Treated wastewater irrigation of tomato: Effects on crop production, and on physico-chemical properties, SDH activity and microbiological characteristics of fruits

Soukaina Ouansafi[1*], Fatima Bellali[2], Hind Maaghloud[1], Mostafa Kabine[1], Fahde Abdelilah[1]

(1. Department of Biology, Health and Environment Laboratory, Hassan II University, Aîn Chock Science Faculty, P.O. Box: 5366, Casablanca, Morocco;

2. Department of Biology, Biological Engineering Laboratory, Sultan Moulay Slimane University, Faculty of Sciences and Techniques, P.O. Box: 523, Beni Mellal, Morocco)

Abstract: The aim of the present investigation was to evaluate the effects of irrigation by treated domestic and industrial wastewater on the crops production of tomato plants, and on the physicochemical properties and the microbiological properties of tomato fruit. Succinate dehydrogenase (SDH) activity was also monitored in the leaves, roots and fruit. The physico-chemical characteristics and microbiological properties of the soil and the irrigation water samples were determined. The results reported in the present study show that the irrigation water source was significantly affect yield of tomato crops and quantitative parameters of tomato fruits (i.e number of fruits and the weight of fresh fruits). However, with treated wastewater, the tomato growth was higher as compared to other treatments. The physicochemical composition of tomato fruits in all treatment was recorded levels below the FAO permissible limits in tomatoes. For the microbiological results, no *Escherichia coli, Fecal Streptococci*, *Vibrio cholerae* and *Salmonella* isolated in both treatment (Treated wastewater and potable water) and total coliforms and fecal coliforms were below to acceptable limits for vegetable (<4 log CFU g^{-1}). So, the microbial quality of tomato fruits was very good.

Keywords: treated wastewater, tomatoes crops, microbiological properties, succinate dehydrogenase activity, fruits

Citation: Ouansafi, S., F. Bellali, H. Maaghloud, M. Kabine, and F. Abdelilah. 2022. Treated wastewater irrigation of tomato: Effects on crop production, and on physico-chemical properties, SDH activity and microbiological characteristics of fruits. Agricultural Engineering International: CIGR Journal, 24(1): 13-25.

1 Introduction

Faced with the threat of water shortage, several European and African countries in the Mediterranean region can no longer meet their populations' continuously increasing food requirements, especially those countries characterized by an arid or semi-arid climate where the quantity of good quality water is not sufficient to irrigate agricultural products. This is the case of Morocco, a country whose Mediterranean climate tends to become semi-arid in certain regions, notably Casablanca-Settat, which alone has a population estimated at 6 861 739 habitants (RIC, 2018). Thus, agriculture has always had the challenge of producing more and while using 80% of the fresh water available. Consequently, some farmers use raw waste water for the

Received date: 2020-11-15 **Accepted date:** 2021-05-09

***Corresponding Author: Soukaina Ouansafi,** Ph.D, Department of Biology, Health and Environment Laboratory, Hassan II University, Aîn Chock Science Faculty, P.O. Box: 5366, Casablanca, Morocco. Tel.: +212664947927. Email: ouansafi.soukaina@gmail.com.

irrigation of their fields even though this practice is not regulated (Mateo-Sagasta et al., 2013). Hence the need to adopt strategies and procedures that protect the environment and that could perfectly meet the crop nutrient requirements. Among these procedures, we find the orientation towards unconventional water resources such as the use of treated wastewater rich in fertilizers which would considerably reduce the quantity of fertilizers used (Vergine et al., 2017).

Work in this direction has been carried out on an international scale, in order to evaluate the different impacts of the use of treated wastewater on the morphological aspects of microbiological contamination of tomatoes. In fact, this water reuse has consequences; there is a danger that groundwater and agricultural soils with their soil fauna could be contaminated by the presence of significant levels of pathogenic microorganisms. There is even a risk that the metabolic activities of plants and their physiological functions may be disrupted (Gatta et al., 2018). Additionally, the use of treated wastewater contaminated by certain parasites can cause several more or less serious public health risks (WHO, 2006).

The aim of the present investigation was to determine the effect of irrigation by treated wastewater (TWW), as compared to potable water (PW) and untreated wastewater (UWW), on tomatoes crops under surface drip irrigation systems. In particular, the objectives of the study were to evaluate the effect of the water's samples (PW, TWW and UWW) on: (i) tomato crop production; (ii) the physicochemical fruits properties; (iii) succinate dehydrogenase (SDH) (EC 1.3.5.1) activity; (iv) the microbiological characteristics of tomato fruit. In this study, the tomato belonging to the *Solanaceae* family has been chosen precisely because it is known to have substantial water demands and a high sensitivity to water stress (Zheng et al., 2013).

2 Materials and methods

2.1 Plant material and culture conditions

The field trial was carried out with the tomato (*Solanum lycopersicum*) and the variety chosen is Tomaland during the growing season of 2017 (April to August). The tomato seeds were placed in alveolate trays containing pot at and irrigated daily with drinking water. As soon as seedlings at the 6-leaf stage were obtained, they were transplanted in each plot and irrigated this time with the irrigation treatments (PW, UWW and TWW). A schematic representation of the agriculture system of tomatoes crop is shown in Figure 1.

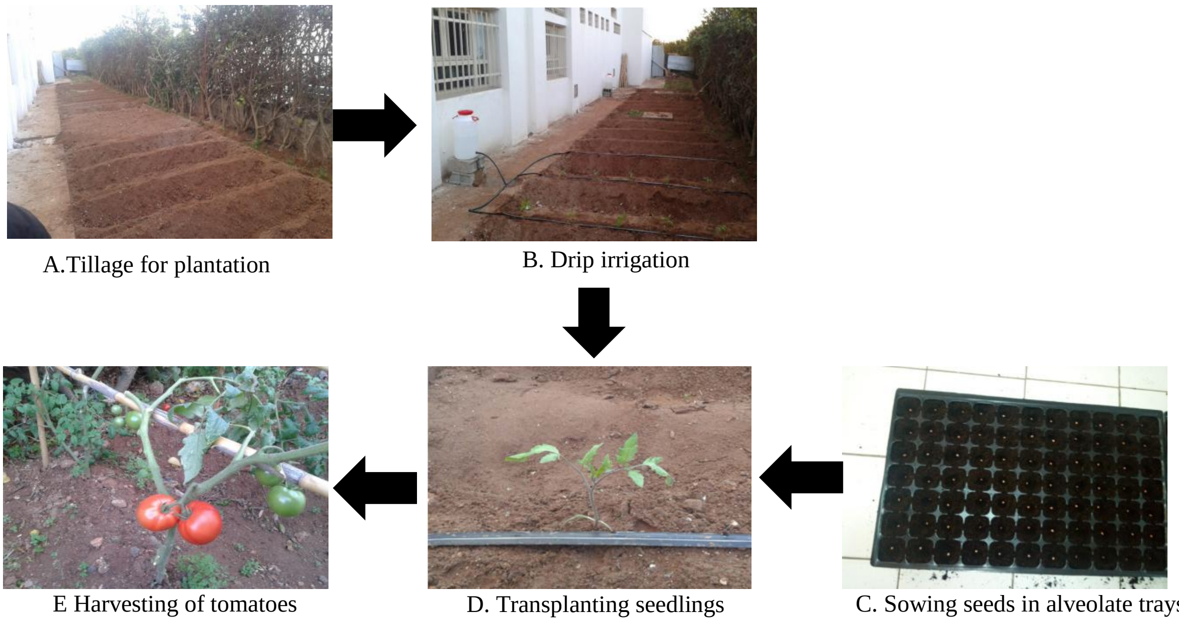


Figure 1 Schematic representation of the agriculture system of tomatoes crop

2.2 Treatments and experimental design

A field trail was carried out at the Ain Chock Faculty of Science of the Hassan 2 University, in Casablanca. In this study, the soil at the experimental site was chosen, because it showed in previous study that it was suitable physicochemical potential and favorable to agricultural activities (Ouansafi et al., 2019).

