



Asian Journal of Plant Sciences

ISSN 1682-3974

science
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Effect of Treated Municipal Wastewater on Forage Yield, Quantitative and Qualitative Properties of Sorghum (*S. bicolor* Speed feed)

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Abstract: This study was carried out to investigate the effect of treated wastewater on yield quantitative and qualitative properties in fresh forage of sorghum. The experiment was conducted at the University of Zabol in Iran during 2007 growing season by using a randomized complete block design and four replications. The treatments were managed for irrigation; with well water during entire period of growing season as control (T_1); well water during entire period of growing season along with NPK (T_2); wastewater during the first half of growing season (T_3); wastewater during the second half of growing season (T_4); wastewater and well water alternately (T_5) and wastewater during entire period of growing season (T_6). Results showed irrigation with wastewater lead to significant increase ($p \leq 0.01$) on total yield, leaf fresh and dry weight, stem fresh and dry weight, stem diameter and leaf number in plant based on Duncan test for comparing, maximum yield (13 ton ha^{-1}) was obtained by irrigation with wastewater and well water alternately, also significant increase in forage qualitative indexes such as WSC, CP, ASH and significant decrease in NDF and ADF. Irrigation with wastewater and well water alternately has shown lower NDF, ADF and favorite CP in comparison with usage of wastewater during the whole growth period.

Key words: Irrigation, forage quantity and quality, municipal wastewater, sorghum, yield

INTRODUCTION

Nowadays the water crisis is one of the challenges that world is faced with. The lack of water resources have encouraged the researchers to use of non ordinary waters including salinity waters, municipal and industrial wastewater. By increasing population, water consumption increases wastewater production as well. Positive use of this new huge resource in agriculture may increase cultivate land, yield and also may decrease the environmental pollution. As FAO has reported, application of wastewater in agricultural context is its best applicability (Pescod, 1992). The plant suitable nutrition and using of sufficient and suitable manure resources lead to increasing the forage quality. This rich resource from nutrient elemental especially nitrate, moreover economic advantage because of decrease chemical manure consume, can improve the forage quality (Shanechi, 2004; Ghanbari *et al.*, 2007). Recently, about 300 hectares of wheat, barley, alfalfa and sorghum farms being irrigated by treated wastewater, just around Zabol in Iran. But

some deficiencies such as weak drainage, wastewater salinity ($EC > 3 \text{ dS m}^{-1}$), low annual rainfall (50 mm) and very high annual evaporation (more than 4500 mm), lead to worry in salinity, sodification and destruction of region soils (Lone *et al.*, 2003). One of the solutions to this problem is to irrigate plant with wastewater and well water alternately at developed stage of growth (Ghanbari *et al.*, 2007). This method could be an appropriate way to put a stop to salt accumulation and to increase the accumulated salt leaching in soil. Generally, in attention to poor pastures of Zabol region and high forage requirement for animal feeding, a proper choice to overcome this deficiency is to utilize these non ordinary water resources. Irrigation with wastewater leads to increasing in forage qualitative yield, about 3 to 3.5 times more than irrigation with well water. Also, it increased the stem length, chlorophyll and flowering rate (Asgari *et al.*, 2007). Irrigating tomato with municipal treated wastewater has increased the total fresh and dry weight of subterranean organ and upper organ in compare with the rest treatments (Erfani *et al.*, 2001). Ghanbari *et al.* (2007)

reported that irrigation with wastewater increased the wheat yield, as well. Day and Tucker (1977) reported that irrigation with wastewater increased the leaf width, grain yield and postponed the sorghum maturity. Although, it had no efficacy on protein content but it decreased some of amino acids and plant fiber (NDF and ADF). Marten *et al.* (1980) in a research on yield of corn and canary grass have perceived that irrigation with wastewater has significant increase on digestibility and dry matter of corn in comparison with canary grass, although amount of protein in canary grass is more than corn. Crude protein increase in wheat grain has been reported by irrigation with wastewater (Ghanbari *et al.*, 2007). The effect of treated municipal wastewater on growth and chemical compound of canola has no significant effects on protein and fat percentage; however the protein percentage in the treats with low wastewater quantity and fat percentage in the treats with high wastewater quantity were more than the rest (Kiziloglu *et al.*, 2007). Wastewater because of high nitrate and other nutrient elements lead to increase the leaf to stem ratio. Eventually protein and stem fiber percentage will increase and decrease respectively, because of enhancement in water contains and WSC; Consequently NDF decrease in forage and silage is resulted. It has been shown that nitrogen fertilizer lead to increase in CP, crude fat and Ash and also decrease in celluloses, hemicelluloses, K, P, Ca, Mg, S in grasses; in addition, little decrease in Zn, B and increases of Cu, Co, NO₃ attraction is reported (Vuckovic *et al.*, 2005).

