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# Effect of utilization of treated wastewater and seawater with Clinoptilolite-Zeolite on yield and yield components of sorghum

Fakhroddin GhassemiSahebi[a], Omolbani Mohammadrezapour[b], Masoomeh Delbari[c], Abbas KhasheiSiuki[d], Henk Ritzema[e,*], Ali Cherati[f]

[a] Department of Water Engineering, University of Zabol, Zabol, Iran
[b] Department of Water Engineering, University of Agricultural Sciences and Natural Resources Zabol & Gorgan, Iran
[c] Department of Water Engineering, Faculty of Agriculture, University of Zabol, Zabol, Iran
[d] Department of Water Engineering, University of Birjand, Iran
[e] Water Resource Management Chair Group, Wageningen University, Wageningen, the Netherlands
[f] Department of Soil and Water Research, Mazandaran Agricultural and Natural Resources Research and Education Center, AREEO, Sari, Iran

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ABSTRACT

Under conditions of water shortage, utilizing unconventional waters, such as treated urban wastewater and/or seawater, in combination with soil amendments such as zeolite, can reduce the harmful effects of drought stress on crop yield. To investigate the agronomic and physiological effects of a mix of water qualities and soil treatments on sorghum, a randomized split-plot research was conducted at Gharakheil agricultural research station, Ghaemshahr, Northeast Iran. Six combinations of water qualities and three different use of soil amendments were tested over two seasons in 2016 and 2017. The water quality treatments with increasing salinity included well water as the control (W1); 75 % well water and 25 % seawater (W2); 25 % well water and 75 % seawater (W3); 100 % treated urban wastewater alternating with 100 % seawater (W4); 50 % seawater and 50 % treated urban wastewater (W5) and 100 % treated urban wastewater (W6). The soil amendments were no-zeolite as the control (Z1) and calsic (Z2) and potasic (Z3) zeolite. With increasing salinity, the forage yield decreased significantly. Maximum and minimum forage yield were respectively 129.6 ton.ha$^{-1}$ inW6-Z2 in 2016 and 46.9 ton.ha$^{-1}$ in W3-Z1 in 2017. Irrigation Water Use Efficiency (IWUE) was the highest with the treated urban wastewater in combination with zeolite. All six combinations (W4-Z2, W4-Z3, W5-Z2, W5-Z3, W6-Z2 and W6-Z3) had significantly higher IWUEs (range 2.0–2.4) compared to the control (IWUE = 1.7) and the other soil and water treatments. The combinations of 75 % seawater and no zeolite had by far the lowest IWUE (range 1.1–1.7). The same trends were observed for the Leaf Area Index (LAI) and leaf and stem protein. The use of saline sea water increased the soil salinity levels significantly, but the levels were still well below the FAO threshold values for yield reduction. Overall, we can recommend use of treated wastewater in combination with calsic zeolite soil amendment as the combination that had the best effect on crop yield, IWUE, LAI and leaf and stem protein for sorghum production under the conditions of north of Iran.

## 1. Introduction

Although water covers two thirds the surface of the earth, only a small portion of this water is suitable for human consumption. During the last decades, droughts and water scarcity have become one of the major concerns for governments, organizations, policy-makers, water users and water managers in many parts of the world (Owusu-Sekyere et al., 2017). This concern is obvious in countries like Iran that are in arid and semiarid areas. The current water shortage problem in Iran has been caused by multiple factors, some of which may be related to mismanagement (Madani, 2014), and some of which are related to natural causes such as climate change and persistent drought conditions in recent years. The lack of water resources has increased the use of unconventional waters such as seawater, urban and industrial wastewater.

Wastewater can have a positive effect on the soil and eventually crop growth as it is often rich in organic matter and nutrients such as nitrogen, potassium and phosphorus (Ghanbari et al., 2007). Use of this

* Corresponding author at: Water Resource Management Chair Group, Wageningen University, P.O. Box 47, 6701 EB, Wageningen, the Netherlands.
*E-mail addresses:* Ghasemi.f19@gmail.com (F. GhassemiSahebi), Mohammadrezapour@uoz.ac.ir (O. Mohammadrezapour), Mas_delbari@yahoo.com (M. Delbari), Abbaskhashei@birjand.ac.ir (A. KhasheiSiuki), henk.ritzema@wur.nl (H. Ritzema), Acherati@yahoo.com (A. Cherati).

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huge resource in agriculture may allow increases in cultivated area and yields and may also decrease environmental pollution. Another unconventional water resource is saline water which is widely available. In particular the water of the Caspian Sea is a good option for use in agriculture (Machekposhti, er al., 2017). As the salinity of the Caspian Sea waters is much lower than that of open seas (Dordipour et al., 2004), it can be an alternative water resources for the northern provinces of Iran. However, to use this particular low-quality water, only crops that are salt-resistant can be grown. Sorghum is one such crop.

Sorghum (*Sorghum bicolor (*L.*)Moench*) is the fifth major staple crop worldwide, after maize, wheat, rice and barley (Paterson, 2008.) It is the main staple food for more than half a billion people, particularly in the semi-arid regions such as south Asia and sub-Saharan Africa (Mace et al., 2013). Sorghum is also important for livestock forage, and forage health has a direct effect on human health (Al-Jaloud et al., 1995). Sorghum, with its high tolerance to environmental stresses, such as salinity, drought and heat, can grow relatively well under adverse conditions (FAO, 1985; Teetor et al., 2011). Sorghum in Iran is cultivated both as irrigated and rain fed crop in summer. Mostly tall varieties are sown for fodder production that can resist drought and hot weather.

Recently, one of the issues that attracted the attention of researchers and environmentalists is unconventional waters chemicals and heavy metals especially those which can penetrate into soil, plant and finally food chain (Ashworth and Alloway, 2003). Heavy metals represent a portion of important environmental pollutants which causes pollution problems by increasing their use in products in recent decades. Cadmium in food and the environment is considered not only because of its high toxicity, but also because of its high levels of sustainability, as the most dangerous element in the environment (Perez-Lopez et al., 2008). Since one way of human exposure to cadmium is to get this element through food, assessing and controlling the amount of contaminated food sources and identifying the sources of contaminants and modifying or eliminating them plays a significant role in the health and longevity of humans (Rahimi and Raisi, 1999). Chromium is considered as an environmental pollutant released into the atmosphere due to its use in large industries. Chromium is a well-known human carcinogenic agent, and many reputable organizations have confirmed lung cancer as a result of exposure to it (Tirger et al., 2008). Therefore, the study of the behaviour of these two heavy metals, due to their higher concentrations compared to other heavy elements such as lead and zinc, which could be neglected because of their low density, was considered in this study.

