Produced water reuse for irrigation of non-food biofuel crops: effects on switchgrass and rapeseed germination, physiology and biomass yield

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Abstract

High volumes of flowback and produced water are generated everyday as a byproduct of hydraulic fracturing operations and shale gas developments across the United States. Since most shale gas developments are located in semi-arid to arid U.S. regions close to agricultural production, there are many opportunities for reusing these waters as potential alternatives or supplements to fresh water resources for irrigation activities. However, the impacts of high salinity and total organic content of these types of water on crop physiological parameters and plant growth needs to be investigated to determine their utility and feasibility. [The aim of the present study was to evaluate the response of switchgrass and rapeseed to treated produced water as an irrigation water source.](#) In this greenhouse study, the influence of produced water at four total organic carbon (TOC) concentrations [*1.22, 38.3, 232.2 and 1352.4 mg/l*] and three total dissolved solids (TDS) levels [*400, 3,500, and 21,000 mg/l*] on rapeseed (*Brassica napus L.*) and switchgrass (*Panicum virgatum L.*), two relatively salt-tolerant, non-food, biofuel crops, was

studied. Seedling emergence, biomass yield, plant height, leaf electrolyte leakage, and plant uptake were evaluated. Irrigation water with the highest salinity and TOC concentration resulted in significantly lower growth health and physiological characteristics of both crop species. The organic content of the produced water had a negative impact on biomass yield and physiological parameters of both species. The results of this study could be valuable for regulators and stakeholders in development of treatment standards in which organic matter should be removed to less than 50mg/l to keep leaf EL (cell damage) to less than 50% and a TOC concentration of less than 5mg/l required to keep a sustainable biomass production rate.

Keywords: hydraulic fracturing, produced water, reuse, switchgrass, rapeseed, biofuel

1. Introduction

Large volumes of the injected water during hydraulic fracturing flows back to the surface with the extracted oil and gas (Esmailirad et al., 2015). This water is called flowback at the early well-life stage and produced water later during the life of a well. Approximately 90% of these kinds of water are injected back into the ground in class II wells that are regulated by EPA's *Underground Injection Control* program (Esmailirad et al., 2015; Boysen et al., 2011). However, this method has been linked to induced seismic activity and frequent earthquakes in Oklahoma and Texas, states that contain a large number of injection wells (Frohlich, 2012; Nicholson and Wesson, 1990; Ellsworth, 2013; Keranen et al., 2013). Moreover, drought conditions and competition for freshwater consumption, as well as environmental issues such as chemical spills, CO₂ and other greenhouse gas emissions, can result from trucking, the main water transport method for oil and gas operations (Boschee, 2012). Considering the large volume of produced water generated every day across the nation and the projected growth rate of water-

intensive biofuel production (International Energy Agency, World energy outlook, 2012), using produced water for irrigation may provide a potential alternative to the use of only freshwater resources. High salinity, the total organic matter load, and the presence of toxic organic compounds (e.g., benzene) are the main pollutant constituents in produced water that need to be addressed when considering reuse for irrigation purposes.

In this study, we evaluated the impacts of produced water for irrigation on two non-food biofuel crops, switchgrass and rapeseed. These species can tolerate a broad range of temperature, soil pH, and water salinity conditions, and can grow under salt-stressed, marginal soil conditions (Alexopoulou et al., 2008; Lee et al., 2007; Lynd et al., 1991; McLaughlin et al., 2002; McLaughlin et al., 2005; Sanderson et al., 1996). The salinity of the soil can cause osmotic and specific ion effects (ionic stress) on plant cells that lead to nutrient imbalances affecting important physiological and biochemical processes. These can ultimately inhibit plant growth and development and reduce biomass production (Munns, 2005).

Switchgrass, a native North American, warm-season (C4 metabolism), perennial grass, is rich in natural diversity and adapted to grow over a large portion of the continent with high biomass yield potential, has been targeted for large scale lignocellulosic biomass production (Alexopoulou et al., 2008; Keshwani and Cheng, 2009; Anderson and Sharp, 1995; Chen et al., 2007; Montemayor et al., 2008). With 5–10 year average yields of 12–19 Mg/ha, switchgrass has been at the forefront of biofuel biomass research (Burkhardt et al., 2015). Switchgrass has been adopted to a range of climate and soil conditions from the northern U.S. to the Rocky Mountain regions and Mexico (Mosel and Vogel, 1995; Hitchcock, 1971).

Rapeseed, an industrial oil seed crop, is relatively tolerant to stress conditions including saline soils (Ashraf et al., 1990; He and Cramer, 1992). Grewal (2010) studied the response of wheat,

barley (*Hordeum vulgare*, cv. *Carla INTA*), rapeseed (*Hyola 42*), and chickpea (*Jimbour*) to variable levels of subsoil sodium chloride (NaCl) and determined rapeseed to be the most tolerant to Na⁺ of the four crops. In rapeseed damage to plant tissues due to salt intake can appear during all developmental stages including germination, seedling establishment, vegetative growth, and seed production (Porcelli et al., 1995). Salt stress can also damage cell membranes and inhibit photosynthetic functions (Munns and Tester, 2008) in the rapeseed crop.

While the effects of salinity on many edible and non-edible crops are widely reported in the literature, the effects of the high organic content present in produced water on plant growth and physiological characteristics are not well known. The specific impact of shale-gas-produced water on plant health has not been studied. Burkhardt et al. (2015), Mullins and Hajek (1998), and Vance et al. (2008) reported different dilutions of coalbed-methane-produced water used for irrigation. Burkhardt et al. (2015) studied the effect of varying ratios of produced water and municipal water on soil characteristics, and plant biomass and secondary metabolites of sweet wormwood and switchgrass. They found increasing accumulation of Na⁺ and salts in the soil with increasing concentration of the produced water. An average produced water Na⁺ concentration of 1156 mg/l hampered plant growth.