Fateh *et al.* (2009) in study of organic and chemical fertilizers effects on forage yield and quality of globe artichoke has illustrated that the fertilizers such as N, Zn, Ca and Fe increased CP, WSC. Increase of CP, WSC and decrease of plant fiber is a result of nitrogen fertilizer application. The present study is an investigation of treated municipal wastewater effects on quality and quantity attributes in forage Sorghum. moreover, acquiring the most suitable application of wastewater and well water jointly to achieve desired qualitative and quantitative yield and to put a stop to pollution accumulation in plant and soil is the another objective of this study.

MATERIALS AND METHODS

The study was conducted in the Agricultural Institute of Zabol University Sistan and Baluchistan

Province, Iran (30° 55' N, 61°31' E). The region has an arid climate with 483 m altitude from sea level. Annual mean precipitation and temperature are 55 mm and 21.7°C, respectively. The soil (sandy-loam) properties prior to the experiment are shown in Table 1.

During the 2007 growing season (120 Day), the experiment was conducted out in a randomized complete block design and four replications. The irrigation treatments were well water in all growing stages as a first treatment control (T₁); well water for all growing stages along with NPK application (NPK: 100, 120, 90 kg ha⁻¹) as a second control (T₂); wastewater during the first half of growing period (The stage before flag leaf visible) (T₃); wastewater during the second half of growing period (The stage after flag leaf visible) (T₄); wastewater and well water alternately (T₅) and wastewater during the whole growing period (T₆). The chemical fertilizers, ½ N, total of P and K were applied before sorghum sowing. Each plot was constructed in 3×2.5 m with 1 m distance between plots and 2 m distances between replications. Sorghum was seeded by 25 kg ha⁻¹, 20 cm row distance, 15 cm between plants and 3-5 cm planting depth. Seeds were planted manually using two seeds per hole in May 2007. The forage was harvested at soft dough stage of grain maturity in August 2007.

Collected data include: Yield was obtained by combining the five center rows in 1 m² in each plots, number of leaf, stem diameter, stem length and fresh weight of stem and leaf were measured in 10 sample plants per plots. The dry weight of stem and leaf were measured after drying samples at 60°C for 48 h in an air oven (Horwitz, 2005), qualitative characteristics are DM content (at 105°C for 24 h), residual ash (4 h at 600°C) and OM were measured from difference of DM and Ash. One portion of these samples was dried in an oven at 60°C for 96 h and also it ground through a 1mm sieve, then, it was analyzed for N (Kjeldahl method; 7.021 procedure at Horwitz, 2005), content of cell wall components and content of NDF was determined without sodium sulfite and with a heat-stable amylase (Van soest and Wine, 1967). Acid Detergent Fiber (ADF) was determined by sequential analysis for the residual NDF and expressed exclusive of residual Ash (Van soest and Wine, 1967). Also WSC was analyzed by Spectrophotometer (Deriaz, 1961).

The results of analysis well water and wastewater quality are shown in Table 2 and they have been

Table 1: Soil analysis result for chemical characteristic

Depth (cm)	EC (dS m ⁻¹)	Micro and macro nutrient (mg kg ⁻¹)								OM (g kg ⁻¹)	pH
		Ca+Mg	Fe	Mn	Zn	Cu	N	P	K		
0-30	4.6	20	6.12	9.6	0.31	0.73	3	2.6	17	2.4	8.2

Table 2: Quality of well water and treated wastewater (mg L⁻¹)

Parameter	Well water	Standard limit	Wastewater	Standard limit	Standard limit of pollutants in	
					Agriculture applications	Water resources
pH	7.8	7.6	8.2	7.60	6-8.5	6-8.5
EC (dS m ⁻¹)	2.04	2.61	4.5-5.5	2.97	-	-
Calcium	140	200	90	-	-	75
Magnesium	100	29.5	85.2	-	100	100
Chloride	6.4	<142	999.7	-	600	600
Sulfate	374.4	501.1	739.2	-	500	400
Nitrate	-	-	13.2	-	-	50
Phosphate	-	-	13.5	4.10	-	6
Boron	-	-	3.3	-	-	2
Sodium	253	899.30	915.4	-	-	-
Potassium	-	-	22.62	-	-	-
Copper	-	0.002	0.006	0.20	0.2	1
Manganese	-	-	0.039	-	1	1
Zinc	-	-	0.017	-	2	2
Ferro	-	0.11	0.2	0.33	3	3
Total coliform (MPN 100 mL ⁻¹)	-	-	85	-	1000	1000

