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Effect of Irrigation with Treated Wastewater on Squash Yields, Soil Chemical and Microbial Properties

Hasan Salem Alkhaza'leh [1*], ***Ahmad Moh'd Abu-Awwad*** [2] ***and Mohammed Ibrahim Al-Qinna*** [3]

[1] Department of Lands, Water, and Environment, University of Jordan, Amman, Jordan.
Email: hasan-khazaleh@aabu.edu.jo

[2] Department of Lands, Water, and Environment, University of Jordan, Amman, Jordan.
Email: abuawwad@ju.edu.jo

[3] 3 Department of Land Management and Environment, the Hashemite University, Zarqa, Jordan.
Email: qinna@hu.edu.jo

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ABSTRACT

In this study, the effects of irrigation with treated wastewater (TWW) on soil chemical, microbiological, and yield of squash were investigated. Squash seedlings (Cucurbita pepo) were irrigated using conventional irrigation water (CIW), treated wastewater (TWW), and blended irrigation water (BIW). The drip irrigation system was used to irrigate Squash with CIW, TWW, and BIW. The concentration of all chemical and microbial irrigation water characteristics was falling within the limits of Jordanian standards (JS893/2021), except for turbidity and boron. Pathogens indicators such as Salmonella, and Helminth eggs were not found in TWW. TWW-irrigated plots had significant differences in electrical conductivity, organic carbon, total nitrogen, and sodium adsorption ratio (SAR). Total coliform and Escherichia coli (E. coli) contents increased significantly within TWW-irrigated plots. Squash's fresh yield weight irrigated with TWW showed no significant difference compared with CIW. TWW and BIW treatments had a tendency toward lower fruit numbers than CIW. E. coli was not significantly different on the surface of squash fruits, while Total coliform increased significantly for fruits within the TWW-irrigated plots.

Keywords: Treated wastewater, Escherichia coli, Pathogen, Salinization, Squash, Jordan.

INTRODUCTION

Jordan has one of the lowest water availability rates in the world, since 1964, the Jordanian individual's share of annual water use has decreased from 3600 to less than 100 m^3/capita, which is less than 10% of the estimated worldwide water poverty level of 1000 m^3/capita (MIW, 2019).

Jordan's Ministry of Water and Irrigation adopted National Strategic Plan (2016-2025) that incorporates blended irrigation water (BIW: blended irrigation water with treated wastewater in the water budget for unrestricted reuse in agricultural irrigation. Agriculture uses 52% of the total conventional water in the country; 29% of the irrigation water comes from treated wastewater (MIW, 2020).

Using TWW for irrigation, the main concern is the transmission of infectious illnesses to humans (Pescod,

* Corresponding author.E-mail: hasan-khazaleh@aabu.edu.jo

1992). To safeguard public health and make TWW use in agriculture safe, the Jordan Standards and Metrology Organization (JSMO) developed and issued the Jordanian Standards 893/2021, based on WHO (2006) guidelines that prohibit the use of TWW for irrigating vegetables eaten raw (uncooked). Although wastewater contains beneficial constituents such as organic matter and nutrients that are important to soil productivity, it may contain toxic or unwanted chemical constituents that may influence soil health and crop yield, besides pathogens. (Qadir *et al*., 2007).

In most cases, Coliforms (such as *Faecal*, *Escherichia coli* (*E. coli*)), *Helminth* eggs, and *Salmonella* are indicators of microbial contamination (Mayer *et al*., 2016; Pescod, 1992). Several studies, on the use of TWW in agriculture, were conducted for various crops and studied the effects of TWW irrigation on soil microbiological characteristics (Urbano *et al*., 2017; Akhtar *et al*., 2012; Cirelli *et al*., 2012; Day *et al*., 1962). Some studies show that there is a possibility for the transport of pathogens through the leaf, stem, and cracks, or flaws in the skin (Blumenthal and Peasey, 2002). Although using TWW for irrigation may improve soil fertility and crop yield, some hazards are still a concern such as soil salinity and soil infiltration capacity decline. In arid and semiarid areas, salinization is a common problem. About 995 million hectares around the world areas are suffering from salinity problems (Szabolcs, 1986). Therefore, scientific researchers suggested measures to mitigate these problems (Cirelli *et al*., 2012; Nogueira *et al.*, 2013).