Mullins and Hajek (1998) treated sorghum-sudangrass in a greenhouse study with varying dilutions of produced and deionized water up to 2000 mg/l total dissolved solids based on limits set by Alabama's Department of Environmental Management for irrigation water salinity, and reported forage yield decreased due to water logging. Vance et al. (2008) studied soil properties and vegetation responses resulting from one to four years of saline-sodic water with an EC range of 1.6–4.8 ds/m applied on native range grasslands, seeded grass hay fields, and alfalfa hay fields during two growing seasons. Biomass production was lower in fields irrigated with coal bed

produced water. Zheljazkov and colleagues (2013) studied the effect of produced water from coal bed methane production on spearmint (*Mentha spicata L.*) and peppermint (*Mentha × piperita L.*). The low quality irrigation water adversely affected the soil quality and crop biomass yield by 50%. The characteristics of coal bed methane produced water were greatly different than shale gas produced water (Burkhardt et al., 2015), and the TOC concentration had not been noted.

The aim of the present study was to evaluate the response of switchgrass and rapeseed to treated produced water as an irrigation water source. Given how little is known about the effects of shale oil/gas produced water on plant growth and the growing interest to reuse produced water in irrigation, it is paramount that we understand its effects on soil accumulation and plant health.

2. Material/Methods

2.1 Water treatment processes

To adjust the TDS and TOC concentrations of produced water to the four treatment levels, dilution with CSU (Colorado State University) tap water and/or chemical treatment processes was used. Treatment processes. Water quality of CSU tap water is derived from the surface water and is given in Tables 1-3. Oxidation of Fe^{+2} to Fe^{+3} , electrocoagulation (Water Tectonic, Everett, WA), solid-liquid separation process (dissolved air flotation followed by hollow fiber membrane ultrafiltration (Mann+Hummel, Germany), granular activated carbon for organic matter removal were used to treat the produced water samples.

2.2 Water characteristics

The produced water used in this study was taken from a commercial central processing facility that serves over 500 wells in the Denver-Julesburg Basin. Typically these water samples have high TOC concentrations (>2000 mg/l) and TDS values in the range of 20,000 to 25,000 mg/l. In

order to study the individual effects of these parameters on plants, best and worst case scenarios were defined representing high TDS-TOC and low TDS-TOC, respectively. Also, two other cases were defined to study the effects of the organic content of these waters. These water qualities were achieved by treating produced water to different qualities through a sequence of several treatment processes which included dilution with surface waters. Five different water qualities were chosen:

A. Horsetooth Reservoir water

This water source was used as a control treatment. TDS and TOC levels of control water were 310mg/l and 3.3mg/l, respectively. All the results will be compared to the control experiments.

B. Best quality produced water: low TDS (400mg/l) and low TOC (<5 mg/L). To generate high quality water for irrigation, effluent of a coagulation/ultrafiltration (UF) process is treated with granular activated carbon (GAC) and then diluted using CSU tap water to a TDS level of 400mg/l. The TOC concentration of this sample was under 3mg/l.

C. Medium salt TDS=3500mg/l and low organic matter concentration.

To examine the influence of organic matter on the plants, it was decided to keep the salt content of water at a constant TDS concentration (3,500mg/l) and vary the TOC value. For this aim the effluent of the GAC column was diluted with CSU tap water to reach the TDS level of 3,500mg/l. TOC concentrations for these samples varied from 65 mg/l to 100mg/l.

D. Medium salt TDS =3500mg/l and high organic matter concentration.

Dilution of coagulation/UF effluent with CSU tap water to a TDS level of 3,500 mg/l was used to obtain high organic content water with TDS level of 3500mg/l. TOC concentration of these waters varied between 215mg/l and 235mg/l.

E. Cleaned brine: high TDS and high TOC

A pretreatment method was used to remove suspended solids. To generate this water quality several treatment process included H₂O₂/EC/UF (oxidation with H₂O₂ followed by coagulation and filtration) were used. The TDS concentration was in the range of 15,000 to 20,000mg/l and the TOC concentration was approximately 1,500mg/l.

The purpose of these four water qualities was to determine minimal water quality and treatment required for sustainable biomass growth using produced water. Table 1 through 3 show different characteristics of water samples with standard error (SE) used for irrigation of the rapeseed and switchgrass crops during the 106-day experiment.

2.3 Plant material

Seeds of switchgrass and rapeseed cultivars were purchased from Midwest Laboratories (Omaha NE 68144) and were stored at 4 °C prior to initiation of experiments.

2.4 Seed germination experiment

To evaluate the influence of produced water quality on germination efficiency and seedling emergence rate, seeds were planted in germination boxes placed in growth chambers according to Anderson et al. (2012). Seeds of both species were sown in 10cm diameter Petri dishes on two layers of blue seed germination blotter (Anchor, St. Paul, MN, USA) and imbibed with 15 mL of liquid from each water treatment solution. The Petri dishes were sealed tightly with Parafilm to prevent evaporation. Three replications with 25 seeds of each switchgrass and rapeseed were used for each of the five treatments (Tables 1-3). Seeds in the Petri dishes were incubated in a

programmed germination chamber (VWR international, Cornelius, OR, USA) at 18 °C dark/30 °C light with 16 hour photoperiod (light photon flux density: 25 $\mu\text{m photon m}^{-2} \text{s}^{-1}$, 380–680 nm). All dishes were randomly arranged inside the growth chamber. Germinated seeds were counted at 2d intervals for 21 days. A seed was counted as germinated when the seed coat was broken and the radical emerged. The relative seed germination percentage for each treatment was calculated by dividing the number of germinated seeds in the test solution by number of germinated seeds in the control solution.

