Reclaimed municipal wastewater for forage production

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ABSTRACT

This study aims to evaluate the possibility of using reclaimed municipal wastewater for agricultural purpose. We assessed the validity of municipal wastewater treatment, analyzing its chemical characteristics before and after the biological stabilization by pond treatment (WSP). The reclaimed municipal treated wastewater (TWW) was used to irrigate *Cenchrus ciliaris*. Experiments were carried out in greenhouse, from July 2013 to July 2014, comparing the effects of TWW with the water normally used for irrigation (tap water, TW) on the growth and flowering parameters of *C. ciliaris*. During this study, total coliforms, fecal coliforms, *Escherichia coli*, and *Salmonella* spp. were detected in TW, TWW, soils and plants under irrigation. Our results evidenced that TWW increased plant growth, producing taller plants with respect to TW. Total coliforms and fecal coliforms in TWW, TW, soils and plants were under the threshold recommended by the World Health Organization (WHO). *Salmonella* was never found in TW, TWW, or soil and plants irrigated with TWW. The absence of pathogens suggests that the pond treatment is an effective method to reclaim wastewater, lowering biochemical oxygen demand (BOD), chemical oxygen demand (COD) and pathogens. In this respect, TWW can be used as a valid alternative to freshwater for irrigation of fodder species.

Key words | *Cenchrus ciliaris*, microbial contamination, treated wastewater reuse, vegetable production, water scarcity

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INTRODUCTION

Food and water security is a growing global challenge, especially for marginal environments such as the Middle East and North Africa regions, which receive a mere 1.3% of the world's renewable freshwater but have the highest population growth rate in the world (Karrou & Oweis 2012). These factors, along with the projected effects of climate change, put enormous pressure on agriculture to reduce its share of freshwater use and look for alternative sources to meet the requirements. Municipal wastewater is a useful option for farm production systems as it contains organic matter and nutrients which are essential for plant growth and crop yield improvement (Chavez *et al.* 2012). However, its use needs careful handling to mitigate its detrimental effects on soils, crops and human health. The municipal wastewater has been recycled in agriculture for centuries (Jang *et al.* 2012); the farmers irrigated with diluted, untreated, or partly treated wastewater (TWW); consequently, the lack of appropriate wastewater treatment generated adverse health effects (Jeong *et al.* 2013), associated with the presence of high concentrations of human pathogens, enteric in origin, such as viruses, protozoa, bacteria, and helminths (Agrafioti & Diamadopoulos 2012). In this respect, it is necessary to adequately treat wastewater before its use in the environment, lowering the pathogen concentration to 1,000 most probable number (MPN)/100 mL of fecal coliforms, which is the threshold recommended by the World Health Organization (WHO), for unrestricted irrigation of crops to be eaten uncooked, sport fields and public parks. Therefore, according to its composition and to the international standards of water irrigation quality, the use of municipal wastewater after a reclaimed process may represent the most promising practice for ensuring safe and sustainable food crops in arid and semi-arid regions, with consequent reduction in the use of chemical fertilizers (Hong *et al.* 2014).

On the basis of the above statements, the aim of this paper was to evaluate the suitability of municipal wastewater, reclaimed through the biological stabilization by pond treatment, to be used as nutrient enriched water to irrigate *Cenchrus ciliaris* L. (syn. *Pennisetum ciliare* (L.) Link, Buffel grass). This species, native to dry areas of Africa,

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West Asia and India, has been introduced in arid and semi-arid regions of the world for its high pastoral value (Angassa 2012), and now it is also widely distributed in Tunisia (Kharrat-Souissi *et al.* 2012). Despite its well-known importance as fodder, *C. ciliaris* is a hyper-root-accumulator of heavy metal (Mukhtar *et al.* 2013), and its leaves contain compounds able to inhibit bacterial/fungal growth. In this study, we assessed the effects of irrigation with reclaimed municipal wastewater, in comparison to tap water (TW), on the growth and flowering parameters of *C. ciliaris* during two vegetative cycles, evaluating also the amount of total coliforms, fecal coliforms, *Escherichia coli* and *Salmonella* spp. in reclaimed municipal wastewater, TW, and irrigated soil and plant.

MATERIALS AND METHODS

Sampling and analyses

Reclaimed wastewater was sampled at the outlet of the Sfax wastewater treatment plant, where municipal wastewater was treated with the biological stabilization ponds, at different times and stored at 4 °C before the chemical characterization. In the anaerobic ponds a large concentration of organic and inorganic solids in wastewater is stabilized. The anaerobic digestion that occurs in ponds converts organic load and releases some by-products into water, reducing the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values and pathogen load, as already reported by the Food and Agriculture Organization (FAO 1992).