Vegetable production in Jordan is concentrated in Jordan Valley, where the irrigation water is from King Talal Dam (KTD). Some potential sources of contaminants affecting water quality in KTD include discharges from the Samra wastewater treatment plant (WWTP) and Wadi Rmemeen (Al-Taani *et al*., 2018). This study aimed to investigate the effects of TWW used for agriculture irrigation on soil chemical, and microbial properties, and the squash yield and quality.

Material and methods

Study area and experimental conditions.

The experiment was conducted on a farm located in Deir Alla, at 32.233615°N, 35.603982°E at an elevation of -224 m below sea level (Fig 1). Summers in Deir-Alla are hot and dry, and winters are mild and wet (Kool, 2016); With an annual mean temperature of 23.6°C, the temperature in summer is around 40°C and rarely drops below 20°C in winter. The average total annual rainfall is 285 mm (Kool, 2016; Tarawneh and Kadıo˘glu, 2003).

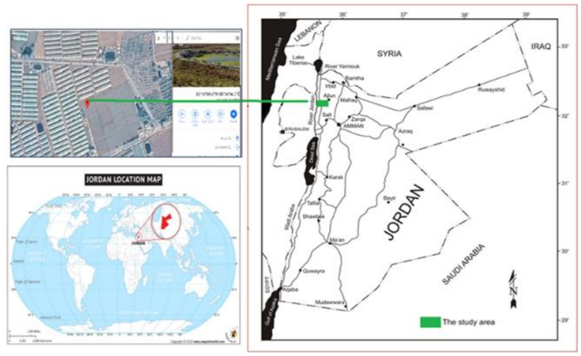

Figure 1. Overview of the study area

A randomized complete block design (RCBD) with three replicates, was used to examine treatments (Fig 2): (i) Conventional irrigation water (CIW); (ii) Blended irrigation water (BIW: 50% CIW blended with 50% TWW); (iii) Treated wastewater (TWW). Three separate tanks and three irrigation pumps were used. Each block was distributed in a random order so that they would not be next to each other. Squash seedlings (*Cucurbita pepo*) were planted in January 2021. Each row was irrigated using drip irrigation covered with plastic mulch.

Temporary plastic tunnels were used, at the time of rainfall, to prevent rainwater from entering treatment plots. Each plot contained three rows, each 7 m long. Plants were spaced 40 cm apart and rows were separated by 130 cm. Each row had an irrigation line, 6 liters hr^{-1} discharge emitters, 40 cm apart. Harvesting squash started in February 2021. TWW was sourced from the secondary stage of the Kufranjah wastewater treatment plant (WWTP). The plant comprises preliminary treatment (screening, grit removal), activated sludge, tertiary treatment, and sludge treatment.

In this study, the fertilizer requirement for squash was applied. The pesticide was used to control the pathogens. For each irrigation event, the irrigation depth was applied and recorded

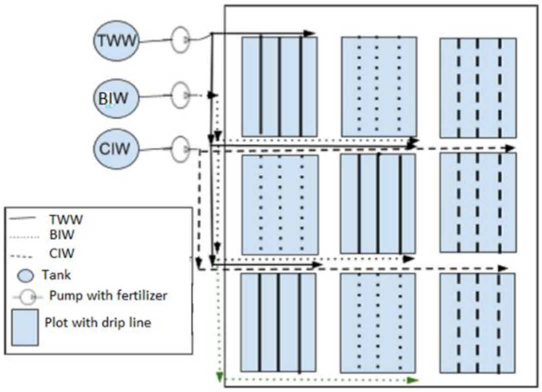

Figure 2. Experimental layout
(TWW: treated wastewater, BIW: blended wastewater, CIW: conventional irrigation water).

Water analyses.

During the experiment (January, February, and March 2021), water samples were collected from the holding tank in clean plastic bottles. Water sampling was conducted whenever the tanks are refilled. To analyze heavy metals, samples were collected in clean bottles and acidified to a pH of 2.0 using nitric acid, according to the American Public Health Organization's standards (APHA, 2017). Electrical conductivity (EC), turbidity (TUR), dissolved oxygen (DO), and pH were immediately measured on-site during sample collection using a conductivity meter (Mettler Toledo, model FP20 Meter); DO was measured using a dissolved oxygen meter (Lovibond SD 400 Optical); A turbidity meter was used to measure TUR (Mettler Toledo FSC402).