The use of zeolites is an appropriate option to improve soil conditions through the absorption of harmful salts, as well as the maintenance of water for the crop under conditions of use of Caspian Sea water or wastewater. Zeolites are aluminosilicate minerals containing exchangeable alkali or alkaline-earth metal cations (normally $Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$) in addition to water in their structural framework. Their physical structure is porous containing interconnected cavities within metal ions and water molecules (Gottardi and Galli, 1985). Zeolite are microporous aluminosilicate frameworks with three-dimensional networks of corner-sharing [TO4] tetrahedra, in which T usually represents silicon or aluminium. The framework composed of purely [SiO4] units is neutral. When Al with a charge of 3+ is an isomorphous substitute for Si with a charge of 4+, the framework becomes negatively charged compensated by extra-framework cations, leading to its cation exchange capacity (Hong et al., 2018). Zeolites can adsorb more than 41 % of their weight in water and they can keep elements such as nitrogen, potassium, calcium, magnesium, and micronutrients in the root environment and then release them according to the crop's needs, ultimately improving crop growth (Mumpton, 1999). Zeolites in soil increase the pH of soil, which reduces the absorption and bioavailability of heavy metals by plants. The increase in pH increases the adsorption of heavy metals and their oxides on the surfaces of zeolites (Shi et al., 2009). Application of zeolites in the soil significantly reduced the Cd uptake by a wheat crop (Chang et al., 1997; Dheri et al., 2007). Moreover, application of natural zeolites increased protein content, biomass, root length, and root dry weight of alfalfa. Zeolite is an efficient amendment to reduce Cd translocation in plant tissues (Hasanabadi et al., 2015). Amendments of zeolite reduced the adverse effects of Cd and Cr and, ultimately, increased dry weight as compared to the control. Therefore, application of zeolites as soil amendment increases the soil pH due to replacement of Na + and H + ions between the soil and the zeolites. Increase in soil pH increased the Cd-ion adsorption at the surface of iron oxide and reduced bioavailability and uptake by plants. In arid and semi-arid environments, materials such as zeolite, can increase a soil's water holding capacity as well as buffer, nutrient absorption and release to avoid damage caused by stress to the photosynthetic apparatus (Polat et al., 2004; Mao et al., 2011). Zeolites appear to be an appropriate option to test for managing soil conditions through the absorption of harmful salts, as well as the maintenance of water for crops under conditions of using Caspian Sea water or wastewater for irrigation.

However, reports of zeolite applications for sorghum have been rare. Furthermore, little is known about biomass quality of sorghum irrigated with combination of wastewater and seawater with zeolite. Therefore, in this work we explored the effect of using zeolite with unconventional water on sorghum yield and quality, as well as soil salinity accumulation in soil profile. The specific objectives of this study are to evaluate the yield and quality of sorghum and soil salinity accumulation in responses to zeolite application in north of Iran.

## 2. Materials and methods

### 2.1. Site and climatic conditions

During 2016–2017, a two-year field investigation was carried out in a 672 $m^2$ (12 × 56 m) sorghum field at the Gharakheil-Agricultural-Research-Station, Ghaemshahr, Mazandaran Province, Iran (36° 29´ N, 52° 46´ E). The mean elevation of the site is 14 m above mean sea level. Based on the DeMarten method (Oliver, 2005), the climate is classified as humid. The annual average rainfall and temperature over the period 1980–2017 are respectively 725 mm and 17 °C, with rainfall events mainly concentrated in autumn and late winter and almost absent in the spring and summer seasons. The total rainfall was higher in 2016 than in 2017 (Fig. 1). Rainfall data were recorded at the agro meteorological station located at Ghaemshahr less than 500 m from the experimental site.

### 2.2. Site conditions and preparation

Before sowing, the soil of the experimental plots was sampled at three depths (0–0.25, 0.25–0.50 and 0.50-0.80 m). Soil texture was classified, using the hydrometer method (USDA Soil Survey Staff, 1975) (Table 1).

Chemical properties of the soil are as follows: pH = 7.30, electrical conductivity (EC), 0.9 dS $m^{-1}$; organic matter, 11.1 g $kg^{-1}$; organic carbon, 6.7 g $kg^{-1}$; nitrogen (N), 0.7 g $kg^{-1}$; phosphorus (P), 4.6 mg $L^{-1}$; potassium (K), 75 mg $L^{-1}$. Prior to planting, the field capacity and permanent wilting point of the soil samples were determined using a pressure plate apparatus in a gravimetric way. Percentage of organic matter was determined with the burning method (Horwitz, 2005), total soil nitrogen by the Kjeldahl method (Rusan et al., 2007), plant available phosphorus by the Olsen method (Ghanbari et al., 2007). The average water table in the experimental field was about 120 cm below the soil surface at the beginning of the growing season, and then it continues to fall to 200 cm below the soil surface by the end of the season. In the late winter of both growing seasons (2016 and 2017), the soil was prepared by ploughing to a depth of 25 cm. Immediately before sowing (May), the soil was tilled using a double-disking harrow. Finally, a field cultivator was used to prepare the seedbed. After the preparation of the land and before planting, certain

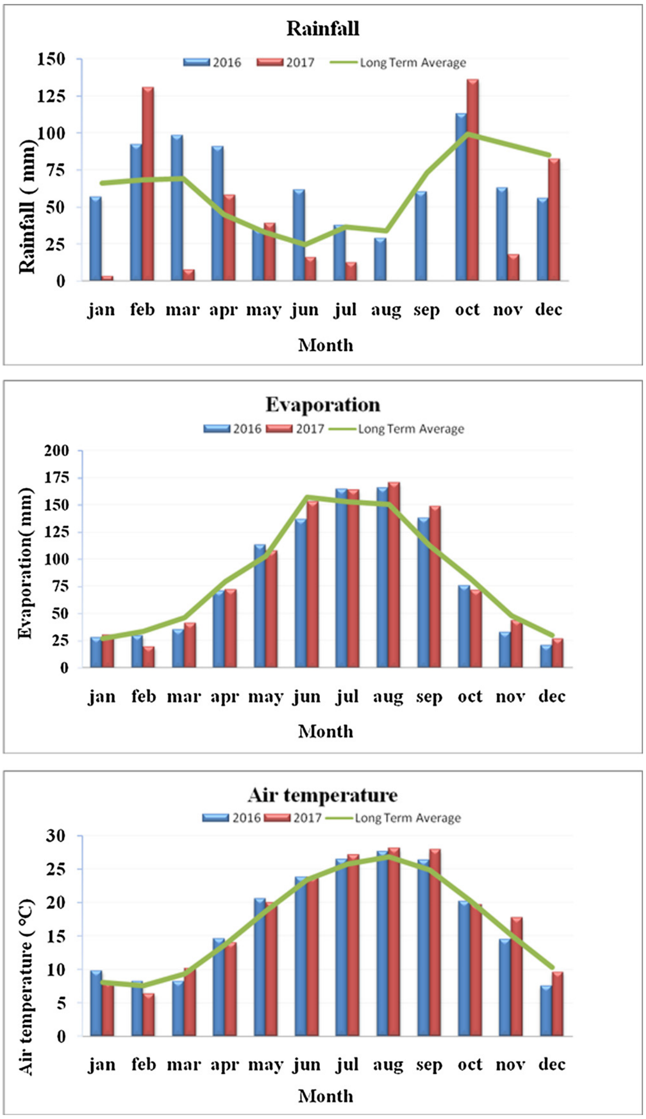


**Fig. 1.** Rainfall, Evaporation and Air temperature during the 2016 and 2017 growing seasons and the long-term average (1980-2017) obtained from Ghaemshahr Meteorological Station.

quantities of zeolite (one kilogram per square meter of soil. Seif et al. (2016) and Najafinezhad et al. (2014)) were spread by hand over the field and ploughed to the depth of 25 cm again. The natural Clinoptilolite-Zeolite were obtained from Semnan region in the north-east of Iran.