Three experimental irrigation treatments were applied to the tomato plants: irrigation with potable water (PW), irrigation with UWW and irrigation with TWW. The PW was from a water source that is commonly applied for crop irrigation in the experimental area. The TWW used in this study was taken from the WWTP in the province of Mediouna. This station receives waste water of various types: domestic, industrial and rainwater. The TWW is discharged into the Oued Hassar stream, used by the population of Mediouna Province for agricultural purposes (Ouansafi et al., 2019).

The experimental field was arranged according to a randomized plot design with three treatments (PW, UWW and TWW), each one replicated three times for a total of 9 plots (3 irrigation treatments × 3 replicates). Each plot was 10 m² (2.5 m wide × 4 m long).

The main plot treatments consist of irrigation systems drip. Each tomato plot comprised three rows of plants and accordingly three irrigation lines with 0.80 m spacing between lines and 0.4 m spacing between drip emitters within drip lines. The space separated two closely spaced plots is 1.5 m to avoid any contact between them. The irrigation amount was controlled, with the head flow was 4 L h^{-1}.

2.3 Water, soil and plant physicochemical analysis

When the seedlings transplanted into the plots were irrigated, three samples of each type of water used were taken in order to perform the necessary physicochemical and microbiological analyzes. All the water samples (PW, UWW and TWW) were analyzed according to standard methods (APHA, 2005) for the physicochemical parameters. The analysis included the physicochemical parameters of pH, electrical conductivity (ECw; µs cm^{-1}), total sus- solids (TSS; mg L^{-1}), biological oxygen demand over 5 days (BOD5; mg L^{-1}), chemical oxygen demand (COD; mg L^{-1}), nitrate-nitrogen (NO_3-N; mg L^{-1}), nitrite-nitrogen (NO_2–N; mg L^{-1}), total phosphorus (Total P; mg L^{-1}), orthophosphorus (PO_4-P; mg L^{-1}), nitrogen Kjeldahl total (NTK; mg L^{-1}), sulphate (SO^{4-}; mg L^{-1}) and Fluorides (F-, mg L^{-1}).

Soil sampling was carried out two days before transplanting. In the plot, eight samples were taken at a depth of 30 cm and then dried, crushed with mortar, then sieved to 2 mm and finally subjected to chemical and biological analyzes. Soil sample from experimental plot was analyzed for the physicochemical parameters. The soil electrical conductivity (mmhos cm^{-1}), pH (H_2O) and pH (KCl) were measured on aqueous soil extracts (ASE) using an electrical conductivity meter and pH meter. The organic Matter (OM, %) was determined by dichromate oxidation method (Walkley and Black, 1934). The ammonium-Nitrogen (NH_4-N, mg kg^{-1}) and nitrate nitrogen (NO_3-N, mg kg^{-1}) were determined according to Bremner and Keeney (1996). The chloride ion (Cl^-, mg kg^{-1}) and sodium ion (Na^+, mg kg^{-1}) in ASE were determined by titration (Richards, 1954). The phosphorus pentoxide (P_2O_5, mg kg^{-1}) was estimated by Olsen's method (Olsen and Sommers, 1982). The potassium oxide (K_2O, mg.kg^{-1}), magnesium oxide (MgO, mg kg^{-1}) and calcium oxide (CaO, mg kg^{-1}) were analyzed by fluorescence through X rays (Öblad et al., 1982). The total carbonate (Total $CaCO_3$, %) was determined according to the method described by (Black et al., 1965) while active carbonate (Active $CaCO_3$, %) was determined by the method of Loeppert and Suarez (1996).

The fruits samples were analyzed for soluble solids content (SSC; %), titratable acidity (TA; %) (AOAC, 1995), and sulfur, Ca^{2+}, Na^+, Mg^{2+}, K^+ and nitrate NO_3^- content. Protein content were estimated by the method of Bradford (1976). Vitamin C was determined by using 2.6- diclorofenol indofenol dye titration method (Ranganna, 1997). Sugar was estimated by the method of Dey (1990). Lycopene content was determined according to Alda et al. (2009) with few modifications. The lipid content was determined by the method of AOAC (2002).

2.4 Plant growth measurements

All the measurements carried out at the plant level concerned twelve individuals and that for each category of plants used.

The leaf length and width, stem length and diameter of tomato plant in each plot were recorded every month. The number of flowers, number of fruits and fresh weight of the fruit was measured at the end of the experiment.

2.4.1 Bacteriological analyses

Microbiological analysis of the water and tomato fruit samples from each experimental treatment included the determination of the parameters: *Total coliform*, *Fecal coliform*, *Escherichia coli*, *Fecal streptococci*, *Vibrio cholera* and *Salmonella* spp., which are useful indicators of contamination (Tallon et al. 2005). However, soil sample was analyzed also for the enumeration of the bacteria: *Escherichia coli*, *Fecal coliforms* and *Mesophilic bacteria*.

Fecal and *total coliform* counts were performed using tube fermentation method (APHA, 2005). *Escherichia coli* were enumerated using membrane filter procedure and mTEC Agar (Difco 0334) as described by EPA (2002). *Salmonella* were identified using MPN technique (El-Lathy et al., 2009). Total Vibrios was detected and enumerated by MPN technique according to Koch (1994) and APHA (2005).

2.4.2 Helminth eggs analysis in irrigation water samples

Helminth eggs was detected in water samples by the technique of Bailenger (1962). Microscopic observation of helminth eggs in sample was performed in Mac Master Counting cell at 100-fold magnification.

2.4.3 Isolation of mitochondria and measurement of succinate dehydrogenase (SDH) activity

The mitochondria of tomato fruit, roots and leaves were isolated using the method of Romani et al. (1969), with slight modifications. Plant material was homogenized in grinding buffer (0.25 M sucrose, 50 mM potassium phosphate (pH 7.2), 6 mM ethylenediamine tetraacetic acid (EDTA), 10 mM β-mercaptoethanol, 0.5% soluble polyvinylpyrrolidone (PVP), and bovine serum albumin (BSA) at 1 mg·mL^{-1}). The homogenate was filtered through two layers of cheesecloth and centrifuged at 2000× g for 10 min. The supernatant was further centrifuged at 10 000g for 15min. The pellet was resuspended in Suc wash medium (0.25 M sucrose, 50 mM potassium phosphate (pH 7.2), and BSA at 1 mg·mL^{-1}) to give the final mitochondrial fraction.