Total suspended solids (TSS) were measured using the filtration method, then drying the filtered sample at 105°C. Total nitrogen (TN as N) was measured using the macro-Kje1dahl method; Ion chromatography (Dionex DX-120) was used to analyze chloride, nitrate, phosphate, and sulfate; Potassium and Sodium were determined by a Flame photometer (Jenway Clinical PFP7); Calcium was measured by EDTA titrimetric method; Magnesium was measured by the difference between calcium and hardness (APHA, 2017). The sodium adsorption ratio (SAR) was calculated using Equation (1), according to Lesch and Suarez (2009); Inductively Coupled Plasma (ICP-OES - Perkin Elmer, Model 2000 DV) was used to measure heavy metals.

$$SAR = \frac{[Na^+]}{\sqrt{\frac{[Ca^{2+}]+[Mg^{2+}]}{2}}} \quad \ldots\ldots\ldots\ldots(1)$$

For microbiological analysis, water samples were collected in sterilized glass bottles with sodium thiosulphate. The samples were kept cold in an icebox below (10°C) during transport. multiple tube fermentation method (MTF) was used to count *E. coli* and *Total Coliforms* (APHA, 2017); The modified-Bailenger method was used to count *Helminth* eggs (Bailenger, 1979; Ayres *et al*., 1996). *Salmonellae* were measured as described by APHA (2017) and Collee *et al.* (1989); The BOD-5Day method was used to determine biological oxygen demand (BOD5), and the Closed Reflux, Titrimetric Method was used to measure chemical oxygen demand (COD) (APHA, 2017).

Soil analyses

Soil samples were collected from each plot at the end of the growing season, between the plants (emitters) for each depth of 0-20 cm, 20-40 cm, and 40-60 cm. Three soil samples were homogenized as composite samples from each plot for each depth. The air-dried soil samples were crushed and sieved using a 2-mm screen. According to the US Salinity Laboratory Staff (1954), the saturation paste extract and soil suspension were prepared, respectively, and were used to measure cations and anions.

ECe (dS m^{-1}) was measured as described by Richards (1954). A pH meter was used to measure the pH of the soil directly in a (1 soil: 1 water) solution (Jackson, 1958). After the soil solution was filtered using a filter paper

(Whatman No.1), sodium was measured directly by flame photometer according to APHA (2017); The sodium adsorption ratio (SAR) was calculated according to equation (1); Kjeldahl digestion was used to measure Total Nitrogen, then by distillation of steam (Jackson, 1958);

The Walkley-Black method was used in the determination of organic carbon. (FAO, 1974; Walkley, 1947). *E. coli* and *Total coliforms* were analyzed by the multiple tube fermentation method according to Turco (1994). The MPN table was used to calculate the most probable number (MPN) according to Cochran (1950). The results were expressed in MPN/g.

Yield and microbiological characteristics

Squash fruits were harvested every four days, from the middle rows of each treatment. Squash fruits were collected, weighed, and enumerated for every plant. For microbial analysis, composite samples (six medium-sized fruits, around 500 gm) were handled from the middle rows of each treatment. Gloves were changed to prevent contamination between plots. To detect *E.coli* / *Total Coliform*, 500 ml sterile of 0.1% buffered peptone water (BPW) aliquots were added to a nylon bag containing the vegetable composite sample. To suspend the microorganisms from the surface of the fruits, the sample was massaged and shaken for 2 minutes in a nylon bag to catch the microorganisms present on the surface of the fruit (Seow *et al.*, 2012). For each Appropriate dilution, 1 ml of sample was spread on chromogenic agar (Brilliance E. coli/coliform; Oxoid), following a 24-hour incubation time at 37°C, pink and violet colonies have been counted. Results were reported as colony-forming units per gram (cfu/g).

