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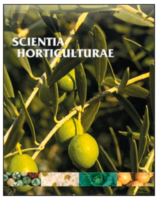

Assessment of the impact of irrigation with treated wastewater at different dilutions on growth, quality parameters and contamination transfer in strawberry fruits and soil: Health risk assessment

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ABSTRACT

Industrial treated wastewater (TWW) could be an attractive alternative fto fresh water use in agriculture due to the shortage in water resource especially in the Mediterranean region. Furthermore, TWW could represent an efficient substitute for crop production thanks to its high load in nutrient. However, its high content of salts or mineral as well as organic pollutants can affect the crop safety, productivity and growth. A simple way to reduce the amount of chloride and salinity could be the dilution. In this study the irrigation of strawberry (*Fragaria x ananassa* cv Camarosa) using diluted TWW at three dilution ratios; D60 (40% TWW) D40 and D20 was performed and compared with the well water (WW) used as control. The use of TWW for the irrigation of strawberry plants was done for the first time in the city of Sfax located in the southeast of Tunisia.

D20 registered the lowest strawberry yield and highest heavy metal contents in fruits compared to the other dilutions. Also, this dilution showed a toxic effect of high accumulation of chlorine, salts and metals by the apparition of brown edges in the leaves. However, D60 recorded the lowest soil salinity, the high plant growth and strawberry yield compared to the WW irrigated plants and showed lowest metal contents in soil, plants and fruits compared to other dilutions. Our study suggests the use of 60% diluted TWW for the irrigation of strawberry in order to obtain fruits matching international safety and quality standards.

1. Introduction

The fast climate change modifies the world weather and temperature, water resources are in consequence vulnerable, especially in arid and semi-arid zones. The rising population induce to an increase in fresh water demand and consumption (Darwall et al, 2018). Water shortages are expected to affect 40% of the population by 2050. Therefore, fulfilling the water scarcity and conserving freshwater are becoming an urgent need all over the world. The agriculture is considered to be the main water consumer with around 70% of the total world water use (Guadie et al., 2021). An important alternative strategy is the use of treated wastewater (TWW) in the agricultural sector in order to decrease the pressure applied on fresh water resources (Hamilton et al., 2007; Uzen et al., 2016). The use of TWW in irrigation has valuable benefits regarding its mitigation strategies to conserve water, nutrient infused and continuous availability (Hamilton et al., 2007; Hanjra et al., 2012; Martinez et al., 2013; Tunc and Sahin, 2015). Actually, TWW is a suitable and sustainable solution in water-scare areas (Petousi et al., 2015; Lyu et al., 2016). Furthermore, the high content of nutrients, organic and minerals matter in TWW can replace the use of fertilizers which reduce considerably the cost of crop production.

In addition to its economic and environmental benefits, TWW has different effects on the physicochemical and biological properties of soil and crop productivity (Cirelli et al., 2012; Mok et al., 2014), since it can contain hazardous elements in which the presence is mainly due to treatment limits of wastewater treatment plants. As examples of these contaminants, heavy metals, personal care products and pharmaceutical compounds can be omnipresent in the final destination of the TWW (Kasprzyk-Hordern et al., 2009; Paz et al., 2016; Burns et al., 2018; Kibuye et al., 2019; de Santiago-Martín et al., 2020). These biological

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and chemical pollutants can be accumulated in soil, absorbed by plants or consumed by animals then contaminate soil, food chain and groundwater and affect the public health (Rekik et al., 2017; Vergine et al., 2017; Guadie et al., 2021).

To ensure crop protection from possible contamination by using TWW for irrigation, a range of permissible limits and the draft limit values on minimum quality requirements for reuse made by the European commission (Rizzo et al., 2018). A robustly risk of heavy metal accumulation may be found in vegetative products after irrigation using raw wastewater, industrial wastewater or improperly TWW (ur Rehman et al., 2019; Guadie et al., 2021). However, the use of tertiary TWW was considered safe regarding their heavy metal contents (Christou et al., 2016; de Santiago-Martín et al., 2020). Moreover, heavy metals, transportation in soil depends strongly with the physicochemical characteristics of the soil like the organic matter, content, pH and EC (Khalid et al., 2018; Shahid et al., 2021). Previous study achieved by Aydin et al. (2015) demonstrated that the phyto-availability of heavy metals decreased by the increase organic matter content of soil, therefore, the use of TWW for irrigation may reduce the metals phyto-availability in agricultural products.

The origin of sewage can affect the development of plant and its productivity yield. de los Santos et al. (2019) used olive mill wastewater for the irrigation of pepper, tomato and strawberry and verified its effects as natural fertilizers and fungicide. An increase of plant growth, fruit size and cumulative yield by at least 20% were found using Table olive wastewater compared to the dripping and spraying applications mode. Christou et al. (2016) revealed the absence of significant effects on strawberry fruits' marketability, taste and antioxidant capacity and heavy metal contents by irrigation using tertiary TWW by different techniques (drip, sprinkler, drips under plastic mulch). They verified the valid alternative for the irrigation using tertiary TWW of strawberry crops, even with sprinklers. Renai et al. (2021) showed that nutritional and nutraceutical quality determined by total soluble polyphenols and total monomeric anthocyanins concentrations in strawberries produced using tertiary TWWs irrigation included in the range of previously reported values for Camarosa fruits purchased in the market or cultivated in research.

In Tunisia, which is a Mediterranean country characterized by a semi-arid to arid climate, the decrease of reuse of fresh water in agriculture has become urgent. Indeed, strawberry is one of the highest consumed fruit that occurs nutraceutical and economical values which increase this crop cultivation in the Mediterranean regions characterizing by a favourite climate for the strawberry crops (Giampieri et al. 2012; Akhatou et al., 2016). Considering the safety of strawberry fruits obtained in previous works using TWW, the effect on soil, strawberry plants and fruits using a TWW in crop irrigations were analysed in this study. Taking into consideration the sensitivity of strawberries to salinity (Suarez and Grieve, 2013), the objective of this study was to evaluate the impact of different dilution percentages of TWW comparing to the WW on strawberry performance in particular on soil, plants and productivity and fruits metal compositions.

2. Material and methods

2.1. Wastewater

Treated wastewater (TWW) used in the current study was collected at the outlet of the basin of an industrial wastewater treatment plant. This industrial area is known for gathering major industries specialised in the manufacturing and production of copper-based sanitary ware as well as the production of fertilizers. Fresh TWW samples were collected at weekly frequency and used to prepare the following dilutions: D20, D40, and D60 with D60 as example, referring to 60% of TWW and 40% of well water (WW).

