



Research Paper

The impact of treated wastewater irrigation on strawberry development, fruit quality parameters, and microbial and chemical contaminant transfer: A health risk assessment

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ABSTRACT

The reuse of treated wastewater (TWW) in agriculture is an important alternative technology for reducing freshwater demand and improving soil physicochemical and biological properties. According to the Jordanian national water policy for 2023–2040, freshwater allocated for irrigation in Jordan's agriculture will be decreased and replaced with TWW. The purpose of this study was to investigate how irrigation with TWW influenced strawberry performance as a model for annual plants, namely, the impact on soil, plants, productivity, heavy metals and microbial contamination. Strawberry plants were irrigated with TWW, surface water (SW), and blended water (BW), as contrasted to domestic tap water (DW). Irrigation with TWW, SW, and BW raised the electrical conductivity, total organic compounds%, total nitrogen%, P, and K in the substrate; the rise in salinity, which is one of the most important abiotic stresses, was related to Cl buildup in the substrates, which has a detrimental influence on plant growth, development, and productivity. The heavy metal hazard index values in strawberry fruits were less than one in all treatments, suggesting that the heavy metal level in strawberry fruits did not exceed the requirement for health protection from serious risk. However, microbial contamination by fecal bacteria such as *Escherichia coli* and *Enterococci* spp. and human pathogens such as *Pseudomonas aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp. increased by TWW irrigation, for example *E. coli* population increased from 1.8×10^2 in DW-irrigated fruits to 2.8×10^3 colony forming unit per gram in TWW-irrigated fruits, which may pose health risks. Accordingly, it is possible to conclude that TWW, SW, and BW can be used to irrigate strawberries, but it must be washed before consumption.

1. Introduction

Water scarcity is estimated to affect 40 % of the world's population by 2050, and water scarcity and freshwater conservation are becoming major global needs (He et al., 2021). Agriculture is thought to be the largest water consumer, accounting for over 70 % of total global water consumption (Rost et al., 2008). The use of treated wastewater (TWW) in agriculture is an important alternative technique for reducing the demand for freshwater resources. Moreover, TWW irrigation may improve soil physicochemical and biological qualities by increasing

organic content, pH, electrical conductivity (EC), and mineral content, potentially eliminating the need for fertigation (Tzortzakis et al., 2020). However, the risk of contamination with hazardous elements such as heavy metals, which may be related to the limited efficiency of wastewater treatment plants (WWTPs), and contamination with human pathogenic microbes, which may come from municipal discharge, are the limitations of using TWW for crop irrigation (Pitoro et al., 2022).

The potential adverse effects on soils, land use, and crops are a major concern for both farmers and stakeholders, which decreases the willingness to use this unconventional resource and limits the development

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of this practice (Tawfik et al., 2023). It is frequently cited that domestic and industrial wastewater exhibits a certain level of microbial pollutants and high salinity that farmers do not consider in the choice of production technology and the management of their cropping systems (Renai et al., 2020). So, inappropriate management of irrigation with wastewater can pose substantial risks to public health and the surrounding environment as a result of its microbial and toxic components (Elgallal et al., 2016.). In general, the most critical aspects in relation to the chemical risks arising from TWW reuse for irrigation are excessive concentrations of salt, heavy metals, nutrients, toxic organic compounds, and organic matter (Qadir et al., 2010; WHO., 2006). The environmental impacts caused by using wastewater in agriculture can be positive and negative. Good agricultural practices, executed with planning and management, reduce the environmental impacts and contamination of water resources, and reuse can be beneficial to the environment. Among the concerns about irrigating soil using TWW are damage to soil quality and crop development, salinity increase, clay dispersion, soil hydraulic conductivity reduction, and the presence of pathogens in the water, which represents a public health risk (Urbano et al., 2017).

The water sector in Jordan suffers from chronic problems and faces many challenges in terms of water supply and sanitation. These challenges can be summed up as a general water shortage, caused in part by the increasing demand due to population growth and conflicts, and it is also caused by increased urbanization, a deterioration in water quality due to pollution phenomena, low access to drinking water and sanitation in rural areas, lack of financial resources, inactive water pricing and lack of cost recovery and asset management applications (Al-Karablieh, 2022; Fragaszy et al., 2022). Consequently, there is room for substantial improvements in terms of administrative procedures, water policies, laws and legislation, as well as technical capacity.

Agriculture contributes 4.7 % to Jordan's gross domestic product (GDP) and employs 3 % of its workers (DOS, 2022, 2021), but it consumes more than 52 % of the country's freshwater (MWI, 2020b). Increased water stress can lead to crop failure, import dependency, and increased costs for consumers and businesses. While the direct value of primary agriculture to Jordan's economy is relatively limited, the overall contribution of the agriculture and food sector is closer to 20–25 % of GDP when considering indirect linkages with other parts of the economy (van den Berg et al., 2016; IRG and Al-Karablieh, 2012).

The long-term TWW use was not fully evaluated for its efficacy and risk in Jordan. The National Water Strategy (2023–2040) states that all TWW should be used for irrigation (MWI, 2023, 2016b, c), and the Ministry of Water and Irrigation (MWI) constructed WWTPs across the kingdom, making this vision a reality. TWW currently makes up 30 % of the irrigation water in Jordan and represents approximately 15 % of the water budget (MWI, 2020a, 2021). Nonconventional water resources, such as TWW, offset the country's dependence on freshwater, especially groundwater. However, current regulations and standards limit the direct reuse of treated effluent in agriculture for the irrigation of fodder crops and fruit trees and prohibit all kinds of vegetables from being irrigated with TWW. Although the effluent quality from most WWTPs in Jordan is suitable for restricted irrigation, the culture among farmers and decision-makers is to restrict use even further (MWI, 2020b, 2016a; Official Gazette, 2016; Al-Omari et al., 2015). However, the use of TWW for irrigation is receiving much attention since it is viewed as a means of closing the water scarcity gap and freeing up freshwater for higher-value municipal and industrial purposes (Bdour and Hadadin, 2005).

The characteristics of Jordanian wastewater differ from those of other countries. The raw wastewater intensity is high in comparison to industrialized countries (Abdulla and Farahat, 2020; Abdulla et al., 2016). This is due to the low average domestic water consumption per capita. Toxic contaminants such as heavy metals and organic micro-pollutants are quite low in Jordanian wastewater. This is because industrial outputs to sewage treatment plants are at a low level. Most WWTP effluents meet Jordanian standards for restricted irrigation but fall short of the standards for unrestricted irrigation (Abdulla et al.,

2016).

