Received: 22 June 2022 | Revised: 3 January 2023 | Accepted: 5 March 2023

DOI: 10.1111/wej.12858

RESEARCH ARTICLE

Water and Environment Journal Promoting Sustainable Solutions CIWEM WILEY

Treated municipal wastewater as option to the use of fresh water for the cultivation of valuable pastoral species Buffel grass (*Cenchrus ciliaris* L.)

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Funding information
Tunisian Ministry of Higher Education and Scientific Research

Abstract

The increasing aridity exacerbated by climatic changes is leading to loss of perennial herbaceous plants *Cenchrus ciliaris*, an apomictic, polyploid grass used as forage in hot and dry areas, and is currently under threat for the increased scarcity of water. In this study, treated municipal wastewater (TWW) was used to irrigate two ploidy levels of *C. ciliaris* for two consecutive years. The objectives were (1) to assess the possibility of using unconventional water for watering *C. ciliaris* and (2) to identify at what extent TWW irrigation affected growth and nutritive properties of *C. ciliaris* that differed for polyploidy level. TWW irrigation positively affected growth and physiological plant parameters. In leaves of TWW watered *C. ciliaris*, the concentration of N_t, P and K significantly increased. The tetraploid *C. ciliaris* showed a better growth and quality than the hexaploid ones, appearing the most suitable cultivar to be irrigated with TWW for feed uses. In short, TWW can be strategically used for irrigating forage species with the double output of ameliorating soil properties, recovering degraded area and improving the nutritive values of fodder species contributing to the promotion of a green and sustainable circular economy, more in climatically under pressure developing countries.

KEYWORDS
Cenchrus ciliaris, physiological parameters, polyploidy, treated wastewater, water resources

1 | INTRODUCTION

Water is severely limited in most of the Mediterranean countries, for the arid climate exacerbated by the extreme climatic change events. Currently, the annual average water resource is about 450 m^3 per capita, and it is forecasted to be barely 315 m^3 beyond 2030 (Aquastat, 2005). In 2030, an increase in annual average temperature of 1.6°C in the south country and of 0.9°C in the north country (IPCC, 2014) is expected, with severe consequence for agriculture, with a decrease in the crop production of about 50% and an estimated yield loss of 20% in arable land (Dorte, 2013). The water scarcity will impact on livestock production with a sharp fall of 80%, both in the centre and in the southern countries (Mahdhi et al., 2019). In this framework, treated wastewater (TWW) for watering could be a pragmatic and sustainable option to water supply for agriculture (Angelakis, 2002). TWW contains a lot of nutrients and organic matter (OM) and if correctly used can improve soil fertility acting on its physical and chemical properties (Xu et al., 2009). A part its benefits, TWW containing pollutant substances, if improperly used could cause damage to soil ecosystem adding to the soil heavy metals and toxic

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organic contaminants (Al-Habahbeh et al., 2021; Keser & Buyuk, 2012) and can heavily contribute to river and basin pollution (Morin-Crini et al., 2022). Therefore, the use of TWW requires great control and precautions, to prevent dangerous consequences for cultivations, environment and human (Gatta et al., 2018; Kalavrouziotis et al., 2008). Interest in *C. ciliaris* L., a perennial (C_4) grass (family *poaceae*), cultivated as pastures and leys in East Africa, Central Africa and Northern Australia (Ashraf et al., 2013; Duke, 1983), arises from the ability of this species to adapt to a broad gamma of habitats and live on better in changeable climates than other crop species (Ghafar et al., 2021; Heslop-Harrison, 2000). Most plants blooming are polyploids, because polyploidy is an omnipresent occurrence in plant evolution (Wendel et al., 2012). *C. ciliaris* is highly polymorphic and variable for several morphological behaviours of agronomic and ecological importance (Mseddi et al., 2004). *C. ciliaris* possesses different ploidy levels: tetraploid ($2n = 4x = 36$), pentaploid ($2n = 5x = 45$), hexaploid ($2n = 6x = 54$) and aneuploid (Fisher et al., 1954); the high level of polyploidy, in the evolutive story of blooming plants, suggests that polyploidy is a key for plant adaptation in natural populations (Van de Peer et al., 2009). Successful polyploidy in general occurs with variations in plant morphology, phenology, physiology and ecology (Levin, 2009) and may generate individuals that can stand fluctuating environments (Prentis et al., 2008; Soltis & Soltis, 2004; Van de Peer et al., 2021). North African is an arid zone marked by climatic aridity that will face in the next decades with serious problems linked to the productivity of pasture land for water scarcity and loss of fodder species (Li et al., 2008). Considering the raising demand of sheep and goat meat, it results necessary to find sustainable solution to improve forage cultivation in arid environment, where food, essentially dependent on the rangelands, can affect the economy of countries that otherwise will be forced to import animal proteins, worsening their economic status (Valone et al., 2002). A previous study (Ben Said et al., 2017) evaluated the possibility of reusing municipal TWW to irrigate the tetraploid *C. ciliaris*, demonstrating that TWW can be a sustainable option to the use of fresh water (FW) for watering this fodder species. In addition, it has been also demonstrated that when irrigated with TWW, 'tetraploid' plants improved their growth than plants irrigated with FW (Ben Said et al., 2016). Considering the wide geographical distribution of *C. ciliaris*, and its wide polymorphism, especially its polyploidy, our hypothesis driven research was related to verify if the different polyploidy responded differently to the TWW irrigation treatments, based on the validated concept that the more is the polyploidy, the better should be the adaptability of a species to different environmental conditions in terms of phytomass production and growth. On the basis of the above statement the aim of the present work was to discriminate and determine the best ploidy level, between 'tetraploid' and 'hexaploid', in tolerating TWW irrigation to make become, more successful the pastoral production in marginal and semi-desert area of Tunisia.

The recourse to TWW to cultivate *C. ciliaris*, a valuable pastoral species, can a double aim to recover degraded land and to save the herding that represents an important economic incoming for the majority of developing countries.

The results of this work would be incontestably relevant for other regions marked by climatic aridity in view of a green and sustainable circular economy.