### 2.3. Experimental design and crop management

A split plot experimental design was used to evaluate the effects of different water qualities (well water, treated wastewater and seawater) on three soil treatments (no zeolite (Z1), calsic zeolite (Z2), and potasic zeolite (Z3)) replicated three times. The water quality treatments with increasing salinity included well water as the control (W1); 75 % well water and 25 % seawater (W2); 25 % well water and 75 % seawater (W3); 100 % treated urban wastewater alternating with 100 % seawater (W4); 50 % seawater and 50 % treated urban wastewater (W5) and 100 % treated urban wastewater (W6). The soil amendments were no-zeolite as the control (Z1) and calsic (Z2) and potasic (Z3) zeolite. The EC of each irrigation treatment is shown in Table 2. The source of the treated wastewater was the nearby Sari Wastewater Treatment Plant that receives urban wastewater. The treatment process consisted of screening, de-gritting, pre-aeration, primary settling, aeration, secondary settling, and disinfection (Ganjegunte et al., 2018). The field experiment consisted of six irrigation treatments with well water during the entire period of growing season as the control. There were three replicates for each irrigation treatment, which were carried out in a randomized complete block design (Table 3). Each plot was 3 × 3 $m^2$ with a 1-meter distance between plots and 1.5-meter distances between replications.

**Table 2**
Salinity of irrigation water of each irrigation treatment.

| No. | Treatments | EC (ds. $m^{-1}$) |
|---|---|---|
| W1 | well water | 0.9 ± 0.05 |
| W2 | 75 % well water + 25 % sea water | 5.2 ± 0.4 |
| W3 | 25 % well water + 75 % sea water | 11.7 ± 0.8 |
| W4 | treated waste water & sea water | 1.1 & 15.2 |
| W5 | 50 % treated waste water + 50 % sea water | 8.1 ± 0.6 |
| W6 | Treated waste water | 1.1 ± 0.08 |

The sorghum (speed feed) was planted manually the last week of May in both 2016 and 2017 at a depth of 5 cm, using two seeds per hole. Crop row and crop spacing was 60 cm × 10 cm. The forage was harvested at the soft dough stage of grain maturity in August.

Soil samples were collected from top to the depth of 80 cm in three levels (0 − 25 cm, 25 − 50 cm and 50 − 80 cm) before each irrigation. Surface irrigation was used with irrigation intervals of 7–12 days, totally 6 and 7 irrigations were given in the 2016 and 2017 growing seasons, respectively. For each irrigation event, the irrigation requirement (in mm) was calculated using:

$$I_n = \sum_{n=1}^{m} \{(\theta\, FC_i - \theta\, BC_i)\, D_i\}\ \mathrm{I_n} = \sum_{\mathrm{n=1}}^{\mathrm{m}} \{(\theta \mathrm{FC_i} - \theta \mathrm{BI_i}) \mathrm{D_i}\} \tag{1}$$

Where $I_n$ is the net irrigation depth (mm) of the nth irrigation event, (θ $FC_i$) is the volumetric soil water content at field capacity ($cm^3$. $cm^{-3}$) of the ith soil layer, (θ $BC_i$) is the average volumetric soil water content of the ith soil layer ($cm^3$. $cm^{-3}$) before irrigation, $D_i$ is the soil layer thickness (mm), i is the soil layer, and m refers to the number of soil layers down to a specific soil depth (m = 3). (θ $BC_i$) was measured before each irrigation event by drying soil samples in an oven. To measure soil moisture, before each irrigation soil samples were taken every 25 cm to a depth of 80 cm for each treatment using an auger. It is assumed that after irrigation, the soil water content was close to field capacity. Irrigation Water Use Efficiency (IWUE) was calculated as the ratio between crop produced (Y in kg.$ha^{-1}$) and the total volume of irrigation water delivered to the plot ($V_{tot}$ in $m^3$. $ha^{-1}$) and was

**Table 1**
Soil properties at the experimental site.

| Depth | Soil texture | Sand | Silt | Clay | Field capacity | PWP[a] | Bulk density |
|---|---|---|---|---|---|---|---|
| (cm) | | (%) | (%) | (%) | ($cm^3$ $cm^{-3}$) | ($cm^3$ $cm^{-3}$) | (g. $cm^{-3}$) |
| 0-25 | Clay Loam | 31 | 31 | 38 | 0.31 | 0.15 | 1.46 |
| 25-50 | Clay Loam | 29 | 32 | 39 | 0.33 | 0.15 | 1.44 |
| 50-80 | Clay Loam | 28 | 32 | 40 | 0.34 | 0.15 | 1.43 |

a) Permanent wilting point.

**Table 3**
The experimental split plot design with three replicates of six water treatments (W1, W2, W3, W4, W5 and W6) and three soil treatments (Z1, Z2 and Z3).

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replica 1 | W3 | | | W6 | | | W5 | | | W1 | | | W2 | | | W4 | | |
| | Z3 | Z1 | Z2 | Z3 | Z1 | Z2 | Z3 | Z1 | Z2 | Z3 | Z1 | Z2 | Z3 | Z1 | Z2 | Z3 | Z1 | Z2 |
| Replica 2 | W6 | | | W5 | | | W2 | | | W4 | | | W3 | | | W1 | | |
| | Z2 | Z1 | Z3 | Z2 | Z1 | Z3 | Z2 | Z1 | Z3 | Z2 | Z1 | Z3 | Z2 | Z1 | Z3 | Z2 | Z1 | Z3 |
| Replica 3 | W1 | | | W2 | | | W3 | | | W4 | | | W5 | | | W6 | | |
| | Z2 | Z3 | Z1 | Z2 | Z3 | Z1 | Z2 | Z3 | Z1 | Z2 | Z3 | Z1 | Z2 | Z3 | Z1 | Z2 | Z3 | Z1 |

W1 = well water, control; W2 = 75 % well water and 25 % seawater; W3 = 25 % well water and 75 % seawater; W4 = 100 % urban treated wastewater alternating with 100 % seawater; W5 = 50 % seawater and 50 % urban treated wastewater; W6 = 100 % urban treated wastewater. Z1 = no-zeolite as control; Z2 = calsic zeolite and Z3 = potasic zeolite.