A major consideration in the use of reclaimed water in Jordan is the potential impact of regulations on the export market of fresh fruit and vegetables and the possibility of restrictions placed by importing countries based on the poor microbiological quality of the irrigation water (Tawfik et al., 2023). The export market for food crops grown in Jordan has suffered from restrictions imposed by some of the importing countries of the Arabian Peninsula because wastewater, when inadequately treated, may have been used to irrigate crops in some parts of Jordan. More recently, standards for exporting crops to Europe have become more rigorous, as they need to be compliant with EU regulations, which shows the importance of addressing the role of wastewater in the water used for irrigation. It is therefore expedient to prepare farmers for the permanent use of TWW for irrigation and how farmers can be informed about alternative cropping patterns and agronomic practices for better adaptation to the expected quality change in irrigation water. The physical and chemical properties of TWW and its effect on irrigation and agricultural practices on soil salinity are determinants of reuse and farmers' acceptance.

Heavy metals, pathogens, nutrients (nitrogen and phosphorus), organic matter, and emerging contaminants (pharmaceuticals, personal care products, etc.) can all be found in wastewater. These contaminants can impact direct and indirect water quality, soil health, and crop performance. Certain contaminants, such as heavy metals, can accumulate in plants, and consuming plants cultivated on contaminated soils or irrigated with wastewater can introduce toxins into the food chain, potentially causing health problems. Therefore, the objective of this study was to investigate how irrigation with TWW, SW, and BW affects strawberry performance as a model for annual plants and salt-sensitive crops namely, cultivar Camarosa (Ferreira et al., 2019; Rivoira et al., 2019; Barroso and Alvarez, 1997). Strawberries are consumed fresh or utilized in desserts and food processing due to their high nutritional value. In Jordan, strawberries are mainly cultivated for fresh consumption for local and international markets during the winter growing season, which lasts from November to March. As a result, strawberry production is increasing to satisfy the increased demand for this fruit (Al-Ramamneh et al., 2013).

2. Material and methods

2.1. Water

The current study used four types of water: (i) TWW was collected at the Abu-Nusier wastewater treatment plant's basin outlet, which discharges to water bodies and wadis, (ii) SW was collected at the King Talal Dam's basin outlet, (iii) blended water (BW) was obtained by mixing SW and TWW samples at a ratio of 1/1 (v/v) collected on a monthly basis to simulate farmers practices that are currently used to minimize freshwater demand, and (iv) clean water was utilized for domestic purposes (DW).

2.2. Strawberry planting

Young fresh strawberry plants (*Fragaria ananassa* cv Camarosa) were transplanted into 10-liter pots (30 × 30 cm diameter × height) filled with 7 kg of a substrate (mixture of clay loam soil, peat moss, and perlite 1:1:1 part) and placed in a plastic house on The University of Jordan campus, Amman, as a representative area for highlands for three growing seasons, from January to June of 2017, 2018, and 2019. The pots were organized in a Randomized complete block design (RCBD), the plants were watered twice a week by drip irrigation with an equal amount, 250 - 500 ml, of treatment water, considering plant age and soil field capacity. Twenty pots were considered for replication in each treatment for each type of the aforementioned irrigation water. The number of leaves and runners per plant and shoot and root dry weights were counted at the end of each growing season.

2.3. Physicochemical and microbiological analysis of water

The four types of irrigation water were analyzed based on the following parameters: pH of the water, EC, total suspended solids (TSS), chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD₅), and phosphate (PO₄³⁻). The bicarbonate ions (HCO₃⁻) were determined using acid (HCl) titration. Nitrate (NO₃⁻) levels were determined using a reflectometric technique and test strips (Ramaswami et al., 2017). Mohr's titration method was used to quantify chlorine ions (Cl⁻). Heavy metals (Cd, Cr, Cu, Mn, Pb, and Zn) were analyzed using a benchtop inductively coupled plasma emission spectrometer (ICPES) (GBC Scientific Equipment Pty Ltd, Victoria, Australia) with argon as the flame gas. Determination of colony forming units (CFU mL⁻¹) was used to estimate the population of fecal, *E. coli*, *Enterococci* spp., and human pathogens (i.e., *Pseudomonas aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp.) after plating the relevant bacterial organisms on selective media using HiCrome™ universal differential medium (Sigma–Aldrich).

2.4. Physicochemical and microbiological analysis of the substrate

Soils used for the cultivation of strawberry plants were analyzed before the irrigation period started (to obtain a “zero level” characterization) and at its end. Substrate samples were obtained from all pots for each treatment every three months and after harvest. The samples were dried at room temperature for one week. For additional testing, 2 mm sieved substrates were chosen. According to the methodology described elsewhere (Belaid et al., 2012), a substrate suspension was generated with a substrate/water ratio of 1/2.5 and 1/5 (w/v) for the pH and EC of the saturated paste measurements, respectively. The suspension was agitated for one hour and decanted for 24 h before measuring the pH in the supernatant with a pH meter and an EC meter.

The Walkley and Black modified method (Verma et al., 2013) was used to determine the total organic compounds (TOC%). The total nitrogen (TN%) was determined using the Kjeldahl method as described elsewhere (Sáez-Plaza et al., 2013). The total phosphorus (P) and potassium (K) in the substrate were assessed according to methods described by Monteiro-Silva et al. (2019). Mohr's titration method was used to quantify Cl⁻.

To assess heavy metal contents, one gram of the substrate was digested with 15 mL of aqua regia solution (mixture of HCl:HNO₃ vol ratio 3:1). The mixtures were placed in a thermostatically controlled water bath, and the temperature was maintained at 95 °C for 2 hr. Then, the mixtures were ultrasonicated for 30 min at 80 °C, filtered with a syringe filter (45 mm), and then diluted to 25 ml with 1 % HNO₃ (Abu-Zurayk et al., 2017). The heavy metals were analyzed using a benchtop inductively coupled plasma emission spectrometer (ICPES). The CFU g⁻¹ of fecal *E. coli* and *Enterococci* spp. and human pathogens *P. aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp. in the substrate was estimated.

2.5. Strawberry plant and fruit evaluation

To compare the development of strawberry plants irrigated with various types of water, measurements and monitoring of morphological and agronomic parameters such as plant height (the tallest part measured using a sewing meter in cm), crown diameter (measured using calipers on the living plant without removing it from the soil in cm), the number of fully developed leaves, the number of developed runners, and chlorophyll content (measured using a chlorophyll content meter (CCM-200; Opti-Sciences, Inc.) were conducted. Data were obtained nondestructively on all plants every 14 days beginning with transplanting. During the production phase, data for morphological, chemical, and physical parameters of fruits, such as fruit fresh weight (g), fruit dry matter (%), total fruit production per plant, total soluble solids (°Brix) using a refractometer (DR201–95; A. Krüss Optronic GmbH), and ascorbic acid (mg 100 mg⁻¹ of fruit fresh weight) as described elsewhere

(Cruz-Rus et al., 2011), were gathered from fruits for each treatment. The plants were harvested at the end of June, and the dry weight (g) of the plants was estimated after separating them into roots and shoots and drying them in an oven at 70 °C for 3 days.