2 | MATERIALS AND METHODS

2.1 | Plant material

C. ciliaris has different levels of ploidy (Kharrat-Souissi et al., 2011). However, the most common Buffel grass in Tunisia are tetraploids and hexaploids; for this reason, in this work, tetraploids and hexaploids have been studied in order to know their eco-physiological responses and their productivity when irrigated with the TWW. In August 2016, seeds of each ploidy level were sown in soil at a 30 cm depth with a total of 160 plants (80 for each ploidy level). Chemical composition of the soil was: K^+ 3.16 mEq L^{-1}, Na^+ 6.64 mEq L^{-1}, $CaSO_4$ * $2H_2O$ 0.44%, total $CaCO_3$ 13% and OM 2.1%. Soil electric conductivity was 3.7 mS cm^{-1}. The study period was from the first week of July 2017 to the third week of July 2018. The experiment took place in the experimental field located at the Olive Tree Institute of Sfax (34°43′ N, 10°41′ E), in Central-Eastern Tunisia and under natural conditions. During the establishment period, individuals were regularly irrigated to ensure maximum survival, with FW pH 7.51, electric conductivity 1.78 mS cm^{-1} and dry residue 1220 mg L^{-1}.

The temperature and relative humidity were determined continually over the experiment with a data-logger (model HOBOU12-012 onset). Air temperature were 11.3°C (minimum), 25.7°C (average) and 37.9°C (maximum); the minimum relative humidity was 23.4%, and the maximum 100%, with a mean of 66.5%.

After 1 year of plant establishment (in June 2017) growth adult plants were cut 3 cm above the soil surface to simulate growth level zero. After the cutting procedure, two irrigation treatments were applied during the whole period (July 2017–July 2018). In each treatment the following irrigation regime was applied: 800 mm FW and 800 mm TWW (Table 1). So 40 plants were irrigated with (FW) and 40 with (TWW). The frequency of irrigation was every 10 days (1st, 10th and 20th day monthly). In order to verify the effects of irrigation with TWW on soil chemical properties, soil samples were collected in August 2017 and after harvesting, in August 2018. The soil samples were air dried, sieved at 2 mm and then chemically analysed. Standard methods have been used for soil analysis (Sparks et al., 1996).

2.2 | Shoot growth and yield

The shoot height (in cm) was detected weekly utilizing a graduated ruler on six plants for each treatment. Shoot phytomass (SP) and root phytomass (RP) were obtained by splitting the roots from shoots under water. Then, SP (as g per plant) and RP (as g per plant) were detected by weighting them after drying in oven at 80°C for 48 h.

TABLE 1 Physicochemical properties of the treated wastewater (TWW) and fresh water (FW) used for irrigation.

Parameters	Soil	FW	TWW	Significance	Tunisian limits
Clay	9.20 ± 0.05	nd	nd		
Silt	5.80 ± 0.02	nd	nd		
Sand	84.30 ± 0.05	nd	nd		
Texture[a]	Loamy-sand	nd	nd		
OC %	1.2				
OM %	2.1				
$CaCO_3$ (g kg^{-1})	130				
N_t (g kg^{-1})	2.5				
pH	7.70	7.46 ± 0.19	7.66 ± 0.32	ns	6.50–8.50
EC (dS m^{-1})	3.7	4.30 ± 0.06	5.45 ± 0.75	*	7
TDS (g L^{-1})		0.82 ± 0.03	1.71 ± 0.10	*	2
NH_4-N (mg L^{-1})		0.02 ± 0.01	9.14 ± 0.62	*	
NO_3-N (mg L^{-1})		0.88 ± 0.06	1.63 ± 0.44	*	
NO_2-N (mg L^{-1})		0.05 ± 0.02	0.06 ± 0.02	ns	
PO_4-P (mg L^{-1})		0.11 ± 0.02	0.26 ± 0.06	*	
BOD_5 (mg L^{-1})		0	22.50 ± 3.01	*	30
COD (mg L^{-1})		0	75.66 ± 2.25	*	90
Na^+ (mg L^{-1})		290.33 ± 1.5	355.16 ± 3.31	*	300
Ca^{2+} (mg L^{-1})		188.45 ± 0.86	292.23 ± 4.87	*	
Cl^-(mg L^{-1})		1530 ± 0.04	1969 ± 0.04	*	600
Mg^{2+} (mg L^{-1})		125.56 ± 1.16	84.73 ± 3.32	*	
K^+ (mg L^{-1})		26.16 ± 0.75	33.55 ± 2	*	50
Pb^{2+} (mg L − 1)		0	<0.004	*	0.10
Cd^{2+} (mg L^{-1})		0	<0.004	*	0.005
Zn^{2+} (mg L^{-1})		0.10 ± 0.01	0.42 ± 0.01	*	5
Mn^{2+} (mg L^{-1})		0.18 ± 0.01	0.49 ± 0.01	*	
HCO_3^- (mg L^{-1})		223 ± 1.41	351.16 ± 21.2	*	600
SO_4^- (mg L^{-1})		31.20 ± 1.03	33.56 ± 6.97	*	1000

Note: Data represent mean values ± standard deviation.
Abbreviations: BOD, biological oxygen demand; COD, chemical oxygen demand; EC, electrical conductivity; FW, fresh water; nd, not determined; ns, not significant; TDS, total dissolved solids; TWW, treated wastewater.
[a]USDA classification.
*Statistically significant at $p \leq 0.05$.

The sampling was carried out at the end of the test ($n = 10$), and the relationship between RP and SP (RP/SP ratio) was calculated as Rate = (RP/SP) * 100.

2.3 | Leaf mineral content

Leaves were collected by the top of the plant; four plants per treatment for each ploidy level were used for experimental purposes. They were taken after harvesting (August 2018) and stored in a portable cooler. Subsequently, the leaves were oven-dried (80°C) and analysed as described by Pauwels et al. (1992). Mineral element (P, K, Na, Cl, Mn, Zn, Cd and Pb) analysis was conducted after dry-ashing at 450°C in a muffle oven (HEROTEC) and digestion of the ashes with 1 M HNO_3. Total nitrogen (N_t) was determined using the Kjeldahl method; K^+ and Na^+ were determined by atomic emission spectrophotometry (JENWAY PFP7); heavy metals (Mn^{++}, Zn^{++}, Cd^{++} and Pb^{++}) by atomic absorption spectrophotometry (Perkin Elmer A Analyst 300, Perkin Elmer Inc., Willesley, MA, USA). P in leaves was detected by a vanado-molybdate colorimetric method by using a JENWAY 6405 UV/Vis spectrophotometer. Finally, Cl^- was analysed titrimetrically with $AgNO_3$ (Karaivazoglou et al., 2005).