The irrigation water samples (TWW and TW) were taken and immediately analyzed (except for metal) according to the Tunisian standard methods, and analyzed for chemical parameters according to *Standard Methods* (APHA). The measured parameters were: pH, electrical conductivity (EC), BOD, COD, total dissolved solids (TDS), suspended matter (SM), total coliforms and fecal coliforms, anions, cations, and total P and N. Samples for metals were preserved with nitric acid and then analyzed by atomic absorption spectroscopy (GBCmod. 908). Water samples were taken nine times for each experimental cycle and collected randomly in four replicates using 1 L sterile glass bottles and stored at +4 °C before microbiological analysis. Soil samples were collected in quadruplicate at 10 cm depth, 10 days after the end of each irrigation cycle. Soil samples were placed in sterile plastic bags, brought to the laboratory and stored at +4.0 °C. Microbiological analysis was done within 24 h from sample collection. The analyses were performed on sub-samples collected at every pot after each crop cycle, under the drippers. The leaves of *C. ciliaris* were harvested by hand from the crown portion nearest the drippers (the worst case condition in terms of potential contamination), stored at +4.0 °C and transported to the laboratory in sterile plastic bags. Using a sterile knife, 200 g of leaves were aseptically weighed into a sterile jar and 500 mL of sterile water was added. The jars were shaken by machine for 15 min and the solutions were used for chemical analysis. Microbiological analyses, including total coliforms, fecal coliforms, *E. coli* and *Salmonella*, were carried out on water, soil and leaves of *C. ciliaris*. The densities of colonies were expressed as $\log_{10}$ colony forming unit (CFU) 100 mL^{-1} for waters, $\log_{10}$ CFU 100 g^{-1} for soils and CFU g^{-1} for plant material. The same water samples were also analyzed for *Salmonella* spp., with their detection performed according to a procedure consisting of a 'pre-enrichment' stage using a buffered peptone water solution, a non-selective culture medium to revitalize the microorganism. An inoculum culture was prepared in Selenite and Cystim medium and incubated at 36 °C for 48 h. After the incubation period, several cultures were inoculated and incubated in parallel on SS-agar gel to identify and enumerate any *Salmonella* colonies.

Plant material

C. ciliaris belongs to the grass family (Poaceae), subfamily Panicoideae, tribe Paniceae. It is an erect, deep-rooted, tussock forming, summer-growing perennial grass. It is an apomictic, polyploid grass with three ploidy levels: tetraploid ($2n = 4x = 36$), pentaploid ($2n = 5x = 45$) and hexaploid ($2n = 6x = 54$) (Kharrat-Souissi *et al.* 2012). Stems of *C. ciliaris* can be either erect or decumbent. The leaves are linear blades, green to bluish green, slightly pilose, 3–30 cm long and 4–10 mm wide. Flowering inflorescences of *C. ciliaris* are dense, cylindrical and 2–13 cm long by 1.0–2.6 cm wide; each inflorescence has 30–50 involucres bracts. The fruit is an ovoid caryopsis that is 1.4–1.9 mm long by 1.0 mm broad.

In this work we used hexaploid *C. ciliaris*, widely distributed in the dry zones of the South of Tunisia and well adapted to drought conditions. Seeds were collected in the National Park of Bou Hedma (Centre) (Mezzouna: latitude 34 °28 N, longitude 09 38 ° E).

Experimental design

The experiment was conducted in polyethylene pots maintained in a non-air-conditioned, arched-roof plastic greenhouse, with ceiling height of 2.5 m on the sides and

6.5 m in the central part, and open sides to maintain natural ventilation. The structure was installed in an area belonging to the Olive Tree Institute of Sfax (34 °43N, 10 °41E), located in Central-Eastern Tunisia. The experiment has been conducted during two cycles of vegetation growth. In August 2012, six seeds from hexaploid *Cenchrus ciliaris* were sown per pot (pot capacity 20 L, diameter 30 cm, depth 30 cm) with a total of 80 pots. Chemical composition of the potting soil was 3.16 mEq L^{-1} of K, 6.64 mEq L^{-1} of Na and 0.44% of $CaSO_42H_2O$. Total $CaCO_3$ of the soil was near 13%, organic matter percentage was 3.7% and electric conductivity was 3.7 mS cm^{-1}. The experiments were carried under environmental conditions with natural sunlight and temperature. The averages for the relative humidity and the temperature ranged between day and night from 50 and 80% and from 22 ± 8 to 11 ± 5 °C, respectively. The average of photosynthetically active radiation was 750 ± 1,120 μmol/m^2/s during the course of the experiment. After germination, only one plant was left in each pot. During establishment, individuals were regularly irrigated to ensure maximum survival. Pots were irrigated with TW with a pH of 7.51, an electric conductivity of 1.78 mS cms^{-1} and a dry residue of 1,220 mg Ls^{-1}. A year after plant establishment (in August 2013) adult plants were cut 3 cm above soil surface to simulate level growth zero during the summer season. After the cutting procedure, two irrigation treatments were applied during July 2013–July 2014 with two growth cycles: the first cycle from July to November and the second from March to July 2014. During this time in each treatment the following total amount of water was applied: 800 mm in T1 (TW) and 800 mm in T2 (TWW). So 40 pots were irrigated with TW and 40 with TWW. The frequency of irrigation was on a 10-day basis (1st, 10th and 20th day of each month). Since *C. ciliaris* occurs naturally in areas with average annual rainfall from as low as 100 mm up to about 1,000 mm, we chose 800 mm as irrigation rate.