expressed in kg. $m^{-3}$ (Ali and Talukder, 2008):

$$WUE = \frac{Y}{V_{tot}} \tag{2}$$

Crops were harvested to determine their total nitrogen uptake (N in the stem and leaves) and the leaf area index. Three plants per plot were harvested. The oven-dried (at 70 °C) weight of each part of a plant (stem and leaves) was determined. Total nitrogen content was determined using Kjeldahl's method automated with the Kjeltec (Nelson and Sommers, 1980). Cadmium (Cd) and Chromium (Cr) concentrations in the dry matter of forage was determined using a Shimadzo AA-670 atomic absorption spectrophotometer (Sparks, 1996). Procedures for heavy metal analysis in plants and soil: Harvested plant samples were chopped into small pieces, packed in paper bags and dried in oven at 80 °C for 48 h. After complete drying, the samples were finely grinded into powdered using an electric grinder. One gram each of the dried samples was digested with 15 ml of concentrated nitric acid (HNO3) overnight. Digested samples were then heated up to 250 °C until white fumes were produced and heating was continued for another thirty minutes, allowed to cool down to room temperature. Twenty-five ml of distilled water was added to each digested sample. The concentrations of Cd and Cr were detected in the samples via Atomic Absorption Spectrophotometer (Hitachi Z-8100, Japan) at their respective wavelengths. For soil samples, one-gram dry soil sample was weighed and digested in 15 ml of concentrated nitric acid overnight followed by acid digestion carried out in a fume hood till the appearance of reddish brown flames. The digested soil samples were allowed to cool down at room temperature and then diluted with 25 ml distilled water and subsequent filtration with filter paper. The concentrations of Cd and Cr were detected in the samples via atomic absorption spectrophotometer at their respective wavelengths as described earlier (Ullah et al., 2011; Madiha et al., 2012). The dry matter was determined on sampled plants by using a dry-oven (at 65 °C for 48 h). At the end of the sorghum cycle (the first week of August in 2016 and the second week of August in 2017) all plants were harvested, and the above ground biomass production was determined. Leaves area of sorghums in one square meters were measured and the Leaf Area Index (LAI) was determined as follows (Yoshida, 1981):

$$LAI = LA \times A^{-1} \ (m^2 . m^{-2}) \tag{3}$$

$$LA = L \times W \times 0.75 \tag{4}$$

where: LAI is the leaf area index ($m^2 m^{-2}$), LA the area of leaves of sorghums in one square meters of land ($m^2$), and A the land area ($m^2$) occupied by the crop, L the length of leaf (m), W the maximum width of the leaf (m). Length and maximum Width of leaf was measured by ruler. At harvest, the above-discussed parameters, as well as crop yield, were determined for each treatment. Crops were harvested on August 3, 2016 (Day 65) and on August 12, 2017 (Day 79).

### 2.4. Statistical analysis

Appropriate standard errors of means were calculated using the analysis of variance (ANOVA) procedure (SAS Institute Inc., 1988). Duncan's test was applied to compare measured parameters from plants that had experienced different irrigation treatments ($p \le 0.05$).

## 3. Results and discussion

### 3.1. Analysis of variance

Effects of variables of year (Y), water qualities (W) and zeolites (Z) on Yield, LAI (Leaf area index), leaf and stem protein and IWUE were analysed for significance at the ($P < 0.05$) or ($P < 0.01$) levels. Significant effects of Y, W and Z were observed on each parameter ($P < 0.01$), except Y doesn't exerted significant effects on Leaf protein. The Y × W interaction exerted significant effects on LAI, Dry IWUE, Leaf protein and Stem protein ($P < 0.01$). The Y × W interaction didn't have a significant effect on Yield. The interaction of Y × Z application didn't reveal significant effects on parameters ($P < 0.01$). Significant effects of the W × Z interaction were observed for each parameter ($P < 0.01$) except Leaf protein. The Y × W × Z interaction had a significant effect ($P < 0.01$) on yield, Dry WUE and Stem protein (Table 4).

+ +Table 4. Summary of the analysis of variance (ANOVA) sorghum for the year (Y), water qualities (W), zeolite (Z), and their possible interactions on biomass yield, LAI, Dry IWUE, Leaf protein and Stem protein in 2016 and 2017.

### 3.2. Effect of unconventional waters and zeolites on Sorghum yield, Irrigation Water Use Efficiency and leaf area

#### 3.2.1. Yield

The average yield of the six water treatments (W1, W2, W3, W4, W5 and W6) during 2016 and 2017 were 85.5, 83.3, 64.1, 105.7, 108 and 116.7 ton.$h^{-1}$, respectively (Table 3). For the entire experiment, maximum yield (129.6 ton.$h^{-1}$) was obtained in the W6-Z2 treatment in 2016 and minimum yield (46.9 ton.$ha^{-1}$) in the W3-Z1 treatment in 2017 (Table 5). A higher average yield for all treatments was observed in 2016 (99.3 ton.$ha^{-1}$) compared to 2017 (91.83 ton.$ha^{-1}$) that can be partially attributed to the slightly more evenly distributed rainfall in 2016 (110 mm higher). Previous studies have shown that amount of irrigation water significantly influences sorghum production in semi-

**Table 4**
Summary of the analysis of variance (ANOVA) sorghum for the year (Y), water qualities (W), zeolite (Z), and their possible interactions on biomass yield, LAI, Dry IWUE, Leaf protein and Stem protein in 2016 and 2017.

| Parameter | Y | W | Z | Y * W | Y * Z | W * Z | Y * W * Z |
|---|---|---|---|---|---|---|---|
| Wet forage weight | ** | ** | ** | ns | ns | ** | ** |
| LAI | ** | ** | ** | ** | ns | ** | ns |
| Dry IWUE | ** | ** | ** | ** | ns | ** | ** |
| Leaf protein | ns | ** | ** | ** | ns | ns | ns |
| Stem protein | ** | ** | ** | ** | ns | ** | ** |

ns: No significant effects.
* Significant effect at $P < 0.05$ level.
** Significant effect at $P < 0.01$ level.

**Table 5**
The wet forage weight of sorghum in 2016 and 2017. All means in each year with the same letter(s) are not significantly different at 0.05 level of probability.

| | | Yield (T. h$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Year | Treatment | W1 | W2 | W3 | W4 | W5 | W6 | Avg | **Avg2016-17** |
| 2016 | Z1 | 88.3 | 82.4 | 57.2 | 95.3 | 95.2 | 104.3 | 87.1 $^{c}$ | |
| | Z2 | 90.2 | 89.1 | 75.8 | 117.8 | 121.1 | 129.6 | 103.9 $^{a}$ | |
| | Z3 | 89.7 | 91.0 | 75.1 | 114.6 | 116.3 | 125.1 | 102.0 $^{b}$ | |
| 2017 | Z1 | 80.9 | 76.8 | 46.9 | 82.1 | 88.5 | 95.4 | 78.4 $^{c}$ | **82.8** $^{c}$ |
| | Z2 | 82.1 | 77.5 | 71.6 | 110.6 | 115.5 | 129.0 | 97.7 $^{a}$ | **100.8** $^{a}$ |
| | Z3 | 81.7 | 83.1 | 57.7 | 113.7 | 111.4 | 116.6 | 94.0 $^{b}$ | **98** $^{b}$ |
| Avg 2016 | | 89.4 $^{c}$ | 87.5 $^{c}$ | 69.4 $^{d}$ | 109.2 $^{b}$ | 110.9 $^{b}$ | 119.6 $^{a}$ | 99.3 | |
| Avg 2017 | | 81.6 $^{c}$ | 79.1 $^{c}$ | 58.7 $^{d}$ | 102.1 $^{b}$ | 105.1 $^{b}$ | 113.7 $^{a}$ | 91.8 | |
| **Avg 2016-17** | | **85.5** $^{d}$ | **83.3** $^{d}$ | **64.1** $^{e}$ | **105.7** $^{c}$ | **108** $^{b}$ | **116.7** $^{a}$ | **93.9** | **93.9** |

arid regions (Saeed and El-Nadi, 1998; Cotton et al., 2013). Cosentino et al. (2012) reported that sorghum produced 21.1 ton.ha$^{-1}$ dry matter under 334 mm irrigation conditions versus 7.5 ton.ha$^{-1}$ dry matter with 80 mm irrigation. Seif et al. (2016) also reported that application of zeolite has significant effect on forage yield.