The seedling germination rate was estimated using a modified Timson Index:

$$\text{Germination rate} = \sum G/t$$

Where G is the percentage of seed germination at 2-day intervals and t is the total germination period (Whitney, 1988; Khan and Ungar, 1984; Timson, 1964). The greater the value the more rapid the rate of germination.

2.5 Greenhouse plant growth study

A crop growth study was performed in a heated and humidified greenhouse at Colorado State University to evaluate the feasibility of flowback and produced water for irrigation on the switchgrass and rapeseed crops. The greenhouse temperature varied in the range of 22±3 °C, relative humidity was adjusted to vary between 50 and 70%, and light exposure was about 10 h per day. Seeds were sown into one-inch deep holes in 40 cm diameter round pots in mid-December 2015 and the plants were harvested the first week of April 2016. A total of 30 pots including 15 rapeseed and 15 switchgrass were studied. The pots were randomly arranged in one block of 15 pots of each species, with three replicates for each treatment. To avoid boundary edge effects due to radiation variation from differences in shading on each treatment, barrier

layers of extra planted pots were placed between the two crop species and around the perimeter of the three blocks of treatment pots. During the first five weeks after planting, the pots were irrigated with water from Horsetooth Reservoir. For the remainder of the experiment, plants were irrigated using five different qualities of treated hydraulic fracturing produced water. The irrigation frequency was three times a week for 20 minutes during establishment and during the treatment application period.

2.6 Biomass measurements

At the end of plant growth period (106 days), all the plants were cut 2 cm above of the soil surface, put in paper bags, dried at 60 °C for 48 hours, and weighed to determine above ground biomass. Since the rapeseed plant is an annual, roots were also pulled out of the soil, and rinsed with DI water, and dried as described above. Switchgrass plant crowns and roots were not removed and left in the pots for a second growth cycle.

2.7 Physiological measurements

At the end of the experiments on day 106, physiological measurements were performed on harvested shoots and the above ground biomass of both species, and rapeseed roots. Plant survival, height, growth, and biomass were measured for each pot. Scanning electron microscopy was done on the leaves of rapeseed and switchgrass. Leaf firing percentage was determined weekly by visually estimating the burned leaf area as an indication of salt injury (Kim et al., 2012). A 1 to 9 visual rating scale is used with 1 being complete wilting, 100% leaf firing, complete dormancy or no plant recovery; and 9 being no wilting, no leaf firing, 100% green-no dormancy, or 100% recovery. Plant survival was determined based on the presence of one or more green tillers for each pot at the time of harvest. Leaf electrolyte leakage (leaf EL) was measured according to the method of Marcum (Morris et al., 1998) with some modifications. The

top second third of switchgrass and rapeseed mature leaves were cut into 2-cm segments. After being rinsed three times with distilled water, 0.2 g of leaf segments were placed in a plastic tube containing 20 mL of distilled water. The test tubes were agitated on a shaker for 24 hour and the solution conductivity (C1) was measured with a conductivity meter SR60IC (VWR International, PA USA). Leaf samples were autoclaved at 120 °C for 30 min, and the conductivity of the solutions containing killed tissue were measured (C2). The relative leaf EL was calculated as $(C1/C2) \times 100$.

Oven dried roots and shoots were ground using a mechanical grinder to pass through a screen with 425µm openings. Mineral analyses including Na, K, Mg and Ca were conducted on ground roots and shoots using a SpectrAA 40 atomic absorption spectrometer (Varian Analytical Instrument, Palo Alto, CA). The selectiveness of ion accumulation for K⁺ over Na⁺ was estimated from ion content according to the following equation (Marcum, et al., 1998):

$$\text{Selectiveness } S = [K^+/K^++Na^+]_{\text{leaves}} / [K^+/K^++Na^+]_{\text{medium}}$$

The higher S value indicates a greater selectivity absorption capacity that excluded Na⁺ and absorbed K⁺ by either the shoot or root system.

The salt crystals on the surface of adaxial leaves in switchgrass and rapeseed at 33.8 ds/m EC were examined with scanning electron microscopy (JEOL JSM-6500F field emission scanning electron microscope, Massachusetts, USA). Three of the first fully extended mature leaves, that were the fourth leaves from apex, were selected for microscopic investigation.

2.8 Organic analyses

Fresh plant tissues of both rapeseed and switchgrass were cut into 2cm segments and frozen at -20 °C until the day of extraction and analyses. Total nitrogen (N) and Total carbon (C) were analyzed with a Leco FP-528 (Horneck and Miller, 1998) and Leco Chn analyzer (Saint Joseph, MI, USA), respectively. Soluble carbohydrate (glucose, fructose, and sucrose) analyses were conducted on switchgrass and rapeseed shoots based on Smith (1969). Carbohydrates and lignin were determined using a sequential procedure described by Soil, Water and Plant Testing Laboratory at CSU. Briefly, soluble carbohydrates were extracted with 80% (vol/vol) ethanol and analyzed by a colorimetric method by Smith et al. (1969) using a Thermo spintronic scientific 20Dplus (Madison, WI, USA).