2.2. Strawberry plantation

Young fresh strawberry plants (*Fragaria x ananassa* cv Camarosa) were transplanted into 5 litre PVC pots (23 cm diameter, 17.6 cm deep) filled with 5 kg of substrate (soil mixed with peat (1/3; 2/3) and placed in a greenhouse in the National Engineering School of Sfax starting from January to June 2018. Since strawberry requires cool and moist climates, the climatic condition in the greenhouse were set to have a temperature of 25/12 °C (day/night).

Four experimental series of eight pots were considered for each type of irrigation water; one series of pots was irrigated with WW and three other series were irrigated with diluted TWWs at 60% (D60), 40% (D40) and 20% (D20). Harvest was performed at the end of June and the plants were washed, divided into two parts (areal parts and roots), and stored at -20 °C for analysis. After each harvesting, fruits were washed with distilled water and dried using paper towel.

2.3. Physicochemical and microbiological analysis of well water and treated wastewater

Water pH was determined using a Metertoldo type pH metre. The electrical conductivity (EC) was measured according to AFNOR (NF 27,888) using an Inolab WTW conductivity metre.

Total suspended solids (TSS), total phosphorus (Pt), chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were measured using the AFNOR standard methods. Five days BOD was determined by placing the sample in an Oxitop system (WTW).

Bicarbonate's ions were measured by acid (HCl) titration. Nitrates were estimated using reflectometric method with test strips. Chlorine ions were measured using Mohr's titration method.

Anions and cations were determined using aqua regia acid digestion followed by ion chromatography and Furnace Atomic Absorption Spectrometry for the determination of trace metal contents.

Faecal coliforms (FC), total coliforms (TC) and faecal streptococci (FS) as well as E. coli were determined according to water standard methods (NF T90–411 and ISO 4832).

2.4. Physicochemical analysis of the substrate

Substrate samples were collected after each three months and after the harvest from all pots. The samples were dried for one week at room temperature. 2 mm sieved substrates were chosen for further characterizations. For the pH and EC of the saturated paste measurements, substrate suspension was prepared with a ratio of substrate/water ratio 1 / 2.5 and 1/5 (W/V) respectively according to the protocol used by Belaid et al. (2012). The suspension was stirred for one hour and left 24 h for decantation, the pH was then measured in the supernatant using a laboratory pH metre. Organic matter was determined by the Walkey and Black modified method (Verma et al., 2013). The sample was oxidized with concentrated sulfuric acid in the presence of potassium dichromate. The quantity of excess $K_2Cr_2O_7$ was dosed back by a standard solution of Mohr's salt (ferrous sulphate) in the presence of redox indicator (ferroin). Total nitrogen was analysed using the Kjeldahl method using Büchi B-315 apparatus. This method started with the mineralization of organic matter which was carried out using concentrated sulphuric acid in the presence of a catalyst (K_2SO_4 and $CuSO_4$). After mineralization the nitrogen transform into NH_4^+. A distillation step in the presence of soda was necessary to transform the ammonium ions into ammonia. The latter was then collected in a boric acid solution in order to be trapped and then neutralized with a calibrated solution of strong acid (HCl or H_2SO_4) (Sáez-Plaza, et al., 2013). Total phosphorus contained in the substrate can be measured after mineralization by sulfonitric acid attack of a sample in the presence of ammonium molybdate forming a complex phosphomolybdic anion, which after reaction of the acid with ascorbic acid ($C_6H_8O_6$, 99%) to give a blue colour. The optical density is measured at 880 nm. Chlorine was determined in saturated substrate paste extract using the Mohr's titration method.

To assess heavy metal contents, 1 g of substrate was calcined at 500 °C in a muffle furnace for two hours. The sample was taken up to 10 mL of 50% hydrofluoric acid and dried again in a Teflon beaker on a sand bath. The residue obtained was dissolved by adding 7.5 ml of hydrochloric acid and 2.5 ml of nitric acid. The beaker was covered with a watch glass and then dried on a hot plate until the redhead vapours, indicator of the complete mineralization, disappear. The solution obtained was then brought to a final volume of 10 ml with distilled water. Mineralization blanks was carried out jointly, the metals were then analysed by Flame Atomic Absorption Spectrometer (Belaid et al., 2012).

2.5. Determination of chlorophyll content

Plant growth data were measured each end of month from January to June. Leaves and fruits were also counted.

The chlorophyll a and b contents were determined according to the method of Lichtenthaler and Wellburn (1983). The method consists of the extraction of chlorophylls from 100 mg of fresh leaves in 80% acetone. The extract was centrifuged then the recovered supernatant was adjusted to a volume of 2 ml with acetone (80%). The chlorophylls a, b and total chlorophyll contents were evaluated with a spectrophotometer (HACH DR 4000/U) at different wavelengths: 645 and 663 nm.

The chlorophyll a and b contents are expressed in µg / g fresh weight as follows:

Chlorophyll a = (12.7 × OD 663 nm) - (2.6 × OD 645 nm) × V / M
Chlorophyll *b* = (22.9 × OD 663 nm) - (4.68 × OD 663 nm) × V / M
Total chlorophyll = chlorophyll a + chlorophyll *b*
Where:
V: volume of acetone added to the supernatant.
M: mass of the fresh leaves.
OD: Optical density.

2.6. Plants and fruits metal contents

All plant and fruits samples were washed with distilled water and were dried at 60 °C for one day then crushed and sieved at 500 µm on a nylon sieve. To determine the mineral contents (Mn, Zn, Cu, Cr and Ni), fruit and plant samples were calcinated in a muffle oven at 450 °C. Ashes were then digested with 1 M nitric acid and the suspension was filtrated using cellulose filter. Filtered samples were then analysed using atomic absorption spectrometry.

2.7. Fruit dry matter, total soluble solids, and ascorbic acid content

Dry matter was determined by drying strawberry fruits until constant mass at 70 °C in ventilated oven. Total soluble solids were measured using a refractometer and expressed in % of strawberry fresh weight. Ascorbic acid (AA) was determined using a refractometer (Merck, Darmstadt, Germany) with an AA test strip and expressed in mg of AA in 100 mg of fruit fresh weight.

2.8. Health risk assessment

Possible risks to public health by human intake of heavy metal contamination from fruits and vegetables was determined using the U.S. Environmental Protection Agency's Health Risk Handbook. Health risk assessment indices were given by the target hazard quotient (HQ) and the hazard index (HI) that measured for carcinogenic and non-carcinogenic heavy metals as described in equations (A.1) and (A.2).