2.4 | Gas exchange analysis

Leaf gas exchange measurements including net photosynthesis rate (A_n), transpiration rate (T_r) and stomatal conductance (g_s) were

measured at midday of fair-weather days (between 11:30 AM to 13:30 PM) on the youngest mature leaves using LCi portable device of photosynthesis (ADC Bio-Scientific Ltd., USA). The sampled leaves were fully exposed to sun, and were obtained at 25, 35, 50, 65, 80, 95, 110, 125, 140, 155 and 200 days after tiller initiation (DAT) in 2017 and 2018.

2.5 | Statistical analysis

The statistical analysis was conducted using the 'SPSS 19' software, adopting an analysis of variance ANOVA, linear model generalized to two treatment factors and ploidy levels. The differences between means were compared by Duncan's multiple range test at 5% level of probability.

3 | RESULTS

3.1 | Soil chemical analysis and vegetative growth

The soil was a loamy-sand (USDA) with (9.2% of clay, 85% sand and 5.8% of silt) with 130 g kg^{-1} total $CaCO_3$, 21 g kg^{-1} OM, 2.5 g kg^{-1} N_t and a pH of 7.7. TWW and FW significantly differed each other's (Table 1). TWW contained more cations, anions and heavy metals and had a higher COD and BOD content than FW. All the chemical parameters were under the limit allowed by Tunisian rule excepting chloride (Table 1).

One month after the treatments (August 2017), soil irrigated with TWW and FW did not show significant differences in the concentrations of cations and heavy metals. Increasing the time of treatment (August 2018), N_t, K and P concentrations significantly increased in soil irrigated with TWW (Table 2). The increase was highly significant ($p \leq 0.001$) for K and N_t. After 2 years of irrigation with TWW an important and significant increase in Cl^- (181.1 mg kg^{-1} vs. 98.0 mg kg^{-1}) and Na^+ (181.30 mg kg^{-1} vs. 141.9 mg kg^{-1}) was observed in respect to FW treatment. At the starting of the experiment, heavy metal concentration ranged from 114 to 112 mg kg^{-1} for Mn and from 20.1 to 24.2 mg kg^{-1} for Zn, respectively (Table 2). In August 2018 (Table 2), irrigating with TWW remarkably increased Mn (138.7 mg kg^{-1} vs. 122.3 mg kg^{-1}) and Zn (32.4 mg kg^{-1} vs. 26.5 mg kg^{-1}). For the both ploidy levels in 2017, after the irrigation treatment, shoot growth increased significantly ($p \leq 0.05$), and it was higher in TWW irrigated plants than in FW irrigated ones. Significant differences were also observed in August 2018 (Figure 1). Comparing the two ploidy levels, the 'tetraploid' plants had higher shoot length (ranging from 63.3 to 95.6 cm) than the 'hexaploid' (ranging from 41.3 to 71.6 cm).

3.2 | Yield

For both ploidy levels, in each year, significant differences were found in SP, RP and RP/SP ratio. SP (Figure 2) of both ploidy levels increased under TWW irrigation. In 2017, phytomass of 'tetraploid' plants were 59 g $plant^{-1}$ in TWW treatment compared with 42.4 g $plant^{-1}$ irrigated with FW ($p \leq 0.01$). In 2018, SP increased significantly under TWW (71.2 g $plant^{-1}$) compared with FW (55 g $plant^{-1}$) irrigation. In 2017, 'hexaploid' plant phytomass was 44.5 g $plant^{-1}$ with TWW and 34.3 g $plant^{-1}$ with FW irrigation ($p \leq 0.01$). As for the 'tetraploid', 'hexaploid' SP increased over time and was again significantly higher with TWW (57.3 g $plant^{-1}$) than FW (44.9 g $plant^{-1}$). The same behaviour was observed for RP (Figure 3). RP was the highest under TWW treatment especially in

TABLE 2 Effects of irrigation with fresh water (FW) and treated wastewater (TWW) on the amount of soil ions.

	August 2017			August 2018		
Parameters	FW	TWW	Significance	FW	TWW	Significance
N_t (g kg^{-1})	2.5	2.5	ns	2.6	11.3	***
P (mg kg^{-1})	63.4	65.3	ns	66.6	59.6	*
K (mg kg^{-1})	114.2	114.8	ns	186.4	323.1	***
Na (mg kg^{-1})	104.1	113.3	ns	181.3	141.9	**
Cl (mg kg^{-1})	63.3	64.0	ns	181.1	98.0	***
Zn (mg kg^{-1})	20.1	24.2	ns	32.4	26.5	*
Mn (mg kg^{-1})	112	114	ns	138.7	122.3	*
Cd (mg kg^{-1})	<0.004	<0.004	ns	<0.004	<0.004	ns
Pb (mg kg^{-1})	<0.004	<0.004	ns	<0.004	<0.004	ns

Note: Data represent mean values of four replicates. Significance referred to the comparison between treatments (FW and TWW) within the same year. Differences between means are according to LSD test. Abbreviation: ns, non-significant.

*$p \leq 0.05$.

