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Effects of Irrigations with Treated Municipal Wastewater on Phenological Parameters of Tetraploid *Cenchrus ciliaris* L.

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Abstract

This study was conducted to investigate the use of treated municipal wastewater (TWW) in agriculture. Experiments have been carried out from July 2013 to July 2014, irrigating *Cenchrus ciliaris* with TWW or tap water (TW). The study, conducted under greenhouse conditions, compared the effect of TWW with the water normally used in irrigation, on the growth, phenological and phytomass production of *C. ciliaris* a species with high pastoral value. Firstly, our results evidenced that all the chemical parameters of TWW fell in the range of values permitted by Tunisian regulation except chloride. Additionally, TWW increased plant growth during the growth cycle, producing taller plant with respect to TW. All plants irrigated with TWW showed a better performance than plants irrigated with TW only. Similarly, TWW irrigations had positive impacts on flowering parameters during the reproductive cycle. Therefore, treated wastewater can be used as an alternative water resource in irrigation of annual fodder species, with the dual purpose of preserving fresh water and of increasing soil fertility as well as crop productivity.

Keywords: *Cenchrus ciliaris*; Pastoral species; Phenological and phytomass production; Waste water

Introduction

The volume of water used in the world increased more than twice the growth rate of the population and the growing number of regions reached a certain limit that made it impossible to provide reliable services and water supply for different uses FAO [1]. Population growth and economic development are placing unprecedented pressure on water resources, renewable but limited, particularly in arid regions. World total water resources is 1.4 billion m^3 DSİ [2] and only 1% of this amount is used as potable water [3]. Due to its arid and semi-arid climate, Tunisia is facing water scarcity problems, where the estimated available freshwater is only about 450 m^3 /citizen/year [4]. Since half of the world population lives in urban sections EC [5], the demand for fresh water is increasing every day and the production of municipal wastewater is increasing as well. Thus, the availability of good-quality water for irrigation is threatened Alobaidy, et al. [6] and irrigated agriculture faces the challenge of using less water, in many cases of poorer quality, to irrigate lands which provide food for an expanding population. The municipal wastewater has been recycled in agriculture for centuries as a means of disposal in cities such as Berlin, London, Milan and Paris AATSE [7]. However, in recent years wastewater has gained importance in water-scarce regions. In the most of these cases, the farmers irrigate with diluted, untreated, or partly treated wastewater. The lack of appropriate treatment and management of wastewater generated adverse health effects [8]. In this respect, it is necessary to adequately process wastewater before its use in the environment. Therefore, the usage of municipal treated wastewater for irrigation purpose, according to their composition and to the international standards of water irrigation quality, seems to be the most promising practice that may help to ensure safe and sustainable food crops in arid and semiarid regions. On the basis of the above statements, the aim of this paper is to evaluate the suitability of treated municipal wastewater to irrigate *Cenchrus ciliaris* L. (syn. *Pennisetum ciliare* L.) Link, Buffel grass). This species, native to dry areas of Africa, West Asia and India has been widely introduced in arid and semi-arid regions of the world for its high pastoral value [9-13]. Despite its importance as fodder, leaf of *C. ciliaris* contains compounds able to inhibit the bacterial/fungal growth much more than the standard drug used, representing an environmentally safe alternatives for plant disease control [14]. Additionally, *C. ciliaris,* is a hyper-root-accumulator of heavy metal and could be used for phytoremediation purpose [15]. *C. ciliaris*, has been used also in traditional medicines to relieve kidney pain, cure wounds, sores and tumors [16]. Due to its economic potentiality, in this study we used a tetraploid *C. ciliaris,* that are widely distributed in the most humid areas of Tunisia Kharrat-Souissi et al. [17], to evaluate the fertilizer potential of treated municipal wastewater.