The choice of biomarker fell on the succinate dehydrogenase SDH (EC 1.3.5.1), a key enzyme involved in two vital processes (the krebs cycle and the respiratory chain), and whose physiological roles strongly influence the phenomenon of photosynthesis, the function of stomata, and plant root system elongation (Huang and Millar, 2013).

The SDH (EC 1.3.5.1, complex II) activities of the mitochondrial suspension was measured by the methods of Frenkel and Patterson (1973), with some modifications. Isolated mitochondria were used in a 1-mL reaction mixture containing 100 mM potassium phosphate (pH 7.2), 10 mM KCN, 6 mM phenazine methosulfate, 20 mM succinate, and 0.2 mM dichlorophenol indophenol. The reaction was started by the addition of 100 µl of the mitochondrial preparation to the reaction mixture. SDH activity was determined spectrophotometrically at 600 nm.

2.4.4 Statistical analysis

Results were expressed as average ± SEM (standard error mean) and statistically analyzed using Minitab.16. A statistical analysis was performed by using one-way analysis of variance of (ANOVA) followed by Tukey’s Multiple Comparison Test. Dunnett’s test was performed to detect statistical differences of microbial levels between all the studied samples. Significant differences were considered when $p< 0.05$.

3 Results and discussion

3.1 Soil sample properties and qualities

3.1.1 Physicochemical properties of the soil sample

The results of the physicochemical properties of soil at the experimental field are shown in Table 1. The average pH values of the sample were in pH (H_2O) 7.89 and in pH (KCl) 7.49 which indicate the soil was slightly alkalic. The soil pH affects the mobility and bioavailability of heavy metals in the soil (Violante et al, 2010). Thus, by increasing the soil pH, the mobility of heavy metals decreased due to the precipitation of hydroxides, carbonates or the formation of insoluble organic complexes (Violante et al., 2010).

The conductivity value in soil sample was relativity higher than values reported by Moroccan Standards. Increasing the conductivity in soil enhance the solubility of HM, which can be causing larger availability of metals for plants (Singh et al., 2009). The mean of organic matter (2.17%) was found same than the Standard Limits. Additionally, the average values of total $CaCO_3$, active $CaCO_3$, NH_4-N, NO_3-N and MgO were 11.4, 4.2, 1, 9 and 246.5 mg kg^{-1} respectively. These values were within the safe limits. In comparison with the tolerance limit of Moroccan Standards for agricultural soils, it was found that the average values of CaO, Cl^-, P_2O_5 and K_2O were higher than the limit values.

Table 1 Average values of the physicochemical parameters of the soil samples and reference limits

Parameters	Unit	FS	Limit values **
Organic Matter	%	2.17± 0.02	1.5-3
EC 1/5	mmhos cm^{-1}	0.691 ± 0.01	< 0.5
pH (H_2O)	-	7.89 ± 0.01	6.5-7.5
pH (KCl)	-	7.49±0.01	5.3 < pH
Total $CaCO_3$	%	11.4±0.1	< 15
Active $CaCO_3$	%	4.2±0.01	< 10
CaO	mg kg^{-1}	7210±46.37	2500-5000
NH_4-N	mg kg^{-1}	1±0.02	-
NO_3-N	mg kg^{-1}	9±0.01	20-30
Cl^-	mg kg^{-1}	174.3±0.82	60-150
Na^+	mg kg^{-1}	188.7±6.15	200-480
P_2O_5	mg kg^{-1}	68.7±6.08	30-60
K_2O	mg kg^{-1}	629.6±29.49	140-360
MgO	mg kg^{-1}	246.5±29.64	250-500

Note: Average ±SD. **: limit values according to Moroccan standards of agricultural soils. The means are the average of height samples. FS: Faculty Soil.

3.1.2 Microbial contamination in soil sample

The average number of *mesophilic bacteria* and *total coliforms* in the soil sample were 4.76×10^6 and 3.11 log CFU g^{-1} respectively (Table 2). The *Escherichia coli* were absent in the samples, indicating that soil samples were not contaminated and it can reduce the possibilities for contamination of the fruit by microbial of the soil (Bernstein et al., 2007).

Table 2 Bacteriological parameters in soil sample

Bacteriological parameter	Unit	FS
Escherichia coli	log CFU g^{-1}	0±0.0
Faecal coliform	log CFU g^{-1}	3.11±0.6
Mesophilic bacteria	----------------	4.76×10^6±0.3

Note: The data are presented as the average ± S.D

3.2 Irrigation water qualities

3.2.1 Physicochemical properties of irrigation water

The physicochemical proprieties of PW, TWW and UWW, which were used for irrigation of tomatoes, are shown in Table 3. The results showed the UWW was characterized by higher concentration of nutrients than TWW. The average pH value of UWW indicated slightly acidic nature (pH=6.9) with very high electrical conductivity (> 3000 µs cm^{-1}) as compared to PW and TWW (Pescod, 1992). The electrical conductivity (EC) (1032, 1225, 906 and 1099 µs cm^{-1}) indicated the salinity of the water (Rattan et al., 2005).

The average values of the Biological Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD) in UWW were 525 mg $L^{-1}$and 1439.7 mg L^{-1} respectively. These values were very high when compared to the FAO value (Pescod, 1992) and the S.E.E.E values (S.E.E.E, 2007). The average values of Nitrate-nitrogen (NO_3–N), Orthophosphorus (PO_4-P) and Total Phosphorus (Total P) in PW and TWW were 3.44 mg L^{-1}, 0.0092 mg L^{-1}, 0.0095 mg L^{-1} and 21.8 mg L^{-1}, 5.1 mg L^{-1} and 8.58 mg L^{-1} respectively. In comparison with the tolerance limit of pH for irrigation water, it was found that these values are equivalent the norms limits. However, the NO_2–N of TWW was significantly higher than for UWW. The amount considerable of NO_2–N and phosphate in TWW can provide an important source of nutrient for plant growth and soil fertility. The average values of Fluorides (F^-), the all water samples range from

0.005, 0.22 to 0.62 mg L^{-1} which are equivalent to the standard values.