Measurements of morphological and phenological traits were conducted across two growth cycles, from the beginning of the vegetative activity (in August 2013) until the end of this activity (in June 2014). Daily and weekly observations were obtained during both the first and second growth periods.

Vegetative growth phase

Tillering in the first growth cycle took place between the end of August and the appearance of the first spike (between 23 August 2013 and 28 November 2013). Tillering in the second growth cycle was observed between 7 April 2014 and 9 July 2014. During the tillering phase, we measured the following tiller and leaf characteristics from the first and second growth periods: leaf number; leaf length; tiller number; tiller length; plant height and plant diameter.

Reproductive growth phase

This period begins with the appearance of the first spike. It stops either in winter (first growth period) or in the beginning of summer when the tuft dries up (second growth period). The following parameters were observed: spike number; seeds number and inflorescence lengths.

At the end of each growth cycle, sections of 3 cm from above the soil surface including stems, leaves and cobs were collected from each plant. The plant material was dried in the oven at 80 °C for 48 h, and subsequently weighed to obtain dry matter.

Statistical analysis

Data were analysed for mean values and standard deviation by using SPSS program (version 19). A one-way analysis of variance was used to compare the effects of each irrigation water source on plant growth characteristics. Differences between means were tested for significance ($p < 0.05$) by Tukey's test.

RESULTS AND DISCUSSION

Irrigation water properties

Note: TWW (Table 1) contained high concentrations of Cl^- and Na^+ and high amounts of total and fecal coliforms; thus, if used for irrigation without preliminary treatments it could have negative consequences for crop and human health. The biological treatment of wastewaters improved their quality from a chemical point of view, decreasing the concentration of Cl^- and Na^+, and breaking down the polluting power. All the chemical parameters were within the limits permitted by Tunisian regulation except for chloride.TW and TWW had different physical and chemical characteristics: TWW was characterized by higher N (especially as N-NO_4^+), P, K^+, Na^+, Ca^{2+}, Cl^-, $SO4_2^+$, HCO_3^- and organic carbon than TW. The high salt content in TWW was the cause of its highest EC value and TDS content (Table 2). Mg^{2+} was the only cation significantly lower (85.70 mg L^{-1}) in TWW than in TW (126.20 mg L^{-1}) (Table 2). The higher nutrient

Table 1 | Physico-chemical characteristics of raw wastewater

Characteristics	**Units**	**Wastewater**
pH (20 °C)		7.40 ± 0.2
EC (20 °C)	(mS cm^{-1})	7.11 ± 1.9
TDS	(g L^{-1})	2.20 ± 0.01
COD	(mg L^{-1})	382 ± 0.7
BOD	(mg L^{-1})	167 ± 0.2
HCO_3^-	(mg L^{-1})	504.13 ± 0.5
SO_4^{2-}	(mg L^{-1})	398.66 ± 0.9
N total	(mg L^{-1})	108.03 ± 1.4
N-NO_3^-	(mg L^{-1})	18.96 ± 0.06
N-NO_4^+	(mg L^{-1})	72.3 ± 0.03
N-NO_2^-	(mg L^{-1})	97.99 ± 0.04
P total	(mg L^{-1})	26.22 ± 0.9
K^+	(mg L^{-1})	60.45 ± 0.1
Na^+	(mg L^{-1})	379.15 ± 0.04
Cl^-	(mg L^{-1})	2,129 ± 0.08
Ca^{2+}	(mg L^{-1})	149 ± 0.03
Mg^{2+}	(mg L^{-1})	131 ± 0.01
Pb^{2+}	(mg L^{-1})	0.19 ± 0.01
Cd^{2+}	(mg L^{-1})	0.02 ± 0.00
Zn^{2+}	(mg L^{-1})	0.49 ± 0.01
Mn^{2+}	(mg L^{-1})	0.81 ± 0.01
SM	(mg L^{-1})	25.77 ± 0.03
Total coliforms	(UFC/100 mL)	$6.3\ 10^6 \pm 2.04\ 10^4$
Fecal coliforms	(UFC/100 mL)	$3.9\ 10^5 \pm 2.24\ 10^4$

EC, electrical conductivity; TDS, total dissolved solids; COD, chemical oxygen demand; BOD, biological oxygen demand; SM, suspended matter. Data represent mean values ± standard deviation.

amount in TWW compared with TW indicates that TWW may represent an important source of nutrients for plants and can contribute to crop growth. The addition of TWW to the soil can increase short-term fertility for the provision of nutrients readily available for plants and long-term fertility due to the organic matter added to soil. Both TW and TWW were alkaline, and TWW had the highest pH value. The heavy metal content (Cd, Zn, Cr, and Pb) was lower than the toxicity limits (<0.004 mg/L). The results obtained evidenced that the chemical properties of treated municipal wastewater met the Tunisian standards for wastewater reuse.