**$p \leq 0.01$.

***$p \leq 0.001$.

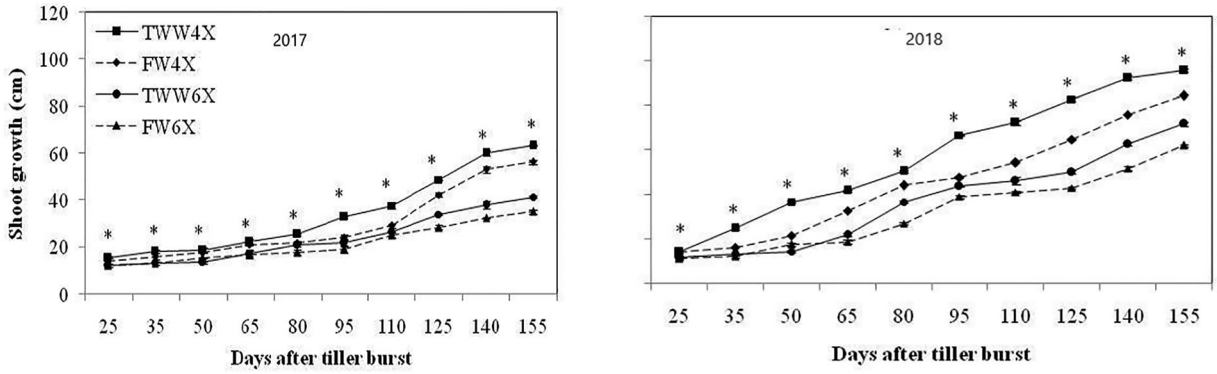


FIGURE 1 Shoot length (cm) of the two ploidy (4X: tetraploid, 6X: hexaploid) *Cenchrus ciliaris* irrigated with fresh water (FW) and treated wastewater (TWW) over 2 years of experiment. Bars indicate standard error ($n = 10$). Asterisks indicate significant differences between ploidy levels ($p < 0.05$).

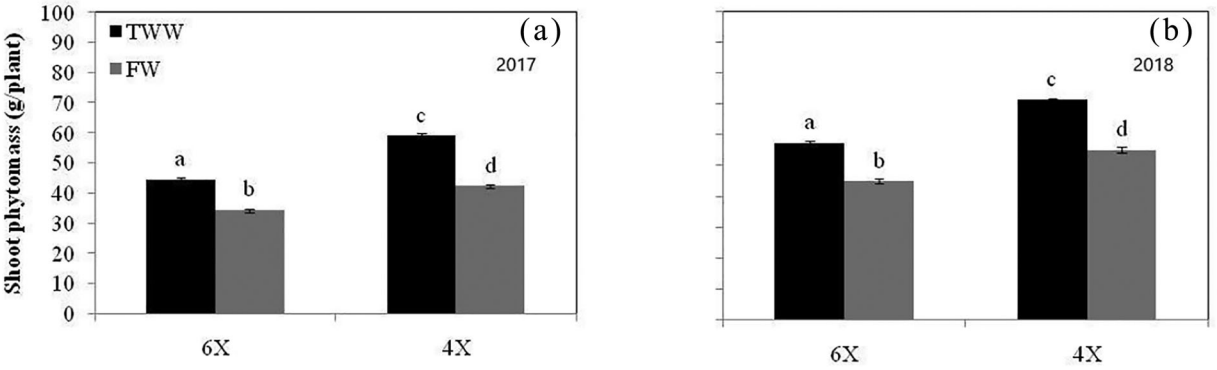


FIGURE 2 Shoot phytomass (g) of the two ploidy (4X: tetraploid, 6X: hexaploid) *Cenchrus ciliaris* irrigated with fresh water (FW) and treated wastewater (TWW) over 2 years of experiment. Bars indicate standard error ($n = 10$).

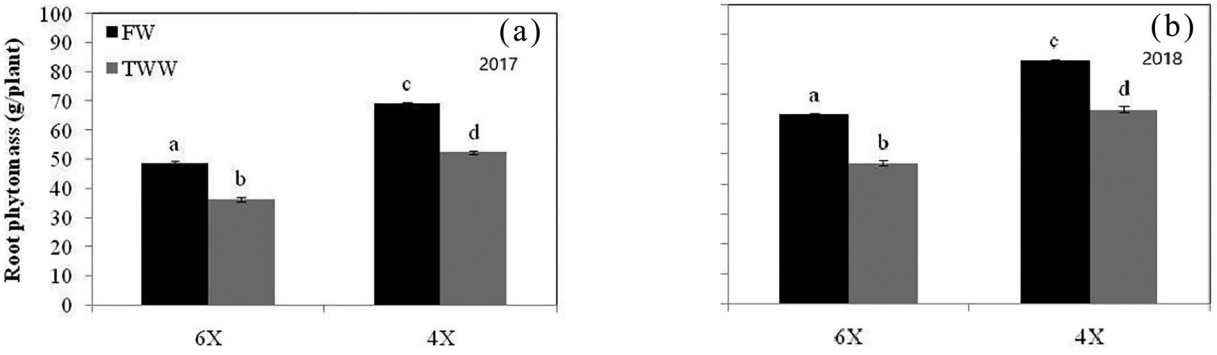


FIGURE 3 Root phytomass (g) of the two ploidy (4X: tetraploid, 6X: hexaploid) *Cenchrus ciliaris* irrigated with fresh water (FW) and treated wastewater (TWW) over 2 years of experiment. Bars indicate standard error ($n = 10$).

'tetraploid' plants over the whole experimental time (August 2017–2018). Additionally, RP/SP ratio, an index indicating a balanced plant growth, was always significantly higher in TWW irrigated plants, for both the ploidy levels, than in FW irrigated ones (Figure 4). The RP/SP ratio increased significantly under TWW treatment and mainly in the 'tetraploid' plants.

3.3 | Leaf macroelement and heavy concentrations

Data on mineral profile of *C. ciliaris* (Table 3) evidenced a greater amount of N_t, P and K 'in tetraploid' leaves compared with 'hexaploid' ones. N_t in 'hexaploid' plants ranged from 1.3% to 1.95%, and it was significantly lower than in 'tetraploid' (ranging from 1.7% to