Hazard quotient can be identified as the proportion of the probable exposure to a chemical or element and level that does not presents any expectable negative risk. If the quotient is lower than1, this implies no predictable risk resulting from the exposure to the mentioned element. However, when THS is higher than 1, hazardous health problems are expected (Bermudez et al., 2011).

Additive risks can be resulted from the display to many contaminants. Therefore, the hazard index (HI) presents the sum of the target hazard quotients that assesses generally the effects of two or more pollutants. Similar to the THQ, a HI higher than 1 demonstrates adverse health impacts from cons consumption contaminants from a foodstuff.

$$THQ = \frac{C_M \times D_{Fuit} \times E_F \times E_D}{B_w \times AT_n \times RfD} \tag{A. 1}$$

$$HI = \sum \mathrm{THQ} \tag{A. 2}$$

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 represents the exposure frequency (365 d $year^{-1}$), E_D is the exposure duration (74.68 years) and B_w is the mean human body weight (60 kg). AT_n represents the average time of exposure to heavy metals (E_D × 365 days $year^{-1}$) (Qureshi et al., 2016, Chen et al 2021). The oral reference doses (RfD) are the daily oral allowed dose of heavy metals without inducing any harmful impacts throughout the lifespan (Akoto, et al., 2014). RfD of Cr, Mn, Zn, Cu and Ni are 1.5, 0.14, 0.3, 0.04 and 0.02 mg kg^{-1} d^{-1} respectively (Ferré-Huguet et al., 2008; Guadie et al., 2021).

2.9. Statistical analysis

The influence of irrigation with TWW on strawberry crops and soil was evaluated using a statistical assessment implying comparison of result's mean, and analysis of regression and correlation data by a software IBM SPSS Statisticas 20 and using Tukey's post hoc test.

3. Results

3.1. Characteristics of the irrigation waters

Physico-chemical characteristics of the used TWW are presented in Table 1. The TWW is characterized with a neutral pH (pH=7.84). In term of electrical conductivity, Table 1 show a value of 5 mS/cm which is considered high for an effluent. Yet, this value remains below the Tunisian law (NT 106.03) related to the reuse of TWW in agriculture. The TWW used in the current study was characterized with concentration of COD and BOD_5 equal to 106.9 and 37 mg of O_2/L, respectively. These values are above the values dictated by the Tunisian law (NT 106.03) related to the reuse of TWW in agriculture which are 90 and 30 mg of O_2/L, respectively for COD and BOD_5.

According to Table 1, potassium, total phosphate, and nitrogen which are essential for the growth of strawberry are present in sufficient concentration. In term of heavy metals, TWW demonstrate in the cases of Mn (3.4 mg/L), Cr (14.3 mg/L), and Cu (4.6 mg/L) values above those dictated by the Tunisian law (NT 106.03) related to the reuse of TWW in agriculture which are 0.5, 0.1, and 0.5 mg/L, respectively for Mn, Cr, and Cu. In Table 1, the physicochemical characteristics of the different dilutions are also demonstrated. Table 1 shows also that concentrations of certain parameters such as COD and BOD decreased after the dilution to match Tunisian Law (NT 106.03). On the microbiological level, analysis on WW as well as TWW demonstrated the total absence of FC, TC, FS as well as E. coli.

3.2. Substrate analysis

In Table 2, the physicochemical characteristics of the substrate at the initial status is demonstrated. The substrate used in this study was made my mixing sandy soil and peat (1/3; 2/3). In Table 2 it is shown that the substrate is characterized with a neutral pH (pH=7.1), low conductivity (0.67 mS/cm) and TOC (27.5%).

The substrate was also characterized with high concentration of potassium (3 g/kg), magnesium (1.514 g/kg), sodium (1.447 g/kg), and

Table 1
Average physicochemical characteristics of well water (WW) diluted TWW, D60, D40 and D20 used for strawberry irrigation.

	WW	TWW	D20	D40	D60	NT 106.03
pH	7.11±0.2	7.84±0.28	7.63±0.4	7.52±0.45	7.45±0.4	6.5- 8.5
EC (mS/cm)	0.48±0.17	5.17±0.39	4.57±0.41	3.43±0.4	2.69 ±0.18	7
TSS (mg/L)	2.23±0.22	30±2.22	25±1	18.8±0.77	12.5 ±1	30
HCO3 (mg/L)	103.78±1.7	283.31±2.79	232.2±2.58	176±2.19	117.6±2.95	-
COD (mg/L)	0	106.9±3.28	89.1±2.1	65.2±2.75	44.2±2.88	90
BOD5 (mg/L	0	37±2.72	30.3±2.91	22.2±1.73	15.2±1.57	30
Pt (mg/L)	0.6±0.18	15.4±0.78	12.8±1.29	9.6±0.4	6.4±0.69	-
NTK (mg/L)	1.8±0.38	56.23±1.97	46.1±1.69	35.1±1.74	23.4±2.89	-
Cl^- (mg/L)	257±18.68	1780±55.66	1435.5±30.7	1002.5±11.35	741.7±11.39	-
K (mg/L)	5±0.85	30.5±2.42	25±1.78	19.1±2.16	12.7±1.85	-
Mg (mg/L)	28.3±1.57	81.42±3.22	67.9±2.63	50.9±3.02	33.9±3.38	-
Na (mg/L)	31.8±1.93	204±1.71	170±1.76	127.5±1.71	85±1.56	-
Ca (mg/L)	69.2±1.61	107.25±0.83	89.4±0.97	67±1.38	44.7±2.17	-
SAR (m_{eq}/L)	n.d.	3±0.13	2.5±0.17	1.9±0.16	1.3±0.15	-
Mn (mg/L)	n.d.	3.4±0.23	2.73±0.13	2.05±0.15	1.37±0.18	0.5
Zn (mg/L)	n.d.	2.6±0.32	2.07±0.25	1.56±0.22	1.04±0.18	5
Cr (mg/L)	n.d.	14.3±1.38	11.5±0.49	8.63±0.4	5.75±0.4	0.1
Cu (mg/L)	n.d.	4.6±0.1	3.71±0.2	2.78±0.08	1.86±0.1	0.5
Ni (mg/L)	n.d.	0.17±0.06	0.14±0.05	0.1±0.01	0.07±0.01	0.2
Faecal coliform	n.d	n.d	n.d	n.d	n.d	-
Total coliform	n.d	n.d	n.d	n.d	n.d	-
Streptococcus	n.d	n.d	n.d	n.d	n.d	-
E. coli	n.d	n.d	n.d	n.d	n.d	-

n.d.= not detected

Table 2
Main physicochemical characteristics of the used substrate for strawberry cultivation in pots.