Growth and flowering parameters

Crops irrigated with TWW showed a better growth during the two growth cycles than those irrigated with TW (Table 3). In both cycles of growth the quality of irrigation water significantly affected plant heights. However, a significant increase in terms of height was observed in the two growth cycles. The greatest height (64.45 cm) was observed in wastewater irrigated plants, in November (end of the first cycle of growth). The results evidenced that the plants irrigated with TW were shorter than plants irrigated with reclaimed wastewater. Similar results on tomato were reported by Cirelli *et al.* (2012), who observed that crops irrigated with TWW produced taller plants and a yield increase of about 20% compared with those grown with pump water alone. Our results showed that the diameter of plants irrigated with TWW was larger than the diameter of those irrigated with TW in both growth cycles. The largest diameter (52.60 cm) was observed in wastewater irrigated plants, in November. Leaf length, in the first cycle, was increased more by TWW than TW, while no significant differences in leaf length were observed in the second cycle between the TWW and TW irrigated plants. The highest leaf length (26.93 cm) was observed under TWW irrigation, in July. The greatest leaf number was detected in plants irrigated with TWW in both growing cycles. The irrigation with TWW increased leaf number and growth of *C. ciliaris* mainly during the second vegetative cycle. These results are in agreement with those of Oliveira-Marinho *et al.* (2013) indicating an increase in the leaf number of *Rosa hybrida* irrigated with TWW. An increase in leaf number was also reported for *Gossypium hirsutum* (Alikhasi *et al.* 2012) when irrigated with biologically TWW. Except the first two months of each growth cycles, the tiller length for all plants irrigated with TWW showed a better performance with respect to plants irrigated with TW. Concerning tiller number, in the second cycle, TWW increased tiller number compared with TW, while no significant differences in tiller number were observed in the first cycle between the TWW and TW irrigated plants. The highest tiller number (10.80) was observed under TWW irrigation, in July.

TWW not only increased the growth and the flowering power of *Cenchrus ciliaris* but also intensified its physiological processes (Table 3). All plants irrigated with TWW showed, during the second growth cycle, a better performance than plants irrigated with TW, even if TWW contained a higher concentration of chloride. These results confirmed previous findings of Celaya *et al.* (2015) that identified *Cenchrus ciliaris* as a suitable plant to be used for bioremediation of surface saline soils, or marine sediments, just for its ability to grow in presence of 1–2% NaCl. Only in August did the quality of irrigation water not cause

Table 2 | Chemical characteristics of TWW and TW

Characteristics	**Units**	**TWW**	**TW**	**Tunisian regulation**
pH (20 °C)		7.60 ± 0.10	7.51 ± 0.11	6.50–8.50
EC (20 °C)	(mS cm^{-1})	5.6 ± 0.02	4.30 ± 0.03	7.00
TDS	(g L^{-1})	1.77 ± 0.02	0.93 ± 0.01	2.00
COD	(mg L^{-1})	74.00 ± 0.01	0	90.00
BOD	(mg L^{-1})	20.00 ± 0.01	0	30.00
HCO_3^-	(mg L^{-1})	356.00 ± 0.3	223.30 ± 0.20	600.00
SO_4^{2-}	(mg L^{-1})	354.00 ± 0.7	67.50 ± 1.5	1,000
N total	(mg L^{-1})	53.80 ± 1.20	–	30.00
N-NO_3^-	(mg L^{-1})	13.40 ± 0.01	0.97 ± 0.01	–
N-NO_4^+	(mg L^{-1})	35.6 ± 0.01	2.67 ± 0.04	–
N-NO_2^-	(mg L^{-1})	4.00 ± 0.02	0.04 ± 0.01	–
P total	(mg L^{-1})	9.44 ± 0.11	0.45 ± 0.02	0.05
K^+	(mg L^{-1})	33.80 ± 0.09	26.00 ± 0.05	50.00
Na^+	(mg L^{-1})	430.00 ± 0.01	297 ± 0.01	300.00
Cl^-	(mg L^{-1})	1,767 ± 0.04	1,340.00 ± 0.2	600.00
Ca^{2+}	(mg L^{-1})	298.50 ± 0.01	188.20 ± 0.0	–
Mg^{2+}	(mg L^{-1})	85.70 ± 0.01	126.20 ± 0.03	–
Pb^{2+}	(mg L^{-1})	<0.004	0	0.10
Cd^{2+}	(mg L^{-1})	<0.004	0	0.005
Zn^{2+}	(mg L^{-1})	0.33 ± 0.01	0.5 ± 0.01	5.00
Mn^{2+}	(mg L^{-1})	0.65 ± 0.01	0.13 ± 0.03	–
SM	(mg L^{-1})	12.20 ± 0.02	2.30 ± 0.02	
Total coliforms	(UFC/100 mL)	nd	0	–
Fecal coliforms	(UFC/100 mL)	nd	0	–

