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Integrative Effects of Treated Wastewater and Synthetic Fertilizers on Productivity, Energy Characteristics, and Elements Uptake of Potential Energy Crops in an Arid Agro-Ecosystem

Nasser Al-Suhaibani [1], Mahmoud F. Seleiman [1,2,*], Salah El-Hendawy [1,3], Kamel Abdella [1], Majed Alotaibi [1] and Ali Alderfasi [1]

[1] Plant Production Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia; nsuhaib@KSU.EDU.SA (N.A.-S.); mosalah@ksu.edu.sa (S.E.-H.); kkamel58@yahoo.com (K.A.); malotaibia@KSU.EDU.SA (M.A.); aderfasi@gmail.com (A.A.)
[2] Department of Crop Sciences, Faculty of Agriculture, Menoufia University, Shibin El-kom 32514, Egypt
[3] Agronomy Department, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt
* Correspondence: mseleiman@ksu.edu.sa or mahmoud.seleiman@agr.menofia.edu.eg; Tel.: +966-553153351

Abstract: Using wastewater in agriculture is a desirable alternative source of irrigation and is gaining attraction worldwide. Therefore, this study was designed to assess the effect of treated municipal wastewater (TWW) and groundwater (GW), along with half and full doses of the recommended NPK dose on the plant growth, total biomass, gross energy, and macro- and trace element content and uptake of safflower (*Carthamus tinctorius* L.), canola (*Brassica napus* L.), and triticale (X *Triticosecale Wittmack*) grown in old and virgin soil as potential bioenergy crops. The results showed that crops planted in old or virgin soil irrigated with TWW had higher values of plant height, leaf area per plant, total chlorophyll content, total biomass, and gross and net energy contents compared to those irrigated with GW grown in virgin soil. Similarly, crops grown in old soil irrigated with TWW showed higher concentrations in dry matter and uptake for both macronutrients (N, P, and K) and trace elements (B, Zn, Mn, Cu, Cd, Pb, and Ni) compared to those planted in virgin soil and irrigated with GW. Furthermore, the application of the recommended half dose of NPK in old and virgin soil irrigated with TWW showed occasionally comparable results to that of a full recommended dose of NPK for most of the measured parameters. Importantly, the recommended half dose applied to old soil irrigated with TWW resulted in a significant improvement in all measured parameters compared to virgin soil irrigated with GW, along with a full recommended dose of NPK. Briefly, TWW can be used to irrigate crops grown for bioenergy purposes, since it did not pose any harmful effect for energy crops. In addition, it provides additional nutrients to soil and thus decreases the required rate of synthetic fertilizer by up to 50% without any significant decreases in the final production of crops.

Keywords: treated wastewater; chemical fertilizer; potential energy crops; productivity; gross energy; trace elements

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1. Introduction

Agriculture represents the main consumer of freshwater sources globally and consumes approximately 70% of freshwater withdrawn from rivers, lakes, and aquifers. However, global climate change is disrupting water cycle patterns and leading to extreme water scarcity in different parts of the world [1–4]. Thus, a search for alternative irrigation sources is believed to be essential to ensure food, feed, and fuel security and to preserve natural water sources [5–8]. Hence, using treated wastewater (TWW) in the agriculture sector is becoming a desirable alternative source of irrigation [6,8,9], especially in countries confronted with water shortages [5,10,11]. The use of TWW in agriculture benefits the environment, human health, the economy, and it can reduce the pressure on freshwater sources used in agriculture [8,10,12,13]. Additionally, TWW is a potential source of macro-

(N, P, and K) and trace-elements (Ca, Mg, B, Mg, Fe, Mn, and Zn) [8,14–16] and therefore makes it possible to reduce the use of synthetic fertilizers [8,16]. However, TWW can still contains some trace elements such as Zn, Ni, Cu, Pb, Cd, and Cr.

A recent study by Chojnacka et al. [17] showed that the reuse of treated municipal wastewater in the agricultural sector or for other purposes could cover 100% of both phosphorus and potassium requirements for crops due to the nutrient contents. The use of TWW can decrease environmental pollution, particularly the indirect return of P to water bodies, which causes eutrophication conditions in water bodies [10,16]. The use of TWW can improve the stabilization of soil aggregates (sand, silt, and clay), decrease the compaction of soil, and increase the water holding capacity (WHC) of different types of soils [6,18] through improving soil organic matter (OM) [19]. However, the stability of soil aggregates and WHC depend on the percentage and composition of OM in TWW as well as the soil texture. For example, the application of TWW can enhance the aggregate stability of sandy–clay soil while also decreasing the aggregate stability of clay-textured soil [20]. Depending on the amount of OM contributed, many studies have shown that organic soil carbon and macro- and trace-elements increase in soil irrigated with TWW [10].

In recent decades, the world has recorded an uncontrolled and unprecedented use of fossil fuels which has significantly increased greenhouse gas emissions, global climate change, and health-related hazards. Thus, alternative energy sources are being investigated [21–25]. The alternative renewable sources of fossil energy such as wind, solar, hydropower, geothermal, and biomass are considered vital for reducing the dependence on fossil fuels and the environmental concern, as well as for coping with global climate change [22,23]. The use of bioenergy crops for energy production is one of such alternative renewable sources that can be a potential option to replace the existing fossil fuels with long-term positive future outcomes [8,22,23,25]. Bioenergy from agricultural biomass can be generated from a wide variety of biomass sustainable resources such as food and non-food crops and agricultural residues [8,22,23]. Generally, bioenergy crops are fast-growing crops and produce a higher biological yield (i.e., yield and straw yields). Energy crops have an energy potential with less CO_2 emissions, and they can be grown in marginally or low-fertile soils [26]. Energy is directly generated from bioenergy crops by combustion or gasification or by being converted into liquid fuels such as ethanol, biodiesel, and biogas [8,22,23,25]. Using bioenergy crops as a source of energy can promote renewable energy production and can replace the current fossil fuel-based energy generation. Thus, the concept of bioenergy crops is gaining significant attention in the scientific and research communities for its renewability and environmentally beneficial potential [25].

With regard to climate change mitigation strategies in bioenergy cropping systems, triticale (X *Triticosecale* Wittmack), safflower (*Carthamus tinctorius* L.), and canola (*Brassica napus* L.) can play important roles as potential bioenergy crops, since they can grow on marginal lands with low inputs in terms of irrigation and fertilizers. Triticale has a high adaptability and tolerance to abiotic and biotic stresses [27]. It has a well-developed extensive root system, which can allow it to grow well in low-fertile, marginally fertile, and sub-standard soils as well as in dry areas [28,29]. Triticale has lower production costs, much less susceptibility to biotic stresses, and can produce high grain and biomass yields even in marginal environments compared to other crops [28]. Triticale is mainly cultivated for its grain as a fodder crop. However, recently it has been grown for bioenergy production [28]. Triticale biomass material used for bioethanol production has a high ratio of energy efficiency in traditional agricultural systems at conventional tillage and N fertilization requirements of 40–80 kg per hectare [30]. Safflower is an oilseed crop and its seeds can be used for flavoring and coloring foods [31]. Recently, safflower has started being used as a potential source for bioenergy production. It is a suitable crop for bioenergy production due to the high tolerance for biotic and abiotic stresses, as well as its adaptability to grow in marginal lands [32]. It also requires low inputs in terms of irrigation and fertilizer [33]. Oğuz et al. [34] indicated that due to sustainability and fuel properties, safflowers can become an important and economic feedstock for the biodiesel fuel industry.

Safflower seeds have higher (40%) oil content compared to the other feed stocks used for the production of biodiesel. Therefore, safflower could be a suitable option for raw material for bioenergy [35]. Likewise, canola is considered a suitable crop for biodiesel feedstock, since its seeds contain a high oil percentage [23,36]. Rapeseed is grown worldwide due to its economic value, as well as its ability to grow under a wide range of climate conditions and in different types of soils.

Synthetic fertilizers are quick sources of plant nutrients. However, the proper use of synthetic fertilizers is essential to maximize plant growth and yield. Farmers use synthetic fertilizers at high rates to get high yields. However, high application of synthetic fertilizers can contaminate water bodies and environment [37]. The application of TWW in agriculture may not only fulfill the water needs of plants, but can also be considered as a cheap source of several macro- and trace elements such as N, P, K, Zn, Cu, and Mn, which lead to savings in the external supply of synthetic fertilizers [38,39]. However, in general, the concentrations of these nutrients in TWW depend upon the quality of the wastewater, the water supply, and the type and degree of wastewater treatment. TWW can provide plants with essential nutrients and organic matter, which enhances plant growth by improving the physio-chemical properties of the soil [38]. Generally, TWW contains up to 40 mg N L^{-1} and up to 20 mg P L^{-1}. This can add about 200 and 100 kg N and P ha^{-1}, respectively [40]. The application of TWW with a dose of chemical fertilizer 33% less than recommended improves the yields of celery (*Apium graveolens*) and lettuce (*Lactuca sativa* L.) on par with 100% of the recommended amount of fertilizer [41]. Montemurro et al. [39] reported that irrigation with TWW can make up for a 54% reduction of N fertilizer in fennel (*Foeniculum vulgare*) and lettuce. This shows that reductions in synthetic fertilizer could be possible when TWW is used as the main source of irrigation.