Salmonellae were measured by suspending the microorganisms from the surface of the fruits as described above (Seow *et al*., 2012). Then, filtering the composite sample through a 47 mm and 0.45 um, HA membrane filter, Millipore Corp, as described by APHA (2017). The filter membrane was then thoroughly blended with 100 ml of sterilized BPW (0.1 %), and then the sample was selectively enriched. The 0.1 ml of samples were streaked after enrichment and Biochemical and serological tests were used to confirm the isolates (colonies), according to Collee *et al*. (1989). The results were reported as colony-forming units per gram (cfu/g).

Helminth eggs were detected by preparing homogenate (BPW) as described above, after sediment for roughly 24 hours. The top water was removed, and the remaining wash water was centrifuged for 5 min at 2000 g. The sediment was carefully collected, and then they were gently shaken by hand in a saline physiological solution containing Lugol and were subsequently examined by light microscopy (Bailenger, 1979). Results were reported in terms of H. eggs/g.

Statistical analyses

Treatment effects were determined using analysis of variance (ANOVA). When the F ratio was significant, the Tukey-Kramer HSD (honestly significant difference) was used to compare the mean values of all parameters (0.05 probability level). Statistical analyses were performed with the program JMP software (Version 12, 2015, SAS Institute Cary, NC, USA). Deviation from the mean is presented in the tables of water quality as standard deviation.

Results and discussion

Irrigation water characteristics

Chemical and biological analyses were done to assess CIW and TWW content (Table 1). Most of the average characteristics of TWW and CIW used for irrigation (in mgL^{-1}) were within the limits in FAO recommended concentrations (Pescod, 1992), and the technical regulation of reclaimed domestic wastewater use, number 893/2021 in Jordan (JSMO, 2021). The turbidity of TWW (25 NTU) was higher than the maximum limits for irrigation (10 NTU). High TUR and TSS in TWW could cause emitter clogging, particularly if micro-irrigation is used (Li *et al*., 2013; Pescod, 1992). TWW electrical conductivity (2.06 dSm^{-1}) was 3.27 times higher than CIW electrical conductivity (0.63 dSm^{-1}). In general, TWW salinity is 1.5 – 2 times higher than freshwater salinity, according to Chen *et al*. (2013).

Table 1. Characteristics of irrigation water in the study.

Parameters	Conventional irrigation water (CIW)		Treated wastewater (TWW)		Jordanian Standards (JS 893/2021/Class 1)
ECw (dSm^{-1})	0.63	±0.05	2.06	±0.53	2.3
pH	7.69	±0.12	7.60	±0.16	6-9
Cl (mg L^{-1})	97.82	±2.67	202.91	±88.76	400
SO_4 (mg L^{-1})	58.62	±4.87	79.89	±16.88	500
HCO_3 (mg L^{-1})	57.96	±4.76	351.89	±134.89	400
P-PO_4 (mg L^{-1})	0.00	±0.01	18.69	±11.40	30
N-NO_3 (mg L^{-1})	3.52	±2.60	12.02	±3.18	30
N-NH_4 (mg L^{-1})	0.00	0.00	86.03	±16.83	N/A
K (mg L^{-1})	6.11	±1.26	47.44	±10.52	N/A
B (mg L^{-1})	2.08	±0.15	0.44	±0.07	1.0
Ca (mg L^{-1})	20.57	±1.72	91.67	±19.52	230
Mg (mg L^{-1})	23.97	±3.49	37.57	±8.57	100
Na (mg L^{-1})	74.22	±10.87	200.56	±27.02	230
SAR	2.39	±0.78	4.46	±0.29	9.0
T-N (mg L^{-1})	-	-	9.92	±1.60	N/A
TSS (mg L^{-1})	-	-	41.70	±12.80	50
TUR (NTU)	-	-	25	±27.00	10
BOD5 (mg L^{-1})	-	-	25.44	±4.44	30
COD (mg L^{-1})	-	-	59.92	±9.01	100
DO (mg L^{-1})	-	-	4.09	±3.58	>2
Cu (ppm)	< 0.008	-	< 0.008		0.2
Fe (ppm)	< 0.013	-	0.06	±0.00	5.0
Zn (ppm)	< 0.017	-	< 0.017	-	5.0
Mn (ppm)	< 0.017	-	< 0.017	-	0.2
Cd (ppm)	< 0.009	-	< 0.009	-	0.01
Cr (ppm)	< 0.005	-	< 0.005	-	0.1
Ni (ppm)	< 0.01	-	< 0.01	-	0.2
Pb (ppm)	< 0.008	-	< 0.008	-	0.2
TC (MPN100mL^{-1})	< 1.1	-	>1600000	-	N/A
E. coli (MPN 100mL^{-1})	< 1.1	-	57333	±38911	100
Salmonella (MPN L^{-1})	ND	-	ND	-	N/A
Nematode Eggs	ND		ND	-	≤ 1