EC, electrical conductivity; TDS, total dissolved solids; COD, chemical oxygen demand; BOD, biological oxygen demand; SM, suspended matter. Data represent mean values ± standard deviation.

significant differences in the number of spikes (Table 4). The largest number of spikes (34.40) was observed in July and during the second vegetation cycle in TWW irrigated plants. This high number of spikes could be attributed to the increase in the absorption of macro and micro nutrients from the TWW. Several researchers attributed the increase in crop production to the increase in the nutrient availability (Mousavi & Shahsavari 2014; Noor *et al.* 2014). The seed number of plants irrigated with TWW was larger than plants irrigated with TW in both growth cycles. The largest seed number (64.80) was observed in wastewater irrigated plants, in July, the end of the second growth cycle. Wastewater appeared to be an important source of nutrients with the potential of increasing plant productivity, as already demonstrated for beans and fruit trees by Oliveira *et al.* (2013). According to these authors, the use of wastewater in fertigation brought benefits not only to the environment but also to the farmers by reducing the costs of chemical fertilizer application. Moreover, Silva *et al.* (2012) showed increased Mombaça grass productivity as a function of the sewage dose applied. Our data showed also an increase in inflorescence length, in both cycles, in presence of TWW. The highest inflorescence length (13.40 cm) was observed under TWW irrigation, in July. Biomass of hexaploid *C. ciliaris* irrigated with TWW was significantly higher than that irrigated with TW (Figure 1). A similar behavior was reported by Zema *et al.* (2012) in fodder species irrigated with treated urban wastewater. The irrigation with TWW significantly increased plant biomass over time. These results suggest that the application of TWW may add nutrients and bacteria to the soil, increasing biodiversity and abundance of soil organisms that are important to maintain agro-ecosystem services mainly in arid and semiarid regions. Our results are in agreement with the findings of

Table 3 | Effects of irrigation with TW and TWW on growth parameters of hexaploid *C. ciliaris* from July (2013) to July (2014)

		First cycle					**Second cycle**				
Parameter	**Irrigation water**	**July**	**August**	**September**	**October**	**November**	**March**	**April**	**May**	**June**	**July**
Plant height (cm)	TW	8.45	17.32	32.50	53.65	61.35	12.05	17.05	28.52	45.25	50.55
	TWW	8.05	22.30	41.40	56.45	64.45	13.80	21.85	36.70	55.30	56.45
	Significance	$0.245^{n{\cdot}s}$	0.000**	0.000***	0.000**	0.000**	0.000**	0.000**	0.000**	0.000***	0.000**
Plant diameter (cm)	TW	16.65	21.70	29.55	34.75	42.15	15.05	28.15	33.30	35.55	38.40
	TWW	20.45	28.40	35.00	43.45	52.60	18.40	33.80	35.60	37.30	49.15
	Significance	0.000**	0.000**	0.000**	0.000**	0.000***	0.000**	0.000**	0.000**	0.000**	0.000**
Leaf length (cm)	TW	6.470	12.750	18.550	19.520	21.470	4.980	13.970	22.280	25.580	27.270
	TWW	7.550	12.430	24.000	24.530	24.960	4.910	14.550	23.170	26.180	26.930
	Significance	$0.026^{n{\cdot}s}$	$0.544^{n{\cdot}s}$	0.000**	0.000**	0.000**	$0.827^{n{\cdot}s}$	$0.336^{n{\cdot}s}$	$0.058^{n{\cdot}s}$	$0.266^{n{\cdot}s}$	$0.239^{n{\cdot}s}$
Leaf number	TW	5.50	8.10	12.20	15.20	16.30	3.60	7.00	12.80	15.20	18.50
	TWW	5.80	16.90	21.90	23.50	24.40	3.50	8.50	14.40	19.90	24.00
	Significance	$0.382^{n{\cdot}s}$	0.000**	0.000**	0.000**	0.000**	$0.673^{n{\cdot}s}$	0.000**	0.001*	0.000**	0.000**
Tiller length (cm)	TW	0.00	1.50	5.00	9.10	14.40	0.00	2.80	6.40	10.40	16.70
	TWW	0.00	2.60	8.00	13.80	19.80	0.00	3.50	9.00	15.30	20.15
	Significance	.	$0.102^{n{\cdot}s}$	$0.002^{n{\cdot}s}$	0.000**	0.000**	.	$0.242^{n{\cdot}s}$	0.000**	0.000**	0.000**
Tiller number	TW	0.00	1.30	2.60	3.50	5.50	0.00	1.90	3.20	4.50	6.60
	TWW	0.00	1.80	2.60	4.90	6.80	0.00	1.90	4.30	5.60	10.80
	Significance	.	$0.299^{n{\cdot}s}$	$0.313^{n{\cdot}s}$	$0.012^{n{\cdot}s}$	$0.005^{n{\cdot}s}$	.	$1.000^{n{\cdot}s}$	0.000**	0.000**	0.000**

n.s., not-significant; ***$p < 0.0001$; **$p < 0.001$; *$p < 0.01$.