The study of the effect of irrigation with treated wastewater on the growth, and production of plants is one of the promising aspects, under the projected climate change for the next time IPCC. Although, the ecophysiological aspects under natural rainfall conditions of *Cenchrus ciliaris* have been widely studied in Tunisia Visser et al. [18], it should be noted that no study of this species has irrigations was carried out in conditions with the treated wastewater. In this context, the present study conducted under greenhouse conditions compared the application of treated municipal wastewater with the ground water, normally used in irrigation, on the growth, phenological and phytomass production of *C. ciliaris.*

Materials and Methods

Municipal wastewater

Treated wastewater were sampled at the outlet of the Sfax wastewater treatment plant, where municipal wastewater was treated with the biological stabilization bonds, at different times and stored at 4°C before the chemical characterization. Effluent samples were analyzed for pH and electrical conductivity (ECw) using a pH meter (AFNOR

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standard method N° NF T 90-008 AFNOR [19] and a conductimeter (AFNOR N° NF EN 27888 AFNOR [19]) respectively. Chemical oxygen demand (COD), suspended solids (SS), biochemical oxygen demand (BOD) and total phosphorus were measured according to the standard methods (AFNOR N° NF T 90-018, NF EN 872, NF T 90-103, NF EN 1189 AFNOR [19]. Heavy metal contents were measured following standard methods (APHA, 2005) Cations and anions were measured using ionic chromatography while carbonates and bicarbonates were estimated by titrating an aliquot of the effluent samples with HCl (AFNOR N° NF EN ISO 9963-2 AFNOR [19].

Plant material

4X *Cenchrus ciliaris* tetraploid (2n = 4x = 36), more adapted to wet areas in the extreme north of Tunisia Kharrat-Souissi et al. [17], were collected randomly from Morneg (south of the city of Tunis: latitude 36° 73 N, longitude 10° 24 E).

Experimental design

This experiment was carried out under a shelter greenhouse in the experimental field of the Olive Tree Institute of Sfax, (34° 43 N, 10° 41 E) in Central-Eastern Tunisia. The planting of tetraploid level, took place in August 2012 in pots under semi-controlled conditions. The pots were 20 L capacity, 30 cm in diameter and 30 cm in depth. The substrate used is a natural postural soil. Each pot contained one plant. Tap water was used during the installation containing 1.3 g/l of NaCl. The photon flux in the greenhouse varied between 163 to 389 µmol / m²/s the temperature ranged from 13.3 to 28.3°C with a photoperiod of 12-14 h. The relative humidity ranged from 43% to 83% and the evaporation ranged between 88.5 and 268.5 mm. One year after planting (in June 2013), that is to say as soon as we got adult plants, a cutting (3 cm) from above the soil surface was conducted for each plant to simulate the zero level of growth during the summer season. After the cutting procedure, two irrigation treatments were applied, after cutting, during July 2013-July 2014 with two growth cycles: the 1th cycle from July to November and the 2th one from March to July 2014. The frequency of irrigation was on a ten-day basis (1st, 10th, and 20th day of each month). The irrigation of plants was as follow:

T1: 800 mm tap water (TW).

T2: 800 mm treated wastewater (TWW).

At the end of the vegetative growth (6 months), the plant growth parameters in terms of height and tuft diameter, number of leaves, length of leaves, were detected. Additionally, reproductive parameters in terms of number of cobs per growth unit, total number of ears per individual, were measured monthly. At the end of the growth cycle (about 6 months), 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 hours, and subsequently weighed to obtain phytomass.

Statistical analysis

Statistical analysis was conducted using the "SPSS 19" software, adopting an analysis of variance ANOVA, linear model generalized to two treatment factors. The mean values of all parameters were compared using the Dunnett test.