Based on the above background, it was hypothesized that the use of TWW could reduce the application of synthetic fertilizers, particularly N, P, and K fertilizers. Therefore, the primary objectives of this study were to (1) evaluate the safety of TWW as a source of irrigation for three potential bioenergy crops fertilized with half and full doses of the total recommended NPK in order to reduce the use of synthetic fertilizer for the sake of environmental safety; and (2) covering part of the increasing demand for freshwater by using TWW for irrigation of energy crops. To achieve the abovementioned objectives, the impact of long- and short-term irrigation with TWW on growth, biomass yield, energy production, and concentration and uptake of macro- and trace elements of different field crops intended for bioenergy production were investigated and compared to the impacts of groundwater use.

2. Materials and Methods

2.1. Locations of the Study

Different field experiments were conducted during the winter seasons of 2019/2020 and 2020/2021 to investigate the impact of irrigation with TWW and GW on the growth and energy traits, biomass yield, and macro- and trace-element analysis of three field crops (safflower, canola, and triticale) fertilized with half and full doses of the recommended NPK in two different soil types (old and virgin soils) at the Research Station of the College of Food and Agriculture Sciences of King Saud University, Riyadh, Saudi Arabia (24°25′ N, 46°34′ E, 400 m a.s.l.).

The first soil was an old field soil that had been used for cultivation with different field crops during winter and summer seasons and had been irrigated with TWW for the last 15 years. The second soil was named a virgin soil, since it had not been cultivated for the last 35 years. It was divided into two parts, where the first part was irrigated with TWW and the second part was irrigated with GW during our study. The TWW was provided by the Southern Plant of Riyadh Wastewater Treatment Plant, where domestic and municipal wastewater were the main sources. Different processes were used to produce TWW, including an activated sludge, trickling filters, and rotating biological contactors, followed by a single tertiary treatment method in the form of sand filters.

2.2. Soil Analysis

Prior to sowing, nine soil samples were collected from each soil type at three depths (0–20, 20–40, and 40–60 cm) to analyze the chemical and physical traits of the soil (Table 1). Then, the soil samples were air-dried for 24 h and passed through a 2 mm sieve prior the analysis. The soil elemental analysis (i.e., total N, P, K, Fe, Mn, Cu, Cd, Pb, Ni, Co, and Zn) and the soil physical traits (i.e., EC, pH, OM, saturation percentage, and field capacity) were measured using the standard methods described by Cottenie et al. [42] and Burt [43].

Table 1. Physico-chemical properties and elemental analysis of old cultivated and virgin soil before sowing.

Physico-Chemical Soil Traits		
Parameters	**Old Cultivated Soil**	**Virgin Soil**
pH (soil paste 1:5)	7.80	7.91
Saturation percentage (%)	28.02	23.16
EC (dS m^{-1})	3.60	3.75
Organic matter (%)	0.52	0.40
$CaCO_3$ (%)	28.81	30.72
Field capacity (%)	18.24	15.03
Wilting point (%)	7.04	7.96
Sand (%)	56.65	58.90
Silt (%)	28.46	26.52
Clay (%)	14.89	14.58
Texture	Sandy loam	Sandy loam

Macro and trace elements analysis										
Soil	**Elements Depth**	**N**	**K**	**P**	**Fe**	**Mn**	**Cu**	**Cd**	**Co**	**Zn**
		g kg^{-1} DM			**mg kg^{-1} DM**					
Virgin soil	0–20 cm	0.53	1.40	0.03	1.44	41.23	4.23	5.22	0.00	6.97
	20–40 cm	1.40	1.55	0.05	0.93	49.57	2.03	4.63	0.00	4.80
	40–60 cm	0.36	0.50	0.03	1.00	49.17	1.47	4.75	0.15	5.10
Old cultivated soil	0–20 cm	5.87	1.67	0.06	1.49	44.47	4.93	8.96	0.00	11.50
	20–40 cm	3.07	2.43	0.09	1.39	55.83	10.02	9.20	0.64	9.10
	40–60 cm	3.00	0.90	0.07	1.38	56.80	9.03	8.22	1.97	9.70

2.3. Water Analysis

Different water samples (TWW and GW) were collected at different dates during our investigation to perform physico-chemical measurements. The trace elements, including microelements and heavy metals, present during the TWW irrigation are shown in Table 2. The analysis of GW was pH 7.20, EC 3.80 dS m^{-1}, Ca^{2+} 201.00 mg L^{-1}, Mg^{2+} 121.28 mg L^{-1}, Na^{+} 312.21 mg L^{-1}, K^{+} 21.15 mg L^{-1}, and Cl^{-} 382.82 mg L^{-1}.

Table 2. Analysis of treated wastewater (TWW) used to irrigate the crops in the current study.

Parameter	Value	Parameter	Value
Cr (mg L^{-1})	<0.001	pH 7.1	7.1
Cd (mg L^{-1})	<0.0001	EC (dS m^{-1})	1.8
Cu (mg L^{-1})	<0.001	NH_4^+ (mg L^{-1})	3.3
Pb (mg L^{-1})	<0.001	NO_3^- (mg L^{-1})	5.9
Ni (mg L^{-1})	<0.001	PO_4^{3-} (mg L^{-1})	4.2
Zn (mg L^{-1})	0.041	K^+ (mg L^{-1})	15.9
Al (mg L^{-1})	0.034	Ca^{2+} (mg L^{-1})	98.0
B (mg L^{-1})	0.609	Mg^{2+} (mg L^{-1})	30.1
Co (mg L^{-1})	<0.001	Na^+ (mg L^{-1})	282.0
Fe (mg L^{-1})	0.051	Cl^- (mg L^{-1})	261.1
As (mg L^{-1})	<0.001	Mn (mg L^{-1})	0.019

2.4. Sowing Process

The experimental areas were divided into 72 plots with an area of 16 m^2 (4 m × 4 m) for each plot. The seeds of safflower (cv. Kharjia 1), canola (cv. Pactol), and triticale (cv. TR383) were sown in 15 November in the 2019 and 2020 seasons. The distance between hills was 20 and 10 cm, and that between rows was 50 and 40 cm for safflower and canola, respectively. Four seeds were sown in each hill, and later the seedlings were thinned after 15 days of sowing into one plant per hill in both crops. In triticale, the seeding rate was 150 kg ha^{-1}, with a 15 cm distance between each row.

2.5. Experimental Design and Treatments

The experimental design for each potential bioenergy crop (safflower, canola, and triticale) irrigated with TWW in two types of soil or GW in virgin soil and fertilized with half and full doses of the recommended NPK was a split-plot design with a randomized complete block arrangement and four replications (Figure 1). The three irrigation water treatments, namely irrigation with TWW in old soil (L1 + TWW) and in virgin soil (L2 + TWW) and irrigation with GW in virgin soil (L3 + GW), were placed in the main plots, while the two doses of synthetic fertilizer treatments (half and full doses of the recommended NPK) were randomly distributed in the subplots (Figure 1). The full recommended dose of NPK for the investigated crops was 150–60–60 kg N–P_2O_5–K_2O ha^{-1}, as recommended by the Ministry of Environment, Water and Agriculture, Saudi Arabia, for the local region of the investigation. The nitrogen (urea), potassium (potassium sulphate) and phosphorus (single superphosphate) synthetic fertilizers used in the current investigation contained 46% N and 50% and 18% P_2O_5, respectively. Phosphorus fertilizer was applied at 25 days after sowing (DAS), while potassium fertilizer was applied in two equal doses (50% for each) at 25 and 70 DAS. However, nitrogen fertilizer was applied in three doses. The first, second, and third doses were applied at 25, 50, and 70 DAS, amounting to 20%, 40%, and 40% of the total amount of N fertilizer, respectively.

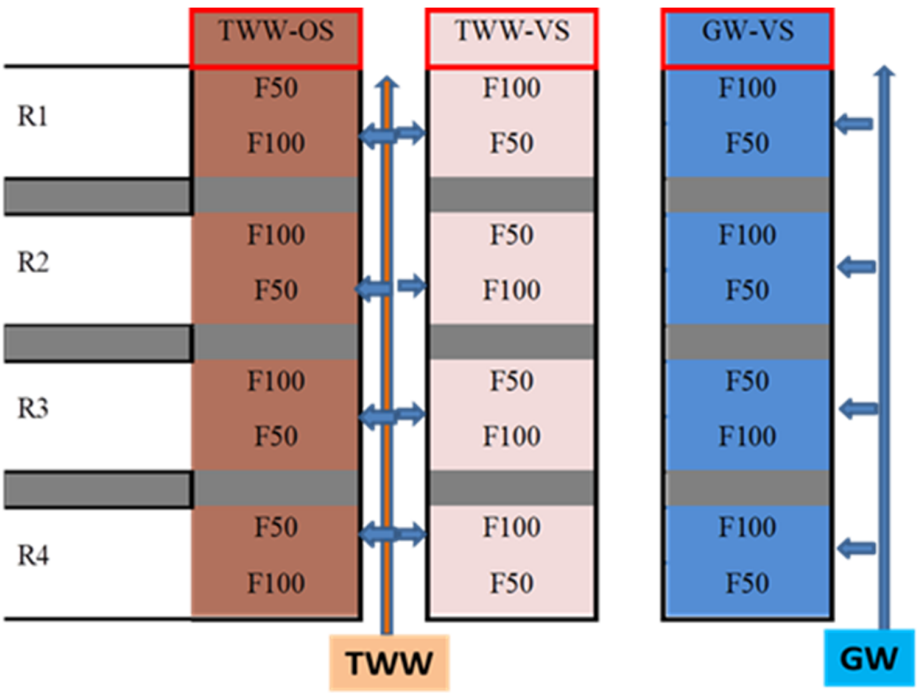

Figure 1. Layout of an experimental design that applied for each crop in each season and includes three irrigation treatments (irrigation with treated wastewater in old cultivated soil (TWW-OS), irrigation with treated wastewater in virgin soil (TWW-VS) and irrigation with groundwater in virgin soil (GW-VS); two fertilizer treatments {half (F50) and full recommended dose of NPK (F100)}. R, replication.