Table 4 | Effects of irrigation with TW and TWW on flowering parameters of hexaploid *C. ciliaris* from July (2013) to July (2014)

Parameter	Irrigation water	First cycle					Second cycle				
		July	August	September	October	November	March	April	May	Juin	July
Spike number	TW	0.00	0.80	2.70	5.60	8.10	0.00	2.80	6.60	22.90	22.90
	TWW	0.00	1.10	6.90	12.60	14.40	0.00	10.10	26.20	34.40	34.40
	Significance	.	0.611$^{n.s}$	0.000**	0.000**	0.000**	.	0.000**	0.000**	0.000**	0.000**
Seed number	TW	0.00	19.7	22.30	33.90	40.30	0.00	18.40	31.40	40.20	48.2
	TWW	0.00	26.5	28.5	39.70	45.60	0.00	29.20	36.40	49.20	64.80
	Significance	.	0.000**	0.000**	0.000**	0.000**	.	0.000**	0.000**	0.000**	0.000**
Inflorescence length	TW	0.00	2.10	4.20	6.85	10.10	0.00	2.55	6.10	8.70	11.30
	TWW	0.00	2.00	6.85	9.65	12.90	0.00	2.65	8.90	11.20	13.40
	Significance	.	0.736$^{n.s}$	0.000**	0.000**	0.000**	.	0.691$^{n.s}$	0.000**	0.000**	0.001*

n.s., not-significant; $^{**}p < 0.001$; $^{*}p < 0.01$.

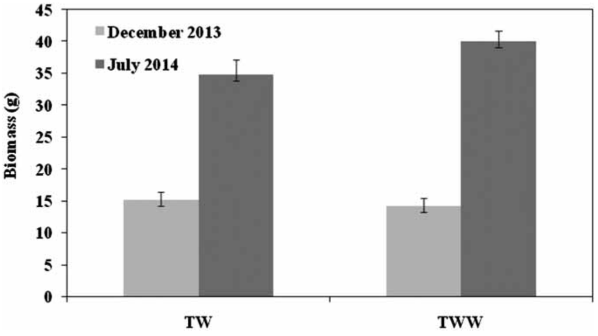

Figure 1 | Effects of tap water (TW) and treated municipal wastewater (TWW) on growth of hexaploid *Cenchrus ciliaris,* during the experimental time (June 2013–July 2014). Growth of *Cenchrus ciliaris* is expressed as dry matter (g).

Bloom (2015) showing that microbial activities were significantly higher in soils irrigated with urban wastewater than in those irrigated with fresh water.

Microbiological quality of the water, soil and plant material

The pathogen load in terms of total and fecal coliforms was completely lowered by the decontamination treatment (Pond) (Table 1). TWW did not contain pathogens. Even if TWW showed the highest levels of total coliforms (Figure 2), fecal coliforms (Figure 3) and *E. coli* (Figure 4), the number of

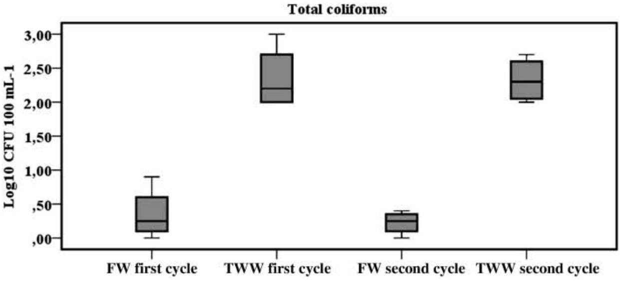

Figure 2 | Total coliforms ($\log_{10}$ CFU 100 mL^{-1}) detected in TW (tap water) and TWW (treated wastewater) during the first and the second cycle.

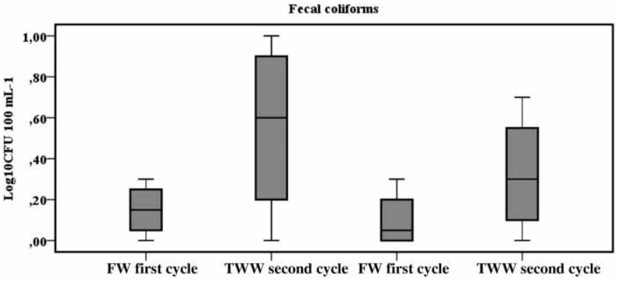

Figure 3 | Fecal coliforms ($\log_{10}$ CFU 100 mL^{-1}) measured in TW (tap water) and TWW (treated wastewater) during the first and the second cycle.