2.9 Water quality analysis

All water quality analyses were done using Standard Method procedures (2013). TOC, gravimetric TDS and pH analyses were conducted at the Colorado State University Environmental Engineering water quality laboratory. An independent, EPA certified laboratory provided analysis of cations and anions. Gravimetric TDS was measured based on standard methods of water and wastewater developed by American Public Health Association and American Water Works Association, Water Pollution Control Federation, and Water Environment Federation. The ionic TDS concentration was calculated by summing the anion and cation concentrations. A Hach HQ40d pH meter (Hach, Loveland) was used to measure pH in samples. Also, TOC was measured using Standard Methods 2540D 5130B. Cations were measured by EPA 2007 method 6010 C using a Varian ICP-AES, Liberty, California, USA.

2.10 Soil properties and laboratory analyses

The soil used in this study was a clay loam. Soil properties are shown in Table 4. Cations and anions, GRO, DRO, and BTEX were measured.

Analysis of the soils was done by the Soil, Water and Plant Testing Laboratory at CSU, Fort Collins, CO after plant harvest. Methods used for soil analysis were as follows: pH using a 1:1 saturated paste according to Watson and Brown (1998); nitrate according to Carson (1980); EC and soluble salts using the saturated 1:1 paste according to Dahnke and Whitney (1988); SAR was done according to procedures defined by United States Salinity Laboratory Staff (1954); P was measured using the bicarbonate method (Richards, 1954); and sulfate, K, Ca, Mg, and Na by Brown and Warncke (1988). Micronutrients (Zn, Fe, Mn, Cu, and B) were quantified according to Whitney (1988).

At the end of the growing cycle, the soils of the rapeseed pots were mixed and subsamples were taken from each pot at root depth for compounds of interest including BTEX, gasoline and diesel range hydrocarbons using purge and trap and methanol extraction methods by LC-MS and GC-MS following SW-8260 and 5035.

2.11 Statistical Analyses

The plant growth experiments were a 2 × 5 factorial design (two crop species and five water qualities, respectively). All data were subjected to analysis of variance (ANOVA), with the measured effects of water quality, species, and interaction of water quality × species (SAS 8.1, SAS Institute Inc., Cary, NC). Seed germination percentages were conducted as a 2 × 5 factorial combination as above. Seedling rate of germination conducted as a 2 × 5 factorial with the

measured effects of water quality, species, time, and interactions of water quality $\times$ species, species $\times$ time, water quality $\times$ time, and water quality $\times$ species $\times$ time. Whenever interactions are not important, the main factors are discussed. The main effects of each treatment were considered significant if $p \leq 0.05$, unless otherwise indicated. Mean separations were done using Tukey Pairwise Comparisons when ANOVA indicated there is a difference between treatments or interactions.

3. Results/Discussion

3.1 Seed germination

Switchgrass and rapeseed seed germination percentages were both adversely affected by increasing salinity concentration ($p < 0.0001$), with rapeseed being more tolerant to salinity and TOC concentration than switchgrass (Figure 1). For both species, water quality E with EC = 34.4ds/m and TOC = 1352 mg/l led to less than 5% seedling emergence, as was expected (Anderson et al., 2015; Kim et al., 2012; Kim et al., 2012). It is important to note that the higher TOC concentration (TOC = 232 mg/l) in water quality D versus water quality C (TOC = 38 mg/l) resulted in a significant decrease in the number of germinated seeds in both rapeseed and switchgrass (Table 5).

There was a significant correlation between salinity and both germination rates and relative germination rate estimated by the Timson Index ($r^2 = 0.69$, $p < 0.0001$, and $r^2 = 0.6$, $p < 0.0001$). Germination rate and relative germination were also highly correlated ($r^2 = 0.88$, $p < 0.0001$).

As Figure 2 illustrates, the rapeseed seed germination rate was more rapid than switchgrass across all water quality treatments. The rate in irrigation water D dropped by 50% compared to irrigation water C, indicating the importance of the produced water derived organic matter

concentration in irrigation water samples on germination rate and also the number of germinated seeds.

Although irrigation waters except irrigation water E resulted in no difference in relative germination percentages for both switchgrass, and canola, there were differences in germination rates (Table 5). There was no difference among control water and irrigation water B and C regarding relative germination and germination rate for either species (Table 5). However, irrigation water D and E were both different than the other water qualities, confirming the observation of strong negative influence of TOC on the seeds emergence and the germination rate of switchgrass and rapeseed species. This is an important observation regarding the high organic concentration of produced water and its influence on germination rate. Irrigation water E, with the highest EC and TOC values, not surprisingly showed the lowest germination rate and relative germination percentages for either species. These results suggest that when establishing new crops, poorer quality produced water should not be used.

3.2 Soil analyses

3.2.1 Organic

Only soils from the rapeseed pots were analyzed for BTEX compounds, diesel range organics (C_{10} - C_{28}) and gasoline range organics (C_6 - C_{12}). Switchgrass crops will be studied for the second growth, therefore soils from those pots were not analyzed. None of these organic compounds were detected in any of the soil samples. Biodegradation or volatilization of BTEX compounds likely occurred in all four of the soil sample types. Biodegradation of BTEX has been well studied ([Debez et al., 2004](#); [Farhadin et al., 2008](#); [Young et al., 2005](#); [Beller et al., 1995](#);

Chakraborty et al., 2004; Cunningham et al., 2001) and can occur within the time period of the growing cycle.

3.2.2 Mineral

Electroconductivity of the soil at the end of the growing cycle (day 106) was plotted versus the irrigation water quality type (A-E) for each of five treatments in Figure 3. Soil electroconductivity increased compared to initial soil measurements collected before starting the experiments (Fig. 2).