2.6. Weather Conditions

The data of weather conditions, including the amount of precipitation (mm), temperature data (maximum, minimum, and average temperatures (°C)), and relative humidity (RH; %) are shown in Table 3.

Table 3. Monthly agro-climatological data at the experimental location during the growing seasons and the long-term (from 1981 to 2020).

Support / Surface	Precipitation (mm)		Maximum Temperature (°C)		Minimum Temperature (°C)		Average Temperature (°C)		Relative Humidity (%)	
	S1	*S2*	*S1*	*S2*	*S1*	*S2*	*S1*	*S2*	*S1*	*S2*
November	0.31	0.27	35.37	34.82	5.05	5.84	20.21	20.33	41.69	41.21
December	0.02	0.10	27.40	27.46	4.01	4.95	15.71	16.21	45.31	44.36
January	0.10	0.05	29.34	28.65	1.23	2.01	15.29	15.33	40.19	40.68
February	0.00	0.00	33.79	32.43	1.21	2.08	17.50	17.26	28.44	29.26
March	0.00	0.00	35.80	36.19	6.98	7.01	21.39	21.60	25.69	26.02
April	0.98	1.02	39.91	38.63	14.13	15.11	27.02	26.87	31.50	30.24
May	0.01	0.00	42.91	41.77	19.21	19.94	31.06	30.86	17.69	17.05
Long-term 1981–2020										
November	0.23		32.55		8.68		20.62		36.59	
December	0.19		27.84		3.22		15.53		44.90	
January	0.22		27.64		0.99		14.32		43.26	
February	0.26		30.86		2.73		16.80		35.72	
March	0.28		34.86		6.84		20.85		31.72	
April	0.16		39.05		12.24		25.65		26.57	
May	0.06		43.33		19.03		31.18		17.56	

S1, Season 2019/2020; S2, Season 2020/2021.

2.7. Measurements

2.7.1. Growth Traits and Biomass Yield

Safflower, triticale, and canola plant samples were manually collected at flowering stage for measuring growth traits (i.e., plant height, leaf area per plant, and total chlorophyll with SPAD. The total chlorophyll for the topmost fully expanded leaves on the main stem was read by Soil Plant Analysis Development; SPAD (Model: SPAD-502; Minolta Sensing Ltd., Osaka, Japan) between 10:00–12:00 a.m. From each plot, leaves of five plants were used to measure the total chlorophyll, then the average of the five readings were calculated for each replication.

At the flowering stage, five plants were collected from the second row of each plot to record the plant height, which was measured from the soil surface to the top of the plant; cm. Also, the green leaf area plant^{-1} (cm^2) were measured from the five plants using LI-COR (LI-3000C, Portable Leaf Area Meter, LI-COR Inc., Lincoln, NE, USA).

At physiological maturity for each crop, about 6.0 m^2 from the middle of each plot was harvested to record the whole crop biomass (from soil surface to the top of the plant; biological yield). Then, different crop parts were well mixed and crushed and oven-dried for 72 h at 65 °C. Later, the oven-dried crops were weighed to record dry weight of plant biomass, then were ground into a powder with 0.5 mm. Finally, crop samples were stored at room temperature for further analyses such as macro- and trace elements as well as energy traits.

2.7.2. Elemental Analysis

Macro- (N, P, and K) and trace elements (B, Mn, Cu, Zn, Cd, Pb, and Ni) were chemically analyzed in the whole plant biomass of safflower, triticale, and canola following the details described by Seleiman et al. [21]. A powder of plant sample (300 mg) was placed in PTFE Teflon tubes (CEM, Matthews, NC, USA). Then, nitric acid (67%; 6 mL) and hydrogen peroxide (30%; 1 mL) were directly added into the crop sample for the digestion in the microwave. After the digestion, the samples were filtered using Whatman paper, and then were diluted using a distilled water up to 50 mL. Then, the diluted samples were used to analyze the above-mentioned elements using iCAP 6200 (Thermo Fisher Scientific, Cambridge, UK). On the other hand, the total N was analyzed from 200 mg DM of whole plant biomass following the Dumas combustion method as explained by Seleiman et al. [21].

The uptake of macro- and trace elements per ha were calculated based on the concentration of each element in the plants multiplied by the productivity of biomass yield per ha as follows:

$$\textit{Element uptake}\left(\text{kg ha}^{-1}\text{ DM}\right) = \text{Element concentration in biomass}\left(\text{g kg}^{-1}\right) \times \text{Biomass yield}\left(\text{kg ha}^{-1}\right) \times 0.001$$

2.7.3. Energy Analysis

The energy content in ground biomass of safflower, triticale, and canola (0.5 g) was analyzed by the adiabatic bomb calorimeter (Parr 1241EA, Parr Instrument Co., Moline, IL, USA) following the described procedure of Seleiman et al. [22]. Biomass samples were compacted by the pellet press (Parr Instrument Co., Moline, IL, USA) to form pellets. Then, pellets were used to measure energy content (MJ kg^{-1}) using the complete combustion with a limit excess for O_2 at 3.04 MPa in a sealed steel bomb. The standards were the pellets of the benzoic acid (1.0 g; Parr Instrument Co., Moline, IL, USA). On the other hand, the gross energy (GJ ha^{-1}) for safflower, triticale, and canola was calculated as follows:

$$\text{Gross energy}\left(\text{GJ ha}^{-1}\right) = \frac{\text{Energy content}\left(\text{MJ Kg}^{-1}\right) \times \text{Biomass yield}\left(\text{Kg ha}^{-1}\right)}{1000}$$

2.8. Statistical Analysis

Data obtained from the main effects of three different irrigation treatments and two doses of fertilizer treatments and their interaction on growth, biomass productivity, gross and net energy contents, and concentrations and uptake of macro- and trace elements of each potential bioenergy crop were statistically analyzed through a multivariate ANOVA using PASW statistics 21.0 (IBM Inc., Chicago, IL, USA). A Tukey's test was used to show the significant differences between the treatments' means at a 0.05 probability. The standard error of the mean (S.E.M.) is also used above columns as error bars to show the significance between the treatments. The combined analysis was carried out for the data of two years to test the homogeneity of error variance assuming season is a random effect, and irrigation sources and synthetic fertilizers are both fixed factors. The output of the analysis indicated a homogenous variance across two years for the investigated traits, and therefore the data obtained from the two years were combined and analyzed.

3. Results

The outcomes of the study (Table 4) revealed that the soil types, along with irrigation sources, greatly affected safflower height. Safflower sown in old cultivated soil and irrigated with TWW (L1 + TWW) showed a higher plant height—a 21.42% increase in plant height compared to safflower grown in virgin soil and irrigated with GW (L3 + GW). Similarly, the plant height of safflower sown in virgin soil and also irrigated with TWW (L2 + TWW) was less than that of L1 + TWW but greater than that of L3 + GW. The individual effect of NPK doses had no significant effect on the plant height of safflower (F50 = 154.15 cm and F100 = 158.40 cm). Similarly, canola and triticale of L1 + TWW

showed an increase in height by 26.17% and 18.26%, respectively, when compared to those of L3 + GW (Table 4). Moreover, the sowing of canola and triticale on L2 + TWW resulted in an increase in plant height when compared to L3 + GW, but less than that of L1 + TWW (Table 4). The effect of NPK doses on the plant height of canola and triticale was also not significant.

Table 4. Effects of individual factors of water resources and fertilization treatments on growth and total biomass traits of safflower, canola, and triticale.

Parameters / Treatments	Plant Height (cm)	Total Chlorophyll (SPAD Value)	Leaf Area Plant^{-1} (cm^2)	Total Biomass (t ha^{-1} DM)
Safflower				
Water treatments				
L1 + TWW	171.03 a	52.50 a	370.85 a	26.10 a
L2 + TWW	156.94 b	50.13 b	319.03 b	17.23 b
L3 + GW	140.86 c	41.73 c	228.90 c	13.83 c
Significance	**	**	**	**
Fertilizer treatments				
F50	154.15 a	47.17 b	295.87 b	18.52 b
F100	158.40 a	49.07 a	316.65 a	19.59 a
Significance	ns	*	**	*
Canola				
Water treatments				
L1 + TWW	144.50 a	49.79 a	2933.00 a	19.75 a
L2 + TWW	128.31 b	44.94 b	2358.38 b	14.64 b
L3 + GW	114.53 c	38.75 c	1668.38 c	11.88 c
Significance	**	**	**	**
Fertilizer treatments (F)				
F50	127.18 a	41.85 b	2235.00 b	14.85 a
F100	131.05 a	47.14 a	2404.83 a	15.99 a
Significance	ns	**	**	ns
Triticale				
Water treatments				
L1 + TWW	91.90 a	52.05 a	210.75 a	11.39 a
L2 + TWW	86.67 b	53.00 a	195.12 b	7.73 b
L3 + GW	77.71 c	49.30 b	122.37 c	6.17 c
Significance	**	**	**	**
Fertilization (F)				
F50	85.00 a	49.84 b	172.33 b	8.23 a
F100	85.85 a	53.06 a	179.83 a	8.63 a
Significance	ns	**	**	ns

Old soil with treated wastewater (L1 + TWW), virgin soil with treated wastewater (L2 + TWW), virgin soil with groundwater (L3 + GW), half-dose NPK (F50), full-dose NPJ (F100). ns = Probability (p) $\geq$ 0.05; * = $p \leq 0.05$; ** = $p \leq 0.01$. Values followed by the same letter were not significantly differed.