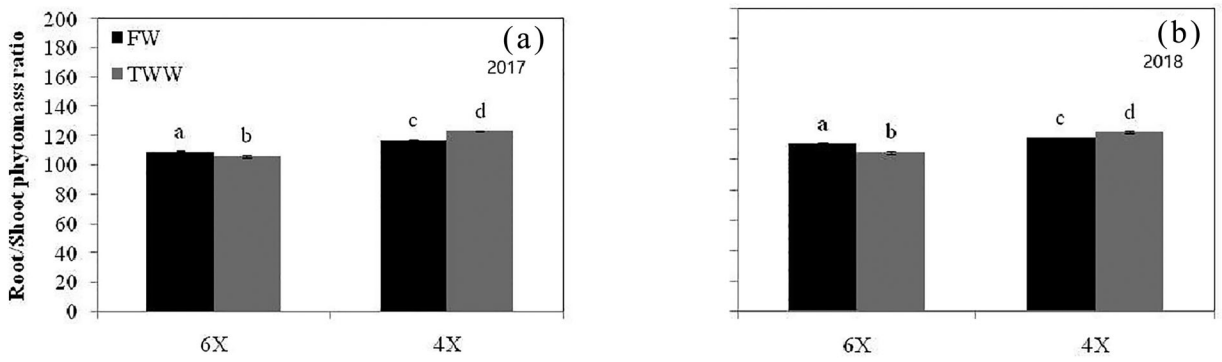


FIGURE 4 Root/shoot ratio in two ploidy *Cenchrus ciliaris* (4X: tetraploid, 6X: hexaploid) irrigated with fresh water (FW) and treated wastewater (TWW) for 2 years. Bars indicate standard error ($n = 10$).

TABLE 3 Chemical composition of leaves of the two ploidy levels of *Cenchrus ciliaris* irrigated with fresh water (FW) or treated wastewater (TWW).

Parameters	Treatment	Hexaploid	Tetraploid	Significance
N (%)	FW	1.3	1.7	**
	TWW	1.9	2.15	***
P (%)	FW	0.14	0.25	***
	TWW	0.23	0.64	***
K (%)	FW	1.1	1.8	**
	TWW	1.8	2.51	***
Na (%)	FW	0.08	0.09	ns
	TWW	0.09	0.09	ns
Cl (%)	FW	0.09	0.09	ns
	TWW	0.08	0.09	ns
Zn (mg kg^{-1})	FW	12.3	14.01	***
	TWW	17.4	19.43	***
Mn (mg kg^{-1})	FW	28.7	30.74	***
	TWW	59.3	62.5	***
Cd (mg kg^{-1})	FW	<0.004	<0.004	ns
	TWW	<0.004	<0.004	ns
Pb (mg kg^{-1})	FW	<0.004	<0.004	ns
	TWW	<0.004	<0.004	ns

Note: Data represent mean values of 10 replications for each treatment. Significance referred to the comparison between ploidy (tetraploid and hexaploid) *C. ciliaris* within the same crop season. Differences between means are according to LSD test.
Abbreviation: ns, non-significant.
**$p \leq 0.01$.
***$p \leq 0.001$.

2.15%) (Table 3). Phosphorous concentration ranged from 0.25% to 0.64% for 'tetraploid' and from 0.14% to 0.23% for 'hexaploid' plants. P requirements for beef cattle grow to assure a balanced daily diet must be 0.30% in leaf dry matter. K^+ concentration ranged from 1.8% to 2.51% and from 1.1% to 1.8%, for 'tetraploid' and 'hexaploid' plants, respectively (Table 3). The increased N_t, P and K amounts in *C. ciliaris* leaves were correspondent with the increased amount in available nutrients in TWW treated soils (Table 2). No significant differences were noticed in Na and Cl leaf concentrations between the two ploidy levels of *C. ciliaris* (Table 3). In detail, Na changed between 0.08% and 0.09% and Cl between 0.08% and 0.09% in both treatments (Table 3).

The concentration of Zn in leaves was statistically different ($p \leq 0.01$) between the two ploidy levels (Table 3) ranging from 10 to 30 mg kg^{-1}.

The concentration of Mn in leaves were significantly higher ($p \leq 0.001$) (Table 3) in 'tetraploid' cultivar ranging from 30.74 to 62.5 mg kg^{-1} than 'hexaploid' plants in which the concentration

ranged from 28.7 to 59.3 mg kg^{-1} (Table 3). At last, leaf concentrations of Pb and Cd were beneath the analytical detection limit of 0.004 mg kg^{-1}. These low values in soil (Table 2) and leaves (Tables 3) were the result of the low concentration present in both FW and TWW (Table 1).

3.4 | Gas exchange

Seasonal changes of diverse gas exchange parameters including A_n, g_s and T_r are shown in Figure 5. The two ploidy levels, after 2 years of experiments, showed in early growing season high g_s values that then decreased at 70–85 DAT, for increasing again until 110–120 DAT. After this, it decreased until 140 DAT showing a final increase near the harvesting (Figure 5a,b). There was a significant difference in g_s values between the two ploidy levels at all times of measurements in 2017 and 2018. In 2017, the maximum values of g_s were in plants TWW irrigated (0.96–0.98 mol m^{-2} s^{-1}) at 140 and 155 DAT. Significant differences between 'tetraploid' and 'hexaploid' plants were observed ($p < 0.05$). In 2018, the maximum values (0.98 mol m^{-2} s^{-1} at 155 DAT and 0.97 mol m^{-2} s^{-1} at 80 DAT) were detected in 'tetraploid' plants. For both ploidy levels, during the 2 years of experiment, the A_n evidenced similar seasonal variations as those observed in g_s under different irrigation treatments (Figure 5c,d). The TWW irrigated plants had the highest and statistically significant A_n values in comparison with FW irrigated plants at all times of measurements in 2017 except for 95 DAT in 2018. Furthermore, the 'tetraploid' plants showed the highest value of A_n for all measurements in 2017 and 2018 (Figure 5c,d). The maximum A_n values for TWW irrigated 'tetraploid' plants (28.5 µmol m^{-2} s^{-1}) were observed at 155 DAT in 2018. The TWW irrigated plants, for both ploidy levels, showed the highest value of T_r in both years (Figure 5e,f). The FW irrigated plants had the lowest and significant values compared with the TWW irrigated ones over the two experimental years. The maximum T_r values for TWW irrigated 'tetraploid' (5.75 mmol m^{-2} s^{-1}) and TWW irrigated 'hexaploid' plants (4.05 mmol m^{-2} s^{-1}) were observed at 155 and 110 DAT in 2017. These values were maintained in both plants in 2018. The g_s value in different times of measurements was linearly correlated to A_n ($R^2 = 0.82$–0.78, $p < 0.05$), and it increased with increasing A_n (Figure 6). Therefore, the higher A_n value in TWW irrigated plants than FW was the reason for the positive effects of TWW. The T_r value in different times of measurements was also, linearly correlated to A_n ($R^2 = 0.65$–0.73, $p < 0.05$), and it increased with increasing A_n (Figure 7). Therefore, the positive linear relationship between