Table 3 Physico-chemical analysis of treated wastewater (TWW), potable water (PW) and untreated wastewater (UWW) used for the tomato irrigation

Physicochemical parameters	Unit	PW	UWW	TWW	Limit values
pH	-	7.56 ± 0.07[a]	6.97±0.065[c]	7.39±0.8[b]	6.5- 8.4*
TSS	mg L^{-1}	0± 0[a]	453±10.15[c]	29.7± 4.16[b]	200*
EC	μs cm^{-1}	1366 ±36.75[a]	5230 ±50[c]	3913.7± 71.49[b]	12000*
COD	mg L^{-1}	0±0[a]	1439.7±16.50[c]	86.7± 10.79[b]	90*
BOD_5	mg L^{-1}	0±0[a]	525.7±13.01[c]	22.39± 7.24[b]	30*
NTK	mg L^{-1}	0±0[a]	91.7±9.07[c]	22.01± 2.81[b]	-
Total P	mg L^{-1}	0.0095±0.0005[a]	14.2±1.17[c]	8.58± 0.82[b]	-
PO_4-P	mg L^{-1}	0.0092±0.001[a]	10.06 ±0.975[c]	5.1± 0.4[b]	2**
SO_4^-	mg L^{-1}	89± 5.29[a]	361.3 ±8.0829[c]	275.7± 0.64[b]	250*
NO_3-N	mg L^{-1}	3.44± 0.46[a]	35.61± 0.82[c]	21.8± 1.05[b]	30*
NO_2-N	mg L^{-1}	0.001±0.01[a]	0.07± 0.01[c]	0.1± 0.025[b]	5*, 3**
F^-	mg L^{-1}	0.005± 0.0008[a]	0.62±0.047[c]	0.22± 0.102[b]	1*

Note: The data are presented as the average ± S.D; different letters indicate significant differences by the Tukey multiple comparison test ($p < 0.05$). *: (S.E.E.E; 2007) **: (Pescod, 1992)

3.2.2 Microbiological properties of irrigation water

The water samples used for irrigation were analysis whether they contained Bacteroides and Helminths (Table 4). The bacteria identified in the UWW and TWW included Escherichia coli, total coliforms and *fecal coliforms* while PW showed no growth of bacterial. Also, the Helminths eggs were detected in the UWW but not in the PW and TWW. Salmonella sp., *fecal streptococci* and vibrio cholera were not detected in all water samples tested. The values of the total coliforms and fecal coliforms obtained for TWW are well below the standard limits for irrigation water (1000 100 mL^{-1}). Theses lowly values of the coliform obtained for TWW could be attributed after chlorine treatment. In addition, total coliforms and fecal coliforms count presented significant different among the three irrigation water treatments. Therefore, the TWW was not contaminated.

Table 4 Average number of bacteria and helminths detected in water samples

Type of microorganism	PW	UWW	TWW	Limit value
Helminth eggs (Eggs L^{-1})	0±0.0	5*±0.3	0*±0.6	Absence [b]
Total coliforms (CFU 100 mL^{-1})	0±0.0	37*±0.9	16*±0.5	1000 100 mL^{-1} [a]
Fecal coliforms (CFU 100 mL^{-1})	0±0.0	17*±0.7	7*±0.4	1000 100 mL^{-1} [a]
Escherichia coli (CFU 100 mL^{-1})	0±0.0	10*±0.5	4*±0.6	a
Fecal Streptococci (CFU 100 mL^{-1})	0±0.0	0±0.0	0±0.0	a
Vibrio cholerae (CFU 450 mL^{-1})	0±0.0	0±0.0	0±0.0	Zero per 450 mL [b]
Salmonella sp (CFU 5000 mL^{-1})	0±0.0	0±0.0	0±0.0	Zero per 5 L [b]

Note: The data are presented as the means ± S.D; The asterisk indicates significant differences from potable water according to Dunnett's test ($p < 0.05$).[a]: (Pescod, 1989). [b]: Moroccan limit standards.

3.3 Tomatoes production

Four months after transplanting, the tomato plants irrigated with different types of water were evaluated for different agronomical parameters (diameter, length, weight, number). Figures 2 and 3 show a significant increase in leave and stem diameters by the use of TWW irrigation treatments. The highest value was observed in tomato plants irrigated with TWW (5.5 and 4 cm respectively), whereas UWW irrigation treatment had the lowest diameter. The highest stem length was observed in the tomato plants irrigated with TWW and PW (73.8, 73 cm respectively), while were higher than plants irrigated with UWW (65 cm). Plants irrigated with UWW have shown the lowest leaves length (9.5 cm), relative to those irrigated with the TWW (12 cm).

The number of fruits, number of flowers and the weight of fresh fruit per tomato plants are the most important yield attributes in tomato (Pandey et al., 2006). The data pertaining to fresh fruit weight per tomato plants was presented in Table 5. Tomato plants irrigated with PW have shown the highest fresh fruit weight (162 g) followed by those irrigated with TWW (155 g). However, tomato plants irrigated with UWW have shown the lowest fresh weight (140 g). Thus, from the data it is evident that there was significant beneficial effect of TWW when compared with PW or UWW on number of fruits and flowers and weight fruit per tomato plants. Effect of irrigation water with TWW on production of tomato plants due to the content of phosphorus (P) and potassium (K) contained in water and soil. Sarief (1985) showed that the increase in yield of plants is more influenced by the availability of nutrients P and K. Novizan (2005) indicated also the P element functions more for accelerating flowering and increasing fruit production. These results showed that irrigation with TWW may be improving the tomato production.