The results showed that the safflower, canola, and triticale with L1 + TWW increased the total chlorophyll (SPAD value) by 25.81%, 28.49%, and 5.58%, respectively, over those of L3 + GW. In terms of the SPAD value, the maximum total chlorophyll in safflower (52.50), canola (49.79), and triticale (52.05) was recorded for treatment L1 + TWW, followed by L2 + TWW, while the minimum total chlorophyll in safflower, canola, and triticale was observed for treatment L3 + GW (Table 4).

The effect of NPK doses enhanced the total chlorophyll (Table 4). The full dose of the recommended NPK significantly increased the total chlorophyll in all of the tested crops compared to the half dose. Similarly, the interactive effect of NPK doses with irrigation treatments enhanced the total chlorophyll in all tested crops. Crops sown with TWW and fertilized with the half (F50) or full dose (F100) of the recommended NPK showed a significantly higher total chlorophyll content by 25.81% for safflower, 28.49% for canola,

and 5.58% for triticale compared to those with L3 + GW and fertilized with either half or full doses of the recommended NPK (Figure 2). Similarly, the sowing of safflower, canola, and triticale on L2 + TWW and received half or full doses of NPK had higher total chlorophyll than that of L3 + GW with the same doses of NPK.

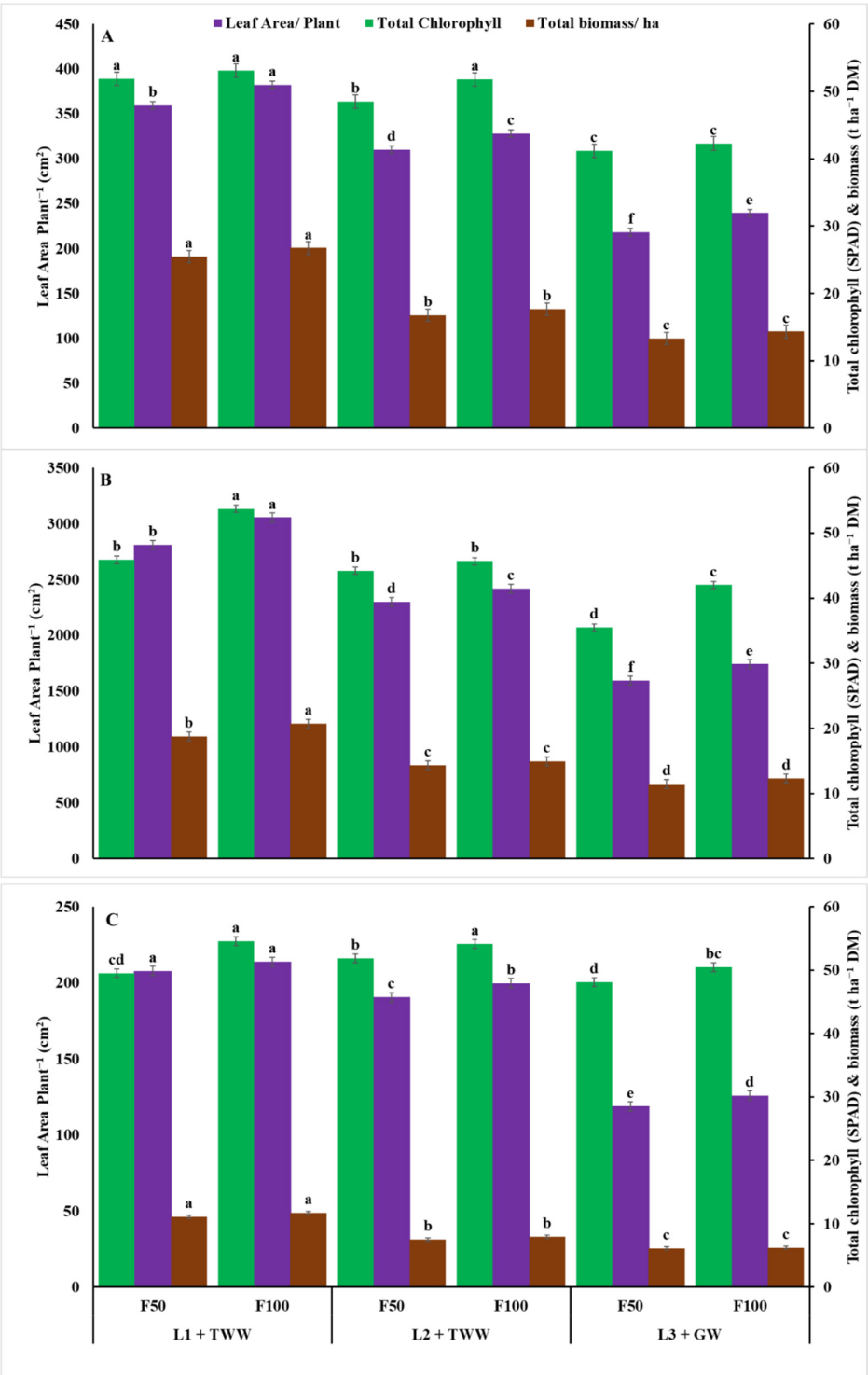

Figure 2. Interaction effects of water resources and fertilization treatments on total chlorophyll, leaf area per plant and total biomass of safflower (**A**), canola (**B**), and triticale (**C**). Old soil with treated wastewater (L1 + TWW), virgin soil with treated wastewater (L2 + TWW), virgin soil with groundwater (L3 + GW), half-dose NPK (F50), full-dose NPK (F100). Values followed by the same letter were not significantly differed for each trait.

The results indicated that the irrigation treatments and NPK fertilizer doses significantly influenced the leaf area per plant of the three tested crops (Table 4). The tested crop plants produced higher leaf area plant^{-1} (safflower +62.01%, canola +75.79%, and triticale +72.22%) with L1 + TWW, followed by L2 + TWW, while the lowest leaf area per plant was observed with L3 + GW. The use of the full dose of NPK significantly enhanced the leaf area per plant in all of the tested crop plants (safflower +7.0%, canola +7.6%, and triticale +4.3%) compared to the half dose of the recommended NPK. However, the interactive effect of NPK doses with irrigation treatments showed that the interaction of L1 + TWW treatment with either the half or full dose of the recommended NPK resulted in a significant increase in leaf area per plant by 62.01% for safflower, 75.80% for canola, and 88.72% for triticale compared to those of L3 + GW treatment and the same doses of NPK (Figure 2).

Likewise, safflower, canola, and triticale with L1 + TWW produced a significantly higher total biomass (safflower +88.7%, canola +66.2%, and triticale +84.6%), followed by L2 + TWW (Table 4), compared to the minimum biomass of all crops grown with L3 + GW treatment. The main effect of NPK doses on biomass yield was also significant for safflower, but non for canola or triticale (Table 4). However, the interaction effect of NPK doses with irrigation treatment on biomass yield for all tested crops was significant (Figure 2). Safflower, canola, and triticale produced a higher biomass by 51.48%, 34.90%, and 47.35% compared to L3 + GW with the half dose of NPK, respectively, when the L1 + TWW treatment was combined with either the half or full dose of the recommended NPK.

The results showed that, in general, irrigated plants with TWW resulted in 3.84–12.36% more energy content for tested crops compared to those irrigated with GW (Table 5). The plants of safflower, canola, and triticale with L1 + TWW had an energy content of 17.24, 16.82, and 17.02 MJ kg^{-1} DM, respectively, when compared to those plants with L3 + GW (safflower 15.75, canola 14.97, and triticale 16.39 MJ kg^{-1} DM). A similar trend was recorded for gross energy (Table 5), where 41.43%–61.73% more gross energy was obtained from L1 + TWW treatment compared to L3 + GW treatment. Although the individual effect of NPK doses was significant, either the half or full dose of the recommended NPK applied to crops with L1 + TWW treatment had statistically equal energy contents, which were significantly higher than those with the L3 + GW treatment with the same quantity of NPK fertilizer (safflower 15.70–15.80 MJ kg^{-1} DM, canola 14.75–15.19 MJ kg^{-1} DM, and triticale 16.31–16.48 MJ kg^{-1} DM). This showed that the half dose of the recommended NPK was sufficient for the three tested crops when planted with L1 + TWW treatment. A similar trend was observed for the gross energy of safflower, canola, and triticale (Figure 3), where 86.76%–106.48% more gross energy was recorded when the tested crops were sown in old soil irrigated with TWW and fertilized with either a 50% or 100% dose of NPK compared to virgin soil irrigated with GW that received the 100% NPK dose.