The average crop yields in the treated wastewater treatments (W4, W5 and W6) were significantly higher than the yield in the control treatment (W1) by 23.6 %, 26.3 %, and 36.5 %, respectively. There was no significant effect between the W2 and W1 treatments, but the yield in W3 was significantly lower compared to W1 (25 %). Nadia (2005) and Ghanbari et al. (2007) both reported similar increases in yield with irrigation with wastewater instead of well water, for both sorghum and wheat. This was attributed to the high amount of nutrient in the wastewater.

The treatments with zeolites significantly increased the forage fresh yield of sorghum (Table 5). In both years, crop yields in the Z2 (average 100.8 ton.h$^{-1}$) and Z3 (average 98 ton.h$^{-1}$) treatments were respectively 22 % and 18 % higher that the control (Z1 with an average yield of 82.8 ton.ha$^{-1}$). Naseri et al. (2012) and Bernardi et al. (2011) have reported similar increases in corn and sorghum yields by applying zeolite. According to Torkashvand and Shadparvar (2013) application of zeolite could be beneficial with respect to increased water holding capacity of soil. Similarly, Ahmed et al. (2010) suggested that zeolite application not only increase the uptake of N, P and K but also increase the efficiency of their use by plants. Valadabadi et al. (2010) reported significant improvement in the yield of rapeseed by the application of Zeolite under drought stress. Similar type of improvement was reported by Najafinezhad et al. (2014) in corn crop.

### 3.2.2. Irrigation and water use efficiency

In the growing season in 2016 rainfall was 110 mm more than in 2017 (Table 6). Consequently, total irrigation in 2017 was about 12 % more than in 2016. Daily reference evaporation (ETo) varied between 0.1–8.6 mm.day$^{-1}$ with a mean of 5.9 mm.day$^{-1}$ totalling 329 mm.day$^{-1}$ in the 2016 and Daily reference evaporation (ETo) varied between 1.2–9.4 mm.day$^{-1}$ with a mean of 5.9 mm.day$^{-1}$ totalling 466 mm.day$^{-1}$ in the 2017 growing season.

The two-year averages of the Irrigation Water Use Efficiency (IWUE) of the treated wastewater treatments (W4, W5 and W6) were significantly higher than the average in the control (W1) by respectively 11.7, 17.6 and 29.4 % (Table 7). The average value of W3 was significantly lower than the average value W1 by 29.4 %. With increasing salinity from 5.2–11.7 ds.m$^{-1}$ the IWUE decreased. The average IWUE of various water qualities in 2016 across all Z applications reached 1.82 kg.m$^{-3}$, which was 10 % greater than that of 2017.

Treatments of zeolites significantly affected the IWUE of sorghum. The two-year averages of the IWUE for the zeolites treatments (Z1, Z2 and Z3) were 1.53, 1.89 and 1.83 kg.m$^{-3}$, respectively. The two-year average IWUE of calsic zeolite was 3.2 % and 23.5 % higher than that of Z3 and Z1, respectively. In both years, Z2 and Z3 treatments had maximum IWUE, while the lowest IWUE was related to Z1, the no-zeolite control.

Irrigation gifts are in line with FAO data but the values of the IWUE were well above the range (0.6 and 1.0 kg.m3) for a good commercial yield (http://www.fao.org/land-water/databases-and-software/crop-information/en). The range of IWUE was rather high compared to other studies as well, e.g. Mastrorilli et al., 1999 and Steduto et al., 1997. This can be attributed to the combined effects of the use of zeolite and because irrigation was based on the actual measurement of the soil water content before irrigation. As a consequent, the irrigation interval was often more frequent compared to the normal practices in Northern Iran (JavaniJouni et al., 2018). Reduced water availability is harmful to plant development because it reduces cell division and prevents bloating due to reduced cellular inflammation (Valentia et al., 1992). In this way, the leaf area and subsequently the amount of dry matter decrease (Wan et al., 2010).

### 3.2.3. Leaf area index

The average Leaf Area Indexes (LAI) of the six water treatments (W1, W2, W3, W4, W5 and W6) during 2016 and 2017 were 6.04, 5.71, 4.43, 6.17, 6.19 and 7.38 cm$^{2}$.cm$^{-2}$, respectively (Table 8). The average values from treated wastewater treatments (W4, W5 and W6) were significantly higher than the average for the control (W1), by 2.1 %, 2.5 %, and 22.1 %, respectively. Day and Tucker (1977) reported similar results that irrigation with wastewater increased the leaf width. There were no significant effects between W2 and W1 that showed this salinity of water (W2) had not such effect on the leaf area of sorghum. The average value of W3 was significantly lower than the average value W1

**Table 6**
Rainfall, Irrigation and Pan evaporation (mm.day$^{-1}$) in the growing seasons of 2016 and 2017.

| | 2016 | | | 2017 | | |
|---|---|---|---|---|---|---|
| Month | Rainfall | Irrigation | Pan evaporation | Rainfall | Irrigation | Pan evaporation |
| May | 8.4 | | 28 | 0.3 | | 42.3 |
| Jun | 50.7 | 283 | 151 | 8.4 | 412 | 183 |
| July | 66.8 | 467 | 136 | 7.1 | 443 | 178 |
| August | 0 | 141 | 14 | 0.1 | 145 | 63 |
| Total | 126 | 891 | 329 | 16 | 1000 | 466 |

**Table 7**
Irrigation Water Use Efficiency (IWUE) in 2016 and 2017. All means in each year with the same letter(s) are not significantly different at 0.05 level of probability.