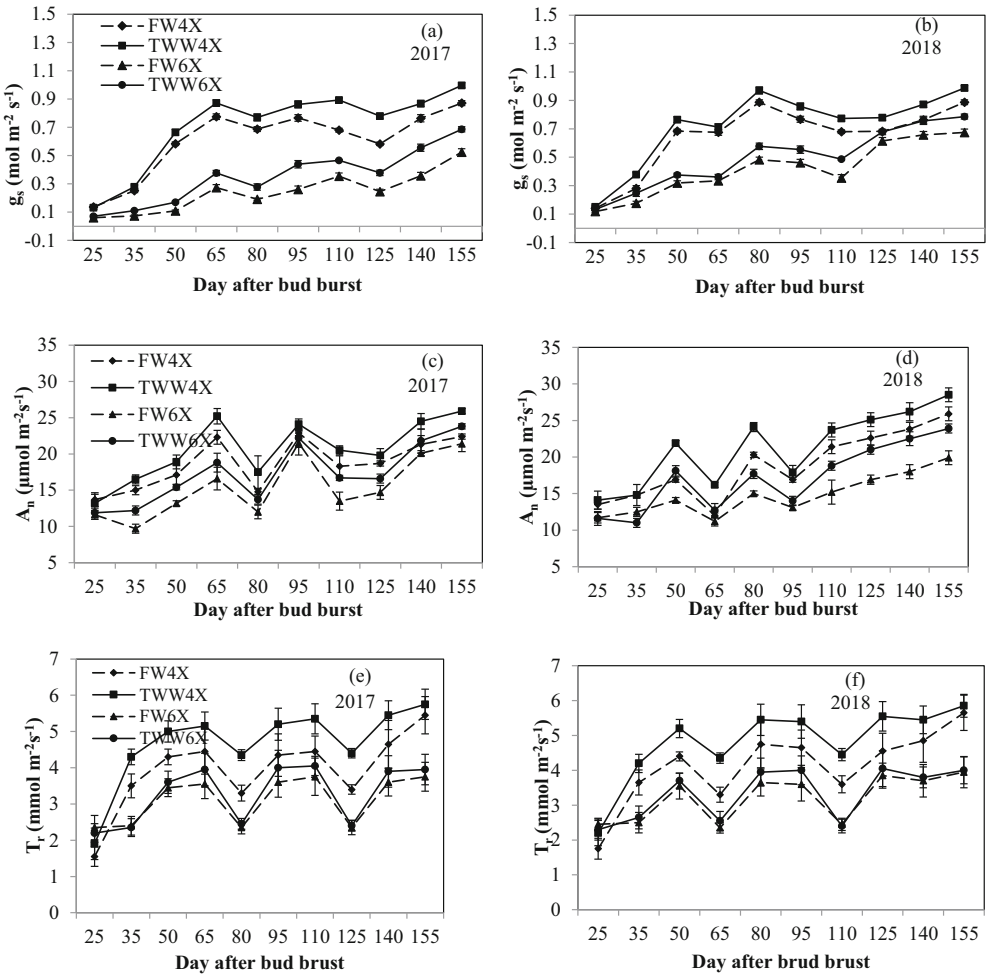


FIGURE 5 Temporal variations of gas exchange parameters including (a,b) stomatal conductance (g_s), (c,d) net photosynthesis (A_n) and (e,f) transpiration rate (T_r) in two ploidy *Cenchrus ciliaris* (4X: tetraploid, 6X: hexaploid) irrigated with fresh water (FW) and treated wastewater (TWW) for 2 years. Asterisks indicate significant differences between ploidy levels ($p < 0.05$).

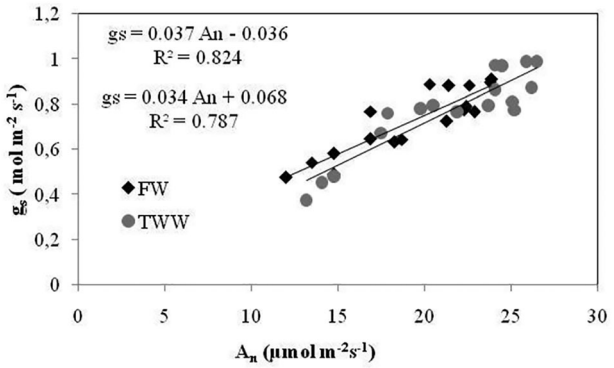


FIGURE 6 Relationships between net photosynthesis (A_n) and transpiration rate (T_r) in different irrigation water (FW: fresh water, TWW: treated wastewater), data ($n = 16$) are from eight times of measurements in 2017 and eight times of measurements in 2018 for each irrigation water.

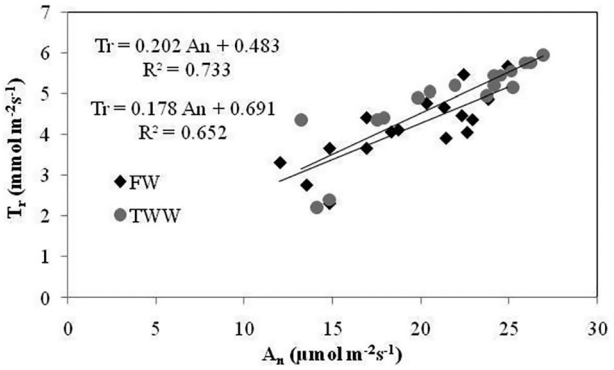


FIGURE 7 Relationships between transpiration rate (T_r) and net photosynthesis (A_n) in different irrigation water (FW: fresh water, TWW: treated wastewater), data ($n = 16$) are from eight times of measurements in 2017 and eight times of measurements in 2018 for each irrigation water.