ECw: electrical conductivity water; SAR: sodium adsorption ratio; BOD5: biochemical oxygen demand at 5 days; COD: chemical oxygen demand; TSS: TIN: total inorganic nitrogen; total solid suspended; DO: dissolved oxygen; NTU: Nephelometric Turbidity Units; TUR: turbidity; MPN: most probable number; CFU: colony-forming unit; ND: not detected

Boron (B) content of CIW (2.08 mg L^{-1}) was 4.73 times higher than TWW (0.44 mg L^{-1}) and exceeded the maximum limits for irrigation in JS893/2021 (1.0 mg L^{-1}) and FAO (2 mg L^{-1}). Squash is moderately tolerant to B (2.0-4.0 mg L^{-1}) (Pescod, 1992).

Table 1 indicates that TWW contains significant amounts of total nitrogen (T-N), phosphorous (P-PO_4), and Potassium (K) compared with the CIW, which are necessary for plant development and growth. In Jordan, wastewater can supply about 75% of the fertilizer needs of typical farms (Carr *et al*., 2011). Heavy metals including nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), zinc (Zn), and lead (Pb) were measured in irrigation water and were within acceptable values.

On the other hand, microbial pollution is one of the significant issues, which is directly related to the health risks of using TWW for agricultural irrigation. In terms of *Total Coliforms*, *E. coli*, *Salmonella*, and *Helminth* eggs, the microbiology quality of water was assessed. No microorganisms were detected in the CIW, while the mean concentrations of *E. coli* in TWW (5.7×10^4 MPN 100 ml^{-1}) were greater than the limit (100 MPN 100 ml^{-1}) required for irrigating vegetables according to JS 893/2021. *Salmonella* and *Helminth* eggs were absent in TWW (Table 1). These findings agreed with several studies that found no Salmonella in municipal TWW (Lonigro *et al*., 2016; Cirelli *et al*., 2012).

The absence of salmonella could indicate that the treated water eco-environment is harsher, more complex, and more dynamic. Numerous environmental conditions influence Salmonella's ability to survive and persist in water (Wanjugi and Harwood, 2013). In addition, the removal of suspended solids in WWTP aids in the control of pathogenic organisms and viruses and makes disinfection more effective. Because disinfectants such as chlorine and ozone react with organic compounds, thus pathogens become protected from disinfectants (Winward *et al*., 2008). These results reflect the treatment effectiveness of Kufranjah WWTP. These results agreed with the findings reported by Karpiscak *et al*. (2001).

Soil characteristics

Soil chemical properties

The impact of TWW irrigation on the soil's chemical properties is mainly reflected by the electrical conductivity (ECe). Soil salinity is undoubtedly a fundamental factor for soil suitability for crop production. Soil salts comprise a variety of dissolved chemicals that contribute to salinity stress, such as $CaSO_4$, $MgCl_2$, NaCl, Na_2SO4, $MgSO_4$, Na_2CO_3, and KCl (Munns and Tester, 2008). Squash is moderately sensitive to moderately tolerant to salt stress depending on the cultivar or growth stage (Francois, 1985).

Table 2. Some soil chemical proprieties [*].

Treatments	Soil Depth (cm)	ECe (dSm^{-1})	pH	TOC (%)	T-N (%)	SAR
TWW	0-20	1.15 a	7.83 ab	1.23 a	0.15 a	5.4 a
BIW		0.82 b	7.75 b	1.20 a	0.12 b	4.5 b
CIW		0.49 c	7.95 a	0.96 b	0.11 b	3.4 c
TWW	20-40	1.30 a	7.89 a	1.87 a	0.11 a	8.3 a
BIW		1.12 b	7.85 a	1.73 ab	0.09 a	6.4 c
CIW		0.70 c	7.80 a	1.27 b	0.07 a	7.5 b
TWW	40-60	1.75 a	7.77 a	1.22 a	0.09 a	13.8 a
BIW		1.62 a	7.80 a	0.98 a	0.08 a	9.2 b
CIW		1.1 b	7.75 a	0.64 a	0.06 a	9.8 b