| | | | DRY IWUE (kg.ha. $m^{-3}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Year | Irrigation + rain ($m^3$. $ha^{-1}$) | Treatment | W1 | W2 | W3 | W4 | W5 | W6 | Avg | **Avg 2016-17** |
| 2016 | 10091 | Z1 | 1.7 | 1.5 | 1.1 | 1.7 | 1.7 | 1.9 | 1.6 c | |
| | | Z2 | 1.7 | 1.7 | 1.5 | 2.2 | 2.3 | 2.4 | 1.97 a | |
| | | Z3 | 1.7 | 1.7 | 1.5 | 2.1 | 2.1 | 2.3 | 1.9 b | |
| 2017 | 10153 | Z1 | 1.7 | 1.4 | 0.8 | 1.5 | 1.6 | 1.7 | 1.45 c | **1.53 c** |
| | | Z2 | 1.7 | 1.4 | 1.3 | 2.0 | 2.1 | 2.4 | 1.82 a | **1.89 a** |
| | | Z3 | 1.7 | 1.5 | 1.1 | 2.0 | 2.1 | 2.2 | 1.77 b | **1.83 b** |
| Avg 2016 | | | 1.7 | 1.6 c | 1.4 d | 2.0 b | 2.0 b | 2.2 a | 1.82 | |
| Avg 2017 | | | 1.7 | 1.4 c | 1.0 d | 1.8 b | 1.9 b | 2.1 a | 1.68 | |
| | **Avg 2016-17** | | **1.7d** | **1.5e** | **1.2 f** | **1.9 c** | **2.0 b** | **2.2 a** | **1.75** | **1.75** |

by 34.7 %. With increasing salinity from 5.2–11.7 ds.$m^{-1}$ the leaf area decreased 26.6 %. The average LAI of various water qualities in 2016 across all Z applications reached 6.24 $cm^2$. $cm^{-2}$, which was 8.9 % greater than that of 2017. There was no significant difference between treatments W4 and W5, but these treatments (composing of wastewater and seawater) had higher LAI (about 2.3 %) than the control treatment.

The leaf area is one of the most sensitive parts of the plant to salinity (Parida and Das, 2005) and its reduction has been reported with increasing salinity levels of irrigation water in other studies. The osmotic stress caused by salinity, with the increase of the inflammatory pressure threshold for the growth of leaf cells and the reduction of intercellular space, on the one hand, and the formation of ionic poisoning due to the accumulation of sodium and chloride ions and consequently damage to the membrane and protein molecules, on the other, causes reduced leaf area (Croser et al., 2001). Reducing leaf area also reduces light absorption and photosynthesis and ultimately reduces the production of photosynthetic products for leaf growth, so it effects development of new leaves. Treatments of zeolites significantly affected the LAI of sorghum. The average LAI of zeolites application (Z1, Z2 and Z3) across two experimental years were 5.65, 6.30 and 6.02 $cm^2$. $cm^{-2}$, respectively. The two-year average LAI of calsic zeolite was 4.6 % and 11.5 % higher than that of Z3 and Z1, respectively. In both years, Z2 and Z3 treatments had maximum LAI, while the Z1 control had the lowest LAI. Khan et al. (2011) conducted greenhouse experiment and found that LAI and plant height of soybean were significantly enhanced by Z application. Kavoosi (2007) reported that Z application significantly increased N uptake, nucleic acid, amides and hence cell multiplication, which increased leaf area and rice plant height. It indicated that use of Z could increase nutrition uptake and enhance cell multiplication, then resulted in higher LAI and biomass. In this experiment, Z application significantly enhanced sorghum LAI and biomass.

### *3.3. Effects of Water qualities and Zeolites on Sorghum and soil quality components*

#### *3.3.1. Leaf and stem protein*

In both years in terms of water qualities, the highest protein contents were related to the W6 treatment, and the lowest content was related to the W3. An inverse relationship was observed the salinity of the irrigation water and the leaf and stem protein content (Tables 9 and 10). The greatest leaf protein and the greatest stem protein values for all water qualities and both years, was in the treated wastewater treatment (W6) in the second year by 14.83 % and 8.1 %, respectively. This was anticipated since wastewater is full of nutrients and specifically nitrogen, which plays a basic role in protein making in plants (Jacobs et al., 1998). Other studies have also shown a significant increase of protein in sorghum by wastewater application (Ghanbari et al., 2007; Day and Tucker, 1977). The presence of nutrient elements in wastewater can have an important role in increased nitrogen uptake. Possibly, existence of micronutrients in rhizosphere causes to more nitrogen uptake and eventually may enhance protein production; in particular, Cu and Zn have been associated with protein structure and nitrogen metabolism (Jacobs et al., 1998).

Treatments of zeolites significantly affected the protein of leaf and stem. The average percentage of leaf and stem protein of zeolites application (Z1, Z2 and Z3) across two experimental years were 9.1, 9.93 and 9.69 for leaf and 5.33, 6.08 and 5.81 for stem, respectively. In both years, Z2 treatment had maximum protein in leaf and stem, while the Z1 control had the lowest percentage.

In some of the previous studies, nitrogen has been identified as the most important food ingredient affected by salt stress, and their findings indicate a reduction in its absorption due to saline water use (Sato et al., 2006; Sairam and Tyagi, 2004). The most important reason for decreasing nitrogen uptake in treatment W3 could be because of reduction of root permeation, reduction of nitrogen mineralization due to decreased soil microbial activity, reduction of nitrification rate, severe competition of chlorine ion in saline water with nitrate and sodium ion with ammonium for receiving positions in plasma membrane (Kafkafi

**Table 8**
Leaf Area Index (LAI) of sorghum in 2016 and 2017. All means in each year with the same letter(s) are not significantly different at 0.05 level of probability.

| | | LAI ($cm^2$. $cm^{-2}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Year | Treatment | W1 | W2 | W3 | W4 | W5 | W6 | Avg | **Avg 2016-17** |
| 2016 | Z1 | 6.22 | 5.44 | 4.47 | 6.25 | 6.27 | 6.96 | 5.94 c | |
| | Z2 | 6.37 | 6.41 | 5.24 | 6.68 | 6.32 | 8.19 | 6.54 a | |
| | Z3 | 6.37 | 6.26 | 4.58 | 6.22 | 6.31 | 7.80 | 6.26 b | |
| 2017 | Z1 | 5.71 | 4.87 | 3.80 | 5.75 | 5.68 | 6.34 | 5.36 c | **5.65 c** |
| | Z2 | 5.78 | 5.66 | 4.51 | 6.19 | 6.51 | 7.69 | 6.06 a | **6.30 a** |
| | Z3 | 5.81 | 5.64 | 3.98 | 5.93 | 6.03 | 7.30 | 5.78b | **6.02 b** |
| Avg 2016 | | 6.32 b | 6.04 c | 4.76 d | 6.38 b | 6.30 b | 7.65 a | 6.24 | |
| Avg 2017 | | 5.77 b | 5.39 c | 4.09 d | 5.96 b | 6.07 b | 7.11 a | 5.73 | |
| **Avg 2016-17** | | **6.04 b** | **5.71 c** | **4.43 d** | **6.17 b** | **6.19 b** | **7.38 a** | **5.97** | **5.97** |

**Table 9**
Leaf protein of sorghum in 2016 and 2017. All means in each year with the same letter(s) are not significantly different at 0.05 level of probability.