compared by suggested standards of Iran environment conservation structure (E.C.O of Iran, 1999) and FAO water qualitative standard has been used (Pescod, 1992). According to this standard, available water had no deficiency for irrigation and evaluation of wastewater pollution (Table 2) showed that magnitude of sulfate, NO₃, PO₄, Ca, Mg and heavy metal concentrations were lower than critical limit (E.C.O. of Iran, 1999). For evaluation of wastewater microbial pollution has used from determinate critical limit (E.C.O. of Iran, 1999).

Concentration of sodium, chloride, Boron and sulfate was higher than optimum limit which it is demonstrate the soil pollution possibility in continuously use of wastewater. Based on FAO standards, Electrical Conductivity (EC) and concentration of Sodium, Chloride and Boron were higher than optimum limit which it may cause toxic outbreak in sensitive plant (Ensink *et al.*, 2007). Due to the high concentration of chloride, rainy irrigation system can not be admissible. Sodium absorption ratio (SAR = 13) in wastewater has measured to determine the existent sodium efficacy of wastewater on soil penetration and structure. Due to low salinity (5>EC>3 dS m⁻¹) of wastewater the possibility of soil sodification would be low, but some long term studies have shown that in this case also it might happen (Kiziloglu *et al.*, 2007).

Collected data were analyzed via SAS Institute Inc. 6.12. First of all data were analyzed by ANOVA to determine significant (p<0.05) treatment effect. The significant difference between individual means was determined by Duncan Multiple Comparison Test.

RESULTS AND DISCUSSION

Effect of wastewater on quantitative properties

Yield: The results in Table 4 show that T₅ and T₆ had the highest yield, but there is no significant difference

between them. Whereas they had significant difference (p<0.05) with the rest; Nadia (2005) have reported increase in sorghum yield at irrigation with wastewater in whole growth period rather than well water and it might be owing to high nitrate amount in municipal treated wastewater. The lowest yield was belonging to T₁ and T₂ treatments. As it was anticipated, the analysis of soil and water showed (Table 1, 2) that the application of NPK fertilizer in this study have led to increase in yield. Since, applying wastewater in different amount and times has led to significant increase in yield, so it shows that NPK application is not the only solution to get maximum yield and there is need to apply other macro and micro elements. It has been reported by some researchers that applying macro and micro chemical fertilizer lead to increase in corn and sorghum yield (Ghanbari *et al.*, 2007).

Leaf fresh and dry weight: Leaf fresh and dry weight were significantly affected by irrigation treatments (Table 3) the comparison of average of fresh leaf weight showed that there is no significant difference between T₆, T₅, T₃ and T₄, but there is significant difference between each of them and control treatment. The lowest fresh weight was belong to control treatment and after that T₄ had lower fresh leaf weight than the rest (Table 4). The sixth and fifth treatments had highest dried leaf weight and T₄ and T₃ treatments had no significant difference and it is in accordance with other researcher's result (Ghanbari *et al.*, 2007; Nadia, 2005).

Stem fresh and dry weight: The highest fresh stem weigh was achieved at treatments which use wastewater and there was no significant difference between them. On the other hand, the lowest fresh stem weight was achieved at T₁ and after that T₂ had the lower fresh stem weight than other with significant difference (p<0.05). Also, there was

Table 3: ANOVA of the effect of irrigation treatments on sorghum quantitative and qualitative yield

SOV	df	Total yield	Leaf number	Stem dry	Stem fresh	Leaf dry	Leaf fresh	CP	WSC	NDF	ADF	Ash
		weight (g)						(%DM)				
Replication	3	1.10ns ¹	2.94ns	6.60ns	11.40ns	6.60ns	6.30ns	3.57ns	0.84ns	6.76ns	0.19ns	0.74ns
Irrigation	2	16.04*	34.90*	52.15*	752.70*	14.96*	134.20*	137.01**	10.16**	24.04**	159.09**	8.43**
Error	6	10.70	22.90	35.00	474.70	11.80	86.22	1.58	1.22	1.95	1.53	1.17
CV (%)		5.70	6.80	12.50	10.40	10.70	8.40	1.97	12.10	3.10	4.40	7.46

ns: Non significant, **p<0.01 and *p<0.05

Table 4: Averages comparison for forage yield and other quantitative characteristics in sorghum