The concentrations of N, P, and K were significantly increased in safflower (N +77.3%, P +46.4%, and K +138.5%), canola (N +25.4%, P +235.5%, and K +2464%), and triticale (N +27.0%, P +115.8%, and K +78.0%) when these crops were sown in old soil irrigated with TWW, followed by virgin soil irrigated with TWW, compared to these macro-elements in safflower, canola, and triticale planted in virgin soil irrigated GW (Table 5). The individual effect of NPK fertilizer doses on the macronutrient concentration was significant in canola and triticale; however, the K concentration in safflower was not affected by NPK doses. On the contrary, the interactive effect of NPK doses with soil locations and irrigation sources depicted that NPK at either the recommended 50% or 100% dose applied to crops grown in old soil irrigated with TWW resulted in higher concentrations of N, P, and K in all of the tested crops (safflower, canola, and triticale) sown in virgin soil irrigated with GW (Figure 4).

Table 5. Effects of individual factors of water resources and fertilization treatments on macro-elements content and energy traits of safflower, canola, and triticale.

Treatments \ Parameters	Energy Content (MJ kg^{-1} DM)	Gross Energy (GJ ha^{-1})	Nitrogen (g kg^{-1} DM)	Potassium (g kg^{-1} DM)	Phosphorus (g kg^{-1} DM)
Safflower					
Water sources (L&WS)					
L1 + TWW	17.24 a	449.93 a	23.59 a	21.92 a	3.09 a
L2 + TWW	16.62 b	286.45 b	15.67 b	9.90 b	2.72 a
L3 + GW	15.75 c	217.91 c	13.30 c	9.19 b	1.25 b
Significance	**	**	**	**	**
Fertilization (F)					
F50	16.49 a	308.29 b	16.95 b	13.53 a	2.29 b
F100	16.58 a	327.91 a	18.07 a	13.81 a	2.41 a
Significance	ns	**	**	ns	**
Canola					
Water sources (L&WS)					
L1 + TWW	16.82 a	332.28 a	27.30 a	20.30 a	2.59 a
L2 + TWW	16.05 a	234.95 b	25.40 b	11.48 b	1.38 b
L3 + GW	14.97 b	177.92 c	21.77 c	5.86 c	0.88 c
Significance	**	**	**	**	**
Fertilization (F)					
F50	15.84 b	237.68 b	22.61 b	11.32 b	1.35 b
F100	16.06 a	259.08 a	27.04 a	13.76 a	1.88 a
Significance	**	**	**	**	**
Triticale					
Water sources (L&WS)					
L1 + TWW	17.02 a	193.98 a	24.27 a	19.07 a	3.01 a
L2 + TWW	15.50 b	119.94 b	21.26 b	14.29 b	2.96 b
L3 + GW	16.39 ab	101.20 c	19.01 c	10.71 c	1.40 c
Significance	*	**	**	**	**
Fertilization (F)					
F50	16.24 a	134.57 b	20.44 b	13.97 b	2.33 b
F100	16.37 a	142.18 a	22.59 a	15.41 a	2.58 a
Significance	ns	**	**	**	*

Old soil with treated wastewater (L1 + TWW), virgin soil with treated wastewater (L2 + TWW), virgin soil with groundwater (L3 + GW), half-dose NPK (F50), full-dose NPJ (F100). ns = Probability (p) ≥ 0.05; * = $p \leq 0.05$; ** = $p \leq 0.01$. Values followed by the same letter were not significantly differed.

The concentrations of the trace-elements (B, Mn, Cu, and Zn) increased in safflower, canola, and triticale when planted in old soil irrigated with TWW, followed by virgin soil irrigated with TWW, while the lowest concentration of these nutrients was recorded in crops grown in virgin soil irrigated with GW (Table 6). The interactive effect of fertilizer sources with soil locations and irrigation sources revealed that the B, Mn, and Zn contents increased in the dry matter of safflower (B 5.03, Mn 17.28, and Zn 88.60 mg kg^{-1} DM), canola (B 4.07, Mn 31.44, and Zn 69.05 mg kg^{-1} DM), and triticale (B 1.95, Mn 31.62, and Zn 86.57 mg kg^{-1} DM) when these crops were sown in old soil irrigated with TWW and fertilized with recommended 100% dose of NPK compared to virgin soil with the same quantity of NPK but irrigated with GW (Table 6). Even 50% of the recommended dose of NPK and TWW applied to the tested crops grown in old soil resulted in a higher concentration of B (safflower 3.85, canola 4.33, and triticale 1.20 mg kg^{-1} DM), Mn (safflower 14.83, canola 29.24, and triticale 2.155 mg kg^{-1} DM), and Zn (safflower 90.45, canola 58.43, and triticale 61.60 mg kg^{-1} DM) in the dry biomass of crops compared to virgin soil irrigated with GW and fertilized with the recommended 100% dose of NPK (safflower B 0.40, Mn 5.58, and Zn 53.30 mg kg^{-1} DM; canola B 0.71, Mn 17.18, and Zn 52.01 mg kg^{-1} DM; triticale B 0.86, Mn 9.15, and Zn 52.92 mg kg^{-1} DM). A similar trend was recorded for the Cu concentration in

the dry biomass of the tested crops; however, the Cu content was non-significant in triticale when irrigated with either TWW or GW.

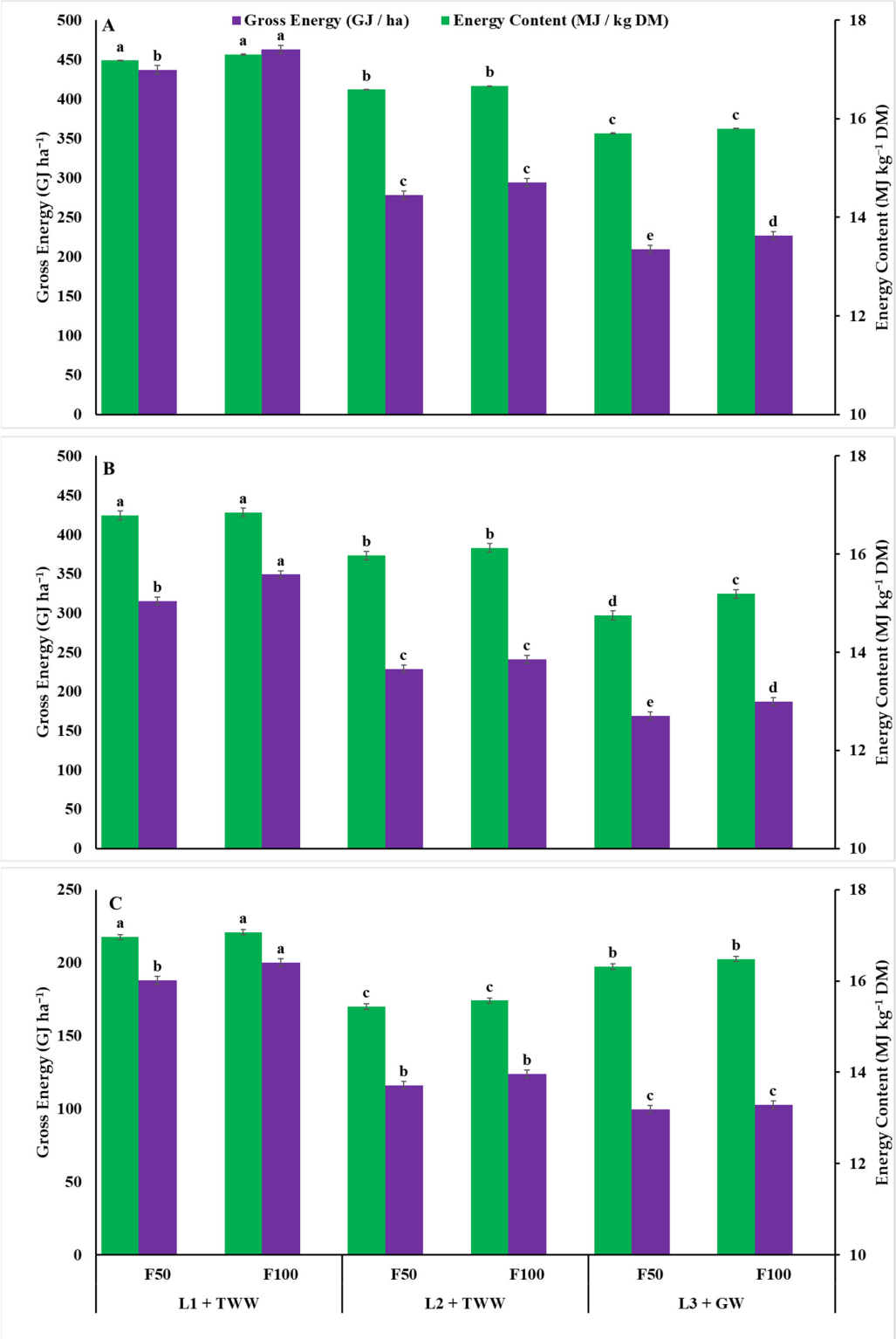

Figure 3. Interaction effects of water resources and fertilization treatments on energy traits of biomass for safflower (**A**), canola (**B**), and triticale (**C**). Old soil with treated wastewater (L1 + TWW), virgin soil with treated wastewater (L2 + TWW), virgin soil with groundwater (L3 + GW), half-dose NPK (F50), full-dose NPK (F100). Values followed by the same letter were not significantly differed for each trait.