[*] Means with the same letters in the same column are not significantly different at the 0.05 probability level, according to the Tukey-Kramer HSD (honestly significant difference) test

The results showed that soil ECe, for the average of all depths, was significantly higher for treatment irrigated with TWW compared to treatments irrigated with BIW and CIW by 18% and 83 %, respectively. In addition, all soil depths' average ECe for treatment irrigated using BIW was significantly higher than that for treatments irrigated using CIW by 55%. The significant increase in soil ECe in the TWW-irrigated plots (Table 2) resulted from the high concentration of salts in the TWW (2.06 dS m^{-1}) compared with CIW (0.63 dS m^{-1}). These results agreed with findings reported by Kaboosi (2017) and Qadir *et al*. (2000). Regardless of irrigation water, the soil ECe increases with increased depth, because of the high solubility of these salts in water.

The pH of the soil samples ranged between 7.75 and 7.95 (Table 2). The results showed no significant values between TWW and CIW irrigated plots. Urbano *et al*. (2015) reported the same finding for five cycles of lettuce fields irrigated using TWW. These findings could indicate that the soil has a buffering effect, thus, the pH value is steady, particularly in clay or organic-rich soil (Masto *et al*., 2009).

Organic matter constitutes a significant part of the soil, and its content is routinely used to evaluate soil fertility (Giusquiani *et al.,* 1995). Organic matter improves soil fertility while also increasing water-holding capacity and improving soil structure and microbial activity (Marinari *et al*., 2000). Soil organic matter plays a key role in global warming. As a result, sewage irrigation became one source of soil organic carbon in cropland, contributing to global carbon circulation (Rattan *et al.*, 2005).

The results showed that soil total organic carbon (TOC), the average of depths, was significantly higher for TWW-irrigated plots compared with treatments irrigated with CIW by 39% (Table 2). The higher concentration of OM in TWW resulted in a significant increase in TOC in the TWW-irrigated plots. These results agreed with the previous findings reported by Bedbabis *et al.* (2014) and Rattan *et al*. (2005). Trost *et al.* (2013) reported a rise of 11% to 35% in soil organic carbon in semiarid regions, regardless of irrigation water type. The results showed that soil TN (Table 2), at the top layer (0-20 cm) was significantly higher for treatment irrigated with TWW compared with treatments irrigated with CIW by 36.4%. Whereas no significant difference in TN for the deeper depths between treatments. These results agreed with the findings reported by Guo *et al*. (2017) and Becerra-Castro *et al*. (2015). TWW irrigation can add the amount of TN to soils as much as, or even more than, what is normally applied by freshwater fertilization (Feigin *et al*., 1991).

The SAR concentration, the average of depths, was significantly higher with TWW-irrigated plots compared with treatments irrigated with CIW by 33% (Table 2). However, the results were below the level for soil to be classified as sodic. These results agreed with the findings reported by Al-Hamaiedeh and Bino (2010), Bedbabis *et al*. (2014), Hentati *et al*. (2014), and Sou *et al*. (2013). Petousi *et al*. (2019) studied the impacts of using secondary TWW irrigation, and the results revealed no significant differences in soil properties compared with control, except for SAR and the EC, which were slightly higher in TWW soil samples. Most of these studies attributed the high SAR to the salinity of TWW, limited rainfall, high evaporation rates, and lack of drainage infrastructure all contributing factors.

Soil microbial characteristics.

The *E. coli* was not detected, while the *Total coliform* was 7 MPN g^{-1} in the soil before the beginning of the experiment. Garc´ıa-Orenes *et al*. (2007) reported that the decrease of soil water content under semiarid conditions could be the main factor in the loss of coliform. After harvesting, *Total coliform* and *E. coli* increased significantly in TWW irrigated plots compared to CIW plots, where *Total coliform* and *E. coli* increased from 18 to 140 and from 1.8 to 31 (MPN g^{-1}) (Table 3), respectively. These results agreed with the findings reported by Petousi *et al*. (2019), Farhadkhani *et al*. (2018), Al-Rashidi *et al*. (2013), Gerba and Smith (2005), and Malkawi & Mohammad (2003).