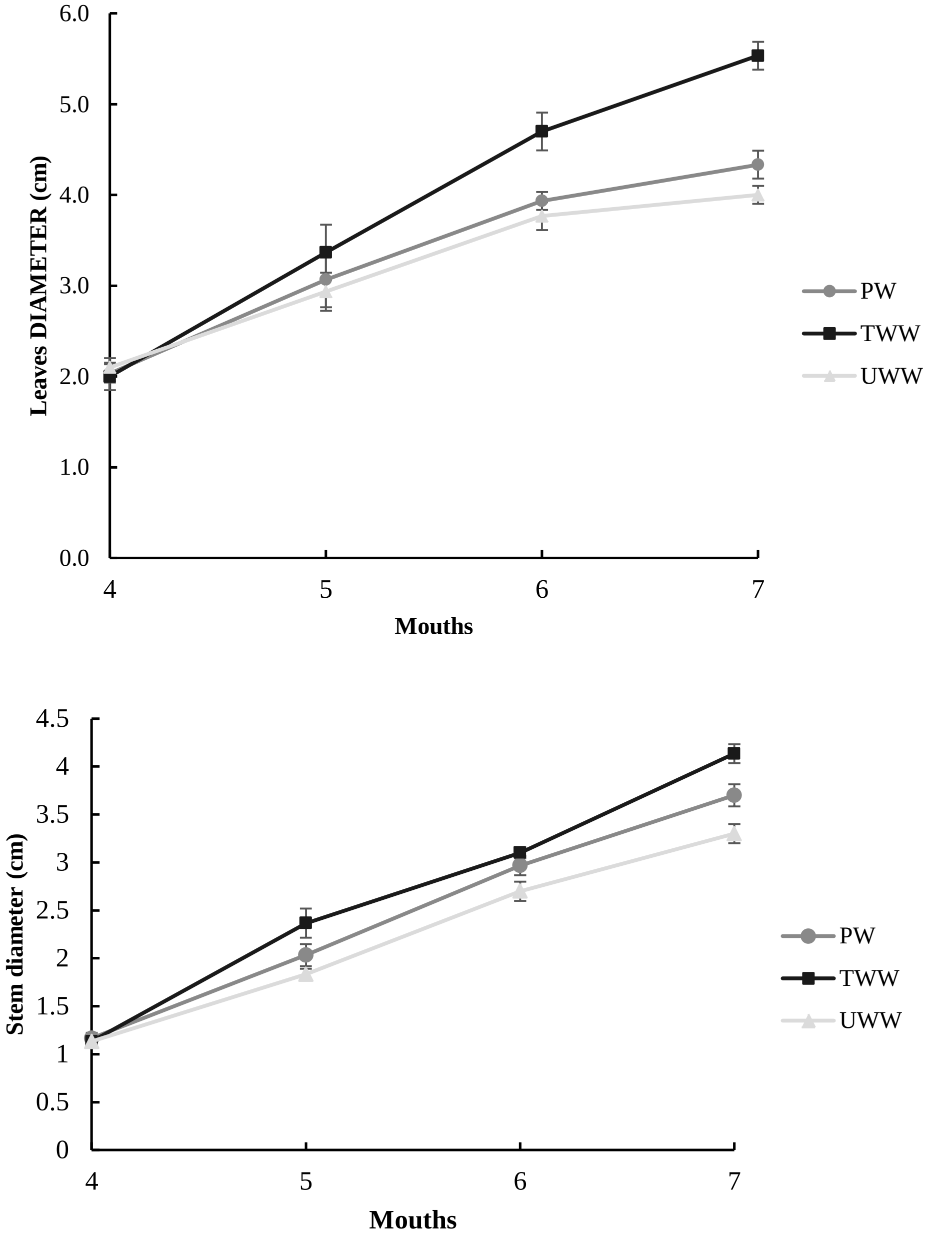
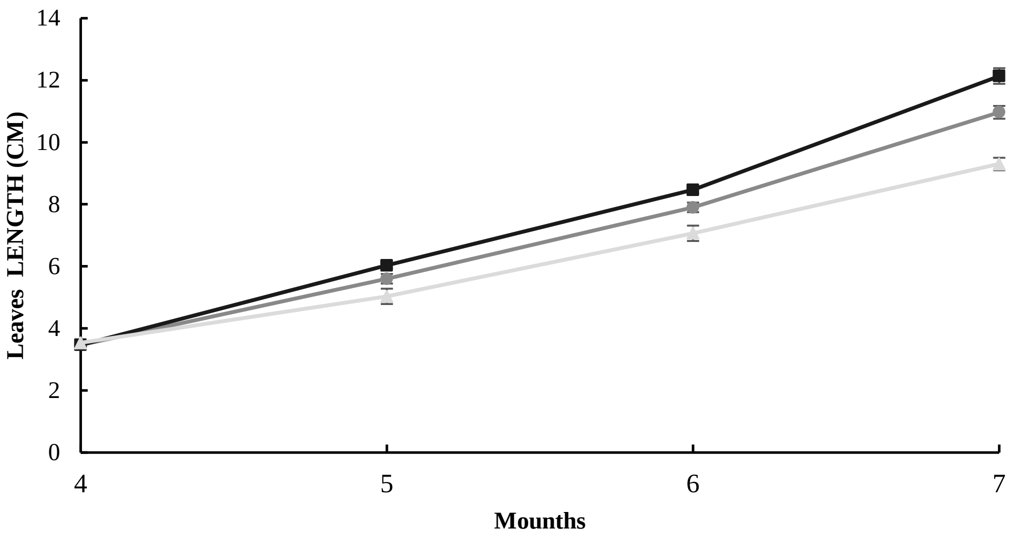


Figure 2 A, Stem and B, leave diameter of tomato plants irrigated with treated wastewater (TWW), potable water (PW) and untreated wastewater (UWW).



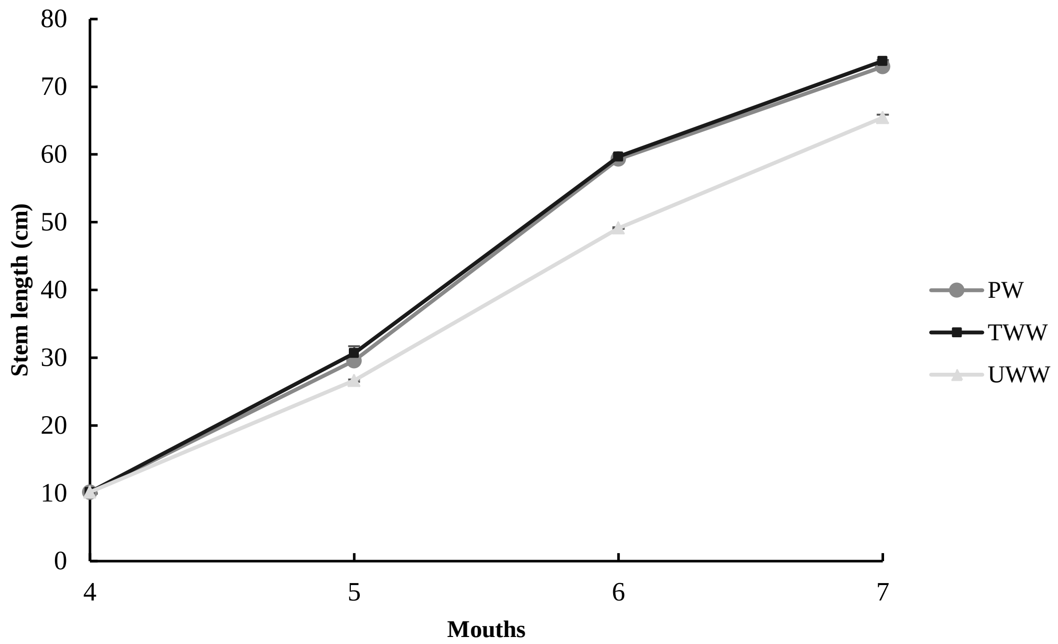


Figure 3 Average values of Leave and stem length of tomato plants irrigated with treated wastewater (TWW), potable water (PW) and untreated wastewater (UWW) along the growing season.

Table 5 Effects of the treated wastewater (TWW), potable water (PW) and untreated wastewater (UWW) used for tomato irrigation on the number of fruit and flowers and the weight of fresh fruit per tomato plants.

Irrigation treatment	Fruit weight per plant (g)	Number of fresh fruits per plant	Number of flowers per plant
PW	162±3.52[a]	22±2 [a]	24± 1 [a]
TWW	155±4.6[b]	18.33±1.52 [b]	22±1 [b]
UWW	140±6[c]	13±1 [c]	18.66±1.53 [c]

Note: The data are presented as the average± S.D; different letters indicate significant differences by the Tukey multiple comparison test ($p < 0.05$).

3.4 Tomato fruit quality

3.4.1 Physicochemical properties of the tomato fruits

Tomato is one of the most popular and important food for Morocco diet. Quality of the tomato fruits depends on chemical components such as acidity, lycopene, Vitamin C, total soluble solids, etc. Effect of irrigating different qualities of water samples on the content of tomatoes nutrients is shown in Table 6. The different water irrigation treatments significantly ($p \leq 0.05$) affected the pH of the tomato fruit, with a higher pH for PW and TWW (4.43 and 4.36 respectively) compared to UWW (3.38). The values of pH in tomato fruit obtained were always within the range of 4, typical to tomato fruit (Balibrea et al., 1997). The titratable acidity ranged from 0.41% to 0.9%. The highest titratable acidity was obtained from tomato fruits irrigated with UWW.