At the end of 106 days, the EC of soil samples of all five treatments (including the control) were higher than the EC of virgin soil (0.5ds/m, Table 4). The salt accumulation in soils of rapeseed and switchgrass pots was relatively low and stable except for irrigation water E (maximum EC 33.8ds/m). Not surprising, maximum soil EC was observed in irrigation water E for both rapeseed and switchgrass at 16ds/m and 18ds/m, respectively.

There were significant differences for soil EC between irrigation water E and the rest of the irrigation waters, with EC increasing to 17ds/m and 22ds/m for rapeseed and switchgrass, respectively (Table 7). Means that do not share a letter are significantly different. As expected, the organic content of irrigation water seemed to not have an effect on soil EC when comparing irrigation water C and irrigation water D. Also, salt accumulation in the switchgrass samples was greater than the rapeseed soil samples. This could be due to less growth of crops in the switchgrass pots (less biomass) and/or measurement errors (different depth/ spot) in the pots.

Soil characteristics of rapeseed pots were analyzed for Na^+ , K^+ , Mg^{+2} , Ca^{+2} , Sr^{+2} and boron at the end of the 106-day growing cycle (Figure 4). Water quality was only significant for Na and boron accumulation ($p < 0.0001$). At the end of 106 days, there was a significant difference in boron concentration between soil E and the rest of the soil samples with B concentration being zero in virgin soil and soil A, B, C and D (Figure 4).

There was no difference among all five treatments at the end of 106 days, except B

Accumulation of boron in soil E with 17mg/kg is due to the high concentration ($\text{B}^{+3}=22\text{mg/l}$) of boron in the produced water sample E (Table 3). Na^+ accumulation was obvious at the end of 106 days for soils B, C, D and E with soil E having the highest concentration at 6400 mg/kg. Ca^{+2} and Mg^{+2} concentrations did not change after irrigation with any of the five water types. Sr^{+2} concentrations in irrigated soils were also higher than virgin and control soils, indicating accumulation of Sr^{+2} in soil samples due to high occurrence in the irrigation waters (treated produced water). This is more pronounced in soil E with Sr concentration at 143.3 mg/kg compared to 110 mg/kg in virgin soil.

3.3Physiological characteristics

Water quality stress on the crop species was statistically significant ($P \leq 0.05$) for electrolyte leakage (EL) and plant height. The interaction of these factors was only significant for plant height. The effects of produced water quality on biomass production and physiological characteristics of rapeseed and switchgrass are discussed below.

3.3.1Plant growth and biomass yield

Effect of water quality on switchgrass and rapeseed biomass is shown in Figure 5. All plants in all treatments survived until harvest. Rapeseed and switchgrass had very similar growth response

patterns for the four produced water irrigation water qualities compared to the control water. Plant growth parameters including aboveground biomass and plant height (for rapeseed and switchgrass) and belowground biomass (for rapeseed) were reduced when the plants were irrigated with irrigation water E containing very high salinity and organic content (Figure 5). These results agree with previous studies such as those of Kim et al. (2012a and 2012b), Anderson et al. (2015), Grewal et al. (2010), and Bilgili et al. (2011) where they reported saline waters (EC range in 10 ds/m - 14ds/m) greatly influenced biomass production of switchgrass and rapeseed. With increasing salinity and TOC concentration of the irrigation water, the decrease of root and height biomass in switchgrass were higher than those in rapeseed. The ratio of root/shoot biomass of rapeseed increased with increasing salinity, a result consistent with previous work on rapeseed by Bilgili et al., (2011).

Crop species and water quality were significant for plant height ($p = 0.001$) while species was not significant for biomass yield. Also water quality had a significant effect on biomass of rapeseed and switchgrass, and below ground biomass and shoot to root ratio of rapeseed ($p < 0.0001$). Pop $\times$ TDS (interaction) was significant only for plant height.

For both rapeseed and switchgrass, plant height was highly correlated with above ground biomass dry weight with $r = 0.62$ and $p < 0.0001$ and $r = 0.69$ and $p < 0.0001$, respectively. The analysis of variance revealed that there were significant negative correlations among growth parameters including biomass and height, and irrigation water treatments with $r = -0.798$, $p < 0.0001$ and $r = -0.534$, $p < 0.0001$, respectively.

373 High salinity and TOC levels of irrigation water E ($EC = 34.4\text{ds/m}$ and $TOC = 1352\text{ mg/l}$)
374 significantly affected the switchgrass biomass yield, decreasing from 47g with irrigation water A
375 (control) down to one eighth of that at 5.9g with irrigation water E (Figure 5a). This response
376 was similar to the response seen for rapeseed with 86% above ground biomass reduction (Figure
377 5a). It is important to note that there was also a significant difference on biomass production
378 (30% reduction) between irrigation water C and irrigation water D. In other words, the greater
379 the produced water organic matter concentration (TOC) in the irrigation water, the less biomass
380 was produced (treatment D compared to treatment C). This significant difference between the
381 two irrigation waters explicitly shows the importance of treatment and removal of the organic
382 matter in produced water.

383 Table 6 and Table 7 show the analysis of means by ANOVA for plant physiological parameters
384 when averaged across treatments and species, respectively. No difference was detected among
385 the species for above ground biomass when averaged across all treatment levels (Table 6). Plants
386 treated with water with the highest EC and TOC (irrigation water E) were significantly shorter
387 than those irrigated with all other waters and were affected by leaf firing at early stages (Table
388 7). The higher organic content of irrigation water D compared to irrigation water C, significantly
389 increased the leaf electrolyte leakage and decreased both the above ground and underground
390 biomass yield (Table 7) when averaged over water quality levels. With increasing salinity,
391 rapeseed root and shoot and switchgrass shoot biomass decreased. Visual observations revealed
392 more developed root mass in control pots with decreasing development with increasing salt
393 concentration (data not shown). The ratio of root/shoot for rapeseed crops decreased with
394 increasing salinity amount in the water treatments (Figure 5d). These results are consistent with

previous studies ([Kim et al., 2012](#); [Burkhardt et al., 2015](#); [Porcelli et al., 1995](#); [Grewal, 2010](#); [Sanderson et al., 1996](#)).