Results and Discussion

Climatic data and water characteristics

Note treated wastewater (Table 1), contained salts, in particular a high concentration of Cl^- and Na^+ that if added to the soil can increase salinity, soil osmotic potential inducing damage to cultivation. Additionally, the high total and fecal coliform content could affect crops and with consequence on human health. The biological treatment of wastewater improved their quality from a chemical point of view decreasing the concentration of Cl^- and Na^+, breaking down the polluting power. The physical and chemical characteristics of TWW and TW and the values admitted by Tunisian regulation are reported in Table 2. All the chemical parameters fall within the value permitted by Tunisian regulation except chloride. The pH of TWW and TW were 7.60 and 7.51, respectively, falling within the limits for *Cenchrus ciliaris* growth (7.0 to 8.0) [20]. The electrical conductivity (EC) was 6.80 dS m^{-1} for TWW and 4.30 dS m^{-1} for TW, indicating, a high and a moderate level of salinity respectively [21,22]. Cl^- concentration in TWW was higher than the threshold values, as reported by Graham and Humphreys [23] in the guidelines for forage plants irrigation. As expected, the concentration of almost all elements was also higher in TWW than in TW, with the exception of Ca^{2+} and Mg^{2+} (Table 2), even if they were present in TWW at concentrations 9 times higher than that contained in the fertilizer normally used in agriculture. Both chemical and biological oxygen demands (COD and BOD5) of TWW were below the Tunisian thresholds for water reuse. According to the chemical parameters detected, the TWW represented a source of nutrients for crops. The content of heavy metals (Cd, Zn, Cr, and Pb) was lower than the toxicity limits (<0.004 mg/L) and it did not exceed the thresholds established by Tunisian regulation [24]. Neither coliforms nor fecal coliforms were detected in the irrigation water, resulting in an environmentally-friendly safe wastewater. The variations of climatic parameters over the experimental period are reported in Table 3. As expected the highest temperature was detected in august and the lowest one in February. The evaporation was the

Characteristics	Wastewater
pH	7.40 ± 0.2
EC	7.11 ± 1.9
TDS	2.20 ± 0.01
HCO_3^-	504.13 ± 0.5
SO_4^{2-}	398.66 ± 0.9
N total	108.03 ± 1.4
$N\text{-}NO_3^-$	18.96 ± 0.06
$N\text{-}NO_4^+$	72.3 ± 0.03
$N\text{-}NO_2^-$	97.99 ± 0.04
P total	26.22 ± 0.9
K^+	60.45 ± 0.1
Na^+	379.15 ± 0.04
Cl^-	2129 ± 0.08
Ca^{2+}	149 ± 0.03
Mg^{2+}	131 ± 0.01
Pb^{2+}	0.19 ± 0.01
Cd^{2+}	0.02 ± 0.00
Zn^{2+}	0.49 ± 0.01
Mn^{2+}	0.81 ± 0.01
SM	25.77 ± 0.03
COD	382 ± 0.7
BOD5	167 ± 0.2
Total coliforms	$6.3\ 10^6 \pm 2.04\ 10^4$
Fecal coliforms	$3.9\ 10^5 \pm 2.24\ 10^4$

Data represents mean values ± standard deviation. EC: electrical conductivity (mS/cm); TDS: total dissolved solids (g L^{-1}); SM: suspended matter (mg L^{-1}); COD (mg L^{-1}): chemical oxygen demand; BOD5: biological oxygen demand (mg L^{-1}); Total coliform and Fecal coliform (UFC/100 mL). anions, cations and total P and N are measured in (mg L^{-1}).

Table 1: Chemical characteristics of wastewater before treatment

highest in august because of the highest temperature and the lowest in December. Brightness, parameter linked to the length of the light cycle during the day, was higher in June and lowest in December. The relative humidity was the highest in august due to the high temperature, and the lowest in January, as a result of due to the scarcity of rain and the cold temperature. All these data reflect the climatic conditions of Mediterranean countries [25].