Significant differences in soluble solid (SS) values were observed among treatments, the highest values were obtained in the fruit irrigated with UWW. The solids content of tomato fruit can be influenced by number of fruits, the rate of assimilate export from leaves; rate of import of assimilates by fruits; and fruit carbon metabolism (Young et al., 1993). Also, the present study shows that the values of K^+, Na^+ and Ca^{2+} obtained were significantly higher in fruit from UWW and TWW irrigation as compared to PW treatments. However, no important differences between treatments in Mg^{2+} and sulfur content in tomato fruits.

Table 6 The average values of physicochemical properties of tomato fruits irrigated with treated wastewater (TWW), potable water (PW) and untreated wastewater (UWW).

	Unit	PW	TWW	UWW
pH		4.43^a	4.36^b	3.88^c
EC	dS m^{-1}	4.15^a	4.29^b	4.76^c
Titratable acidity	%	0.41^a	0.53^b	0.91^c
Soluble solids	%	5.3^a	6.6^b	16.15^c
Total sugar	g 100g^{-1}	2.24^a	3.82^b	5.41^c
Total lipid	g 100 g^{-1}	0.32^a	0.43^b	0.51^c
Protein	g 100 g^{-1}	0.75^a	0.8^b	0.67^c
K^+	mg 100 g^{-1}	221.9^a	222.7^b	229^c
Na^+	mg 100 g^{-1}	7.25^a	12.1^b	18.6^c
Ca^{2+}	mg 100 g^{-1}	8.53^a	9.69^b	15.4^c
NO_3^-	mg 100 g^{-1}	1.42^a	1.13^b	0.85^c
Mg^{2+}	mg 100 g^{-1}	7.15^a	7.09^b	7.06^b
Sulfur	%	0.2^a	0.26^a	0.3^b
Lycopene	mg kg^{-1}	21.26^a	23.03^b	25.01^c
Vitamine C	mg 100 g^{-1}	15.37^a	22.8^b	26.62^c

Note: The data are presented as the average± S.D; different letters indicate significant differences by the Tukey multiple comparison test ($P < 0.05$).

In addition, significant differences between treatments (PW, TWW and UWW) were recorder for protein, lipid, lycopene and vitamin C content in tomato fruit. The results show significant increase in the mean of fat, soluble sugar, vitamin C, and lycopene in fruits for the two treatments (TWW, and UWW) as compared to PW. The value of protein in tomato fruits grown in TWW was significantly higher than the fruits grown in the PW or UWW. This data demonstrates that irrigation with TWW could effectively improve the tomato fruits quality. Lycopene is the most important antioxidant compound of the tomato fruits and the one which determines its red color. Tomato fruits are very healthy as they are a good source of Vitamins C, lycopene and potassium. They have beneficial effects on human health (Brunele Caliman et al., 2010). Lycopene and Vitamin C are a very powerful antioxidant which can help to prevent the development of many forms of cancer (Lee and Kader, 2000).

3.4.2 Enzyme activities in leaves, roots and fruits of succinate dehydrogenase (SDH)

To investigate the effects of irrigation with various water samples on SDH activity in different plant parts, the enzymatic activity was measured in isolated mitochondria. The activity of SDH from leaves, roots and fruits was presented in Figure 4. Figure 4 declared significant change in SDH enzyme activities in tomato plant treated with PW, TWW and UWW. The roots had much lower SDH activity overall (0.27 to 0.45 units/mg protein per min) and there were significant differences between leaves and fruit. Leaves exhibited an increased SDH activity when treated with PW. The fruit irrigated with UWW retained low mitochondrial SDH activity. However, mitochondrial SDH activity doubled in fruit irrigated with TWW compared to UWW.

The increase in SDH activity in leaves is due to a remarkable increase in the energy metabolism of this tissue. However, the decrease in the activity in tomato plants grown under UWW irrigation probably reflects the metabolic changes caused by the stress. The data indicate that the tomato plant under salinity stress

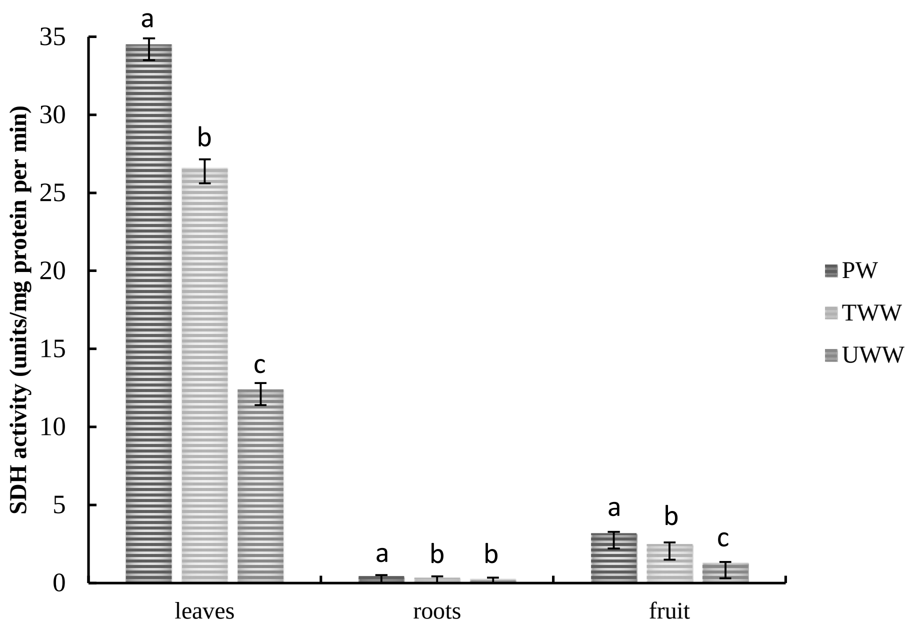


Figure 4 Effects of the treated wastewater (TWW), potable water (PW) and untreated wastewater (UWW) used for tomato irrigation on the SDH activity. Different letters on bars indicate significant differences by the Tukey multiple comparison test (p < 0.05).

3.4.3 Microbial quality of tomato fruit

The microbiological quality of tomato fruits crops is an important aspect for human health. Tomato fruits were classified as acceptable if the bacterial count was less than or equal to 4 log CFU·g^{-1}. The microbiological analysis of the fruit samples irrigated by different water treatments were conducted, as reported in Table 7. The results indicate a good microbiological quality of tomato fruits irrigated with PW. *Escherichia coli*, *fecal coliforms*, *Salmonella spp* were not isolated in any of fruit samples. *Fecal Streptococcus*, *Vibrio cholerae* and *Salmonella spp* were not isolated in the tomato fruits irrigated on TWW and UWW. However, *total coliform* and *fecal coliform* were detected in these samples. For the TWW-treated tomato crops, the level of *fecal coliforms* and *total coliforms* counts were 2.24 and 3.1 log CFU g^{-1} respectively. These values were lower compared with fruit irrigated with UWW.