| | | LEAF PROTEIN (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Year | Treatment | W1 | W2 | W3 | W4 | W5 | W6 | Avg | **Avg 2016-17** |
| 2016 | Z1 | 9.0 | 8.6 | 6.9 | 8.9 | 9.2 | 11.6 | 9.0 c | |
| | Z2 | 9.3 | 9.5 | 8.1 | 9.9 | 9.9 | 13.4 | 10.0 a | |
| | Z3 | 9.2 | 9.2 | 7.7 | 9.5 | 10.2 | 12.4 | 9.7 b | |
| 2017 | Z1 | 8.4 | 9.1 | 6.2 | 8.7 | 8.6 | 14.0 | 9.2 b | **9.1 c** |
| | Z2 | 8.6 | 9.3 | 7.1 | 9.7 | 9.6 | 14.8 | 9.9 a | **9.93 a** |
| | Z3 | 8.5 | 9.0 | 7.1 | 9.3 | 9.8 | 14.4 | 9.7 a | **9.69 b** |
| Avg 2016 | | 9.2 c | 9.1 c | 7.6 d | 9.5 b | 9.8 b | 12.5 a | 9.58 | |
| Avg 2017 | | 8.5 c | 9.1 b | 6.8 d | 9.2 b | 9.3 b | 14.41 a | 9.57 | |
| **Avg 2016-17** | | **8.8 c** | **9.1 c** | **7.2 d** | **9.4 b** | **9.5 b** | **13.4 a** | **9.58** | **9.58** |

**Table 10**
Stem protein of sorghum in 2016 and 2017. All means in each year with the same letter(s) are not significantly different at 0.05 level of probability.

| | | STEM PROTEIN (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Year | Treatment | W1 | W2 | W3 | W4 | W5 | W6 | Avg | **Avg 2016-17** |
| 2016 | Z1 | 6.1 | 4.8 | 4.7 | 5.7 | 5.7 | 6.0 | 5.5 c | |
| | Z2 | 6.3 | 5.3 | 5.0 | 6.3 | 7.2 | 7.2 | 6.2 a | |
| | Z3 | 6.1 | 5.0 | 5.2 | 6.1 | 6.5 | 7.1 | 6.0 b | |
| 2017 | Z1 | 5.1 | 4.8 | 4.1 | 5.3 | 5.8 | 5.8 | 5.2 c | **5.33 c** |
| | Z2 | 5.1 | 5.1 | 4.2 | 6.0 | 7.1 | 8.1 | 5.9 a | **6.08 a** |
| | Z3 | 5.0 | 4.4 | 4.3 | 6.0 | 5.9 | 8.1 | 5.6 b | **5.81 b** |
| Avg 2016 | | 6.2 b | 5.0 c | 5.0 c | 6.0 b | 6.5 a | 6.8 a | 5.91 | |
| Avg 2017 | | 5.1 d | 4.8 d | 4.2 e | 5.8 c | 6.3 b | 7.4 a | 5.57 | |
| **Avg 2016-17** | | **5.6 c** | **4. 9 d** | **4.6 e** | **5.9 c** | **6.4 b** | **7.1 a** | **5.74** | **5.74** |

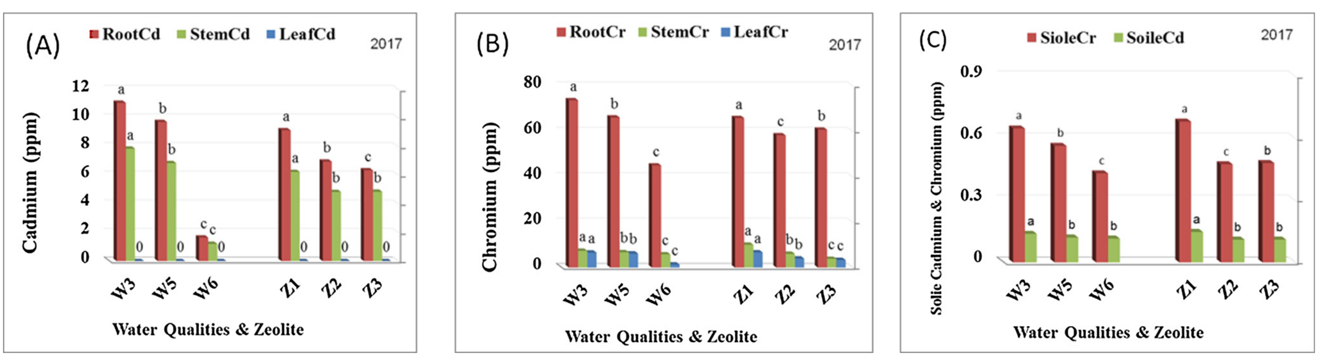


**Fig. 2.** Changes in Cadmium (Cd) and Chromium (Cr) of soil and sorghum by water qualities and zeolite in 2017. All means in each year with the same letter(s) are not significantly different at 0.05 level of probability.

**Table 11**
Average Soil Salinity ($EC_e$ in dS. m$^{-1}$) in soil profile (0-80 cm).

| | Year | W1 | W2 | W3 | W4 | W5 | W6 | Average |
|---|---|---|---|---|---|---|---|---|
| Z1 | 2016 | 0.6 | 1.2 | 2.2 | 1.5 | 1.5 | 0.9 | 1.3 |
| | 2017 | 0.6 | 1.2 | 2.4 | 1.6 | 1.6 | 0.9 | 1.4 |
| | $\Delta EC_e$ | 0.0 | 0.0 | 0.2 | 0.1 | 0.1 | 0.0 | 0.1 |
| Z2 | 2016 | 0.6 | 1.4 | 2.3 | 1.6 | 1.5 | 1.0 | 1.4 |
| | 2017 | 0.6 | 1.4 | 2.4 | 1.6 | 1.6 | 1.1 | 1.45 |
| | $\Delta EC_e$ | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.05 |
| Z3 | 2016 | 0.6 | 1.2 | 2.3 | 1.4 | 1.4 | 0.9 | 1.3 |
| | 2017 | 0.6 | 1.2 | 2.4 | 1.5 | 1.5 | 0.1 | 1.21 |
| | $\Delta EC_e$ | 0.0 | 0.0 | 0.0 | 0.1 | 0.2 | −0.8 | −0.08 |

et al., 1992).

Rubisco is the most important and most abundant soluble protein in a leaf. Every kind of reduction in the protein content of leaf is a sign of reduced Rubisco concentrations, which can be followed to reduce the current photosynthetic activity (Saeidi et al., 2010). The increased rate of protein caused by the application of zeolites could be due to improved photosynthesis and moderating stress conditions caused by the beneficial effects of amendments on soil properties (Najafinezhad et al., 2014). Increased rate of protein due to the application of zeolites have also been reported (Seif et al., 2016; EskandariZanjani et al., 2012; Islam et al., 2011).