Based on these results, switchgrass yields more biomass weight with the same irrigation water quality and seemed to be less sensitive to poorer water quality, indicating that selection of plant species should be considered when using treated produced water for irrigation. Rapeseed aboveground biomass was not affected when using irrigation waters A, B, C and D as the salt concentration in the soil and water increased (below 5.47 ds/m) (Table 6). This is in agreement with data from Porcelli (1995), Francois et al. (1994), and Boem et al. (1994), who had found no effect of salinity up to 10ds/m. However, irrigation water E resulted in a significant decrease in biomass production with salinity at 33.8ds/m. Irrigation water D resulted in significantly lower above and below ground rapeseed biomass yields when compared to treatment C, a result attributed to the greater TOC concentration (salinity concentrations were equal, Table 7). This observation can be explained as the higher concentration of produced water organic compounds in irrigation water D resulted in higher cell damage of plants species, higher leaf EL, and less growth rate and is confirmed in literature ([Bilgili et al., 2011](#); [Francois et al., 1994](#); [Liu et al., 2014](#)).

Although irrigation water B had undergone more treatment processes and contained less salinity and TOC than irrigation water C, there was not a difference between irrigation water B and irrigation water C in plant height, above ground biomass, underground biomass or shoot to root ratio. It is important to point out that treating produced water to the quality of irrigation water B is both time consuming and costly and based on these results, irrigation water C may be sufficient. This remark is of importance in reusing produced water in irrigation of non-food biofuel crops such as rapeseed and switchgrass. Also, establishing treatment requirements for

produced water should be based on removal of salts as well as organic matter, due to the significant influence of TOC concentration of irrigation water on the plants health and physiological parameters and biomass yield.

Based on the physiological characteristics of switchgrass and rapeseed and the germination study, it can be concluded that the rapeseed crop was more tolerant to poor quality treated produced water than switchgrass and biomass production was not affected by the irrigation water quality. This is an important observation as application of produced water for irrigation is being evaluated in multiple shale plays. According to these results, treatment processes that would provide TOC removal to less than 5mg/l is necessary for irrigation of nonfood biofuel crops with hydraulic fracturing-related produced water.

Figure 6 shows salt crystals on the leaf surfaces of rapeseed and switchgrass treated with irrigation water E, and also adaxial salt glands. Abaxial salt glands were often found in the same row with stomata, and adaxial salt glands were located on the walls of ridges, as shown in the leaf cross section of switchgrass (Figure 6a and 6b).

3.3.2 Leaf EL

Leaf electrolyte leakage is a good indication of plant response to an environmental stress. Measurement of this phenomenon is commonly used to evaluate the tolerance of crops to stress conditions such as draught, harsh temperatures, and salinity. The results are shown in Figure 7. Associated TDS and TOC values are shown in Tables 1, 2 and 3.

According to Figure 7, the leaf EL of both rapeseed and switchgrass increased rapidly with increasing salinity and TOC concentration of the water. All four produced water qualities resulted in significantly higher EL values than the control for both rapeseed and switchgrass.

However, the leaf EL was significantly lower for switchgrass with 28.5%, compared to 35.5% for rapeseed when averaged over treatments (Table 6). These values are within the range of reported leaf EL values (Kim et al., 2012; Blum and Ebercon, 1981). By the end of the irrigation season, EL for irrigation water E was more than 3 and 5 times the control level for rapeseed and switchgrass, respectively. The high organic content of irrigation water D relative to irrigation water C (they have equivalent TDS concentrations) significantly increased the leaf EL on both rapeseed and switchgrass species, which shows greater damage to cell membranes for this stress condition (contaminations of irrigation water by high organic loads). Based on the results, leaf EL of switchgrass was 45% when treated with significantly higher TOC water compared to 30% when it was treated with water with the same EC and lower TOC level. The amount of electrolyte leakage of rapeseed at treatment EC = 5.5ds/m and TOC= 35.8mg/l is higher than values reported by Farhoudi et al. (2011) which could be due to the organic content of irrigation water in this study.

For both switchgrass and rapeseed, water quality was significant for leaf EC ($p < 0.0001$, $p < 0.0001$). Analyses of the mean showed a significantly high correlation between leaf electrolyte and irrigation water quality (TOC and TDS) with $r^2=0.83$ and $p < 0.0001$. Leaf electrolyte values significantly decreased from irrigation water A (control) to irrigation water E when averaged over both plant species (Table 7). These results confirm the impacts of organic content of the irrigation water on both rapeseed and switchgrass species with equivalent salinity values (irrigation waters C and D). Based on Figure 7 and Table 7, high TOC concentration was the stress factor, resulting in higher damage in cell membranes and ultimately higher EL values. These observations can be used as guidance for regulators and stakeholders in development of treatment standards in which organic matter should be removed to less than 50mg/l to keep leaf

EL (cell damage) to less than 50% and a TOC concentration of less than 5mg/l required to keep a sustainable biomass production rate.

3.3 Plant uptake

3.3.1 Organic

None of the BTEX, DRO and GRO compounds were detected in any of the samples. This could be explained as high biodegradability and the lack of accumulation of these compounds in the soils.