Parameter	Substrate
pH	7.1 ± 0.45
EC (mS/cm)	0.67 ± 0.14
TOC (%)	27.5 ± 6.07
NTK (%)	0.283 ± 0.03
NH4+ (%)	0.039 ± 0.005
Cl^- (mg/kg d.w.)	43.75 ± 3.16
K (g/kg d.w.)	3 ±0.007
Mg (g/kg d.w.)	1.514 ± 0.003
Na (g/kg d.w.)	1.447 ±0.005
Ca (g/kg d.w.)	5.478 ± 0.006
Fe (mg/kg d.w.)	788 ± 8.72
Mn (mg/kg d.w.)	57 ± 3.2
Zn (mg/kg d.w.)	18.19 ± 4.45
Cr (mg/kg d.w.)	26.03 ± 4.49
Cu (mg/kg d.w.)	11.07 ± 3.84
Ni (mg/kg d.w.)	2.01 ±0.49

calcium (5.478 g/kg), these concentrations are probably high due to their import from peat. Heavy metals such as Fe, Mn, Z, Cr, Cu, and Ni were detected at various level of abundance. Fe was dominating with a concentration of 788 mg/kg of dry weight. The concentration of the remaining metals was ranged between 2 and 57 mg/kg of dry weight.

3.3. *Effect of the dilutions on plant growth parameters*

3.3.1. *Morphological plant growth*

The analysis of the plant lengths at the end of the experiment revealed no significant difference between plants irrigated with WW and those irrigated with D60 dilution. Results presented in Fig. 1 show that the mean plants heights at the end of the experiment was 20.12 cm and 17 cm, respectively for plant treated with WW and D60 dilution.

On the other hand, a significant difference was notified when comparing results from WW with D40 and D20 dilutions. Heights were lower in plants irrigated with D40 (14.43 cm) and D20 (12.43 cm) with reference to the control and D60.

In term of leaves number, results present in Fig. 2 show that the number of leaves was higher during the whole period of cultivation in plants irrigated with WW.

Fig. 2 shows that in plant irrigated with the dilution D60 demonstrated the highest leaves number followed by plants irrigated with D40 with no significant difference. Yet, plants irrigated with the D20 were characterized by low leaves number. This low number of leaves could be explained by the higher concentration of metals in D20, as heavy metals can delay the metabolism in plant tissues if present in inhibitory concentration causing chlorosis and a sharp reduction in leaves number (Singh et al., 2016). The toxic effect of D20 was also demonstrated in Fig. 3.

3.3.2. *Total chlorophyll content*

The analysis of the chlorophyll content in strawberry leaves is demonstrated in Fig. 4. The determination of this parameter gives an idea on the chlorine or other trace element uptake by plant (Aggarwal et al., 2012; Ahmali et al., 2020).

The results in Fig. 4 show that there is no significant difference between the chlorophyll content in leaves from plant irrigated with D60 and D40 with respect to plant irrigated with WW. Mean results were 274.9 μg/g FW, 265.5 μg/g FW, and 266.1 μg/g FW, respectively for WW, D60, and D40.

However, a lower chlorophyll content was observed in pots irrigated with D20 dilution where the chlorophyll content was significantly lower with a value of 218 μg/g FW.

3.3.3. *Fruit yield and quality*

The effect of the irrigation with WW and the various dilutions on strawberry yield, dry matter (%), total soluble solids (%), and ascorbic acid (mg AA/ 100 g FW) was studied, and results were reported in Table 3.

Strawberry mean production was significantly higher in plant irrigated with WW (118 g per plant) compared with plants irrigated with D20 (48.24 g per plant) and D40 (74.32 g per plant) ($p< 0.01$). However, no significant difference was recorded between the yield of plants irrigated by WW and D60.

The same behaviour was notified for dry matter. Dry matter content was higher in plant irrigated with WW (23.60 %) compared with plants irrigated with D60 (16.62 %) and D40 (14.86 %). Mean production was very low for plants irrigated with D20 (9.65%).

As shown in Table 3, plant yield and dry matter were affected by the toxicity of the effluent, as their concentration decrease with the increase of the effluent pollutant’s concentrations.

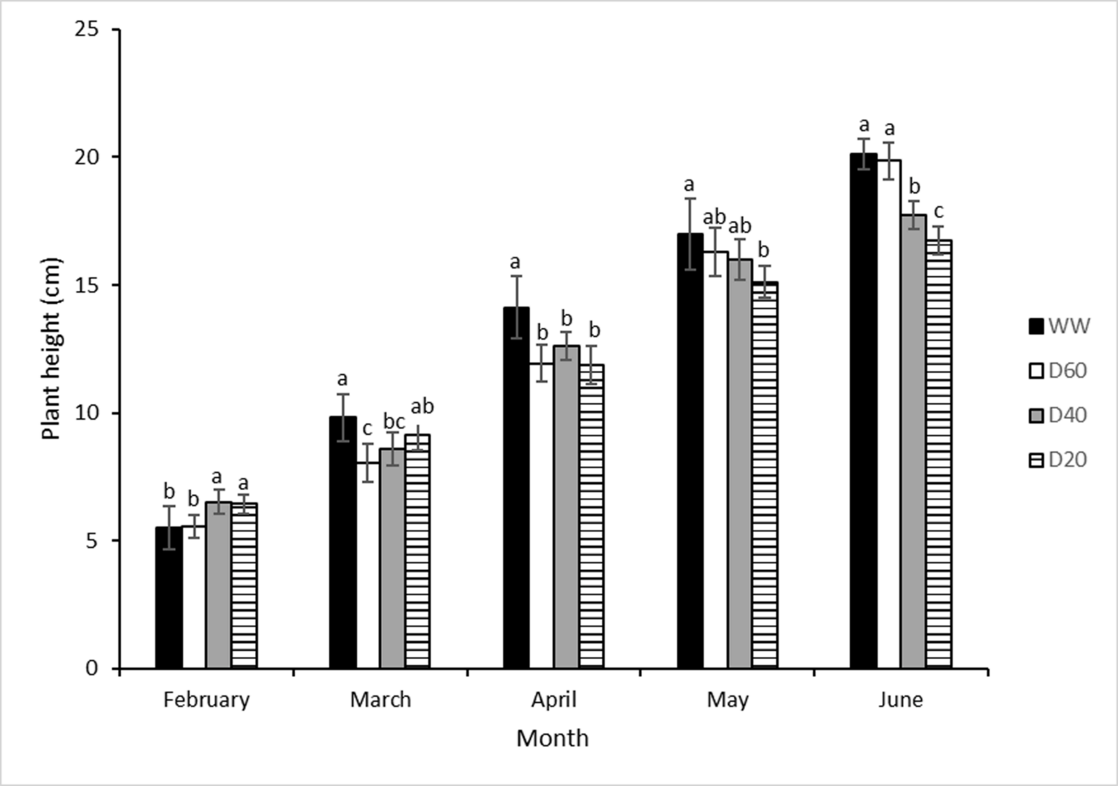

Fig. 1. Effect of well water (WW) diluted treated wastewater D60, D40 and D20 on strawberry plant height according to time. Bars with different letters refer to differences (ANOVA, Tukey's test, $p < 0.05$) between treatments, where the same letter indicates no significant difference.