2.6. Plant and fruit microbial and heavy metal contamination

The CFU per cm² of fecal *E. coli*, *Enterococci* spp., and human pathogens *P. aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp. on strawberry leaves was estimated monthly as described elsewhere, and the CFU g⁻¹ of *E. coli*, *Enterococci* spp., *P. aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp. on strawberry fruits was estimated at the harvest time (Al-Karablieh et al., 2022). At the time of harvest, the strawberry leaves and fruits were washed with distilled water, dried at room temperature for one day, and then calcinated in a muffle oven at 450 °C. The ashes were digested with 1 M nitric acid to obtain a suspension, which was filtered with a syringe filter (45 µm) and finally analyzed by ICPES to evaluate the heavy metal contamination.

2.7. Health risk assessment

To assess potential threats to human health from heavy metal contamination in strawberry fruits, the target hazard quotient (THQ) was estimated based on Eq. (A.1). The THQ is defined as the percentage of possible exposure to a chemical element pollutant and level that does not pose any expected negative risk. If the THQ is < 1, there is no risk of exposure to the specified element. If the THQ is > 1, adverse health effects are expected (Bermudez et al., 2011). The presence of several pollutants can result in additive dangers. As a result, the hazard index HI is the sum of the THQ, which measures the overall impacts of two or more pollutants. If HI is > 1, it indicates that consuming pollutants from a food has a negative influence on one's health (Chen et al., 2021).

$$THQ = C_M \times D_{Fruit} \times E_f \times E_D / B_w \times AT_n \times RfD \quad (\text{Eq. A.1})$$

where C_M is the concentration of metal in the fruit (mg kg⁻¹), D_{Fruit} is the daily consumption of fruit (0.057 kg d⁻¹ person⁻¹), E_f is the exposure frequency (365 d year⁻¹), E_D is the exposure duration (74.68 years), B_w is the mean human body weight (60 kg), AT_n is the average time of exposure to heavy metals (E_D × 365 days' year⁻¹), and the oral reference dose (RfD) is the daily oral allowed dose of heavy metals without inducing any harmful impacts throughout the lifespan. The RfDs of Cd, Cr, Cu, Mn, Pb, and Zn are 0.001, 0.003, 0.04, 0.14, 0.004, and 0.3 mg kg⁻¹ d⁻¹, respectively (Guadie et al., 2021; Naseri et al., 2021).

2.8. Statistical analysis

SAS software was used to perform analysis of variance on data collected for comparisons of parameters measured in the three growing seasons in a manner recommended for an RCBD. As the seasonal effect was found to be insignificant, data from the three growing seasons were combined and analyzed in a manner recommended for an RCBD. Least significant difference at the 0.05 probability level ($P < 0.05$) was used to compare the means. The data presented are means ± standard errors.

3. Results

3.1. Characterization of the used waters

The physicochemical and microbiological characteristics of the used waters are presented in Table 1. BW, TWW, DW, and SW have slightly alkaline pH values of 7.6, 7.8, 8.0, and 8.4, respectively. These values are within Jordanian regulations for reclaimed water. The TWW has an electrical conductivity of 6 mS cm⁻¹, which is considered high for a WWTP effluent. The COD and BOD of BW and SW are higher than those of TWW, although all of them remain within Jordanian reclaimed water

Table 1
Average physicochemical and microbiological characteristics of the water used in irrigation ± standard deviation*.

Parameter	Treated wastewater	Surface water	Blended water	Domestic water	Jordanian standards**
pH	7.8 ± 0.3 ^a	8.4 ± 0.4 ^a	7.6 ± 0.2 ^a	8.0 ± 0.1 ^a	6–9
EC (mS cm ⁻¹)	6 ± 0.3 ^a	4 ± 0.2 ^b	4 ± 0.8 ^b	3 ± 0.2 ^c	1–3
TSS (mg L ⁻¹)	35 ± 1 ^a	25 ± 1 ^b	32 ± 4 ^a	1 ± 0.2 ^c	50
COD (mg L ⁻¹)	55 ± 2 ^a	60 ± 1 ^a	59 ± 2 ^a	ND	100
BOD (mg L ⁻¹)	15 ± 2 ^b	27 ± 2 ^a	20 ± 4 ^a	ND	30
HCO ₃ ⁻ (mg L ⁻¹)	368 ± 8 ^a	315 ± 6 ^b	336 ± 17 ^a	89 ± 2 ^c	400
NO ₃ ⁻ (mg L ⁻¹)	12 ± 1 ^b	16 ± 2 ^a	15 ± 1 ^a	1 ± 0.1 ^c	30
NH ₄ ⁺ (mg L ⁻¹)	14 ± 1 ^a	13 ± 1 ^a	13 ± 0.4 ^a	1 ± 0.1 ^b	45
PO ₄ ³⁻ (mg L ⁻¹)	13 ± 2 ^c	45 ± 2 ^a	31 ± 3 ^b	2 ± 0.1 ^d	30
K (mg L ⁻¹)	31 ± 3 ^a	16 ± 2 ^b	24 ± 6 ^a	6 ± 1 ^c	NL
Cl ⁻ (mg L ⁻¹)	350 ± 15 ^a	250 ± 21 ^b	320 ± 40 ^a	265 ± 18 ^b	400
Cd (mg L ⁻¹)	0.02 ± 0.01 ^c	12 ± 2 ^a	6 ± 3 ^b	ND	0.01
Cr (mg L ⁻¹)	0.04 ± 0.01 ^b	0.2 ± 0.03 ^a	0.06 ± 0.01 ^b	0.02 ± 0.002 ^c	0.1
Cu (mg L ⁻¹)	0.3 ± 0.02 ^a	0.3 ± 0.05 ^a	0.3 ± 0.01 ^a	0.03 ± 0.01 ^b	0.2
Mn (mg L ⁻¹)	0.2 ± 0.02 ^a	0.2 ± 0.04 ^a	0.2 ± 0.02 ^a	0.1 ± 0.01 ^b	0.2
Pb (mg L ⁻¹)	0.4 ± 0.03 ^b	4 ± 0.1 ^a	3 ± 0.1 ^a	0.03 ± 0.01 ^c	0.2
Zn (mg L ⁻¹)	4 ± 2 ^b	9 ± 1 ^a	8 ± 2 ^a	0.7 ± 0.1 ^c	5
<i>E. coli</i> (CFU mL ⁻¹)	934 ± 45 ^c	1737 ± 114 ^a	1235 ± 127 ^b	ND	<100
<i>Enterococci</i> spp. (CFU mL ⁻¹)	123 ± 15 ^c	332 ± 38 ^a	237 ± 35 ^b	ND	NL
<i>P. aeruginosa</i> (CFU mL ⁻¹)	103 ± 10 ^c	330 ± 35 ^a	226 ± 26 ^b	ND	NL
<i>Salmonella</i> spp. (CFU mL ⁻¹)	79 ± 10 ^a	99 ± 15 ^a	88 ± 18 ^a	ND	NL
<i>Staphylococcus</i> spp. (CFU mL ⁻¹)	152 ± 12 ^b	251 ± 15 ^a	211 ± 14 ^a	ND	NL

* Means within a row followed by different letters are significantly different according to LSD at 0.05 probability level ($P < 0.05$).