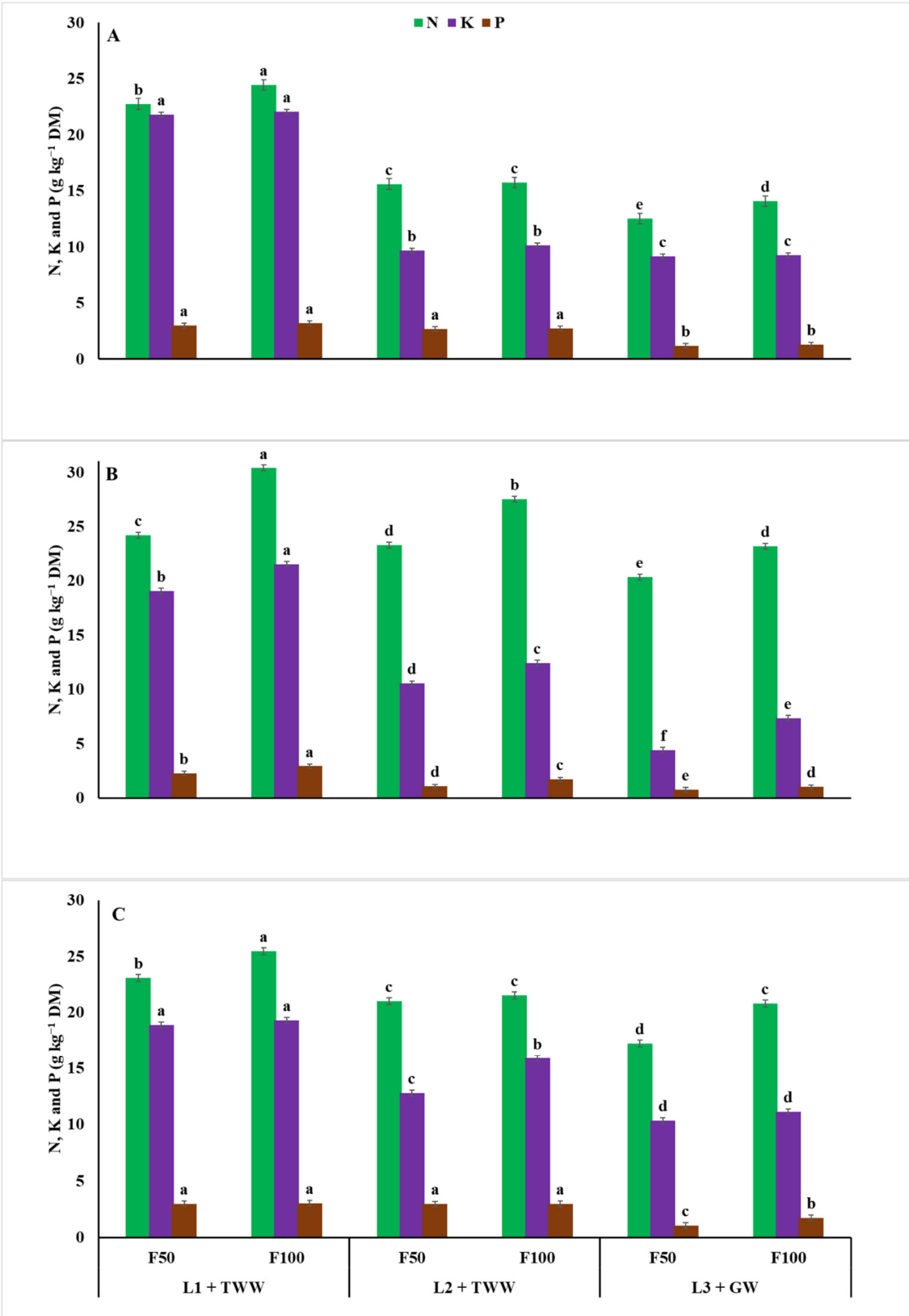

Figure 4. Interaction effects of water resources and fertilization treatments on nitrogen (N), potassium (K) and phosphorus (P) contents in biomass of safflower (**A**), canola (**B**), and triticale (**C**). Old soil with treated wastewater (L1 + TWW), virgin soil with treated wastewater (L2 + TWW), virgin soil with groundwater (L3 + GW), half-dose NPK (F50), full-dose NPK (F100). Values followed by the same letter were not significantly differed for each trait.

Table 6. Interaction effects of water resources and fertilization treatments on trace-elements content of safflower, canola, and triticale.

Treatments	Parameters	B	Mn	Cu	Zn	Cd	Pb	Ni
		(mg kg^{-1} DM)						
				Safflower				
L1 + TWW	F50	3.85 b	14.83 b	13.95 a	90.45 a	1.45 a	9.14 a	48.06 a
	F100	5.03 a	17.28 a	8.22 b	88.60 b	1.45 a	9.82 a	49.84 a
L2 + TWW	F50	0.71 c	7.18 c	4.12 d	66.34 c	1.04 a	7.46 b	44.23 b
	F100	0.86 c	8.75 c	7.55 c	58.77 d	1.10 a	7.37 b	45.73 b
L3 + GW	F50	0.31 d	4.95 d	0.32 e	54.43 e	0.96 a	2.90 c	36.71 c
	F100	0.40 d	5.58 d	0.32 e	53.30 e	0.99 a	2.95 c	38.08 c
Significance		**	**	**	*	ns	**	*
				Canola				
L1 + TWW	F50	4.33 a	29.24 a	2.34 b	58.43 b	1.06 a	9.42 ab	45.78 b
	F100	4.07 a	31.44 b	2.54 a	69.05 a	1.09 a	10.10 a	48.78 a
L2 + TWW	F50	1.00 b	9.58 e	1.25 c	57.08 b	0.98 a	8.78 b	45.32 b
	F100	1.20 b	10.20 e	2.23 b	57.05 b	1.03 a	9.14 ab	45.56 b
L3 + GW	F50	0.32 c	15.30 d	0.86 d	51.48 c	0.35 b	3.53 d	36.19 c
	F100	0.71 c	17.18 c	0.86 d	52.01 c	0.35 b	4.82 c	35.54 c
Significance		**	*	**	**	*	**	*
				Triticale				
L1 + TWW	F50	1.20 b	21.55 b	0.34 a	61.50 b	1.01 a	9.10 ab	45.98 a
	F100	1.95 a	31.62 a	0.35 a	86.57 a	1.05 a	10.73 a	46.54 a
L2 + TWW	F50	1.12 b	14.86 c	0.25 a	59.54 b	0.97 a	8.27 b	43.10 c
	F100	1.17 b	15.43 c	0.23 a	60.52 b	1.01 a	8.21 b	44.92 b
L3 + GW	F50	0.67 c	4.62 e	0.24 a	51.63 c	0.31 b	3.55 c	35.00 d
	F100	0.86 c	9.15 d	0.21 a	52.92 c	0.36 b	4.15 c	35.33 d
Significance		**	**	ns	**	*	**	*

Standard error of means (S.E.M); old soil with treated wastewater (L1 + TWW), virgin soil with treated wastewater (L2 + TWW), virgin soil with groundwater (L3 + GW), half-dose NPK (F50), full-dose NPJ (F100). ns = Probability (p) ≥ 0.05; * = $p \leq 0.05$; ** = $p \leq 0.01$. Values followed by the same letter were not significantly differed.

The results of our study showed that the concentration of heavy metals (Cd, Ni, and Pb) increased, but less so than the permissible limits in the dry biomass of the tested crops when irrigated with TWW compared to GW. The interactive effect of the soil location along with irrigation sources and NPK doses was non-significant for Cd in all of the tested crops, and Ni in safflower and triticale. However, the Pb content significantly increased in safflower (9.14–9.32 mg kg^{-1} DM), canola (9.42–10.10 mg kg^{-1} DM), and triticale (9.10–10.73 mg kg^{-1} DM) when sown in old soil irrigated with TWW and fertilized with 50% and 100% of the recommended dose of NPK rather than virgin soil with the same amount of fertilizer and irrigated with GW (safflower 2.90–2.95, canola 3.53–4.82, and triticale 3.55–4.15 mg kg^{-1} DM).

The results indicated that the uptake of macronutrients (N, P, and K) increased in safflower, canola, and triticale when sown in old soil irrigated with TWW rather than virgin soil irrigated with GW. An individual dose of NPK (50% and 100% NPK dose) had a significant effect on N, P, and K uptake, meaning that the recommended 100% dose of NPK increased the uptake of N, P, and K in plants more than the 50% NPK dose (Table 7). However, when TWW and the recommended 50% or 100% dose of NPK were used in old soil, the uptake of N (safflower 579.10–653.09, canola 454.33–629.35, and triticale 255.71–298.24 kg ha^{-1} DM), P (safflower 75.84–85.33, canola 42.47–60.64, and triticale 32.99–35.60 kg ha^{-1} DM), and K (safflower 554.30–590.11, canola 358.14–446.09, and triticale 208.89–225.77 kg ha^{-1} DM) was significantly higher in the tested crops when compared to those grown in virgin soil irrigated with GW and received the recommended 100% dose of NPK (safflower N 201.63, P 18.64, and K 132.65 kg ha^{-1}; canola N 285.74,

P 12.44, and K 90.43 kg ha^{-1}; triticale N 129.64, P 10.84, and K 69.28 kg ha^{-1}). A similar trend was recorded for the trace-elements (B, Mn, Cu, and Zn), where the uptake of these trace-elements increased when the crops were sown in old soil irrigated with TWW and the recommended 50% or 100% dose of NPK compared to virgin soil fertilized with the recommended 100% dose of NPK and irrigated with GW (Table 7).