Parameters	Treats					
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
Total yield (ton ha ⁻¹)	7.80 ^{dl}	9.300 ^e	11.50 ^b	11.10 ^b	13.00 ^a	12.70 ^a
Leaf fresh weight (g)	18.90 ^e	23.100 ^b	31.50 ^a	31.40 ^a	32.50 ^a	32.10 ^a
Leaf dry weight (g)	8.90 ^e	11.300 ^b	13.30 ^{ab}	12.70 ^{ab}	13.50 ^a	14.30 ^a
Stem fresh weight (g)	37.10 ^e	47.900 ^b	65.10 ^a	51.00 ^b	68.60 ^a	71.90 ^a
Stem dry weight (g)	14.30 ^b	15.900 ^b	21.00 ^a	20.20 ^a	22.60 ^a	23.10 ^a
Stem diameter (cm)	1.10 ^d	1.210 ^c	1.33 ^b	1.40 ^{ab}	1.42 ^a	1.47 ^a
Leaf number	15.90 ^d	17.300 ^c	19.70 ^b	18.40 ^{bc}	23.50 ^a	22.60 ^a

¹Row means followed by the same letter are not significantly different at 0.05 probability level

no significant difference between T₂ and T₄ (Table 4) and these results imply that irrigation with wastewater in last growth stages could not recover the cells growth reduction and consequently plant growth and stem fresh weight's reduction. Whereas, T₅ and T₆ treatments led to significant enhancement in fresh stem weight (p<0.05), applying chemical fertilizer (NPK) also have caused to increase in fresh stem weight although it was less effective in compare with irrigation with wastewater. The results in dry weight have followed the fresh weight process (Table 4) and in case of increase in stem diameter and dry stem weight of sorghum there was in accordance with results attained by Day and Tucker (1977).

Stem diameter: The results of average comparison (Table 4) showed that wastewater treatments have statistical significant difference (p<0.05) in comparison with control. T₆ and T₅ had the most stem diameter (1.47 and 1.42 cm, respectively) than other treatments. The lowest stem diameter was pertain to T₁ (control) and T₂ and in addition, no significant difference was found between T₃ and T₄, therefore it could be said that stem diameter during plant growth is affected by environmental factors. Such that, the existence of specific nitrogen and potassium in wastewater will improve the plant growth, cell reproduction and plant resistance (Emam, 1995) and eventually stem diameter increases (Day and Tucker, 1977).

Leaf number in plant: The highest number of leaf was found in T₅ and T₆ and on the other hand the smallest number of leaf was pertained to control treatment. Variations between T₃ and T₄ and between T₄ and T₂ were no statistically significant (Table 3). The shortage of

Table 5: Averages comparison for forage properties in sorghum

Parameters (%DM)	Treats						SEM
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	
CP	5.73 ^c	6.52 ^d	6.71 ^c	6.22 ^d	7.00 ^b	7.78 ^a	1.59
WSC	7.44 ^c	9.19 ^b	9.02 ^b	8.98 ^b	8.70 ^b	11.50 ^a	1.22
NDF	46.68 ^a	46.03 ^a	42.88 ^b	47.37 ^a	44.22 ^b	42.67 ^b	1.95
ADF	29.50 ^a	25.20 ^d	27.17 ^c	28.08 ^b	24.83 ^d	24.77 ^d	1.53
Ash	8.88 ^d	9.90 ^{cd}	10.70 ^{bc}	10.00 ^{cd}	11.60 ^{ab}	12.10 ^a	1.17

¹Row means followed by the same letter are not significantly different at 0.01 probability level

nutrient, inclusive of macro and micro nutrients in control treatment may cause to decrease in plant height and number of leaf. The leaf number or leafy index is one of the important factors in forage plants. A researcher has reported increase in leaf number by application of manure and chemical fertilizer in sorghum (AL-Jaloud *et al.*, 1995).

Effect of wastewater on qualitative properties

Crude protein: Results show that there is a very significant increase (p<0.01) in protein percentage in sorghum forage due to wastewater application (Table 3). Moreover, well vegetative growth, yield and yield components increase, leaf number increase, specifically increase of leaf to stem ratio which is strong evidence on presence of nitrogen in plant disposal and it is certainly due to wastewater application such that lead to protein increase (Table 4). We should be noted that increasing of leaf to stem ratio lead to protein increasing because of the higher leaf protein than stem (Shanechi, 2004).