Growth and flowering parameters

The plant growth parameters: plant height, plant diameter, leaf length and number leaf, *Cenchrus ciliaris* tetraploids irrigated with tap water and the treated wastewater over the two growing cycles are shown in Table 4. Crops irrigated with treated wastewater showed a better growth during the two growth cycles than the plants irrigated with tap water. The quality of irrigation water did not affect significantly plant heights in the first cycle of growth. However, a significant increase in terms of height was observed in the second growth cycle, of plants treated with wastewater. The results evidenced that the plants irrigated with tap water were shorter than the plants irrigated with treated wastewater. Similar results were reported by Day et al. [26] who observed that wheat irrigated with wastewater produced taller plants, more heads per unit area, heavier seeds, higher grain yields than wheat grown with pump water alone. They attributed this increase to the nitrogen and phosphorus contained in the added wastewater. In contrast, Carter, et al. [27] for *Celosia argentea* and Grieve, et al. [28] for *Matthiola incana,* observed a regression in the height of these plants grown with wastewater. The diameter of plants irrigated with treated wastewater was larger than the diameter of the plants irrigated with tap water in both growth cycles. The largest diameter (52.60 cm) was observed in wastewater irrigated plants in July, the end of the second cycle. Except for April (p = 0.021), the quality of irrigation water did not cause significant differences in the diameter of plants. Regarding leaf length, in the first cycle, the irrigation with treated wastewater caused an increase in leaf length than irrigation with tap water. While no significant differences in leaf length were observed in the second cycle between the wastewater irrigated plants and the tap water irrigated ones. The highest leaf length (29.72 cm) was observed in treated wastewater irrigated plants in November. Nevertheless, the leaf numbers of plants irrigated with treated wastewater were greater than that of plants irrigated with tap water for both growing cycles. The greatest number of leaves was observed in wastewater treated plants in July. The irrigation with wastewater increased leaf number and the reproductive growth of *Cenchrus ciliaris* mainly during the second cycle. These results are in agreement with that of Oliveira-Marinho et al. [29] indicating an increase in the leaf number of *Rosa hybrida* 'Atmosphere' irrigated with wastewater with different salinity levels. An increase in leaf number has also been reported for *Arachis hypogaea* Saravanamoorthy and Kumari [30], *Sorghum bicolor* Khan et al. [31] and *Gossypium hirsutum.* Alikhasi et al. [32] when irrigated with biologically treated wastewater. TWW (containing high salt concentrations) increased not only the growth of the flowering

Characteristics	TWW	TW	Tunisian regulation
pH	7.60 ± 0.10	7.51 ± 0.11	6.50-8.50
EC	5.6 ± 0.02	4.30 ± 0.03	7
TDS	1.77 ± 0.02	0.93 ± 0.01	2
HCO_3^-	356.00 ± 0.3	223.30 ± 0.20	600
SO_4^{2-}	354.00 ± 0.7	67.50 ± 1.5	1000
N total	53.80 ± 1.20	-	30
$N\text{-}NO_3^-$	13.40 ± 0.01	0.97 ± 0.01	-
$N\text{-}NO_4^+$	35.6 ± 0.01	2.67 ± 0.04	-
$N\text{-}NO_2^-$	4.00 ± 0.02	0.04 ± 0.01	-
P total	9.44 ± 0.11	0.45 ± 0.02	0.05
K^+	33.80 ± 0.09	26.00 ± 0.05	50
Na^+	297 ± 0.01	430.00 ± 0.01	300
Cl^-	1767 ± 0.04	1340.00 ± 0.2	600
Ca^{2+}	98.50 ± 0.01	188.20 ± 0.02	-
Mg^{2+}	85.70 ± 0.01	126.20 ± 0.03	-
Pb^{2+}	< 0.004	0	0.1
Cd^{2+}	< 0.004	0	0.005
Zn^{2+}	0.33 ± 0.01	0.5 ± 0.01	5
Mn^{2+}	0.65 ± 0.01	0.13 ± 0.03	-
SM	12.20 ± 0.02	2.30 ± 0.02	
COD	74.00 ± 0.01	0	90
BOD5	20.00 ± 0.01	0	30
Total coliforms	nd	0	-
Fecal coliforms	nd	0	-

Data represents mean values ± standard deviation. EC: electrical conductivity (mS/cm); TDS: total dissolved solids (g L^{-1}); SM: suspended matter (mg L^{-1}); COD (mg L^{-1}): chemical oxygen demand; BOD5: biological oxygen demand (mg L^{-1}); Total coliform and Fecal coliform (UFC/100 mL). anions, cations and total P and N are measured in (mg L^{-1}); TW: tap water; TWW: treated wastewater; nd, Undetected.