** Jordanian standards 893–2014, reclaimed water for irrigation of cooked vegetables. It is prohibited to use reclaimed water to irrigate vegetables.

NL: Indicates that the parameter is not listed, ND: Indicates that the parameter is not detected.

standards. Nitrates, ammonia, and phosphate are present in appropriate concentrations, except for phosphate concentrations in BW and especially in SW, which are greater than the standards. Heavy metal concentrations in the SW are high, with values of 12, 0.2, 0.3, 0.2, 4, and 9 mg L⁻¹ for Cd, Cr, Cu, Mn, Pb, and Zn, respectively, while the TWW satisfies Jordanian reclaimed water standards, except for Cu and Zn in some cases, which are slightly higher than the limit. The SW contains more *E. coli*, *Enterococci* spp., *P. aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp. than the TWW, with FCU mL⁻¹ values of 1.7×10^3 , 3.3×10^2 , 3.3×10^2 , 9.9×10^1 , and 2.5×10^2 , respectively. As a result, the values of most parameters of BW were between those of TWW and SW.

3.2. Characterization of the substrate

The physicochemical and microbiological characteristics of the substrate before and after planting are presented in Table 2. The substrate before planting had a pH of 7.4, a high conductivity (1 mS cm⁻¹), and a TOC of 24 %. The substrate pH did not change much after planting; however, the conductivity of the SW-irrigated substrate reached 4, and the TOC% reached 48, 40, and 35 % in the TWW-, BW-, and SW-irrigated substrates, respectively. Before planting, the substrate contained a high concentration of P and K (23 and 317 mg kg⁻¹, respectively) that could have been imported with peat moss. However, following planting, the concentration rose in substrates irrigated with TWW, SW, and BW, while it decreased in substrate irrigated with DW. The substrate possessed a low concentration of Cl⁻ (2.5 mg kg⁻¹) before planting, which rose in the substrates following planting and irrigation with various types of irrigation water to reach a maximum in the TWW-irrigated substrate (47 mg kg⁻¹).

Heavy metals such as Cd, Cr, Cu, Mn, Pb, and Zn were detected at various concentrations in the substrate before planting and increased in the substrates following planting and irrigation with TWW, SW, and BW, reaching a maximum in the SW-irrigated substrate, followed by the TWW-irrigated substrate and the BW-irrigated substrate. On the other hand, the concentrations of heavy metals in the DW-irrigated substrate were lowered. The tested microbes were detected at various population densities in the substrate before planting and increased in the substrates following planting and irrigation with various types of irrigation water, reaching a maximum in the SW-irrigated substrate, followed by the TWW-irrigated substrate, the BW-irrigated substrate, and the DW-

Table 2
Physicochemical and microbiological characteristics of the substrate before and after planting ± standard deviation.

Parameter	Substrate before planting	The substrate after planting*			
		Treated wastewater	Surface water	Blended water	Domestic water
pH	7.4 ± 0.1 ^a	7.6 ± 1 ^a	7.5 ± 0.5 ^a	7.4 ± 0.4 ^a	7.3 ± 0.3 ^a
EC (mS cm ⁻¹)	1 ± 0.2 ^c	3 ± 0.3 ^b	4 ± 0.2 ^a	3 ± 0.3 ^b	1.7 ± 0.1 ^c
TOC (%)	24 ± 1 ^d	48 ± 1 ^a	35 ± 2 ^c	40 ± 1 ^b	20 ± 2 ^d
TN (%)	3 ± 0.5 ^c	7 ± 2 ^b	10 ± 1 ^a	8 ± 2 ^{ab}	2 ± 2 ^c
P (mg kg ⁻¹)	23 ± 4 ^b	29 ± 4 ^a	35 ± 5 ^a	31 ± 6 ^a	17 ± 2 ^b
K (mg kg ⁻¹)	317 ± 19 ^c	604 ± 25 ^a	515 ± 23 ^b	619 ± 21 ^a	328 ± 16 ^c
Cl ⁻ (mg kg ⁻¹)	2.5 ± 0.1 ^f	47 ± 4 ^a	30 ± 4 ^c	40 ± 3 ^b	26 ± 4 ^d
Cd (mg kg ⁻¹)	2.4 ± 0.6 ^c	3 ± 1 ^c	6 ± 2 ^a	3 ± 2 ^b	2.1 ± 0.4 ^c
Cr (mg kg ⁻¹)	19 ± 3 ^b	19 ± 2 ^b	20 ± 3 ^a	19 ± 3 ^b	16 ± 2 ^c
Cu (mg kg ⁻¹)	13 ± 2 ^b	13 ± 1 ^b	16 ± 2 ^a	14 ± 2 ^{ab}	12 ± 3 ^b
Mn (mg kg ⁻¹)	43 ± 4 ^a	45 ± 4 ^a	50 ± 5 ^a	47 ± 4 ^a	39 ± 4 ^a
Pb (mg kg ⁻¹)	35 ± 7 ^a	39 ± 5 ^a	42 ± 4 ^a	40 ± 4 ^a	31 ± 3 ^b
Zn (mg kg ⁻¹)	45 ± 3 ^a	47 ± 5 ^a	52 ± 5 ^a	48 ± 5 ^a	40 ± 5 ^b
<i>E. coli</i> (CFU g ⁻¹)	3186 ± 786 ^c	4569 ± 864 ^b	9523 ± 915 ^a	5888 ± 646 ^b	3572 ± 159 ^c
<i>Enterococci</i> spp. (CFU g ⁻¹)	200 ± 15 ^c	567 ± 98 ^a	652 ± 25 ^a	481 ± 99 ^b	225 ± 63 ^c
<i>P. aeruginosa</i> (CFU g ⁻¹)	60 ± 17 ^d	352 ± 94 ^b	625 ± 98 ^a	367 ± 74 ^b	125 ± 29 ^c
<i>Salmonella</i> spp. (CFU g ⁻¹)	19 ± 6 ^c	237 ± 58 ^b	364 ± 48 ^a	214 ± 43 ^b	40 ± 24 ^c
<i>Staphylococcus</i> spp. (CFU g ⁻¹)	26 ± 8 ^c	258 ± 67 ^b	589 ± 131 ^a	299 ± 105 ^b	49 ± 18 ^c

* Means within a row followed by different letters are significantly different according to LSD at 0.05 probability level ($P < 0.05$).

irrigated substrate, with the lowest build-up in population densities.