The highest protein percentage was found at T₆ treatment up to 26% more that control treatment (Table 5). In fact, it was anticipated since wastewater is full of nutrient and specifically nitrogen with basis role on protein making in plants (Table 2) (Jacobs *et al.*, 1998). Several studies have shown significant increase of crude protein in sorghum and corn by wastewater application (Mohammad and Rusan, 2007; Ghanbari *et al.*, 2007; Day and Tucker, 1977). Presence of nutrient elements in wastewater can have an important role in nitrogen uptake increase. Possibly, existence of micro nutrients in rizospher causes to more nitrogen uptake and eventually may enhance protein production, on the other hand some elements namely Cu and Zn associate with protein structure and nitrogen metabolism (Jacobs *et al.*, 1998).

Water soluble carbohydrate: Wastewater application increased WSC in sorghum (Table 5). The increase of structural and non-structural carbohydrate have correlation with yield increase and high vegetative growth (Table 4). Day and Tucker (1977) have reported similar result about wastewater efficacy on WSC increase in sorghum. Wastewater treatments had significant efficacy ($p < 0.01$) on WSC in forage sorghum (Table 5) and treatment of T_6 had highest WSC. According to inverse relationship between WSC and NDF, the decrease of NDF due to wastewater application may increase WSC in this treatment (Smith *et al.*, 2002). No significant difference between T_2 , T_3 , T_4 and T_5 was found in term of WSC amount but they had increase in compare with control treatment. The lack of required nutrition for carbohydrate production could be a reason for small amount of WSC in control treatment (Jacobs *et al.*, 1998; Smith *et al.*, 2002).

Neutral detergent fiber: Irrigation with wastewater has caused a significant decrease ($p < 0.01$) in NDF. Wastewater because of high nitrogen and other nutrient has caused to increase in leaf to stem ratio and consequently, forage protein enlargement also decrease in stem fiber because of the water content and solution carbohydrate enlargement and eventually result in the NDF decrease on forage. The similar result about the reduction of plant fiber in sorghum and forage crops with wastewater application is reported by some researchers (Mohammad Rusan, 2007; Day and Tucker, 1977). The treatment of T_1 , T_2 and T_4 had highest NDF (46.68, 46.03, 47 and 37%, respectively), the treatment of T_3 , T_5 and T_6 had significant decrease ($p < 0.01$) than the rest of treatments (Table 5). It seems that, high nitrogen percentage and potassium in wastewater which they have positive role in nitrogen uptake and protein structure, may increase the protein and decrease the structural carbohydrate (cellulose and hemi cellulose) in forage sorghum. The other researchers have reported that wastewater decrease the plant fiber percentage, as well (Tas, 2005; Smith *et al.*, 2002).

Acid detergent fiber: Results showed that wastewater application decreased the ADF in the plant (Table 5). Raise in vegetative growth specifically stem and leaf dry weight and stem diameter in result of wastewater application may led to ADF decrease (Table 4). Vuckovic *et al.* (2005) in a research have shown that application of nitrogen fertilizers led to decrease of cellulose and hemi cellulose in pasture grasses.

Ash: The results showed that wastewater application led to significant increase ($p < 0.01$) in forage Ash percentage

(Table 5). There is no doubt that, existence of nutrients in wastewater has caused to vegetative growth raise and consequently increases mineral and organic mater in plants (Table 2 and 4). Ash enlargement at T_5 and T_6 demonstrate that plant have used the wastewater nutrient (Table 5). The control treatment had lowest ash percentage than other treatments. Wastewater application has led to increase in mineral elements up to 25.2% than control treatment. The treatment of T_2 and T_4 had no significant difference in ash percentage. In fact, it was found that T_4 and T_2 treatments have provided the same value of nutrient for plant requirement.

CONCLUSION

Irrigation with wastewater because of high nutrient content increases the quantitative and qualitative properties in forage sorghum. Wastewater application has caused to increase in WSC, CP, Ash and also decrease in NDF and ADF which is favorite property of forage and in consequence of that the forage digestibility increase because of low cellulose and hemi cellulose. Wastewater application led to more forage quality than control and T_2 treatment. Irrigation with wastewater and well water alternately (T_5) and wastewater during the whole growth period (T_6) had highest forage qualitative. Based on this study, for municipal wastewater application project, increasing forage quality yield, soil and environmental conservation, we recommend utilize the municipal wastewater of Zabol in the form that sorghum was irrigated with wastewater and well water alternately.

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