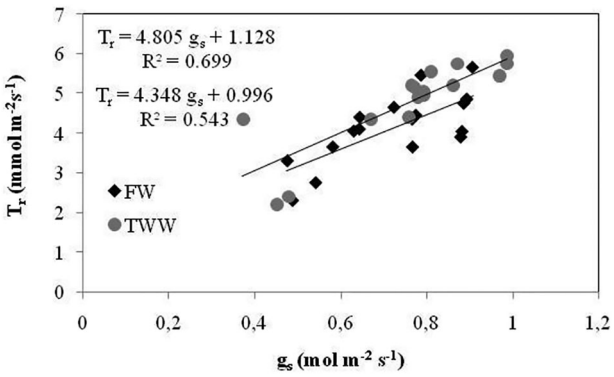


FIGURE 8 Relationships between transpiration rate (T_r) and stomatal conductance (g_s) in different irrigation water (FW: fresh water, TWW: treated wastewater), data ($n = 16$) are from eight times of measurements in 2017 and eight times of measurements in 2018 for each irrigation water.

measured T_r and g_s ($R^2 = 0.54$–0.69, $p < 0.05$) in water irrigation treatment was found in both experimental years (Figure 8). In general, between the two irrigation treatments, TWW irrigated plants showed the highest A_n, g_s and consequently T_r values both in 2017 and 2018.

4 | DISCUSSION

Up to now, the recycle of wastewater for agricultural practice has been considered harmful for the possible introduction of pollutants to the environment, spread of diseases, generation of odour and possible negative effects on animal and human health. Baydan et al. (2017) demonstrated that conventional animal products such as milk and meat include residues of veterinary drugs and environmental contaminants that consequently can cause consumer illness such as allergy, immunosuppression, cancer, teratogenicity, mutagenicity and genotoxicity. Additionally, wastewater contains many different types of pathogens that can represent a great risk for human and animal health. However, wastewater, for its richness in biodegradable organic material (carbon and nitrogen) along with macronutrients and micronutrients, may have beneficial effects on soils and crops representing a valid alternative to FW In this study, the municipal wastewater had been treated with the biological stabilization ponds that reduced the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values and pathogen load (Ben Said et al., 2017), making them more suitable and safer for irrigation purpose. Accumulation of OM in soil through irrigation with wastewater can result in an improvement of soil chemical and physical properties, with consequent positive effects on environment and plants. This work evidenced a positive effect of TWW on soil, showing a growing increase in soil nutrients, increasing the time of watering, in agreement with previous studies of Heidarpour et al. (2007) and Bedbabis et al. (2010) indicating that after 4 years of irrigation, TWW decreased the pH and increased OM and ions amounts (Licata et al., 2021); the results were perfectly in agreement also with previously findings of Bredai et al. (1996) and with a recent research of Mojid et al. (2019), evidencing as the degree of changes in soil properties is anyway a function dependent from soil and wastewater type. Wastewaters (municipal and not) can be utilized for ameliorating physicochemical soil properties and the strong relationship that exists between wastewater and soil organic carbon can be useful to design and manage soil irrigation with wastewater. In TWW irrigated soils, Mn and Zn increased as already reported by Bahri & Houmane (1987), and their values were overtime within the natural background levels in soil (Bordean et al., 2014; Mendoza et al., 2021). Heavy metal concentration increased in TWW treated soils. Many authors evidenced that the increased heavy metals in soils were associated with reduced microbial populations, their diversity and activity; cytotoxic effect on soils; and phytotoxicity in plants leading to reduced plant growth and chlorosis (AL-Huqail et al., 2022). The hazard of heavy metals can be contrasted by the gain of OM content in soil which consequently, allowing a major detention from soils, decreases the danger that toxic elements can reach deep layers or superficial groundwater (Khaskhoussy et al., 2022). Based on the

results obtained, it is possible to see that in spite of the soil enrichment with toxic metals, the gain of OM avoided the circulation of heavy metals at toxic levels.

The two *C. ciliaris* genotypes responded differently to the diverse irrigation treatments, evidencing a better growth for TWW irrigated tetraploid level. The photosynthetic cycle in C4 plants is more complex and supercharges photosynthesis by concentrating CO_2 around ribulose-1,5-bisphosphate carboxylase significantly reducing the oxygenation reaction comparison to C3. C4 plants have in addition a better modularity with complex mechanism coordinates the reactions of the metabolic pathways than that of C3 plants (Bai et al., 2021), and different responses of C3 and C4 have been already evidenced, by other authors versus different types of pollutants (AbdElgawad et al., 2020). Authors evidenced that C4 plants were better adapted in contaminated sites, whereas C3 plants were less adapted; this is because C4 plants are much more efficient at capturing carbon dioxide and biomass production and have a better antioxidative system (Sonowal et al., 2018). The higher yield obtained for TWW tetraploid irrigated plants was for the highest content of nutrient such as N_t, P and K that mimed the fertigation as already demonstrated in a previous study on commercial apple orchards in Southern Greece by Gasparatos et al. (2011). *C. ciliaris* being a C4 plant has a greater photosynthetic nitrogen use efficiency and can metabolize the high levels of nitrogen added with TWW. These results confirmed previous findings showing that the genotypes responded differently to irrigation with different water sources and evidenced that the 'tetraploid' were the most suitable and productive genotype under TWW irrigation, highlighting the possibility of selecting the genotypes most adapted to be irrigated with TWW. Both cultivars meet the dietary requirement of livestock that has to be in the range of 1.7–4.2 (% dry matter) (Boddey et al., 2004). Our findings evidenced that the 'tetraploid' *C. ciliaris* had enough P whose content was within the optimal P range of values (0.15% and 0.65%) reported in literature for *C. ciliaris* by Manzoor et al. (2013). Ramirez et al. (2004) in north-eastern Mexico and Ganskoop & Bohnert (2000) in North Texas, United States, showed that in the seven most prominent regions, where forage is cultivated, the grasses had a level of P inadequate for meeting the cattle needs, evidencing how forage can loss quality in respect to the arid Mediterranean climate.