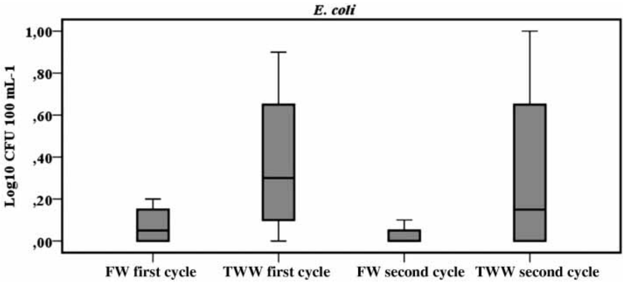

Figure 4 | *E. coli* ($\log_{10}$ CFU 100 mL^{-1}) measured in the TW (tap water) and TWW (treated wastewater) during the first and the second cycle.

CFU was always within the limits allowed by Tunisian regulation for TWW reuse.TW and TWW did not contain *Salmonella* spp. After the first cycle of irrigation with TWW, soils showed the highest levels of total coliforms (Figure 5), fecal coliforms (Figure 6) and *E. coli* (Figure 7), varying from a minimum of 1.90 $\log_{10}$ CFU 100 g^{-1} to a maximum of 3.69 $\log_{10}$ CFU 100 g^{-1}, from 1.90 to 3.71 $\log_{10}$ CFU 100 g^{-1} and from 0 and 1.18 $\log_{10}$ CFU 100 g^{-1}, respectively. *Salmonella* spp. were not found in the irrigated soils. In the second cycle, the total coliforms, fecal coliforms and *E. coli* increased, ranging from a minimum of 2.18 to a maximum of 4.27 $\log_{10}$ CFU g^{-1}, from 2.55 to 4.24 $\log_{10}$ CFU g^{-1} and from 0 to 1.5 $\log_{10}$ CFU g^{-1}, respectively.

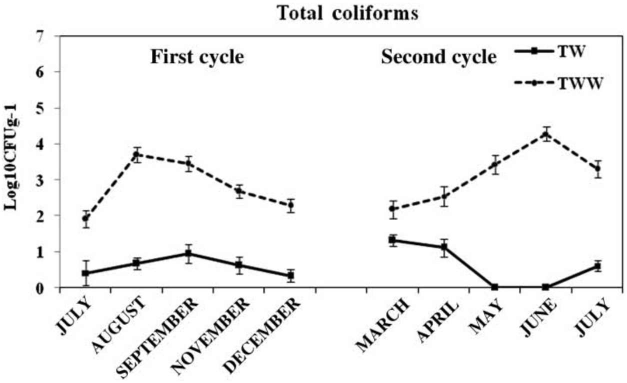

Figure 5 | Total coliforms ($\log_{10}$ CFU g^{-1}) recorded in soil irrigated with TW (tap water), and TWW (treated wastewater): the values are the mean of 10 samples and each point was the mean of four replicates. The samples were collected from July 2013 to July 2014.

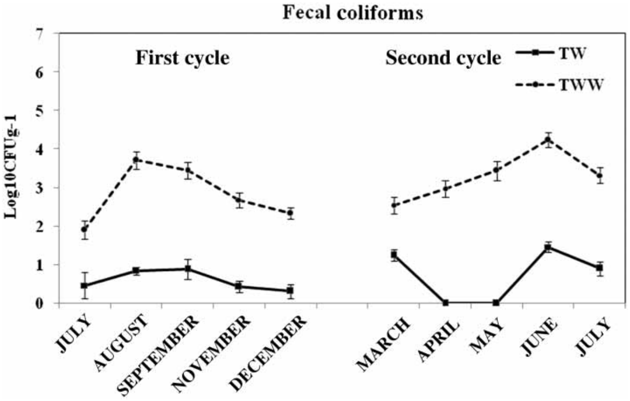

Figure 6 | Fecal coliforms ($\log_{10}$ CFU g^{-1}) recorded in the soil irrigated with TW (tap water), and TWW (treated wastewater): the values are the mean of 10 samples and each point was the mean of four replicates. The samples were collected from July 2013 to July 2014.

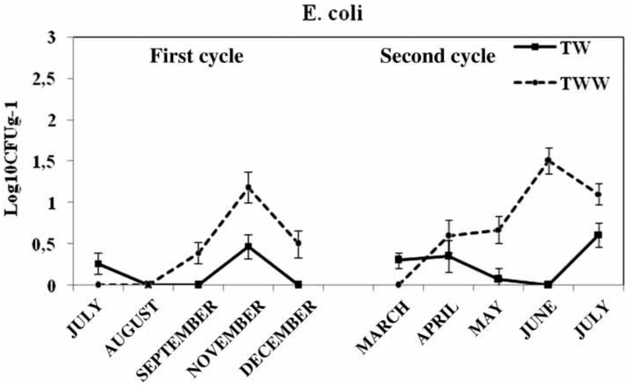

Figure 7 | *E. coli* ($\log_{10}$ CFU g^{-1}) recorded in the soil irrigated with TW (tap water), and TWW (treated wastewater): the values are the mean of 10 samples and each point was the mean of 4 replicates. The samples were collected from July 2013 to July 2014.