Table 2: Chemical characteristics of the irrigation waters used in the experiment

Cycle	1th Cycle					2th Cycle				
Months	July	Aug	Sept	Oct	Nov	Mar	Apr	May	June	July
Temperature(°C)	27.2	28.3	26.1	24.47	16.7	17.3	18.7	21.6	23.7	25.6
Evaporation (mm)	243.4	268.5	183.2	169	126.1	181	170.2	198.4	223.3	241.1
Brightness(µmol/m²)	380,3	319	245	225	174	232	248	301	389	379,9
Relative humidity (%)	82	83	71	67	60	73	75	78	77	81

Table 3: Mean temperature (°C), evaporation (mm), insulation (µmol/m/s²), and relative humidity (%) registered monthly in the greenhouse over the experimental period

Parameter	Irrigation water	1th cycle					2th cycle				
		July	August	September	October	November	March	April	May	June	July
Number of ears /UC	TW	0	0	0	0.1	0.1	0	1.4	2.5	3.2	3.2
	TWW	0	0	0.1	0.5	0.7	0	2.3	3.6	4.5	4.7
	Significance	.	.	0.331 n.s	0.054 n.s	0.004 n.s	.	0.001*	0.000**	0.000**	0.000**
Number of ears /plant	TW	0	0	0	0.3	0.3	2.8	9.6	16.7	23.8	23.8
	TWW	0	0.1	0.1	1.2	1.8	4.2	14.9	29.2	41.5	43.3
	Significance	.	0.331n.s	0.331 n.s	0.122 n.s	0.037 n.s	0.002 n.s	0.000**	0.000**	0.000**	0.000**

UC: unit of growth; n.s.: not-significant; **p < 0.001; *p < 0.01

Table 4: Effects of irrigation with tap and treated wastewater on growth parameters of tetraploid *Cenchrus ciliaris* L from July (2013) to July (2014).

power of *Cenchrus ciliarisbut* also intensified it (Table 5). All plants irrigated with treated wastewater showed a better performance than irrigated plants with tap water only during the second growth cycle even if the TWW contained a high concentration of chloride. Sun et al. [33] identified *Cenchrus ciliaris* as suitable plants to be utilized for bioremediation in surface saline soil or marine sediments, for its ability to grow in soil with (1-2% NaCl). The quality of irrigation water did not result in significant differences in the number of ears in the first cycle of treatment. The largest number of total ears per individual (43.03) was observed in July in irrigated plants with treated wastewater. Similarly, the highest value in the tap water irrigation was also observed in July (23.80). Regarding the number of ears per UC, the quality of irrigation water did not result in significant differences only in April, May, June and July during the second cycle of treatment which corresponded to the reproductive cycle of this species. The largest number of ears per UC (4.70) was observed in plants irrigated with treated wastewater in July. By taking into consideration all together these data, one can deduce that the treated wastewater increased the number of ears in tetraploid plants during the second cycle. Irrigation with treated wastewater had no negative effects on growth and flowering. These results were in agreement with those of Gerhart, et al. [34] on *Prosopis chilensis*, *Sophora secundiflora*, *Malephora* spp., *Cercidium* sp., *Leucophyllum* spp., *Rosmarinus officinalis*, *Acacia stenophylla*, *Caliandra californica* and *Dalea greggii;* and with those of Banon et al. [35] on *Lantana camara*.