### 3.3.2. Cadmium (Cd) and Chromium (Cr) of soil and sorghum

The Cd concentration in Seawater and TWW was 0.104 and 0.0001 (mg.L$^{-1}$) and The Cr concentration in Seawater and TWW was 0.109 and 0.005 (mg.L$^{-1}$) respectively. The Cd and Cr concentrations increased when using seawater and decreased when using zeolite in both the soil and the plant (Fig. 2). Maximum concentration of Cd and Cr was seen in the root of sorghum rather than stem and leaf and It was clear that maximum reduction of Cd and Cr concentration was in W6 and treatment with zeolites. There were no significant different between concentration of Cd in soil in W5 and W6 treatments and also Z1 and Z2 treatments. The W6 treatment had the lowest amount of Cd concentration in soil and also in plant (Fig. 2A and 2C). The Z3 treatment had the lowest concentration of Cr in stem and leaf (Fig. 2B).

Both Cd and Cr are considered environmental pollutants. Cd is a nonessential, toxic element that has inhibitory effects on crop growth

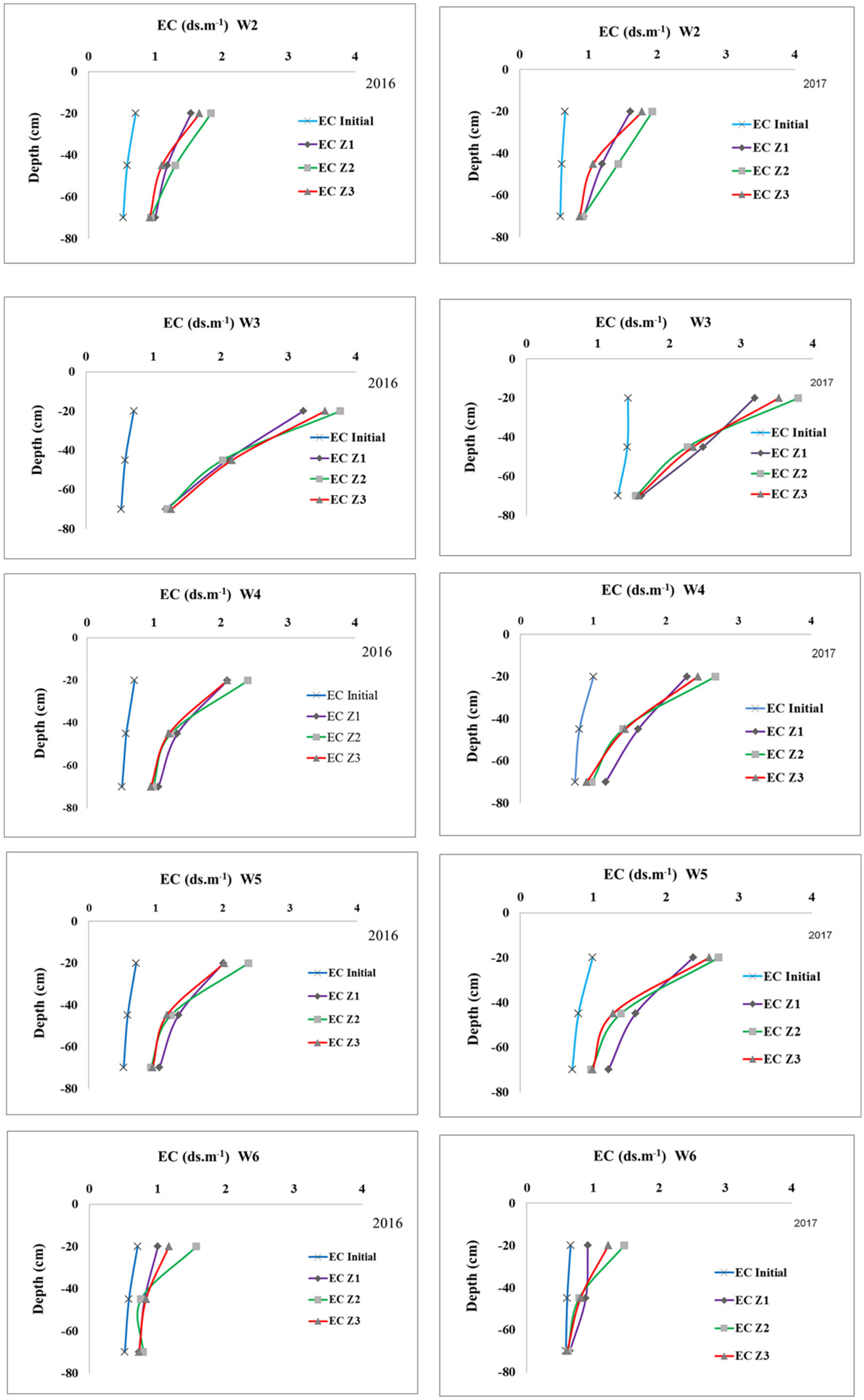


**Fig. 3.** Soil salinity ($EC_e$ in $dS.m^{-1}$) in the soil profile in W2 to W6 treatments in 2016 and 2017.

and chlorophyll synthesis. Cr ranks seventh in abundance in the earth crust. Various studies have documented the positive role of zeolites in stabilizing and reducing the absorption of heavy metals by roots (Gworek, 1992; Rahakova et al., 2004). Zeolites are mostly used for removing heavy metals because of their high capacity for cation exchange and ion adsorption in wide ranges. This leads to a reduction in the Cd concentration in soils containing zeolite, reducing absorption by the crop (Ponizovsky and Tsadilas, 2003). Eshghi et al. (2010) have stated that zeolite can decrease the Cd accumulation in soybean shoots. Rangasamy et al. (2013) reported that, the zeolite material can adsorb chromium very effectively and can be recommended for water and wastewater treatment.

#### 3.3.3. Soil salinity

The accumulation of salt in the root zone is one of the most important environmental hazards that can lead to reduced crop yield and soil degradation. In all treatments soil salinity levels were low (Table 11), far below the FAO threshold values for yield reduction (FAO, 2018). However, the treatments with more than 25 % seawater (W3, W4 & W5) did result in a slight increase in soil salinity over the two-year period, thus there is a risk for salinization using these alternative irrigation water mixtures. Treatments with a higher salinity level in the irrigation water show an increase in soil salinity in the soil profile, especially in the top layer. Fig. 3 shows the soil salinity profile in treatments W2 to W6 for 2016 and 2017. In the zeolite-containing treatments (Z2 and Z3), the amount of salt accumulation in the upper layer of the soil is greater. This would be due to zeolite's capacity to adsorb salts. Although rainfall in 2017 was lower than in 2016, resulting in a 12 % higher irrigation gift in 2017 (Table 6), this did not significantly increase soil salinity levels.

## 4. Conclusions

Irrigation with wastewater increased the quantitative and qualitative properties in forage sorghum. With increasing salinity, the forage yield decreased significantly. The combination of treated wastewater and calsic zeolite had the best effect on crop yield, IWUE, LAI and leaf and stem protein of sorghum. Wastewater application resulted in an increase in protein, which is an important property of forage. Both zeolites with low salinity of water positively influenced the biomass yield and the quality components of sorghum. Under saline water conditions, protein and forage fresh yield were decreased. To address the increasing water shortage issues being faced in north of Iran, we would recommend using treated urban wastewater in combination with calsic zeolite to optimize production of sorghum.

## Declaration of Competing Interest

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

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agwat.2020.106117.

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