including the usual fungicide treatment to avoid crop damage. To rule out this variable, we would need to repeat this part of the trial without the fungicide application.

Zinc is involved in respiration and nitrogen metabolism. In addition, it leads to chlorophyll formation. The same as copper, Zn provides resistance against plant pathogens. On the other hand, tryptophan is a precursor of indole acetic acid (IAA) and zinc has a role in its synthesis [27]. Thus, zinc has indirect role in IAA (growth hormone auxin) synthesis. The auxin level in zinc-deficient plants is very low. Zinc deficiency results in malformed or stunted leaves, caused by oxidative degradation of the growth hormone [26]. Moreover, it is also associated with important enzymes and plays an essential role in maintaining the structure and function of DNA transcription factors.

On the other side, zinc toxicity is increased by acidic soils. Leaf deformation, necrotic speckling and interveinal chlorosis are the most typical signs of zinc toxicity. Additionally, it can stop calcium from entering the shoot apex [28].

During the first growing season studied, the zinc concentration in leaves decreased in both irrigation treatments (Figure 3). When comparing this curve to that of the following year's growing season, it remains stable in this case. What is noteworthy is that the amount of zinc in leaves of vines irrigated with treated wastewater was always higher than that determined in the leaves of control plots. Once more, we can state that using reclaimed wastewater to irrigate crops will increase their nutritional value in terms of this nutrient (Zn).

Therefore, there were no nutritional differences between the control vines and the reclaimed wastewater-irrigated vines. In any event, it was found that the content of some nutrients (Mg, Mn and Zn) had clearly improved. In this sense, the treated wastewater provided a significant fertilizing contribution in terms of nutrients useful for plant growth.

5. Conclusions

In locations with limited water supply, new water resources must be found. Unconventional water resources are increasingly needed to sustain appropriate water supplies in areas that are increasingly vulnerable to drought because climate change is putting pressure on rainfall patterns.

Long-term TWW irrigation studies are scarce. Our study aims to contribute to the understanding of the possible outcomes of the use of this water on crops such as vines.

The continued use of treated wastewater in vineyards did not produce nutritional imbalances in macronutrients, as no differences were observed when compared to control vineyards irrigated with conventional well water.

An increase in Mg concentration was even observed in leaves compared to the control. It also improves the concentration of Mn, Cu and Zn. They are important nutrients for the plant's healthy physiological growth, and the higher concentration found in vine leaves when compared to controls means that the crop's nutritional state has improved.

With the precautions regarding the potential damage caused by the increase in electrical conductivity in the soil caused by continuous irrigation with reclaimed water, we could consider irrigation with treated wastewater as another nutritional supplement within the fertilization program.

Soil, irrigation water and tissue analysis should be used to maintain optimum nutrient availability. In any case, it is critical to not overlook the risk of its continuous application in terms of increased soil salinity.

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References

1. Transforming Our World: The 2030 Agenda for Sustainable Development | Department of Economic and Social Affairs. Available online: https://sdgs.un.org/2030agenda (accessed on 19 December 2022).
2. Singh, A. A review of wastewater irrigation: Environmental implications. *Resour. Conserv. Recycl.* **2021**, *168*, 105454. [CrossRef]
3. Ballesteros-Olza, M.; Blanco-Gutiérrez, I.; Esteve, P.; Gómez-Ramos, A.; Bolinches, A. Using reclaimed water to cope with water scarcity: An alternative for agricultural irrigation in Spain. *Environ. Res. Lett.* **2022**, *17*, 125002. [CrossRef]
4. Mannina, G.; Gulhan, H.; Ni, B.J. Water reuse from wastewater treatment: The transition towards circular economy in the water sector. *Bioresour. Technol.* **2022**, *363*, 127951. [CrossRef]