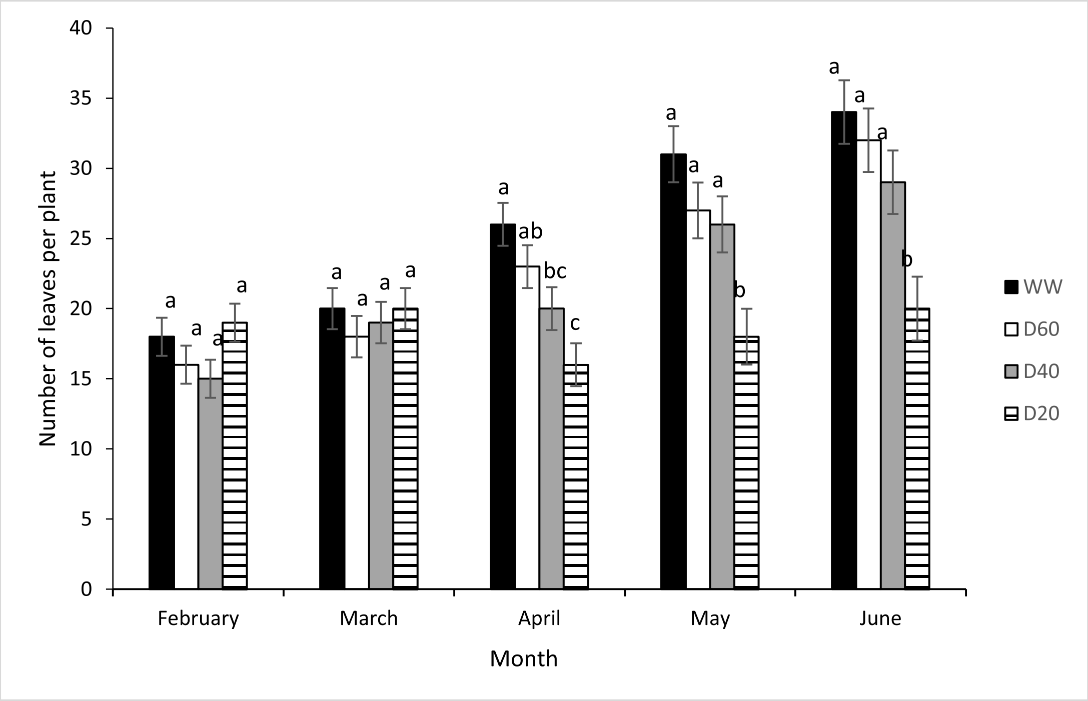

Fig. 2. Effect of well water (WW) diluted treated wastewater D60, D40 and D20 on strawberry leaves number according to time. Bars with different letters refer to differences (ANOVA, Tukey's test, $p < 0.05$) between treatments, where the same letter indicates no significant difference.

3.4. Effect of the dilutions on substrate physicochemical parameters

The evolution of pH, EC, total phosphorus, TOC, NTK, and Cl^- of different substrates was evaluated and the results are demonstrated in Table 4.

As it is demonstrated in Table 4 no significant changes occur between substrate for pH and NTK when treating with the various dilutions. For TOC content, a significant statistically difference ($p<0.05$) was recorded

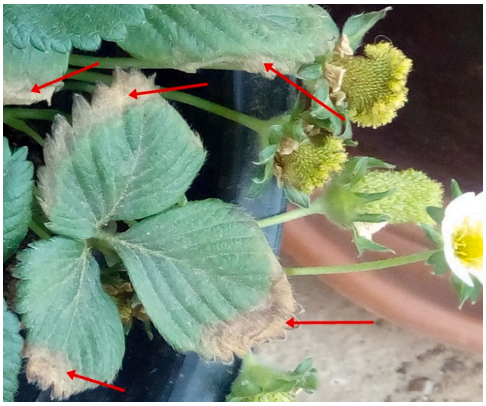

Fig. 3. The apparition of brown edge in strawberry leaves irrigated with D20.

between the substrates irrigated with WW and D20. On the other hand, there is significant statistically differences in EC, P_t and Cl^- between substrate irrigated with WW and different diluted TWWs.

A slighter increase was also observed in the substrate irrigated in D60, such rise proves the fertilizer effect of the TWW. The accumulation of salt in the soil is perfectly correlated to the increasing of EC in the substrate. The increase in such parameters in the substrate after a period of irrigation is totally normal which is usually due to the accumulation of particles present in the irrigation water at the level of the substrate. Similar results were reported by Ahmali et al., (2020). The authors demonstrated an increase in salt content and in the electrical conductivity in the substrate after a period of irrigation using a treated mixture of olive mill wastewater and urban wastewater. El Ghadraoui et al. (2020; 2021) has reported an increase of electrical conductivity, organic content, and total dissolved salts in the substrate of a pilot scale constructed wetland after a period of treatment of highly saline wastewater.

3.5. Heavy metal concentrations in the substrate and in the plant

3.5.1. Heavy metals in the substrate

Table 5 show that no significant difference in substrate heavy metals concentration was observed when irrigating with WW compared to the

Table 3
Effects of well water (WW) diluted treated wastewater D60, D40 and D20 applied for the strawberry plants' irrigation on fruits yield, dry matter, total soluble solids, and ascorbic acid contents. Different letters refer to differences (ANOVA, Tukey's test, $p < 0.05$) between treatments, where the same letter indicates no significant difference.