3.3. Effect of irrigation water on plant growth parameters

Although there were no significant differences in plant height and crown diameters between irrigation water types, the maximum plant height, 22.2 cm, was obtained with DW and the minimum, 16.2 cm, with TWW, as was the maximum crown diameter, 1.5 cm, obtained with DW

and the minimum, 0.8 cm, obtained with TWW. Significant differences in the number of developed leaves and runners were seen between treatments, with DW-irrigated plants having the most, SW- and BW-irrigated plants having no significant differences, and TWW-irrigated plants having the fewest (Fig. 1A).

The chlorophyll content of plants irrigated with various types of irrigation water differed significantly. The chlorophyll concentration of DW-irrigated plants was found to be the highest, at $28 \mu\text{mol m}^{-2}$, and the lowest, at $16 \mu\text{mol m}^{-2}$, in SW-irrigated plants. The shoot and root dry weights of DW-irrigated plants differed significantly from those of TWW-, SW-, and BW-irrigated plants, but no significant differences were found between TWW-, SW-, and BW-irrigated plants (Fig. 1B).

Irrigation water had no effect on total fruit number per plant. Strawberry fresh fruit weight was significantly higher in DW-irrigated plants, with 1270 g per plant, than in SW-irrigated plants, 1035 g per plant, followed by TWW-irrigated plants, 915 g per plant, and BW-irrigated plants, 904 g per plant, with no significant difference between TWW- and BW-irrigated plants (Fig. 1C). Similar behavior was noticed for fruit dry matter (Fig. 1D). Although irrigation water had no influence on total soluble solids, there were significant differences in ascorbic acid concentration. Ascorbic acid concentrations were highest in DW- and SW-irrigated plants, with 50 and 48 mg 100 mg^{-1} , respectively, followed by 40 mg 100 mg^{-1} in BW-irrigated plants, and lowest in TWW-irrigated plants, with 35 mg 100 mg^{-1} (Fig. 1D).

3.4. Microbes on the plants

The populations of *E. coli*, *Enterococci* spp., *P. aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp. on strawberry leaves and fruits were estimated. Overall, the bacterial population on the leaves was greater than that detected on the fruits. *E. coli* was the dominant bacterium detected on strawberry leaves and fruits independent of water type.

The largest *E. coli* population was detected on the leaves of TWW-irrigated plants, with 8.6×10^3 CFU per cm^2 , followed by those of BW- and SW-irrigated plants, and it was the lowest was on the leaves of

DW-irrigated plants, with 2.5×10^2 . The maximum *E. coli* population detected on strawberry fruits was 2.8×10^3 CFU g^{-1} on TWW-irrigated plants, whereas the lowest was 1.8×10^2 on DW-irrigated plants. The largest population of *Enterococci* spp. was detected on the leaves of SW-irrigated plants, with 9.3×10^2 CFU per cm^2 , and the lowest was on the leaves of DW-irrigated plants, with 1.5×10^2 . The largest population of *Enterococci* spp. detected on strawberry fruits was 4.4×10^2 CFU g^{-1} on SW-irrigated plants, whereas the lowest was 1.7×10^1 on the fruits of DW-irrigated plants. The largest population of *Salmonella* spp. was detected on the leaves of TWW-irrigated plants, with 7.0×10^3 CFU per cm^2 , followed by those of SW- and BW-irrigated plants, and the lowest was on the leaves of DW-irrigated plants, with 2.4×10^2 . The maximum *Salmonella* spp. population detected on strawberry fruits was 2.8×10^3 CFU g^{-1} on TWW-irrigated plants, whereas the lowest was 1.8×10^2 on DW-irrigated plants. *P. aeruginosa* and *Staphylococcus* spp. had the highest populations on the leaves of TWW-irrigated plants, with 1.5×10^3 and 1.9×10^3 CFU per cm^2 , respectively. Similarly, both pathogens had the highest population on fruits of TWW-irrigated plants, with 8.4×10^2 and 1.1×10^3 CFU g^{-1} , respectively (Fig. 2).

3.5. Heavy metals in the plant

The concentrations of Cd, Cr, Cu, Mn, Pb, and Zn in the strawberry leaves and fruits were evaluated. Except for Zn, the data demonstrate a substantial difference in heavy metal concentrations in the leaves of DW-irrigated plants when compared to TWW-, SW-, and BW-irrigated plants. The highest concentrations of heavy metals were found in the leaves of TWW-irrigated plants and SW-irrigated plants, with almost no significant differences, followed by BW-irrigated plants, except for Mn, which had a concentration of 38 mg kg^{-1} , and the lowest concentration of heavy metals was found in the leaves of DW-irrigated plants (Table 3). It is worth mentioning that TWW-, SW-, and BW-irrigated plants showed Na toxicity symptoms, such as lower leaves with necrotic spots, followed by the formation of a marginal necrotic band (Fig. 3). Typically, excess Na levels come from the irrigation water; the Na content in the TWW-,