Table 3. Soil microbial characteristics after harvesting (*)

Treatments	*Total coliform* (MPNgm^{-1})	*E.coli* (MPNgm^{-1})
TWW	140 a	31.0 a
BIW	26 b	1.8 b
CIW	18 b	1.8 b

(*) Means with the same letters in the same column are not significantly different at the 0.05 probability level, according to the Tukey-Kramer HSD (honestly significant difference) test.

Yield and microbiological characteristics.

A-yield

In this study, even though it was no significantly different in yields between treatments irrigated with TWW and CIW (Table 4), squash yield in BIW (27.4 ton ha^{-1}) irrigated plots was tangibly lower than that in TWW (30 ton ha^{-1}) and CIW (31.9 ton ha^{-1}) by 9.5%, and 16.4%, respectively (Table 4). These results differ from the findings by Bouhoum and Amahmid (2002) and Makhadmeh *et al*. (2021) which showed that the squash yield increased in response to irrigation with TWW as compared to control. In contrast, some studies showed no significant differences in crop yield between TWW and freshwater (Vergine *et al*., 2017; Mok *et al*., 2014; Shahalam *et al*., 1998). On the other hand, several studies showed a significant decrease in crop yield (Gao *et al*., 2021; Vergine *et al*., 2017; Elamin and Yaseen, 2008). In this study, the reduction of BIW yield compared to TWW and CIW may be interpreted for several reasons: the difference in the wastewater quality, soil properties, growing season, or management methods.

Table 4. Impact of irrigation water quality on squash yield (*)

Treatments	Yield (ton ha^{-1})	Yield (fruits ha^{-1})
TWW	30.0 a	337,607b
BIW	27.4 b	361,645ab
CIW	31.9 a	367,521a

(*) Means with the same letters in the same column are not significantly different at the 0.05 probability level, according to the Tukey-Kramer HSD (honestly significant difference) test

In this study, the results showed that the number of squash fruits decreased significantly for treatment irrigated with TWW (337,607 fruits ha^{-1}) compared with treatments irrigated with CIW (367,521 fruits ha^{-1}) by 8.14% (Table 4). These results agreed with the findings reported by several authors (Pedrero *et al*., 2018; Nicol´as *et al.*, 2016; Maloupa *et al*., 1997). Maloupa *et al*. (1997) showed the total number of tomato fruits of plants fertigated with TWW effluent decreased by 10%.

B- Microbiological quality:

Microbiologically, the harvested squash was analyzed. *Total Coliforms* were significantly higher on the surface of squash in the TWW irrigated plots (41 cfu g^{-1}) compared with BIW (40 cfu g^{-1}) and CIW (12 cfu g−1), respectively (Table 5). No significant increase in *E. coli* was observed between treatments. These results agreed with the findings reported by several authors, especially regarding drip irrigation techniques (Christou *et al*., 2014; Urbano *et al.,* 2017; Orlofsky *et al*., 2016; Li and Wen, 2016; Lonigro *et al*., 2016; Shock *et al*., 2016; Cirelli *et al.,* 2012).

Pathogen indicators, such as *Salmonella* and *Helminth* eggs, were not found (Table 5). Lonigro *et al*. (2016) reported the same results. Chen *et al*. (2013) reported that there is limited evidence of the spread of the disease using TWW for agricultural irrigation. In Contrast, *E. coli* recorded a significant increase for radishes under the drip and furrow system, but *Salmonella* was absent (Bastos & Mara 1995). The presence of *E. coli* in the CIW irrigated plots could be attributed to different sources of contamination, such as roaming animals, and birds (Venglovsky *et al*., 2006). In addition, contact between TWW irrigated soil and fruits may increase contamination potential (Cirelli *et al*., 2012). The contrast in the different studies' results could be because of the difference in the TWW quality, growing season, or management method.