Our results showed that watering with TWW caused a significant rise in the amount of macro nutrients (N, P and K) in plant tissues, and agreed with findings of Rejeb (1992), Charf et al. (1999) and Bahri (2001). Our findings were also consistent with the study of Tavassoli et al. (2010) in which irrigation with wastewater significantly increased the amount of macro nutrients (N, P and K) in corn forage. The increase was related to the amount of nutrients present in wastewater. The increased N_t, P and K amounts in *C. ciliaris* leaves were correspondent with the increased amount in available nutrients in TWW treated soils. The results of the present study suggested that TWW can be utilized complementarily to N and P fertilization and partially as K sources. On the basis of Pescod data (Pescod, 1992), the TWW utilized in this study can contribute with 294 kg N ha^{-1} $year^{-1}$, 50 kg P ha^{-1} $year^{-1}$ and 190 kg K ha^{-1} $year^{-1}$ in terms of fertilizers. Nevertheless, a great Na and Cl increase was observed in soil TWW treated; scarce concentration of Na and Cl in *C. ciliaris* leaves, independently from the ploidy levels, was observed. Salt tolerance in *C. ciliaris* can be ascribed to the capacity of limiting ion (mainly Na and Cl) uptake by roots and/or ion transport from roots to shoots (Ksiksi & El-Shaigy, 2012), hindering salt translocation rather than salt absorption (Lanza Castelli et al., 2010). Considering that no significant differences between the two ploidy levels has been observed, salinity tolerance together with scarce concentration of Na and Cl in *C. ciliaris* leaves could be explained by the efficient mechanism of Na and Cl exclusion of this grass as already reported by al-Dakheel & Hussain (2016).

It was observed an increase in Mn and Zn, in soil and in leaves irrigated with TWW, but it was inside the usual range identified in plants and considered safe for animal health. The values of Zn in *C. ciliaris* fell in the regular range present in forage species (10–30 mg kg^{-1}) (Madyiwa et al., 2002). Zinc, a necessary element for the correct working of reproductive system (Hambidge et al., 2006), was higher in the 'tetraploid' plants. However, it was lightly lesser than the value of 25 mg kg^{-1} reported by Fernandez-Escobar et al. (1999) as optimal in plant tissues. In a similar study, Al-Absi et al. (2009), irrigating soils with treated effluents, found that the concentrations of Zn and other heavy metals were in the common range for plant tissues. Conversely, Al-Lahham et al. (2007) showed a rise in leaf Zn concentration of sorghum watered with TWW. The lower leaf Zn concentration, found in respect to the previous study, can be elucidated by a modest transport of Zn from soil to leaves, as already demonstrated by Mapanda et al. (2005).

Magnesium, that plays a vital role in living organisms, as it is a necessary element for the activity of more than 300 enzymes of metabolic pathway, was above the optimal range reported in a previous study by Fernandez-Escobar et al. (1999) in 'Picual'. Its highest concentration was detected in the 'tetraploid' cultivar adding value to the use of this grass as fodder species for animal feed (Vance et al., 2003).

Watering with TWW provoked a highly significant increase in yield in both years for the two ploidy levels and mainly for the 'tetraploid' cultivar. Study of Khalilzadeh et al. (2020) on *Salicornia europaea* evidenced how watering with wastewater at reproductive stage caused higher biomass production than plants FW irrigated, confirming our data and highlighting that TWW can serve as fertilizer, providing N, P and K in great amounts. Study of Coelho et al. (2020) on digestate application as fertilizer reported that plant growth responses were highly associated with the input amounts of NPK, confirming our results. In general, between the two irrigation treatments, TWW irrigated plants showed the highest A_n, g_s and consequently T_r values both in 2017 and 2018. These results suggested that TWW could act enhancing the gas exchange capacity via decreasing stomatal resistances while increasing transpiration fluxes. This indicates a positive fertilization effect of TWW, mostly related to the increase in N, P and K content. These nutrients improved the capacity of carbon fixation and growth in plants, as already suggested by studies of Singh & Agrawal (2010) showing that A_n, g_s and T_r were higher in wastewater (containing more nutrients) irrigated plants than in canal water irrigated ones. Additionally, the increase in A_n found in leaves of TWW treated plants was in agreement with previous

findings of Herteman et al. (2011) showing that the wastewater affected the pigment concentration in leaves with consequent increment in chlorophyll content in different plant species which in turn resulted in an increase in A_n in plant leaves.

5 | CONCLUSION

In short, the results confirm that the irrigation with TWW represents a valuable and sustainable alternative to the use of FW above all in regions where it is under shortage. The TWW used for irrigating forage species could improve soil properties, recovering degraded area in line with European directive and green deal guideline, and at the same time may improve the nutritive values of fodder species. Taking in consideration that the request of water for agricultural purpose is foreseen to enhance twice more in 2025 compared to 2000. TWW can be successfully used to face crop production and food security under water scarcity environment. TWW can thus represent, in semiarid and arid regions, an opportunity for the economic development and social well being that have been seen to be closely related to the level of development in agriculture.

In this work, tetraploid *C. ciliaris* showed a better growth and quality and a greater amount of important nutrients N_t, P and K in its leaves watered with TWW giving it greater nutritional value. The tetraploid *C. ciliaris* has been identified as the most suitable cultivar to be irrigated with TWW and to be grown in marginal desert area as promising herding grass. Considering that the great regression of the rangelands is associated with the loss of species that have been dominant over vast areas and are now threatened with extinction for climatic reasons, there is an impellent need of selecting resistant native species to restore damaged or overexploited ecosystems safeguarding the local biodiversity.

In the view of a market-driven action, based on the request of the agricultural sector, this result can contribute to the promotion of a green and sustainable circular economy, more in climatically under pressure developing countries.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge to the Tunisian Ministry of Higher Education and Scientific Research for funding this research project. Open Access Funding provided by Universita degli Studi Mediterranea di Reggio Calabria within the CRUI-CARE Agreement. [Correction added on 24 March 2023, after first online publication: CRUI-CARE funding statement has been added.]

CONFLICT OF INTEREST STATEMENT

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.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Ben Said, I., Muscolo, A., Mezghani, I. & Chaieb, M. (2023) Treated municipal wastewater as option to the use of fresh water for the cultivation of valuable pastoral species Buffel grass (*Cenchrus ciliaris* L.). *Water and Environment Journal*, 37(3), 549–560. Available from: https://doi.org/10.1111/wej.12858