Parameters	WW	D60	D40	D20
Yield plant (g/plant)	118 ± 9.87 ^{a}	107.09 ± 11.1 ^{a}	74.32 ± 8.74 ^{b}	48.24 ± 7.7 ^{c}
Dry matter (%)	23.6 ± 5.59 ^{a}	16.62 ± 5.18 ^{ab}	14.86 ± 5.78 ^{b}	9.65 ± 4.12 ^{b}
Total Soluble solids (%)	7.68 ± 2.42 ^{a}	7.72 ± 2.72 ^{a}	7.76 ± 2.81 ^{a}	7.81± 3.41 ^{a}
Ascorbic acid (mg AA/ 100 g FW)	39.83 ± 7.58 ^{a}	39.45 ± 7.37 ^{a}	38.98 ± 4.88 ^{a}	38.12 ± 5.65 ^{a}

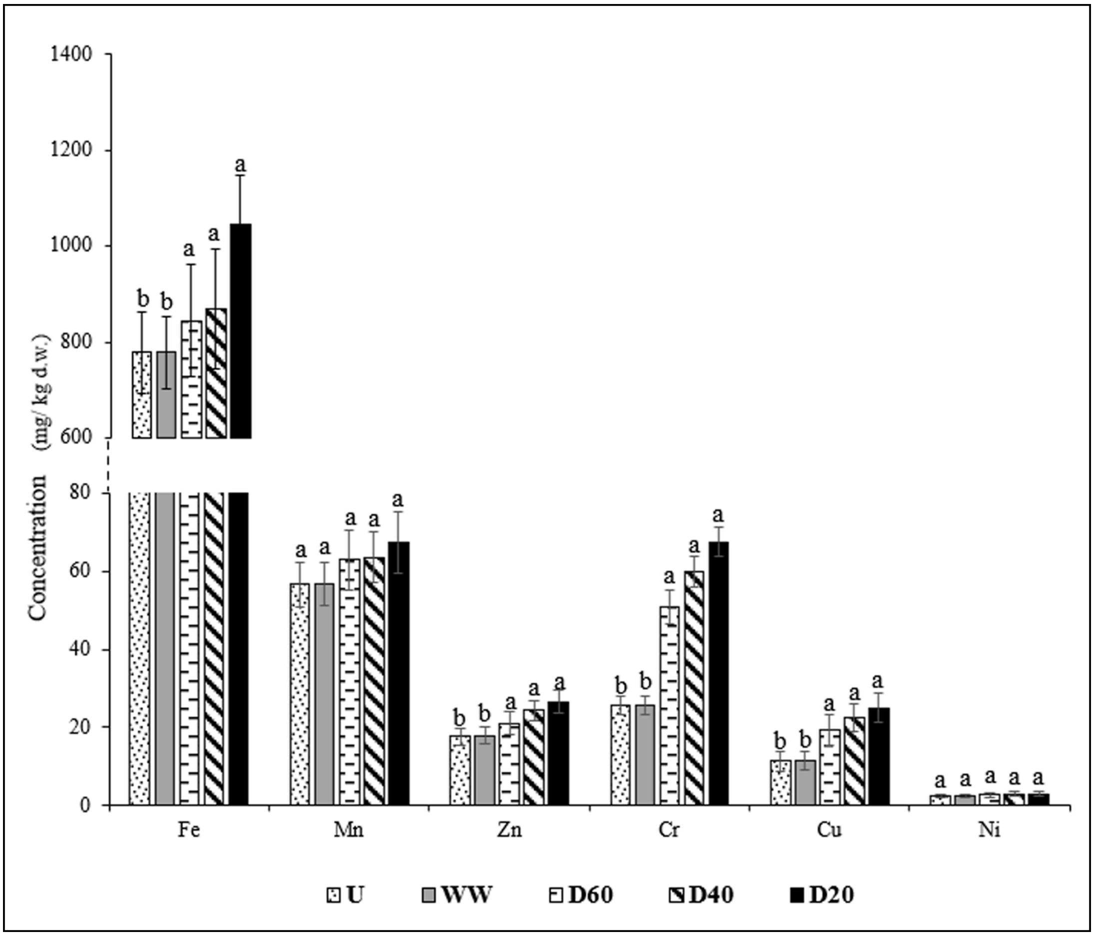

Fig. 4. Metal contents in substrates before (U) and after irrigation with well water (WW) diluted treated wastewater D60, D40 and D20. (mg/kg d.w.). Bars with different letters refer to differences (ANOVA, Tukey's test, $p < 0.05$) between treatments, where the same letter indicates no significant difference.

Table 4
Effects of well water (WW) diluted treated wastewater D60, D40 and D20 applied for the strawberry plants' irrigation on some substrate chemical characteristics.

	pH	EC	Pt (mg/kg)	TOC (%)	NTK (%)	Cl^- (mg/kg)
WW	7.92 ± 1.07	0.76 ± 0.13*	225.46 ± 9.52*	23.67 ± 5.41	0.26 ± 0.06	68.93 ± 5.4*
D60	7.73 ± 1	1.23 ± 0.25*	360.45 ± 8.72*	29.71 ± 7.31	0.3 ± 0.06	134,5 ± 4.7*
D40	7.96 ± 1.24	1.9 ± 0.14*	424.1± 10.26*	29.81 ± 7.77	0.31 ± 0.07	210.9 ± 7.53*
D20	7.92 ± 1.17	2.3 ± 0.16*	479.45 ± 9.09*	31.91 ± 6.9	0.33 ± 0.07	283 ± 5.89*

* Indicate significant differences at a significance level of 5%.

Table 5
Heavy metal (Fe, Mn, Zn, Cu and Ni) contents of strawberry stem and roots irrigated with well water (WW) diluted treated wastewater D60, D40 and D20. Different letters refer to differences (ANOVA, Tukey's test, $p < 0.05$) between treatments, where the same letter indicates no significant difference.

Sample Roots	Fe	Mn	Zn (μg/g d.w.)	Cu	Ni
WW	29.42 ± 6.50 ^{c}	11.64 ±1.72 ^{c}	1.59 ± 0.55 ^{b}	1.76 ± 0.5 ^{b}	0.5 ± 0.14 ^{b}
D60	35.33 ± 6.45 ^{bc}	13.63 ± 1.07 ^{b}	1.59 ± 0.55 ^{b}	2.04 ± 0.67 ^{b}	0.58 ± 0.13 ^{b}
D40	39.11 ± 7.68 ^{ab}	15.51 ± 1.07 ^{a}	2.71 ± 0.49 ^{a}	2.63 ± 0.77 ^{ab}	0.79 ± 0.12 ^{a}
D20	45.02 ± 6.81 ^{a}	16.79 ±1.02 ^{a}	2.98 ± 0.8 ^{a}	3.21 ± 0.74 ^{a}	0.88 ± 0.12 ^{a}
Stem	(μg/g d.w.)				
WW	10.99 ± 2.24 ^{c}	22.25 ± 4.37 ^{a}	5.98 ± 0.87 ^{b}	1.9 ± 0.73 ^{c}	0.76±0.14 ^{b}
D60	14.55 ± 2.97 ^{bc}	24.24±4.14 ^{a}	6.13 ±0.84 ^{b}	2.75 ±0.93 ^{bc}	1.39±0.33 ^{a}
D40	17.55± 2.7 ^{b}	25.43 ± 4.83 ^{a}	6.89 ± 0.96 ^{ab}	3.42 ± 0.98 ^{ab}	1.58±0.26 ^{a}
D20	22.13 ± 3.12 ^{a}	26.96 ± 6.18 ^{a}	7.89 ±0.8 ^{a}	4.51 ± 1.09 ^{a}	1.71±0.34 ^{a}

concentrations in raw substrate. The effect of heavy metals accumulation was significantly higher in substrate irrigated with D20 when compared to the substrate irrigated with D40, D60 and the raw substrate (Table 5).