The leaves collected from *C. ciliaris* irrigated with TWW showed the highest total coliforms values in both cycles, with a maximum of 121 CFU g^{-1} in the first cycle and of 830 CFU g^{-1} in the second cycle (Table 5). Only the plants irrigated with TWW contained fecal coliforms, with a maximum of 93 CFU g^{-1} in the first cycle and of 120 CFU g^{-1} in the second cycle. No *Salmonella* spp. contamination was recorded in all the irrigation treatments, indicating no significant health risk, as it is well known that total coliforms are ubiquitous in agricultural environments. No *E. coli* contamination was observed in leaves of *C. ciliaris* in all the irrigation treatments. Analogous data were obtained for the plant and fruit here, as no *E. coli* was isolated from either, and the levels of fecal coliforms and total coliforms were relatively low and not influenced by the water used for irrigation ($p > 0.05$). These results are in agreement with other studies (Cirelli *et al.* 2012), in which only fruits (tomato and eggplant) directly in contact with the soil were contaminated by fecal bacteria. The good microbial quality of *C. ciliaris* leaves (no *E. coli*, very low fecal and total coliforms, no *Salmonella* spp.) can be seen as the positive consequence of several factors: firstly the interval of time between irrigation and sampling, which may have contributed to reduce the microbial load on the leaves, and secondly the longer time of leaf exposure

Table 5 | Total coliforms, fecal coliforms and *E. coli* (CFU g^{-1}) on leaves of *C. ciliaris* irrigated with TW and TWW, during the experimental time from July (2013) to July (2014)

		Total coliforms		**Fecal coliforms**		***E. coli***	
Cycle	**Irrigation water**	**Minimum (CFU g^{-1})**	**Maximum (CFU g^{-1})**	**Minimum (CFU g^{-1})**	**Maximum (CFU g^{-1})**	**Minimum (CFU g^{-1})**	**Maximum (CFU g^{-1})**
First cycle	TW	0	40	0	0	0	0
	TWW	0	121	0	93	0	2
Second cycle	TW	0	30	0	20	0	0
	TWW	0	130	0	120	0	0

to the UV radiation, which during summer may have disinfected the leaf surface.

Soil under *C. ciliaris* irrigated with TWW showed the highest pollution values with respect to that irrigated with TW. Comparing the *E. coli* contamination level of water with soil, a notable reduction of pollution has been observed in the latter, suggesting that soil can act as a living filter with the potential for self purification through biological processes that reduce microbial concentrations. Similar findings have been reported by Vivaldi *et al.* (2013), using different types of municipal wastewaters to irrigate nectarines in Southern Italy. In agreement with Benami *et al.* (2013), which assessed soil irrigated with TWW and with freshwater, no *Salmonella* contamination was found in soil and crop in both *C. ciliaris* growth cycles. Our findings are also in agreement with recent studies demonstrating the absence of microbial contamination in leaves and fruits of olive trees irrigated with domestic wastewater (Alderson *et al.* 2015; Petousi *et al.* 2015). Soil microbial contamination depends on the survival of pathogens, which is strictly dependent on soil texture, organic matter, moisture, irrigation regime, pH and chemical fertilizer (Bostock *et al.* 2014). Higher clay contents provide a more protective environment, support a larger bacterial biomass overall and extend the survival period of enteric bacteria more than a sandy or loam soil (Kissoudis *et al.* 2014). The low level of organic matter detected in our soil during the experimental time (3.7%) may have created disadvantageous conditions for fecal bacteria. Organic matter is responsible for the survival, and in some instances for the regrowth, of enteric bacteria, increasing the nutrients retention, providing carbon for bacteria and improving moisture retention properties. The occasional contamination of soil irrigated with TW could be attributed to water contamination by roaming wild animals, birds and runoff. Despite this situation, for both growth cycles, no fecal coliforms and *E. coli* contamination has been detected on plant material irrigated with TWW and TW. This result suggests that the reuse of TWW may offer the most effective and efficient way to cope with water shortage for forage production.

CONCLUSION

We can conclude that microbial contamination of the plant–soil system is a function of the irrigation water pollution level, but mainly of soil characteristics and climatic conditions. TWW resulted in low soil contamination and in turn in no plant material contamination. In these sites, reclaimed municipal wastewater seems to be an alternative to TW for forage plant irrigation. However, studies with different types of wastewaters and soils are needed before these results can be generalized, because changes in microbial community are also considerably influenced by soil type and agricultural practices.

TWW reuse in agriculture represents an important strategy for the sustainable use of limited freshwater resources because of its economic potential and environmental benefits.

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