3.3.2 Inorganic

Na^+ , K^+ , Mg^{+2} , Ca^{+2} , Sr^{+2} and boron content of plant tissue of both rapeseed and switchgrass crops were measured. Figures 8 and 9 illustrate the mineral uptake of five cations, Na^+ , K^+ , Mg^{+2} , Ca^{+2} , Sr^{+2} and one anion, boron for both crop species. There was no clear trend either within or between species with respect to the studied monovalent cations. There were no significant changes in Na^+ and K^+ contents in shoot biomass of both switchgrass and rapeseed at all levels of salinity ($P=0.169$). According to Figure 8 and the ANOVA, neither species nor irrigation water quality were significant for Na, K or Na/K.

There was no clear trend in selectiveness between switchgrass and rapeseed in above ground tissues. Below ground tissue analysis revealed positive correlations between Na and Ca, K, Sr, and Mg ($r^2 = 0.78, 0.71, 0.81$ and 0.85 , respectively, all at $P < 0.005$) but only weak or no correlations in above ground tissues. There was a slight difference between plant uptake of both rapeseed and switchgrass for monovalent cations of Na and K, which is associated with salt accumulation with irrigation water E.

Effects of salinity and organic content from four different produced water irrigation water samples plus the control water on the Ca^{+2} , Mg^{+2} and Sr^{+2} content of rapeseed and switchgrass are shown in Figure 9. Plant uptake for these divalent ions was constant through all irrigation treatments. For all studied ions, plant uptake of both rapeseed and switchgrass was higher than the associated measured concentrations in irrigation water samples (Table 2). Based on Figure 9, high occurrence of Mg^{+2} and Ca^{+2} in plant tissues can be related to high concentrations of Ca^{+2} and Mg^{+2} in the virgin soil (Figure 4), since there was very low concentration of Ca and Mg in irrigation waters (Table 2). Although Sr concentration was negligible in all irrigation waters except irrigation water E with $\text{Sr} = 37\text{mg/l}$ (Table 2), plant uptake was constant for all irrigation waters at approximately 29 mg/kg to 36 mg/kg. This can be explained by higher Sr concentrations in the virgin soil samples (Figure 4).

4. Conclusion

This study demonstrated the impacts of produced water reuse in irrigation of rapeseed and switchgrass, two non-food biofuel crops known to have some degree of salt tolerance. A greenhouse study on rapeseed and switchgrass for one growing season was conducted. In this study, seedling emergence, biomass yield plant health were evaluated under high organic content of produced water including BTEX compounds as well as high salinity. Plant health, growth, and physiological parameters were measured. Four different water qualities were developed from oil and gas produced water through different levels of advanced treatment resulting in a range of TDS and TOC concentrations were compared to a typical surface-source irrigation water control. For both rapeseed and switchgrass, biomass yield and plant growth decreased as the quality of water decreased due to an increase in salinity and TOC of treatments water qualities. There were statistically significant differences between the irrigation water with highest TOC and EC rate

(TOC = 1352mg/l, EC=33.8ds/m) and all other irrigation water treatments with lower EC and TOCs. Salt accumulation in soil samples of rapeseed showed significant correlation with water quality. These were clearly very important features for establishment and persistence of perennial energy crops on marginal land affected by salinity. At a constant salinity of 5.47 ds/m (irrigation water C and D) and TOC levels of < 232.3mg/l, compared to TOC of 38.3mg/l in irrigation water, rapeseed and switchgrass physiological parameters such as above ground and underground biomass production were significantly affected. High TOC levels were shown to affect the leaf EL value, which confirms the fact that these crop species are less tolerant to TOC levels from produced water than to low concentrations found in irrigation waters. Thus, organic level of produced water is as important factor as salinity level, if not more. The results indicated that treated produced water to TDS level of 3500mg/l and low organic content, necessary in order to maintain the biomass yield and plant health of both rapeseed and switchgrass. Also it was concluded that treating produced water to medium TDS of 3500mg/l and low TOC level is the optimum treatment regarding cost, scalability and feasibility of reusing produced water in irrigation activities. Not only salinity but also high TOC concentration was the stress factor, resulting in higher damage in cell membranes (high EL values) and less biomass production. The target quality of produced water dictates the cost of treatment and is ultimately a key parameter in the adoption of produced water as an alternative to freshwater resources. These observations can be used as guidance for regulators and stakeholders in development of treatment standards in which organic matter should be removed to less than 50mg/l to keep leaf EL (cell damage) to less than 50% and a TOC concentration of less than 5mg/l required to keep a sustainable biomass production rate.

Acknowledgement

The research described in this paper was funded by the National Science Foundation, NSF, [grant 004180-00002]. Authors would like to thank Dr. Ann Hess, Department of Statistics, Colorado State University, for her support on statistical analyses.

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Sustainable Water Management

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Table 1- Organic characteristics of water samples

Sample	TOC (mg/l)	±SE	Benzene (ug/L)	±SE	Toluene (ug/L)	±SE	Ethyl benzene (ug/L)	±SE	total xylenes (ug/L)	±SE
Irrigation water A	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Irrigation water B	1.2	0.5	1	0.6	1.2	1.2	0.0	0.0	0.0	0.0
Irrigation water C	38.3	19.2	5.8	2.6	7.3	3.6	0.4	0.3	2.1	1.8
Irrigation water D	232.2	41.3	580	0.0	200	0.0	5.8	0.0	30.0	0.0
Irrigation	1352.	108.5	1632	314.2	888	203.9	50.8	10.7	268.0	59.6

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Table 2- Cationic characteristics of water samples