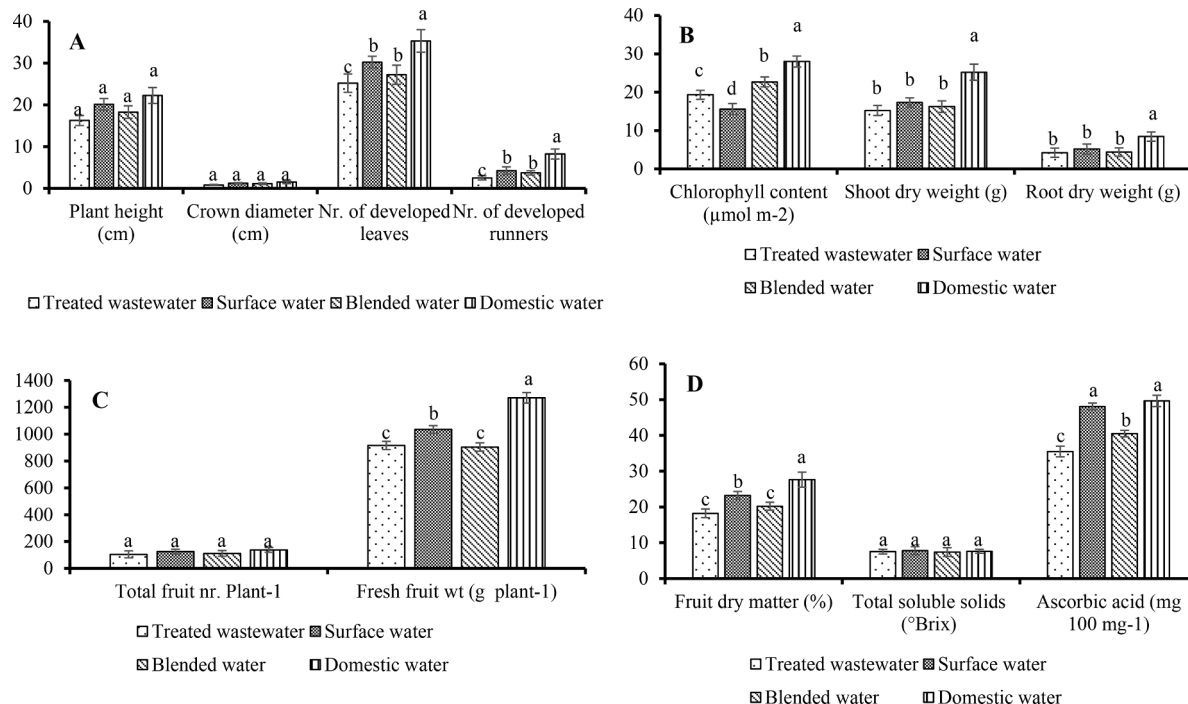


Fig. 1. Effect of irrigation water on strawberry growth parameters. A, Plant height, crown diameter, number of developed leaves, and number of developed runners; B, Chlorophyll content, shoot dry weight, and root dry weight; C, Total strawberry fruits plant⁻¹ and fresh fruit weight plant⁻¹; D, Strawberry fruit dry matter, total soluble solids, and ascorbic acid content. Different letters on bars denote differences (ANOVA, Tukey's test, p 0.05) between treatments, whereas the same letter denotes no significant difference.

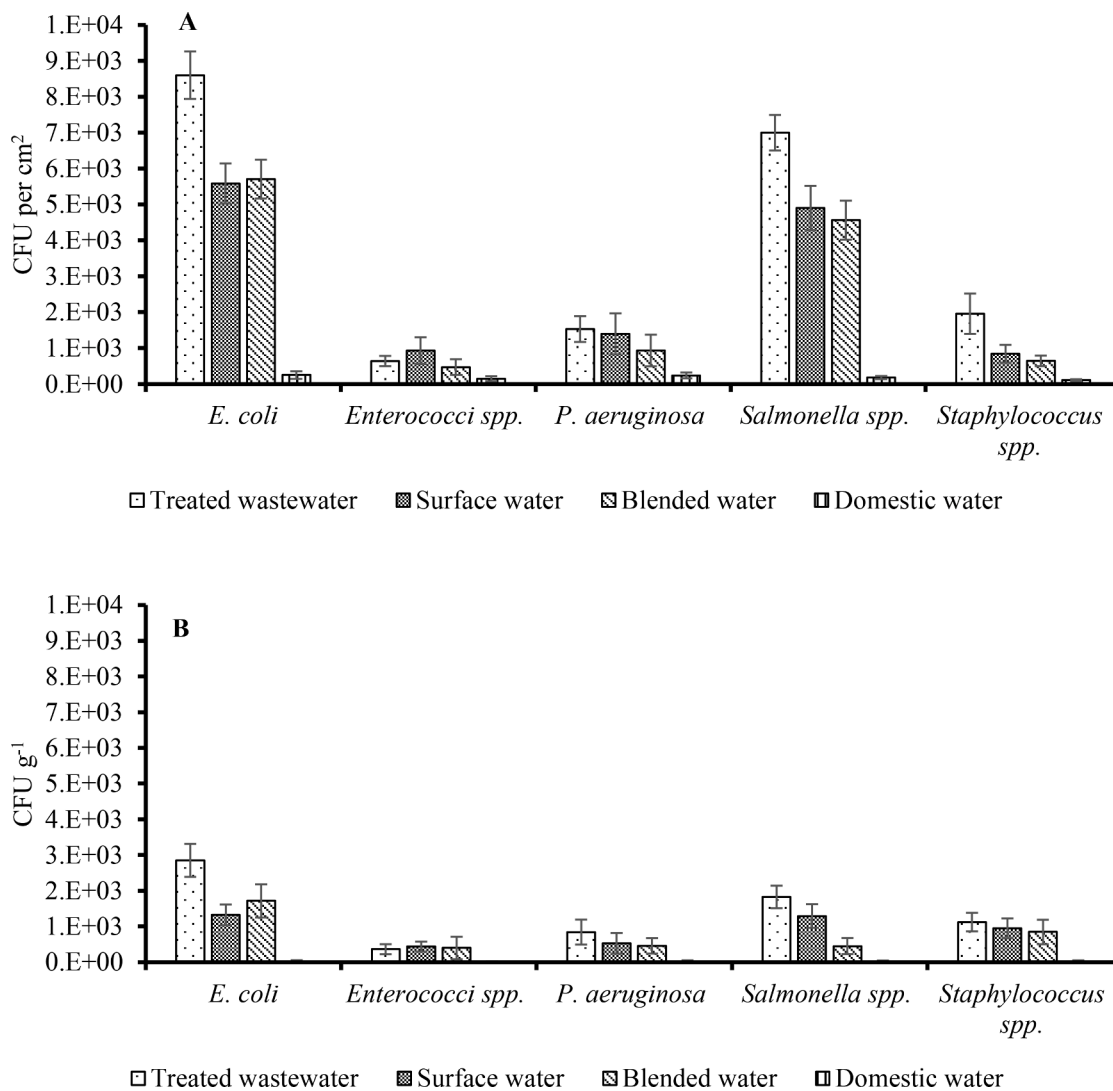


Fig. 2. Effect of irrigation water on fecal, *E. coli* and *Enterococci spp.*, and human pathogens, *Pseudomonas aeruginosa*, *Salmonella spp.*, and *Staphylococcus spp.* populations: A, microbe population on leaf surface; B, microbe population on fruits.

Table 3
Heavy metals concentration in the strawberry leaves (mg kg⁻¹) ± standard deviation*.

Heavy metal	Treated wastewater	Surface water	Blended water	Domestic water
Cd	2.1 ± 0.7 ^a	2.4 ± 0.5 ^a	0.60 ± 0.06 ^b	0.40 ± 0.08 ^c
Cr	3.7 ± 0.1 ^a	3.7 ± 0.3 ^a	3.6 ± 0.2 ^a	2.4 ± 0.2 ^b
Cu	6.3 ± 1.4 ^a	7.2 ± 0.9 ^a	6.1 ± 0.8 ^a	3.1 ± 0.5 ^b
Mn	27 ± 1 ^b	25 ± 1 ^b	38 ± 1 ^a	12 ± 2 ^c
Pb	29 ± 3 ^a	25 ± 2 ^{ab}	25 ± 2 ^{ab}	13 ± 3 ^c
Zn	8 ± 1 ^a	8 ± 2 ^a	7 ± 2 ^a	6 ± 1 ^b

* Means within a row followed by different letters are significantly different according to LSD at 0.05 probability level (P < 0.05).