Table 7. Interaction effects of water resources and fertilization treatments on macro-, trace-elements, and heavy metals uptake (kg ha^{-1}) of safflower, canola, and triticale.

Treatments	Parameters	N	K	P	B	Mn	Cu	Zn	Cd	Pb	Ni
		(kg ha^{-1} DM)									
Safflower											
L1 + TWW	F50	579.10 b	554.30 b	75.84 b	0.098 b	0.377 b	0.355 a	2.303 a	0.037 a	0.233 b	1.223 b
	F100	653.09 a	590.11 a	85.33 a	0.135 a	0.462 a	0.220 b	2.370 a	0.039 a	0.263 a	1.333 a
L2 + TWW	F50	261.84 c	162.53 d	45.33 c	0.012 c	0.121 d	0.069 d	1.114 b	0.017 b	0.125 c	0.742 d
	F100	278.12 c	179.10 c	48.44 c	0.015 c	0.154 c	0.133 c	1.039 b	0.019 b	0.130 c	0.808 c
L3 + GW	F50	167.08 e	121.84 f	16.00 d	0.004 d	0.066 f	0.004 e	0.726 c	0.013 c	0.039 d	0.489 f
	F100	201.63 d	132.65 e	18.64 d	0.006 d	0.080 e	0.005 e	0.764 c	0.014 c	0.042 d	0.546 e
Significance		*	*	*	**	**	**	*	*	**	*
Canola											
L1 + TWW	F50	454.33 b	358.14 b	42.47 b	0.081 a	0.549 b	0.044 a	1.098 b	0.020 a	0.177 b	0.860 b
	F100	629.35 a	446.09 a	60.47 a	0.084 a	0.652 a	0.053 a	1.430 a	0.023 a	0.209 a	1.009 a
L2 + TWW	F50	333.77 d	150.89 d	15.19 d	0.014 b	0.137 e	0.018 c	0.818 c	0.014 b	0.126 c	0.649 c
	F100	411.14 c	185.68 c	25.42 c	0.018 b	0.153 e	0.033 b	0.853 c	0.015 b	0.137 c	0.681 c
L3 + GW	F50	232.88 f	50.22 f	8.58 f	0.004 c	0.175 d	0.010 c	0.589 e	0.004 c	0.040 d	0.414 d
	F100	285.74 e	90.43 e	12.44 e	0.009 c	0.211 c	0.011 c	0.641 d	0.004 c	0.059 d	0.438 d
Significance		**	**	**	*	**	*	**	**	**	*
Triticale											
L1 + TWW	F50	255.71 b	208.89 b	32.99 a	0.013 b	0.239 b	0.004 a	0.681 b	0.011 a	0.101 b	0.509 a
	F100	298.24 a	225.77 a	35.60 a	0.023 a	0.371 a	0.004 a	1.014 a	0.012 a	0.126 a	0.546 a
L2 + TWW	F50	158.01 d	95.80 d	22.18 b	0.008 c	0.112 c	0.002 b	0.448 d	0.007 b	0.062 c	0.324 b
	F100	170.93 c	125.85 c	23.50 b	0.009 c	0.123 c	0.002 b	0.481 c	0.008 b	0.065 c	0.357 b
L3 + GW	F50	105.29 f	62.99 f	6.42 d	0.004 d	0.028 e	0.001 b	0.315 f	0.002 c	0.022 e	0.214 c
	F100	129.64 e	69.28 e	10.84 c	0.005 d	0.057 d	0.001 b	0.330 e	0.002 c	0.045 d	0.220 c
Significance		**	**	*	**	**	*	**	*	**	*

Old soil with treated wastewater (L1 + TWW), virgin soil with treated wastewater (L2 + TWW), virgin soil with groundwater (L3 + GW), half-dose NPK (F50), full-dose NPJ (F100). ns = Probability (p) ≥ 0.05; * = $p \leq 0.05$; ** = $p \leq 0.01$. Values followed by the same letter were not significantly differed.

Likewise, the uptake of heavy metals (Cd, Pb, and Ni) increased in safflower and canola; however, Pb and Ni uptake in triticale was not affected by the soil location, irrigation sources, or NPK dose. Safflower and canola planted in old soil fertilized with the 50% or 100% NPK dose and irrigated with TWW had higher Cd (safflower 0.037–0.039 and canola 0.020–0.023 kg ha^{-1} DM), Pb (safflower 0.233–0.263 and canola 0.177–0.209 kg ha^{-1}), and Ni (safflower 1.223–1.333 and canola 0.860–1.009 kg ha^{-1}) uptake compared to those grown in virgin soil irrigated with 100% of the NPK dose and irrigated with GW (safflower Cd 0.014, Pb 0.042, and Ni 0.546; canola Cd 0.004, Pb 0.059, and Ni 0.438 kg ha^{-1}). A similar trend was recorded for Pb in triticale (Table 7).

4. Discussion

The application of TWW in old or virgin soil resulted in higher values of plant height, leaf area, total chlorophyll content, and biomass of safflower, canola, and triticale compared to the application of GW in virgin soil (Table 4). Furthermore, the application of the half dose of the recommended NPK fertilizer to these tested crops grown in old or virgin soil irrigated with TWW resulted in a remarkable increase in these traits compared to those planted in virgin soil irrigated with GW and fertilized with the full dose of the

recommended NPK. These results indicate that TWW is a potential source of macro- (N, P, and K) and trace-elements (B, Cu, Zn, Mn, etc.), and, if applied to crops, can fulfill the plant nutrient requirement and decrease the use of synthetic fertilizers. Additionally, the old cultivated soil (L1) resulted in better growth and biomass traits compared to the virgin soil (L2 and L3) due to the higher organic matter and the contents of N, P, and K. The use of TWW for irrigation improves soil fertility and the physical and chemical properties [44–46] and could provide OM and nutrients to soils [17], thus improving crop production [47]. Macro- and trace-elements are equally important to plants and play multifarious roles in their growth and development. For example, N plays important roles in energy metabolism, protein synthesis, and cellular multiplication and it is directly related to photosynthetic activity and chlorophyll formation in plants [48,49]. Similarly, P is the key component of DNA and RNA structures, is involved in storing and transporting energy, and helps in root growth, flower formation, and seed development [50,51]. Likewise, K is involved in the stomatal regulation and transportation of plants' reserve substances. It activates various enzymes involved in the metabolism of plants. Studies have shown that in soils that receive TWW for irrigation, the availability of total N, K, and available P increase considerably [52–56]. Similarly, Abd-Elwahed [57] and Xu et al. [58] observed increased total N, available K, and P contents in the top layer of soil for irrigated plants with wastewater. Therefore, the continuous availability of essential nutrients present in TWW enhanced total chlorophyll, photosynthesis rate, and plant growth, which then results in higher maximum leaf area, plant height, and total biomass. TTW also contains the trace-elements (Mn, Zn, Fe, and Cu) and organic matter necessary for plant growth [56,59]. This makes TTW rich in fertilizers that can increase the fertility of soil and enhance crop yield [60–62], since trace-elements have critical importance and play significant roles in plant growth and development. For example, B is needed for the growth of new plant meristem cells. It is also involved in flower formation and pollen germination and helps the absorption of cations [63,64]. Likewise, Zn is involved in the metabolic processes of plants, enzyme activation, protein synthesis, and chloroplast development [13,65], and also takes part in repairing the process of photo system-II by turning over photo-damaged D1 protein [65,66]. Meanwhile, Zn deficiency reduces chlorophyll synthesis, plant growth, and tolerance of plants against stress [67,68]. Similarly, Mn has key roles in nitrogen assimilation, chlorophyll formation, photosynthesis, and respiration. It is also involved in pollen tube growth, pollen germination, root cell elongation, and resistance to root pathogens [4,69]. Cu acts as a component of metalloenzymes, involved in regulation of enzyme activity and acceleration of oxidative reactions [70,71]. Thus, the nutrient elements present in TWW can be used as fertilizer for enhancing the fertility of soil and the growth and production of crops [6,8,16,56]. Therefore, the growing of crops on soil with an adequate amount of nutrients results in the faster and more vigorous growth of plants and, consequently, a higher economic yield [7].