Sample	Na (mg/l)	±SE	K (mg/l)	±SE	Ca (mg/l)	±SE	Mg (mg/l)	±SE	SAR (mg/l)	±SE	Sr (mg/l)	±SE
Irrigation water A	3.6	0.0	0.8	0.0	14.4	0.0	1.8	0.0	1.8	0.0	<0.1	0.0
Irrigation water B	50.0	9.8	5.3	0.4	10.5	0.4	2.1	0.1	5.2	0.9	NA	NA
Irrigation water C	1275.0	246.6	119.0	42.1	13.0	0.8	13.1	2.1	84.2	15.4	0.3	0.1
Irrigation water D	1500.0	408.3	21.0	5.7	61.0	11.4	9.1	1.6	65.6	12.2	NA	NA
Irrigation water E	5175.0	1379.5	100.0	4.1	207.5	8.7	27.8	1.3	127.6	34.9	37.0	0.8

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Table 3- Anionic characteristics of water samples

Sample	TDS	EC	B	±SE	Br	±SE	Cl	±SE	SO4	±SE	PO4
	(mg/l)	(ds/m)	(mg/l)		(mg/l)		(mg/l)		(mg/l)		(mg/l)
Irrigation water A	350	0.6	0.0	0.0	<0.1	0.0	19.2	0.0	<0.5	0.0	<0.5
Irrigation water B	400	0.6	0.0	0.0	NA	NA	90.5	15.9	NA	NA	0.0
Irrigation water C	3500	5.5	7.3	6.4	17.5	0.4	2375.0	433.0	42.0	11.4	0.0
Irrigation water D	3500	5.5	2.6	1.5	NA	NA	2950.0	530.7	NA	NA	0.0
Irrigation	21000	34.4	22.0	0.4	135.0	4.1	10975.0	1591.9	49.0	4.9	0.0

water E

NA: not available, SE: standard deviation

Table 4- Soil characteristics

	Sand	Clay	Silt	pH	EC	OM	NO3	P	SAR
					ds/m	%	mg/l	mg/l	
Clay loam	34%	28%	38%	8.2	0.5	1.0	0.86	1.3	4.2

Table 5- Germination percentage and rate under growth chamber conditions for rapeseed and switchgrass subjected to varying levels of sodicity and salinity from five produced water treatments. Treatment means followed by a different letter are different at *P* less than or equal to 0.05.

Species	Irrigation water treatment	Relative germination		Germination rate	
		%		%	
Switchgrass	A	97.3	a	58.3	a
	B	95.0	a	46.0	b
	C	9.50	a	43.4	b
	D	68.0	a	22.8	c
	E	1.3	b	0.4	d
Rapeseed	A	100.0	a	76.4	a
	B	98.7	a	71.2	a,b
	C	94.7	a	60.0	a,b
	D	88.0	a	48.2	b
	E	8.0	b	0.9	c

Table 6- Growth characteristics of rapeseed and switchgrass species averaged across all water qualities at the end of the growing season. Letter groups represent significant differences ($p = 0.05$) within each variable.

Species	Plant height		Above ground biomass		Leaf EL		EC	
	(cm)		(g)		(%)		(ds/m)	
Rapeseed	73.8	a	28.4	a	35.5	a	17.0	a
Switchgrass	37.1	b	32.4	a	28.5	b	22.0	b

789 Table 7- Growth characteristics of rapeseed and switchgrass species at the end of the growing season. Means
 790 followed by different letters are significantly different ($p = 0.05$).

Species	Irrigation water	Plant height (cm)		Above ground biomass (g)		Underground biomass (g)		Shoot to root ratio		Leaf EL (%)		EC (ds/m)	
Rapeseed	A	44.3	a	46.8	a	14.7	a	3.1	a	19.2	a	3.6	a
	B	36.3	a	42.9	a, b	11	b,c	3.9	a	23.6	a	3.3	a
	C	42.3	a	29.8	a	7.8	b	3.8	a	32.3	b	2.5	a
	D	42.3	a	24.8	b	6.7	c	3.7	a	38.7	b	2	a
	E	19	b	6.2	b	1.9	d	3.3	a	63.6	c	16.1	b
Switch- grass	A	89.3	a	46.8	a	*	*	*	*	10.7	a	3.3	a
	B	81.7	a	42.6	a	*	*	*	*	15.8	b	3.3	a
	C	79.7	a	38.4	a	*	*	*	*	30.4	c	5	a
	D	79.3	a	28.8	b	*	*	*	*	44.5	d	9	a
	E	39	b	6	b	*	*	*	*	50	e	18	a

791 *Underground biomass was not measured

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Figure 1- Effect of water quality on switchgrass and rapeseed seed germination

Figure 2- Effect of water quality on germination rate of switchgrass and rapeseed crops

Figure 3– Effect of five water quality types on soil electroconductivity from pots of rapeseed and switchgrass irrigated with produced water.

Figure 4— Ionic, Ca, Mg, Na, K, Sr and B, content of soils from rapeseed pots for five irrigation waters.

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Figure 5- Effect of water quality on mean plant biomass yield (a), root weight (b) and height (c) of rapeseed and switchgrass in a greenhouse experiment. Error bars are $\pm$ SEM.

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Figure 6. Adaxial leaf surface view by scanning electron microscopy of (a) switchgrass and (b) rapeseed treated with irrigation water E salt crystal.

Figure 7- Effect of water quality on leaf electrolyte leakage for rapeseed and switchgrass crops

Figure 8- Effects of different irrigation water on Na, K and selectivity of K and Na for rapeseed and switchgrass crops

Figure 9- Effects of different irrigation water on Ca, Mg and Sr for rapeseed and switchgrass crops