Table 7 Bacteriological parameters in tomato fruit samples according to the potable water (PW), treated wastewater (TW) and untreated wastewater (UWW) irrigation

Bacteriological parameter (log CFU g^{-1})	Irrigation Treatment		
	PW	TWW	UWW
Total coliform	0±0.0	3.10*±0.1	16.30*±0.3
Fecal coliform	0	2,240*±0.3	9.57*±0.1
Escherichia coli	0	0	1
Fecal Streptococcus	0	0	0
Vibrio cholerae	0	0	0
Salmonella sp	0	0	0

Note: The data are presented as the means ± S.D. The asterisk indicates significant differences from potable water according to Dunnett's test (p < 0.05).

Hazard Analysis and Critical Control Points – Total Quality Management (HACCP-TQM) Technical Guidelines lay down the microbial quality for foods. Food containing < 10^4 CFU g^{-1}, 10^4 –5 ×10^6 , 5 ×10^6 – 5 ×10^7 and > 5 ×10^7 CFU g^{-1} are rated as ‘‘good’’, ‘‘average’’, ‘‘poor’’ and ‘‘spoiled food’’, respectively (Anonymous, 1998).The result indicates the microbiological quality of fruits through irrigation in TWW can be considered safe as per the HACCP-TQM (Hazard Analysis and Critical Control Points-Total Quality Management) guidelines which were less than 4 log CFU g^{-1}. These results join those of (Christou et al.,

2014) who concluded that the tomatoes irrigated with treated wastewater did not present a danger to public health since their microbial load was very low and complied with the required standards and thus the reuse of TWW for agricultural purposes was highly recommended, especially in arid areas. This observation can be seen as the positive consequence the summer month with increased UV radiation exposure of fruit to reduce the effect of TWW on the microbial load of fruits. Also, the drip irrigation method, by reducing the direct contact between the water and the plant, limited the possible contamination of the crop's products. Finally, the time left between irrigation and harvest can contribute to the reduction of the microbial load in tomato fruits.

In another study carried out by Gatta et al. (2015) to examine the microbiological quality of tomato plants and their fruits knowing that these pathogenic organisms can accumulate at ground level (Chen et al., 2008), thus reducing the quality and productivity of crops and even representing a major danger to public health (Palese et al., 2009). Gatta et al. (2015) study revealed that the soil microbial community can be significantly disturbed; however, the tomato plants as well as their fruits did not undergo microbiological contamination exceeding the tolerated limit values despite the use of treated industrial wastewater.

4 Conclusion

In the present study, we wanted to evaluate the use TWW in drip irrigation for the production of crop tomatoes. The aim was to investigate the effects of TWW for irrigation on tomato crop production; and on mineral contents, SDH activity and microbiological properties of tomato fruits. The yield of tomato crops as well as the most important agronomical parameters of tomato fruits (i.e number of fruits and the weight of fresh fruit) were influenced by the irrigation with treated wastewater. The results showed that the growth of tomato plants irrigated with TWW were higher than plants treated with PW, followed by plants treated with UWW. Also, the highest fresh weight was observed in tomato plants irrigated with PW and TWW; and those irrigated with UWW have shown the lowest fresh weight. The results also showed that the physicochemical composition of tomato fruits was influenced by the variety the water in which they were grown. Furthermore, the results show significant increase in the mean of fat, soluble sugar, vitamin C, and lycopene in fruits for the two treatments (TWW, and UWW) as compared to PW. The fruit irrigated with TWW retained high mitochondrial SDH activity (SDH activity doubled as compared with UWW). In addition, the microbiological quality of fruits through irrigation in TWW can be considered safe as per the HACCP-TQM (Hazard Analysis and Critical Control Points-Total Quality Management) guidelines which were less than 4 log CFU.g^{-1}. The results suggested that the treated wastewater can be used for tomato cropping without compromising the quality and safety of the final product. Hence, the treated municipal and industrial wastewater from the WWTP in the province of Mediouna can be used as a valid alternative for irrigation of tomatoes.

References

Alda, L. M., I. Gogoaşă , D. M. Bordean, I. Gergen, S. Alda, C. Moldovan, and L. Nită. 2009. Lycopene content of tomatoes and tomato products. *Journal of Agroalimentary Processes and Technologies*, 15(4): 540-542.

Anonymous. 1998. *HACCP-TQM Technical Guidelines.* Section IV Contamination Levels and Microbiological Control.

AOAC (Association of Official Analytical Chemists).1995. *Official methods of analysis of AOAC International*.16th ed. Gaithersburg, MD: AOAC.

AOAC (Association of Official Analytical Chemists). 2002. *Official methods of analysis of AOAC International*. 17th ed. Gaithersburg, MD: AOAC.

APHA (American Public Health Association). 2005. Standard methods for the examination of water and wastewater. Washington, D.C., USA: American Public Health Association, American Water Works Association (AWWA) and Water Environment Federation (WEF).

Bailenger, J. 1962. Valeur comparée des méthodes d'enrichissement en coprologie parasitaire. *Le Pharmacien Biologiste*, 3: 249-259.

Balibrea, M. E., E. Cayuela, F. Artés, and F. Pérez-Alfocea. 1997. Salinity effects on some postharvest quality factors in a commercial tomato hybrid. *Journal of Horticultural Science*, 72(6): 885-892.

Bernstein, N., S. Sela, and S. Neder-Lavon. 2007. Effect of irrigation regimes on persistence of *Salmonella enterica* serovar Newport in small experimental pots designed for plant cultivation. *Irrigation Science*, 26(1): 1-8.

Black, C. A., D. D. Evans, J. L. White, L. E. Ensminger, and F. E. Clarke. 1965. *Methods of soil analysis*, part 1. Madison, Wisconsin: American Society of Agronomy, Inc.

Bradford, M. M. 1976. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye Binding. *Analytical Biochemistry*, 72(1-2): 248-254.

Bremner, J. M., and D. R. Keeney.1966. Determination and isotope-ratio analysis of different forms of nitrogen in soils: 3. Exchangeable ammonium, nitrate, and nitrite by extraction-distillation methods. *Soil Science Society of America Journal*, 30(5): 577-582.

Brunele Caliman, F. R., D. J. Henriques da Silva, P. C. Stringheta, P. C. Rezende Fontes, G. Rodrigues Moreira, and E. Chartuni Mantovani. 2010. Quality of tomatoes grown under a protected environment and field conditions. *IDESIA (Arica)*, 28(2): 75-82.

Chen, W., L. Wu, W. T. Frankenberger, and A. C. Chang. 2008. Soil enzyme activities of long-term reclaimed wastewater-irrigated soils. *Journal of Environmental Quality*, 37(S5): S36-S42.

Christou, A., G. Maratheftis, E. Eliadou, C. Michael, E. Hapeshi, and D. Fatta-Kassinos. 2014. Impact assessment of the reuse of two discrete treated wastewaters for the irrigation of tomato crop on the soil geochemical properties, fruit safety and crop productivity. *Agriculture, Ecosystems & Environment*, 192: 105-114.