SW-, and BW-irrigated plant leaves ranged between 1405 and 1445 mg kg⁻¹. These values are of concern since in the leaves, values above 1000 mg kg⁻¹ are considered excessive and dangerous for normal plant growth (Whipker, 2014)

The results for the fruit revealed that heavy metal accumulation is greatly influenced by the type of water and the metal itself. For example, SW-irrigated plants accumulate the most Cd, Cr, and Cu in the fruits (1.2, 3.1, and 2.8 mg kg⁻¹, respectively), while BW-irrigated plants accumulate the most Mn (28 mg kg⁻¹) and TWW-irrigated plants

accumulate the most Pb and Zn (17, and 7 mg kg⁻¹, respectively). Overall, DW-irrigated plants accumulate the least amount of heavy metals in their fruits (Table 4).

3.6. Health risk assessment

The THQ and HI health risk assessments were estimated; the results are shown in Table 5. The results showed that DW-irrigated plants had the lowest HI value (0.0027) when compared to TWW-irrigated plants (0.0046), followed by SW-irrigated plants (0.0054) and BW-irrigated plants, which had the greatest HI value (0.0057).

4. Discussion

In terms of physiochemical qualities, the TWW utilized in this study fulfills Jordanian criteria, 893–2006, for utilizing TWW in the irrigation of cooked vegetables but not in terms of microbiological contamination. The SW, on the other hand, does not meet Jordanian criteria in terms of physiochemical qualities, mostly due to heavy metal levels and microbial pollution. When TWW is mixed with SW, the concentrations of heavy metals and microbes are reduced.

Contrary to irrigation with DW, which may bleach the organic content and minerals, irrigation with TWW, SW, and BW increased EC, TOC



Fig. 3. Observed symptoms during the experiment, Na toxicity, on treated wastewater, surface water, and blended water irrigated plants.

Table 4
Heavy metals concentration in the strawberry fruits (mg kg⁻¹) ± standard deviation*.

Heavy metal	Treated wastewater	Surface water	Blended water	Domestic water
Cd	0.40 ± 0.4 ^b	1.2 ± 0.6 ^a	0.30 ± 0.03 ^b	0.27 ± 0.05 ^b
Cr	2.2 ± 0.5 ^b	3.1 ± 0.4 ^a	2.7 ± 0.2 ^b	1.4 ± 0.3 ^c
Cu	1.3 ± 0.2 ^b	2.8 ± 0.5 ^a	2.6 ± 0.4 ^a	1.3 ± 0.3 ^b
Mn	20 ± 1 ^b	27 ± 2 ^{ab}	28 ± 2 ^a	12 ± 2 ^c
Pb	17 ± 2 ^a	13 ± 1 ^b	16 ± 2 ^a	10 ± 2 ^c
Zn	7 ± 2 ^a	6 ± 1 ^a	6 ± 2 ^a	3 ± 1 ^b

* Means within a row followed by different letters are significantly different according to LSD at 0.05 probability level (P < 0.05).

%, TN%, P, and K in the substrate after planting. It has already been observed that irrigation with TWW increases the EC, organic content, and minerals (Bakari et al., 2022; Belaid et al., 2012). The accumulation of Cl in the substrates following irrigation with all water types employed in the study planting might be due to the usage of Cl in the disinfection of TWW and DW. The SW is taken from King Talal Dam, which is a collection of several treatment plants. It has been noted that salinity is one of the most important abiotic stresses, resulting from an increase in the concentration of NaCl and other salts, which causes water stress due

Table 5
Target hazard quotients (THQ) and hazard index (HI) values of strawberry fruits irrigated with different types of water.

Heavy metal	RfD	THQ			
		Treated wastewater	Surface water	Blended water	Domestic water
Cd	0.001	0.00000038	0.00000114	0.000000285	0.0000002565
Cr	0.003	0.00000627	0.000008835	0.000007695	0.000003933
Cu	0.04	0.0000494	0.0001064	0.0000988	0.00004788
Mn	0.14	0.0026866	0.0036309	0.0037772	0.0016226
Pb	0.004	0.00006574	0.00005054	0.0000627	0.0000399
Zn	0.3	0.001881	0.0016245	0.0017955	0.0009975
HI*		0.00468939 ^a	0.005422315 ^a	0.00574218 ^a	0.00271207 ^b

* Means within a row followed by different letters are significantly different according to LSD at 0.05 probability level (P < 0.05).

to an increase in osmotic pressure and a decrease in the water that plants can uptake. This induces a number of negative effects, such as morphological alterations, changes in plant primary and secondary metabolism, plant growth and development, and yield reduction (Moradi et al., 2022).

In fact, sensitive plant growth, such as strawberries, may be adversely harmed when EC is within 2–4 mS cm⁻¹ (Denaxa et al., 2022; Keutgen and Pawelzik, 2009). In this regard, (Al-Shorafa et al., 2014) advised against growing 'Camarosa' and 'Albino' strawberries in irrigation water with an EC of 2.8 mS cm⁻¹ or higher. It has been found that high soil conductivity caused a sharp decline in strawberry growth and fresh fruit yield, with decreases of 29 %, 35 %, and 59 % in total yield when utilizing substrates with initial conductivities of 4, 6, and 8 mS cm⁻¹, respectively (Ondrašek et al., 2006). The increased salt stress reduced strawberry fruit carbohydrates, organic acids, and pigmentations (Denaxa et al., 2022).

The accumulation of heavy metals in the substrate after irrigation with TWW has previously been reported in Jordan but after long-term irrigation (Albdaiwi et al., 2022; Chen et al., 2021; Guadie et al., 2021) however, the substrate irrigated with SW accumulates more heavy metals because the SW itself has a high concentration of heavy metals, which may indicate that contamination with heavy metals occurs after the discharge of TWW from treatment plants to surface running water.

Accumulation of fecal microorganisms, *E. coli* and *Enterococci* spp., in the substrate during TWW irrigation is predicted; this has previously been recorded in soil irrigated with TWW from the same source of TWW, the Abu-Nusair wastewater treatment plant (Al-Lahham et al., 2003). *Salmonella* spp. prevalence in irrigation water from two major sources, namely, King's Abdullah Canal, which receives water from King's Talal Dam, which receives TWW discharged from various wastewater treatment plants, and Wadi Shueib, which receives treated and untreated waste water, has previously been recorded in Jordan (Burjaq and Abu-Romman, 2020) and isolated from the water of the Zarqa River and King's Talal Dam and from vegetables irrigated by such water (Tarazi et al., 2021). This could explain the increase in human pathogens such as *P. aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp. In TWW-, SW- and BW-irrigated substrates.