This difference is obviously due to the concentration of these heavy metals in the irrigation waters. As demonstrated in Table 1, heavy metal concentrations were higher in D20. According the results in Table 1 and Fig. 4, the increase in substrate heavy metals in perfectly correlated with their concentration in the irrigation waters. The lowest heavy metals accumulation in the substrate when using TWW dilutions was observed in the case of D60.

The Ni, Cu and Zn total concentrations in soil increased without reaching 75, 140 and 300 mg kg^{-1}, respectively, which are the maximum permissible limits set by the EU Directive (McGrath et al., 1988). However, since heavy metals tend to accumulate and are persistent pollutant it is clear that the substrate can be used only one more time before reaching the limits.

The added concentration of heavy metals calculated according to the operating conditions (e.g., heavy metal concentration, number of irrigations, and quantity of water per batch) match with a slight difference to the concentration measured in substrate. The major part of heavy metals introduced to each pot were accumulated in the substrate, which indirectly means that only a small fraction was up taking by the plant.

3.5.2. *Heavy metals in the plant*

The concentration of Mn, Zn, Cr, Cu, and Ni in the various part of the strawberry plant (root, stem, and fruit) were evaluated and the results are demonstrated in Table 5.

Table 5 shows that no significant difference in substrate heavy metals concentration was observed when irrigating with WW compared to the concentrations in raw substrate. The effect of heavy metals accumulation was significantly higher in substrate irrigated with D20 when compared to the substrate irrigated with D40, D60 and the substrate before irrigation (Table 4).

When comparing the distribution of heavy metals in the different part of the plant, Table 5 show that all studied metals were found at higher concentrations in aerials parts except from Fe of which higher concentrations were detected in roots. In fact, essential trace elements such as Ni, Cu, and Zn involved in the plant metabolism processes are usually absorbed and translocated in the aerial parts.

For the fruit, results presented in Fig. 5 show that metals accumulation depend strongly on the TWW dilution factor.

Metal's concentration was lower in the fruits harvested from pots irrigated using WW. Cu and Mn concentrations showed high increase with the reduction of the dilution factor compared to Zn, Cr and Ni content. Moreover, the concentrations of Zn, Cu and Ni were lower than the limits set by the WHO/FAO Codex Committee on Food Hygiene.

3.6. *Health risk assessment*

The health risk assessment determined from the target hazard quotients (THQ) and hazard index (HI) was measured and results are presented in Table 6.

Results showed that HI was lower for the strawberry irrigated with well water (0.202) when compared with the one irrigated with D60 (0.243), followed by D40 (0.293). The highest HI value was obtained for D20 (0.34) which contained the higher concentration of heavy metals. However, all HI results were below 1, which evidence the safety of all fruits produced in this experiment according to the standard of the health protection from serious risk. HI was considered as an important way to estimate the possible multiple influences of the presence of heavy metals (Guadie et al., 2021).

Thus, all water resources used in this study did not demonstrate any possible negative health risk for consumers (Chen et al., 2021).

4. Discussion

Table 1 verifies the use of industrial TWW characterized by concentration of Mn, Cr, Cu slightly above the Tunisian law (NT 106.03). The dilution decreased significantly the amount of these elements with the increase in the dilution factor. The irrigations using different diluted TWW induced to the increase of EC, P_t and chloride content in the substrates with regards to results obtained when using WW. These

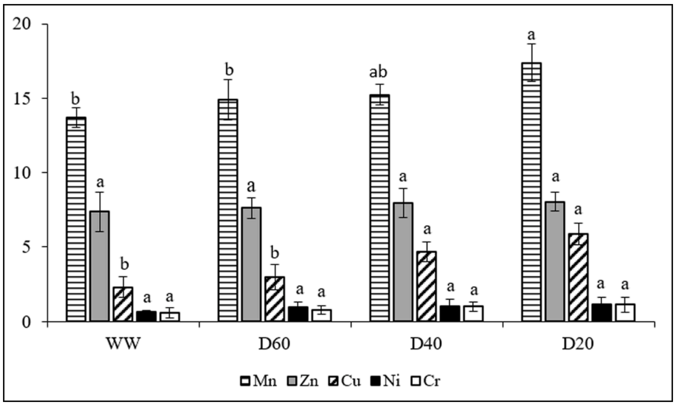

Fig. 5. Heavy metal (Mn, Zn, Cu, Ni and Cr) contents in fruits of plants irrigated with Well water (WW) diluted treated wastewater D60, D40 and D20. Bars with different letters refer to differences (ANOVA, Tukey's test, $p < 0.05$) between treatments, where the same letter indicates no significant difference.

Table 6
The target hazard quotients (THQ) and hazard index (HI) values of strawberries irrigated with Well water (WW) diluted treated wastewater D60, D40 and D20. Different letters refer to differences (ANOVA, Tukey's test, $p < 0.05$) between treatments, where the same letter indicates no significant difference.

	THQ					HI
	Cr	Mn	Zn	Cu	Ni	
WW	0.0003 ± 0.0002^{a}	0.093 ± 0.005^{b}	0.023 ± 0.004^{a}	0.055 ± 0.016^{b}	0.031 ± 0.006^{a}	0.202 ± 0.029^{b}
D 60	0.0005 ± 0.0002^{a}	0.101 ± 0.009^{b}	0.024 ± 0.002^{a}	0.071 ± 0.02^{b}	0.046 ± 0.017^{a}	0.243 ± 0.047^{b}
D 40	0.0006 ± 0.0002^{a}	$0.103\pm0.004a^{b}$	0.025 ± 0.003^{a}	0.111 ± 0.016^{a}	0.052 ± 0.022^{a}	0.293 ± 0.046^{ab}
D 20	0.0007 ± 0.0003^{a}	0.118 ± 0.009^{a}	0.025 ± 0.002^{a}	0.14 ± 0.017^{a}	0.056 ± 0.026^{a}	0.34 ± 0.054^{a}