Effect of municipal TWW on Biomass

Over the experimental time, the sheet dry matter of the plant was weighed at three different periods. The mean values for tetraploid *Cenchrus ciliaris* depended on the origin of irrigation water (Figure 1). Furthermore, the dry matter of tetraploid *Cenchrus ciliaris* irrigated with TWW was higher than those irrigated with TW. Statistical analysis showed significant differences between the average dry matter of the two treatments (TWW and TW) only at the end of the experiment. The irrigation with TWW showed a significant increase in dry mass over time. The irrigation with TW caused significant differences only between the first and second period. 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. This explanation is supported by data of del Mar Alguacil, et al. [36] showing that microbial activities were significantly higher in the soils irrigated with urban wastewater than in those irrigated with fresh water. Additionally, del Mar Alguacil, et al. [36] and Mousavi, et al. [37] showed that irrigation with treated municipal wastewater had a significant positive impact on the growth and quality of orange-tree and maize respectively, supporting the results of this study.

Conclusion

In short, we can conclude that the irrigation with treated wastewater increased plant growth and flowering with respect to tap water during the experimental period. Therefore, as no negative effects were observed on crop vitality and productivity, it seems that the treated wastewater can be used as an alternative source for irrigation of *Cenchrus ciliaris* tetraploid, with the dual purpose of not only saving fresh water for other uses, but also improving soil fertility and productivity in arid and semi-arid regions.

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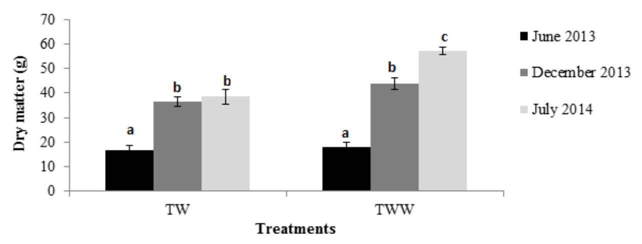

Figure 1: Effects of tap water (TW) and treated municipal wastewater (TWW) on growth of tetraploid *Cenchrus ciliaris,* during the experimental time (June 2013-July 2014). Growth of *Cenchrus ciliaris* is expressed as dry mass (g).

Parameter	Irrigation water	1th cycle					2th cycle				
		July	August	September	October	November	March	April	May	June	July
Plant height(cm)	TW	11.6	22.7	53.9	64.8	67.2	15.3	20.5	34.3	53.8	63.7
	TWW	12	24.3	55.1	66.6	68.9	18.9	25.4	43.4	64.9	69.1
	Significance	0.470$^{n.s}$	0.021$^{n.s}$	0.152$^{n.s}$	0.021$^{n.s}$	0.049$^{n.s}$	0.000**	0.000**	0.000**	0.000**	0.001*
Plant diameter(cm)	TW	16.4	22	24.7	26.3	30.8	17.6	32.7	36.1	39.6	43.1
	TWW	15.6	27.4	33.5	37.2	44.2	22.7	34.3	42.3	51.7	52.6
	Significance	0.145$^{n.s}$	0.000**	0.000**	0.000**	0.000**	0.000**	0.021$^{n.s}$	0.000**	0.000**	0.000**
Leaf length (cm)	TW	10.69	14.02	17.12	21.38	23.17	4.98	13.97	22.28	25.58	27.27
	TWW	12.37	16.18	19.37	24.75	29.72	4.91	14.55	23.17	26.18	26.93
	Significance	0.011$^{n.s}$	0.003*	0.001*	0.000**	0.000**	0.827$^{n.s}$	0.336$^{n.s}$	0.058$^{n.s}$	0.266$^{n.s}$	0.239$^{n.s}$
Leaf number (n°)/ UC	TW	4.2	8	9,30	10.7	12.4	3.8	7.3	13	15.6	19
	TWW	5.3	11.3	14.5	15.8	18.2	3.7	8.6	15.3	20.6	24.9
	Significance	0.080$^{n.s}$	0.000**	0.000**	0.000**	0.000**	0.801$^{n.s}$	0.005*	0.000**	0.000**	0.000**

UC: unit of growth; n.s.: not-significant; **$p < 0.001$; *$p < 0.01$.

Table 5 Effects of irrigation with tap and treated wastewater on flowering parameters of tetraploid *Cenchrus ciliaris* L. from July (2013) to July (2014).

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