Although there were no significant differences in plant height or crown diameters, there were significant differences in the number of developed leaves and runners. These findings were consistent with previous research, which found no significant difference between irrigation with TWW and conventional water in strawberry heights, crown diameter, number of developed leaves, and chlorophyll content (Djillali et al., 2020), and no difference between irrigation with TWW and 60 % diluted TWW in strawberry heights and number of developed leaves (Bakari et al., 2022). However, these results could be explained by a reduction in carbohydrates, growth hormones, enzyme activity, and photosynthesis activity, all of which are directly related to the presence of high salinity (Avestan et al., 2019; El-Banna and Abdelaal, 2018; Garriga et al., 2015). In addition, high heavy metal concentrations generate direct toxic effects such as inhibition of cytoplasmic enzymes and oxidative stress, which destroy cell structures, and indirect deleterious effects such as the substitution of vital nutrients at plant cation exchange sites. These harmful effects, both direct and indirect, reduce plant development and produce stunting, which eventually leads to plant death (Varma and Jangra, 2021; Asati et al., 2016).

The observed decrease in chlorophyll content in TWW-, SW-, and BW-irrigated plants is related to photoinhibition and oxidative damage in the leaves, which could be related to the high Na content of the leaves, which causes marginal leaf necrosis, and/or the high content of heavy metals, primarily Cd and Pb, which accumulated in the leaves of TWW-, SW-, and BW-irrigated plants, as reported in mulberry leaves where Pb and Cd stress caused chlorophyll degradation in the leaves (Huihui et al., 2020). Furthermore, the observed reduction in shoot and root dry weight in TWW-, SW-, and BW-irrigated plants indicates the occurrence of delayed growth of the shoot and root system, which could be related to a delay in the plant metabolism process caused by the inhibitory concentration of metals in the substrates causing chlorosis and reduction in leaf number and root growth, as it has been reported that heavy metal root absorption has a negative impact on root growth (Shahid et al., 2017).

Although there was no effect of water type on fruit quantity, there was a significant effect on fruit weight as total yield and fruit quality as fruit dry matter% and ascorbic acid content. The negative impact of irrigation with TWW, SW, and BW may be attributed to the reduced chlorophyll content, which is associated with the high amount of chlorine and heavy metals in the substrate. According to previous research on strawberry cv. Camarosa, salt stress negatively affects vegetative development, leaf dry weight, chlorophyll content, fruit size, yield, and fruit quality, which can be connected to total soluble solids and ascorbic acid concentration (Garriga et al., 2015). These negative effects can be mitigated by dilution of wastewater with clean water to reduce the concentration of chlorine in irrigation water, aeration, which helps in the breakdown of chlorine compounds and reduces their concentration, and incorporation of organic matter into the soil, which may aid in the breakdown of chlorine compounds.

The THQ and HI remained below 1, indicating that the heavy metal level in strawberry fruits in the study area did not exceed the standard for health protection from serious risk (Yang et al., 2023; Bakari et al.,

2022), which may be related to annual strawberry farming, which gives a short time period for accumulation. As a consequence, based on heavy metal concentrations, none of the water types tested in this investigation constituted a potential health risk to persons.

The presence of fecal microbes, namely, *E. coli*, *Enterococci* spp., and human pathogens, namely, *P. aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp., on the leaves and fruits of plants irrigated with TWW, SW, and BW is a concern to public health. Therefore, TWW, SW, and BW are not viable options for strawberry irrigation, since strawberries are almost always eaten raw. It is indicated that the TWW can be utilized as an alternative for irrigation of cooked tomatoes, but not raw tomatoes (Al-Lahham et al., 2003); however, it was reported that treatment with 2 % lactic acid or acetic acid significantly reduced, *E. coli*, *Salmonella*, mold, and yeast (Wei et al., 2017). Moreover, the wastewater treatment facility should consider regularly monitoring these fecal and human pathogens to avoid contamination.

Due to water shortages, farmers have limited options to cope with water scarcity. Either reducing the cultivated areas, changing the cropping patterns, or accepting the use of TWW for irrigation. However, the majority of the WWTPs' effluent characteristics are consistent with WHO guidelines and Jordanian water quality criteria for restricted irrigation, but they violate unrestricted irrigation standards. Illegal watering methods on crops for raw consumption are also apparently continuing downstream from the treatment plants before the effluent is mixed with surface water.

Therefore, real-time TWW monitoring is required, for the usage of TWW at the final destination. In addition to existing overlaps and weak coordination between affiliated ministries, the lack of human resource capacity building at ministries, and shortages of financial resources and technical equipment might be behind reluctance to implement an integrated TWW management approach. Institutional reform and infrastructure enhancement as well as capacity building are required.

The TWW reuse regulations and standards are appropriate to protect the public health of both consumers and farmers. The major challenge is the enforcement of these regulations and standards. There are several socio-cultural and political factors hindering the successful implementation of established regulations and standards that regulate the use of TWW.

5. Conclusions

In this study, the effects of TWW, SW, and BW irrigation on strawberry productivity and safety, soil fertility and salinity, and metal pollution were compared to those of DW. Irrigation with TWW, SW, and BW increased soil fertility by increasing total NPK and macro- and micronutrients.

According to the examination of several plant development metrics, fruit production, and quality, TWW, SW, and BW could be considered sustainable management strategies for strawberry irrigation. The THQ and HI of heavy metals demonstrated that eating strawberry fruits after irrigation with TWW, SW, and BW poses no health hazards. Microbial contamination with fecal microbes, such as *E. coli* and *Enterococci* spp., and human pathogens, such as *P. aeruginosa*, *Salmonella* spp., and *Staphylococcus* spp., might be responsible for the risk, which may be mitigated by washing before consumption.

Further research will be conducted on perennial plants, such as olive trees, to investigate the impact of wastewater irrigation on perennial plant development, fruit quality parameters, microbiological and chemical contamination and transmission, and health risk assessment.

CRedit authorship contribution statement

Nehaya Al-Karablieh: Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis. **Ibrahim Al-Shomali:** Methodology, Data curation. **Lina Al-Elauimi:** Methodology, Investigation. **Mohammad Tabieh:** Data curation, Conceptualization. **Emad Al-**

Karablieh: Project administration, Funding acquisition. **Madi Al-Jaghbir:** Software, Conceptualization. **Massimo Del Bubba:** Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Data availability

All datasets analyzed in the study are included as tables and figures in the manuscript.

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