Dey, P. M. 1990. Oligosaccharides. In *Carbohydrates Methods in Plant Biochemistry*, vol 2, ed. P. M. Dey and B. Harbourne, ch. 5, 189-219. San Diego: Academic Press.

El-Lathy, A. M., G. E. El-Taweel, M.W. El-Sonosy, F.A Samhan and T. A Moussa. 2009. Determination of pathogenic bacteria in wastewater using conventional and PCR techniques. *Environmental Biotechnology*, 5: 73-80.

EPA (Environmental Protection Agency). 2002. Method 1103.1: *Escherichia coli* (E. coli) in water by membrane filtration using membrane-thermotolerant *Escherichia coli* agar (mTEC). Report EPA 821-R-02-023. Washington DC: Office of Water Environmental Protection, Environmental Protection Agency.

Frenkel, C., and M. E. Patterson. 1973. Effect of carbon dioxide on activity of succinic dehydrogenase in ‘Bartlett’pears during cold storage. *HortScience*, 8(5): 395-396.

Gatta, G., A. Gagliardi, G. Disciglio, A. Lonigro, M. Francavilla, E.Tarantino, and M. M. Giuliani. 2018. Irrigation with treated municipal wastewater on artichoke crop: assessment of soil and yield heavy metal content and human risk. *Water*, 10(3): 255.

Gatta, G., A. Libutti, A. Gagliardi, L. Beneduce, L. Brusetti, L. Borruso, and E. Tarantino. 2015. Treated agro-industrial wastewater irrigation of tomato crop: Effects on qualitative/quantitative characteristics of production and microbiological properties of the soil. *Agricultural Water Management*, 149: 33-43.

Huang, S., and A. H. Millar. 2013. Succinate dehydrogenase: the complex roles of a simple enzyme. *Current Opinion in Plant Biology*, 16(3): 344-349.

Koch, A. L. 1994. Growth measurement. In *Methods for General and Molecular Biology*, 3th ed. P. Gerhardt, R. G. E. Murray, W. A. Wood and N. R. Kriegz, ch. 9, 248-277. Washington, D.C: American Society for Microbiology.

Lee, S. K., and A. A Kader. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, 20(3): 207-220.

Loeppert, R. H., and D. L. Suarez. 1996. Carbonate and gypsum. In *Methods of Soil Analysis, Part 3. Chemical Methods*, ed. D. L. Sparks, ch. 15, 437–474. Madison, Wisconsin, USA: American Society of Agronomy.

Mateo-Sagasta, J., K. Medlicott, M. Qadir, L. Raschid-Sally, and P. Drechsel. 2013. Proceedings of the UN-water project on the safe use of wastewater in agriculture. UNW-DPC Proceedings Series No. 11. Bonn, Germany: UN-Water Decade Programme on Capacity Development (UNW-DPC).

Novizan, I. 2005. Petunjuk Pemupukan yang Efektif. Jakarta: Agro Media Pustaka.

Öblad, M., P. Standzenieks, E. Selin, and J. Dubois. 1982. Application of an energy dispersive X-Ray fluorescence spectrometer to air pollution studies. *Physica Scripta*, 26(4): 257-261.

Olsen, S. R., and S. E. Sommers. 1982. Phosphorus. In *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, 2nd ed, eds. A. L. Page, R. H. Miller, and D. R. Keeney, ch. 24, 403-430. Madison, WI: American Society of Agronomy.

Ouansafi, S., F. Abdelilah, M. Kabine, H. Maaghloud, F. Bellali, and K. El Bouqdaoui. 2019. The effects of soil proprieties on the yield and the growth of tomato plants and fruits irrigated by treated wastewater. *AIMS Agriculture and Food*, 4(4): 921-938.

Pandey, Y. R., A. B. Pun and K. P. Upadhyay. 2006. Participatory varietal evaluation of rainy season tomato under plastic house condition. *Nepal Agriculture Research Journal*, 7: 11-15.

Palese, A. M., V. Pasquale, G. Celano, G. Figliuolo, S. Masi, and C. Xiloyannis. 2009. Irrigation of olive groves in Southern Italy with treated municipal wastewater: effects on microbiological quality of soil and fruits. *Agriculture,* *Ecosystems & Environment*, 129(1-3): 43-51.

Pescod, M. B. 1992. Wastewater treatment and use in agriculture. Irrigation and Drainage Paper 47. Rome, Italy: FAO.

Ranganna, S. 1997. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*. 2d ed. New Delhi: Tata McGraw Hill Publishing Company Limited.

Rattan, R. K., S. P. Datta, P. K. Chhonkar, K. Suribabu, and A. K. Singh. 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. *Agriculture, Ecosystems & Environment*, 109(3-4): 310-322.

RIC, Casablanca-Settat Regional Investment Center, Socio-economic data of the Casablanca-Settat Region. 2018. Repot of Regional Investment Center, Morocco.

Richards, L. A. 1954. Diagnosis and improvement of saline and alkali soils. Agricultural handbook No. 60. Washington, DC: United Stated Department of Agriculture (USDA).

Romani, R. J., K. Y. Ida and L. K. Fisher. 1969. Isolation of tightly coupled mitochondria from acidic plant tissues. *Plant Physiology*, 44(2): 311.

Sarief, E. S. 1985. Kesuburan dan Pemupukan Tanah Pertanian. Bandung: Pustaka Buana.

S.E.E.E (State Secretariat at the Ministry of Energy, Mines, Water and Environment). 2007. Dirctorate of Research and Water Planning, Water quality standards for water irrigation, Rabat.

Singh, A., R. K. Sharma, M. Agrawal, and F. Marshall. 2009. Effects of wastewater irrigation on physicochemical properties of soil and availability of heavy metals in soil and vegetables. *Communications in Soil Science and Plant Analysis*, 40(21-22): 3469-3490.

Tallon, P., B. Magajna, C. Lofranco, and K. T. Leung. 2005. Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution*, 166(1): 139-166.

Vergine, P., A. Lonigro, C. Salerno, P. Rubino, G. Berardi, and A. Pollice. 2017. Nutrient recovery and crop yield enhancement in irrigation with reclaimed wastewater: a case study. *Urban Water Journal*, 14(3): 325-330.

Violante, A., V. Cozzolino, L. Perelomov, A. G. Caporale, and M. Pigna. 2010. Mobility and bioavailability of heavy metals and metalloids in soil environments. *Journal of Soil Science and Plant Nutrition*, 10(3): 268-292.

Walkley, A., and I. A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 37(1): 29-38.

WHO. 2006. WHO Guidelines for the safe use of wastewater, excreta and greywater in agriculture. Geneva: World Health Organization.

Young, T. E., J. A. Juvik, and J. G. Sullivan. 1993. Accumulation of the components of total solids in ripening fruits of tomato. *Journal of the American Society for Horticultural Science*, 118(2): 286-292.

Zheng, J., G. Huang, D. Jia, J. Wang, M. Mota, L. S. Pereira, Q. Huang, X. Xu, and H. Liu. 2013. Responses of drip irrigated tomato (*Solanum lycopersicum* L.) yield, quality and water productivity to various soil matric potential thresholds in an arid region of Northwest China. *Agricultural Water Management*, 129: 181-193.