To reduce the potential of microbial contamination, it is recommended to reduce the exposure of workers to wastewater. Some of the applied measures use drip irrigation, others rely on stopping irrigation before harvest. These could play a significant role in the successful application of TWW for irrigation. A period without irrigation before harvest (1-2 weeks) can allow the die-off of bacteria and viruses to improve the quality of irrigated crops to levels seen in crops irrigated with fresh water, as reported by Vas Da Costa Vargas *et al.* (1996). However, this option is workable for crops that are harvested once (destructive harvested crops), and unworkable for (multiple harvested crops) vegetables that need harvesting daily, because farmers will probably not stop irrigation of leafy salad crops five days or more before harvest (Lamm *et al*., 2002; Aiello *et al*., 2007).

Table 5. Squash's microbiological characteristics (*).

Treatment	Total coliform (cfu g^{-1})	E.coli (cfu g^{-1})	Salmonella (cfu g^{-1})	H.eggs (H.egg g^{-1})
TWW	41 a	1.3 a	ND	ND
BIW	11 b	1.0 a	ND	ND
CIW	12 b	1.3a	ND	ND

(*)Means with the same letters in the same column are not ot significantly different at the 0.05 probability level, according to the Tukey-Kramer HSD (honestly significant difference) test.

Conclusions

Water reuse is essential in Jordan. Treated wastewater contributes significantly to the country's limited irrigation water supply, allowing agriculture to sustain in some areas. The study showed the effects of irrigation with TWW on some chemical and microbial properties of the soil. TWW increased the soil OM and soil TN, specifically in the surface soil. TWW induced a significant accumulation of salinity in the soil layers. Microbiological analysis of soil showed that *Total coliform* and *E. coli* were significantly higher in TWW irrigated plots compared with CIW plots. On the other hand, squash showed significant contamination by Total Coliform. *E.coli* did not show any significant difference between treatments. Pathogens were absent such as Salmonella and Helminth eggs. Although of these results, a period of stopping irrigation should be scheduled before harvest.

Finally, the results of this study indicated that the use of TWW with drip irrigation for squash production could be feasible with good effluent quality. Another key fact is that TWW and BIW treatments tended to lower fruit numbers. However, this fact needs more investigation into the impact of the compositions of wastewater.

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Disclosure statement

The authors reported no potential conflict of interest.

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تأثير الري بمياه الصرف الصحي المعالجة على محصول الكوسا والخصائص الكيميائية والميكروبية للتربة

حسن سالم الخزاعلة[1]، احمد محمد أبو عواد[2]، محمد إبراهيم القنة[3]

[1،2] الجامعة الأردنية، عمان، الأردن.
[3] الجامعة الهاشمية، الزرقاء، الأردن

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ملخص

اجريت هذه الدراسة لتقييم تأثير الري بمياه الصرف الصحي المعالجة على الصفات الكيميائية والميكروبيولوجية للتربه والتلوث الميكروبي وانتاجية محصول الكوسا. تم ري شتلات الكوسا باستخدام مياه الري التقليدية والمعالجة ومياه الري المخلوطة (50%) باستخدام نظام الري بالتنقيط. كان تركيز جميع مكونات مياه الري الكيميائية والميكروبية يقع ضمن حدود المواصفات الأردنية، باستثناء التعكر والبورون. لم يتم العثور على مؤشرات الممرضات؛ السالمونيلا، وبيض الديدان الطفيلية في مياه الصرف الصحي المعالجة. أظهرت تحاليل التربة زيادة معنوية لكل من الموصلية الكهربائية والكربون العضوي والنتروجين الكلي ونسبة امتصاص الصوديوم في الارضي المروية بالمياه المعالجة مقارنة بالمياه التقليدية. زاد محتوى التربة الكلي للبكتيريا القولونية والإشريكية القولونية بشكل معنوي في الأراضي المروية بالمياه المعالجة. لم يظهر وزن المحصول الطازج للكوسا المروية بالمياه المعالجة أي فرق معنوي مقارنة بالمياه التقليدية. تميل معاملات المياه المعالجة والمخلوطة نحو عدد اقل للثمار. لم تكن الإشريكية القولونية مختلفة معنوياً على سطح ثمار الكوسا، بينما زاد عدد القولونيات الكلية معنوياً للثمار داخل الأراضي المروية بالمياه المعالجة. لم يتم الكشف عن بيض السالمونيلا والديدان الطفيلية.

الكلمات الدالة: المياه العادمة المعالجة، الإشريكية القولونية، العامل الممرض، التملح، الكوسا، الاردن