Likewise, safflower, canola, and triticale grown in old cultivated soil irrigated with TWW produced a significantly higher total biomass (safflower 26.10 t ha^{-1}, canola 19.75 t ha^{-1}, and triticale 11.39 t ha^{-1}), followed by virgin soil irrigated with TWW (Table 4), while the lowest total biomass in all crops was recorded when grown in virgin soil irrigated with GW (safflower 13.83 t ha^{-1}, canola 11.88 t ha^{-1}, and triticale 6.17 t ha^{-1}). The results indicate that due to the continuous availability of macro- and trace-elements due to the application of TWW, crops attained higher growth, leaf area, height (Table 4), and, finally, total biomass. Previous studies have shown that TWW increases the microbial biomass, soil organic matter (OM), water holding capacity of soil (WHC), and porosity, favoring plant growth and increasing biomass [18,45,57,72,73]. Similarly, Abd-Elwahed [57] documented that the OM of soil increases after TWW irrigation, which also increases the WHC of soil and the soil porosity and helps plants attain nutrients and higher economic yields [18,45,72,73]. Hence, the use of TWW for irrigation improves soil fertility and the chemical and physical properties of soil [44–46], and can provide soils with OM and nutrients (N, P, K, Ca, Mg, B, Zn, Cu, Mn, etc.), thus improving crop production [17,47,54,55,59,61,62]. Wang et al. [74]

recorded a higher yield of wheat (*Triticum aestivum* L.), maize (Zea mays L.), millet (*Pennisetum glaucum* L.), apples (*Malus domestica*), and rapeseed (*Brassica napus* L.) when irrigated with TWW. They considered that the rise in the yield of tested crops was due to TWW application. Similar outcomes were documented by Tabassum et al. [75] and Akhtar et al. [76]. Seleiman et al. [8] reported an increase in plant height, total biomass, and the gross energy content in maize, sorghum (*Sorghum bicolor* (L.) Moench), and pearl millet (*Pennisetum glaucum* L.) grown as bioenergy crops and irrigated with TWW. In a pot study, Huang et al. [77] evaluated the effects of TWW and freshwater on maize and soybean (*Glycine max* L.) growth, and reported a clearly higher yield of maize and soybean irrigated with TWW than when using fresh water for irrigation. They attributed the increase in yield to the improvement in the physical properties of the soil and the high uptake of nutrients from the soil. Furthermore, in the current study, the improvement in the biomass of the tested crops (safflower, canola, and triticale) irrigated with TWW showed that the use of TWW does not impose any heavy metals stress, which can cause a reduction in the growth and biomass of crops. Similarly, Seleiman et al. [8] reported that the leaf area and total biomass of maize, sorghum, and pearl millet are higher when TWW is applied. Likewise, El-Nahhal et al. [78] reported an increase in the plant height and fresh biomass of maize and Chinese cabbage when irrigated with TWW compared to fresh water. Zema et al. [79] reported an increase in plant height by 25.6%, in leaf area index by 86.7%, and in biomass yield by 63% of *Typha latifolia* L. when TWW is applied compared to fresh water.

The productivity and profitability of bioenergy crops planted for energy purposes is determined by their dry matter yield and energy output. The dry matter yield depends on the agricultural practices, genetic potential of the plants, and the soil and climatic conditions [80,81]. In the current investigation, safflower, canola, and triticale irrigated with TWW resulted in 3.84–12.36% more energy and 41.43–61.73% more gross energy compared to those grown in virgin soil irrigated with GW (Table 5). The increase in the gross energy value of the crops irrigated with TWW was mainly due to the improvement in the total biomass of the tested crops (Table 4 and Figure 2), due to the fact that in bioenergy crops, the biomass yield is the main factor that determines the gross energy yield [22]. Likewise, Seleiman et al. [21] reported that maize, sorghum, and pearl millet show higher total biomass, energy, and gross energy when the tested crops are irrigated with TWW. The enhancement in the biomass of crops is due to wastewater, which supplies readily available nutrients essential for plants for their better growth and development [82]. In the current study, and in another study conducted by Seleiman et al. [8], it was noted that TWW does not place any toxic or heavy metal stress on plants, and consequently, the plants attained higher biomass and resulted in higher gross energy. Similarly, Seleiman et al. [22] observed a slight improvement in the gross energy yield of maize and hemp grown in soil amended with sewage sludge (a solid byproduct of TWW in wastewater treatment plants).

Safflower, canola, and triticale irrigated with TWW showed higher concentrations of macronutrients (N, P, and K), trace-elements (B, Mn, Cu, and Zn), and heavy metals (Cd, Pb, and Ni) in their dry biomass compared to those irrigated with GW (Tables 5 and 6). However, in the current study, the concentrations of heavy metals in the plant dry biomass were below the permissible limits. A similar trend was recorded for uptake of nutrient elements and heavy metals, in that a higher uptake of nutrient elements and heavy metals was recorded when TWW was applied compared to GW (Table 7). The increase in nutrient uptake and their concentrations in safflower, canola, and triticale could be due to the sufficient amount of these nutrients in the plant root zone through TWW irrigation and the high transformation rate of soil nutrient elements via soil microbiological activities, which resulted in the high bioavailability of nutrient elements to plants and consequently led to high concentrations in plant biomass.

Usually, plants obtain mineral nutrients from the soil solution by their roots, but many factors can affect the efficiency of nutrient acquisition. Soil properties such as pH, moisture content, and compaction can negatively affect the absorption of nutrients, or the nutrients may not be available in certain soils or may be present in forms that plants cannot use.

However, TWW alters the physico-chemical and microbiological activities of soil, which, in turn, play essential roles in the cycling of nutrients in soil and increase their accessibility to plants, enhance the decomposition of organic matter (OM), improve the soil structure, and consequently improve the soil fertility [57,73,83–86]. Abd-Elwahed [57] documented that TWW used for irrigation increases soil OM, which improves the WHC of soil and the drainage, and subsequently decreases the soil compaction, which helps plants attain higher economic yields [18,45,72,73]. Thus, TWW not only provides nutrient elements to soil, but also improves the physical and microbiological properties of soil, which increases the nutrient availability of plants. Similar outcomes were documented by Tzortzakis et al. [87], who revealed that irrigating plants with TWW can enhance the availability of N and P in the root zone; consequently, plants can uptake high N and P contents. Furthermore, Faizan et al. [88] reported an increase in N, P, and K in okra leaves when plants are irrigated with TWW rather than GW. Seleiman et al. [8] observed that maize, sorghum, and pearl millet irrigated with TWW show higher concentrations of nutrient elements and heavy metals (N, P, K, Cu, Zn, Fe, Pb, Ni, Co, and Cd) in dry biomass. Similarly, Chen et al. [44], Khaskhoussy et al. [89], and Fang et al. [90] stated that the TWW irrigation resulted in increasing the Zn, Pb, Co, Cu, Cd, and Ni in the soil—although in permissible limits. In the current study, an increase in the uptake of heavy metals was noted—although lower than permissible limits. Similarly, Zhang et al. [91] and Wu et al. [92] showed that there is no heavy metal buildup in soil irrigated with TWW in China. Similarly, Chen et al. [83] reported that heavy metals do not cause problems in the food chain or soil irrigated with TWW for 20 years. Xu et al. [58] documented very little increase in the concentrations of Cr, Zn, Cu, and Ni (but did not pose any toxic effect) when soil is irrigated with wastewater compared to soil irrigated with groundwater.

In the current study, safflower, canola, and triticale showed higher plant height, leaf area, total biomass, energy content, gross energy, etc. when planted in old soil irrigated with TWW and fertilized with the recommended 50% dose of NPK when compared to those grown in virgin soil irrigated with GW that received the recommended 100% dose of NPK. Furthermore, the application of the recommended 50% and 100% doses of NPK in the tested crops grown in old or virgin soil irrigated with TWW showed only slight differences in growth, productivity, and energy parameters in the current study. The application of 100% dose of NPK to the TWW irrigation does not bring further advantage over the 50% dose of NPK added to TWW. As a result, the 100% NPK dose did not present important differences in growth, productivity, or energy traits in the tested crops. As already mentioned, TWW contains various nutrient elements such as N, P, K, B, Fe, Mn, Zn, and Cu, as well as a significant amount of OM [8,56,59]. This makes TWW rich in fertilizer, which can increase soil fertility and enhance the dry biomass of plants when irrigated with TWW, as well as reduce the use of synthetic fertilizers [8,78,87,88]. Similar findings were reported by Seleiman et al. [8], in that TWW, along with the recommended 50% dose of NPK, shows growth, biomass and gross energy content of maize, sorghum, and pearl millet on par with those where TWW and the recommended 100% dose of NPK are used. Similarly, Duarah et al. [93] recorded small difference in the level of NPK uptake when 50% and 100% NPK doses are used after inoculating seeds with phosphorus-solubilizing bacteria. The excessive application of NPK doses can cause salt toxicity, which may account for low nutrient uptake and reduced plant growth [94,95] in wastewater-irrigated crops [8,96]. Therefore, the application of TWW to soil as a source of irrigation can provide additional nutrient elements to the soil and can enhance their uptake in plants. Thus, TWW can reduce the number of mineral fertilizers being used. Moreover, it could help reduce the environmental pollution caused by the overapplication of fertilizers.

5. Conclusions

TWW is an alternative source of irrigation that can be applied to crops grown for bioenergy purposes. Safflower, canola, and triticale irrigated with TWW, whether grown in old or virgin soil, produced a higher height, total biomass, gross and net energy contents,

and uptake and concentrations of macro- (N, P, and K) and trace-elements (B, Mn, Cu, and Zn) and heavy metals (Cd, Pb, and Ni) without any negative effects on the tested crops. As TWW is rich source of nutrients, it thus decreases the synthetic fertilizer rate up to 50% as observed in current study, where half dose of the recommended NPK fertilizer with TWW showed equivalent results with the recommended 100% dose of NPK in all of the measured parameters of the plants (with few exceptions). Thus, TWW is an important source of irrigation and nutrients for plants and protects the environment by reducing the leaching of excessive fertilizers into groundwater.

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