5. Kehrein, P.; Jafari, M.; Slagt, M.; Cornelissen, E.; Osseweijer, P.; Posada, J.; van Loosdrecht, M. A techno-economic analysis of membrane-based advanced treatment processes for the reuse of municipal wastewater. *J. Water Reuse Desalination* **2021**, *11*, 705–725. [CrossRef]
6. Penny, J.S.; Ordens, C.M.; Barnett, S.; Djordjević, S.; Chen, A.S. Vineyards, Vegetables or Business-as-Usual? Stakeholder-Informed Land Use Change Modelling to Predict the Future of a Groundwater-Dependent Prime-Wine Region Under Climate Change. Available online: https://papers.ssrn.com/abstract=4345452 (accessed on 15 February 2023).
7. Simhayov, R.; Ohana-Levi, N.; Shenker, M.; Netzer, Y. Effect of long-term treated wastewater irrigation on soil sodium levels and table grapevines' health. *Agric. Water Manag.* **2023**, *275*, 108002. [CrossRef]
8. Mendoza-Espinosa, L.G.; Burgess, J.E.; Daesslé, L.; Villada-Canela, M. Reclaimed water for the irrigation of vineyards: Mexico and South Africa as case studies. *Sustain. Cities Soc.* **2019**, *51*, 101769. [CrossRef]
9. Mpanga, I.K.; Sserunkuma, H.; Tronstad, R.; Pierce, M.; Brown, J.K. Soil Health Assessment of Three Semi-Arid Soil Textures in an Arizona Vineyard Irrigated with Reclaimed Municipal Water. *Water* **2022**, *14*, 2922. [CrossRef]
10. Sala, F. Model of Vines Production Variation in Relation to Physiological Indices. *Sci. Pap. Manag. Econ. Eng. Agric. Rural. Dev.* **2020**, *20*, 507–516.
11. Mehmeti, A.; Canaj, K. Environmental Assessment of Wastewater Treatment and Reuse for Irrigation: A Mini-Review of LCA Studies. *Resources* **2022**, *11*, 94. [CrossRef]
12. Howell, C.L.; KHoogendijk Myburgh, P.A.; Lategan, E.L. An Assessment of Treated Municipal Wastewater Used for Irrigation of Grapevines with Respect to Water Quality and Nutrient Load. *South Afr. J. Enol. Vitic.* **2022**, *43*, 168–179.
13. Havlin, J.L.; Austin, R.; Hardy, D.; Howard, A.; Heitman, J.L. Nutrient Management Effects on Wine Grape Tissue Nutrient Content. *Plants* **2022**, *11*, 158. [CrossRef]
14. Bhatla, S.C.; ALal, M. *Plant Physiology, Development and Metabolism*; Springer: Singapore, 2018; Available online: http://link.springer.com/10.1007/978-981-13-2023-1 (accessed on 16 December 2022).
15. Walker, H.V.; Jones, J.E.; Swarts, N.D.; Rodemann, T.; Kerslake, F.; Dambergs, R.G. Predicting grapevine canopy nitrogen status using proximal sensors and near-infrared reflectance spectroscopy. *J. Plant Nutr. Soil Sci.* **2021**, *184*, 204–304. [CrossRef]
16. Karimi, R.; Salimi, F. Iron-chlorosis tolerance screening of 12 commercial grapevine (Vitis vinifera L.) cultivars based on phytochemical indices. *Sci. Hortic.* **2021**, *283*, 110111. [CrossRef]
17. Cunha, A.R.; da Katz, I.; de Sousa, A.P.; Martinez Uribe, R.A. Indice SPAD en el crecimiento y desarrollo de plantas de lisianthus en función de diferentes dosis de nitrógeno en ambiente protegido. *Idesia* **2015**, *33*, 97–105. [CrossRef]
18. SPAD-502_Manual. Available online: https://www.konicaminolta.com.cn/instruments/download/manual/pdf/SPAD-502_Manual.pdf (accessed on 14 February 2023).
19. Kalboussi, N.; Biard, Y.; Pradeleix, L.; Rapaport, A.; Sinfort, C.; Ait-mouheb, N. Life cycle assessment as decision support tool for water reuse in agriculture irrigation. *Sci. Total Environ.* **2022**, *836*, 155486. [CrossRef]
20. Shani, U.; Ben-Gal, A. Long-term Response of Grapevines to Salinity: Osmotic Effects and Ion Toxicity. *Am. J. Enol. Vitic.* **2005**, *56*, 148–154. [CrossRef]
21. Cakmakci, T.; Sahin, U. Productivity and heavy metal pollution management in a silage maize field with reduced recycled wastewater applications with different irrigation methods. *J. Environ. Manage.* **2021**, *291*, 112602. [CrossRef]
22. Zhao, Y.; Zhuang, X.; Ahmad, S.; Sung, S.; Ni, S.Q. Biotreatment of high-salinity wastewater: Current methods and future directions. *World J. Microbiol. Biotechnol.* **2020**, *36*, 37. [CrossRef]
23. Basyouni, R.; Dunn, B.; Goad, C. Use of Nondestructive Sensors to Assess Nitrogen Status in Potted Dianthus (*Dianthus chinensis* L.) Production. *Can. J. Plant Sci.* **2016**, *97*, 44–52. [CrossRef]
24. Bell, S.J.; Henschke, P.A. Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.* **2005**, *11*, 242–295. [CrossRef]
25. Jiménez-Ballesta, R.; Bravo, S.; Amorós, J.A.; Pérez-De-Los-Reyes, C.; García-Pradas, J.; Sanchez, M.; García-Navarro, F.J. Soil and Leaf Mineral Element Contents in Mediterranean Vineyards: Bioaccumulation and Potential Soil Pollution. *Water Air Soil Pollut.* **2022**, *233*, 20. [CrossRef]
26. Coll, J.B.; Rodrigo, G.N.; Tamés, R.S. *Fisiología Vegetal*; Ediciones Pirámide: Madrid, Spain, 2005; 568p.
27. Azcón-Bieto, J.; Talón, M. *Fundamentos de Fisiología Vegetal*; McGraw-Hill Interamericana: Madrid, Spain, 2008; 651p.
28. Taiz, L.; Zeiger, E. *Fisiología Vegetal*; Universitat Jaume I: Castellón de la Plana, Spain, 2006; 646p.