changes in the physicochemical parameters might be one of the factors in damaging crop productivity and affecting plant growth parameters especially in the case of shoots irrigated with D20 and D40, in which a significant difference when comparing to the control was demonstrated. In fact, the growth of sensitive plants may be significantly impacted when EC is within an interval of 2–4 mS/cm (Keutgen and Pawelzik, 2009). In this sense, Ondrašek et al., (2006) reported that the high conductivity of the soil induced to a sharp decrease in growth and the fresh fruit yield of strawberry. The author reported a decrease of 29%, 35%, and 59% in total yield when using substrate with an initial conductivity of 4 mS/cm, 6 mS/cm and 8 mS/cm, respectively (Ondrašek et al., 2006). Another study suggested that *Toro* and *Douglas* strawberry cultivars are sensitive to the specific action of the chlorine. These cultivars have demonstrated no negative impact at a soil conductivity level of 2 mS/cm. Yet, if the level is higher than this, the risk of leaf damage increases, due to the higher incidence of the hydric deficit, and also to an increase in sensitivity to the action of chlorine (Barroso and Alvarez, 1997). More in details, the noticed recorded decrease in the plant length with the increase of TWW dilution (Fig. 1) could be explained by the decrease in carbohydrates and growth hormones level, the changes in enzyme activity, or the decrease photosynthesis rate which is directly related to the presence of high concentration of chlorine (Dantas et al. 2005, Memon et al. 2010). Heavy metal might also cause stunted stem length (Srivastava et al., 2012).

At the end of the experiment, leaves from strawberry shoots irrigated with D20 were characterized with brown edges (Fig. 3). This phenomenon can be related to salts accumulation in roots, imbalances in ion ratio, lack of iron or high toxic levels of specific ions (Keutgen and Pawelzik, 2009) as high concentrations of chlorine ions can lead to several growth disorders generally observed at the level of leaves number or the quality of the fruit. These issues are mainly due to the destruction of the leaf metabolism and the reduction of nitrate uptake (Keutgen and Pawelzik, 2009). It was reported in literature that high concentration of chlorine can result in chlorotic discolorations and in the apparition of necrotic leaf edged (Geilfus, 2018).

According to Kato and Shimizu, (1985), the decrease in chlorophyll content was related to the photoinhibition or reactive oxygen species formation. It was reported in the literature that excess in chlorine concentration might lead to a chlorosis at the leaf edges which is often explained by low chlorophyll content (Geilfus, 2018). Ahmali et al. (2020) reported a decrease in olive leaves chlorophyll content when irrigating with water with high content of chlorine. Moreover, the decrease in chlorophyll content could also be related to the high concentration of metals present in the dilutions. In fact, it is reported in the literature that metals such as Hg, Cu, Cr, Cd, and Zn have been found to decrease the chlorophyll content in various plants in most cases (Chandra and Kang, 2016). Maleva et al. (2012) have observed that Mn, Cu, Cd, Zn, and Ni caused a significant decrease in chlorophyll contents in *Elodea densa*. Heavy metals are characterized with high redox potential which can inhibit the reductive steps in the biosynthetic pathways of the chlorophyll (Chandra and Kang, 2016). Heavy metals can also inhibit the production of protochlorophyllide reductase which is a key enzyme involved in the reduction of protochlorophyll to chlorophyll (Küpper et al., 1996). Also, the reduction of production yield with the increase of dilution factor (Table 3) was related to various abiotic stress which can be caused by the presence of significant concentration of heavy metals in soil. It was demonstrated by Keunen et al. (2011) that plant growing in heavy metal-rich medium suffer from decreased yield.

Moreover, in accordance with Sahay et al. (2019), in agriculture, the permissible limits for Cr concentration must be in the range of 75 - 100 mg kg^{-1}. Yet, in this study Cr concentration reached a maximum of 67 mg/ kg d.w. using the most polluted dilution (D20) (Fig. 4) which will allow us to deduce that the irrigation using TWW did not record soil heavy metal~~s~~ contents above preferable levels.

The results in Table 5 show that plant's heavy metal accumulations were pronounced by the irrigation using higher percentage of TWW compared to the WW (D60% < D40% < D20%). These findings were confirmed by previous study in the irrigation of tomatoes conducted by Al-Lahham et al. (2007) in which results approve that for WW: TWW ratios {100%: 0%; 50%:50%; 25%:75%; 0%:100%} metals accumulation increased in the same order of dilution. The high level of metal contents in aerials part was explained by Sahay et al (2019) as a way to reduce these toxic elements from plants by the shedding of old leaves.

The fruits heavy metal contents were lower than the standard limits (WHO/FAO). Therefore, and regarding the unchangeable ascorbic acid and total soluble solids fruit's contents, HI and THQ results below the unit and absence of microbial contamination by Escherichia coli, fecal Streptococci, Salmonella spp. in the used TWW, the suggested dilutions of TWW used in this study produced safe fruits considering the microbial contaminations and strawberry consuming on the public health from serious concern brought by heavy metals (Woldetsadik et al., 2017). These results were found also by Christou et al. (2016) that verified that advanced tertiary treated effluent as a valid alternative for the irrigation of strawberry crops.

5. Conclusion

The possible effects of diluted TWW irrigation in strawberry productivity and safety, soil fertility and salinity and metals contamination, compared to WW were evaluated in this work.

Comparing with the WW irrigated plants, TWW improved the soil fertility by increasing total P, NTK and macro- and micronutrients.

Substrate salinity and metal contents in fruits, plant, and substrate showed a reduction with the dilution factor (D20 > D40 > D60). Substrate irrigated with D60 registered an EC that did not exceed 2 mS/cm, thus the use of D60 showed no significant differences with WW irrigated plants and showed beneficial effects on the soil fertility and fruits safety compared to the other dilutions. According to the evaluation of different physicochemical properties of soil, plants growth and fruits yield and quality, the dilution D60 could be a sustainable management solution to use TWW for the irrigation of strawberry which is a saline sensitive plant. Moreover, the target hazard quotient (THQ) and hazard index (HI) revealed that consummation of strawberry fruits after the irrigation using diluted TWW will not exhibit any hazardous health risks. Therefore, this research insists administrators, environmentalists, and public health employee to use TWW on the irrigation of strawberry plants considering its safety aspect, hence reducing the fresh water shortage risk.

CRediT authorship contribution statement

Zaineb Bakari: Formal analysis, Writing – original draft. **Ayoub El Ghadraoui:** Data curation, Writing – original draft. **Nesrine Boujelben:** Resources, Funding acquisition, Supervision, Writing – review & editing. **Massimo Del Bubba:** Project administration, Resources, Funding acquisition, Supervision. **Boubaker Elleuch:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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