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Effect of varying ratios of produced water and municipal water on soil characteristics, plant biomass, and secondary metabolites of *Artemisia annua* and *Panicum virgatum*

Andy Burkhardt[a], Archana Gawde[b], Charles L. Cantrell[b], Valtcho D. Zheljazkov[a,c,*]

[a] University of Wyoming, Department of Plant Sciences, 1000 University Avenue, Laramie, WY 82071, USA
[b] Natural Products Utilization Research Unit, Agricultural Research Service, United States Department of Agriculture, University, MS 38677, USA
[c] Oregon State University, Columbia Basin Agricultural Research Center, Pendleton, OR 97801, USA

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ABSTRACT

Coal-bed natural gas production in the U.S. in 2012 was 1655 billion cubic feet (bcf). A by-product of this production is co-produced water, which is categorized as a waste product by the Environmental Protection Agency. The effects of varying concentrations of coal-bed methane (produced) water were studied in the greenhouse to elucidate their effects on two species as feedstock for lignocellulosic ethanol production: *Panicum virgatum* L. and *Artemisia annua* L. Two populations of *A. annua* were used, one from Canada and the other from Bulgaria. Plants were treated with varying ratios of produced water: control (0% produced or 100% municipal water), 25% produced, 50% produced, 75% produced, and 100% produced water. With increased concentration of produced water, the soil soluble Na increased, whereas soil soluble Mg and Ca decreased. The Na adsorption ratio (SAR) increased from 2 in the control to 21 in the 100% produced water treatment, and significant interaction was observed between the crop and water terms for electrical conductivity and phosphorus bicarbonate. SAR also increased in soils with both varieties of wormwood and with switchgrass as a result of increased concentrations of produced water. In addition, SAR was influenced by crop; SAR was higher in soil under switchgrass than in soil under the wormwood varieties. The biomass yields had significant interaction between crop and water treatments with Bulgarian wormwood consistently yielding more until the highest concentration of produced water when it decreased below that of Canadian wormwood. Bulgarian wormwood yielded the most with 131 g/pot for the control followed by the 75% (118 g/pot, statistically similar to control), 25% (111 g/pot, statistically similar to control), and 50% (107 g/pot) treatments. Biomass yields were reduced in the 100% produced water treatment. Regression analysis of the Canadian wormwood essential oil yield and constituents revealed weak correlations to increasing treatment of produced water. β-pinene, for which a quadratic yield curve was present, indicated a steep decline and leveling off of β-pinene production at high concentrations of produced water. The study shows increasing accumulation of Na and salts in the soil with increasing concentration of the produced (saline–sodic) water. High concentrations of these ions (average Na concentrations were 1156 mg l^{-1}) and salts in the 100% produced water treatment hampered growth and development of the plants. Implications of this research indicate that produced water could be utilized chemically untreated if diluted with high quality water. Produced water concentrations in dilution can be no higher than 25% in order to avoid deleterious soil and plant growth effects.

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1. Introduction

Coal-bed natural gas has become an important natural resource nationally, with production in the U.S. in 2012 topping 1655 billion cubic feet (bcf) (United States Energy Information Administration, 2014). Water and natural gas intermingled in subsurface coal-beds are pumped out. Once the pressure is relieved, the water and gas

* Corresponding author.
E-mail addresses: valtcho.jeliazkov@oregonstate.edu, valtcho.pubs@gmail.com (V.D. Zheljazkov).

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separate and the gas is piped away. The produced water has variable quality and can either be reinjected into the well, stored on the surface in evaporation ponds, or released into surface waters. The EPA categorizes produced water as a waste product and it must be handled as such (United States Department of Energy, 2002). Depending on water quality from the well, some of the water pumped to the surface can be utilized as supplemental irrigation water. A recent boom in coal-bed methane production in the Powder River Basin of northeastern Wyoming has created large quantities of produced water from well sites; with total water production as of 11 February 2014 at 7.7 bcf (Wyoming Oil and Gas Conservation Commission, 2014). When used for irrigation, due to high sodicity and salinity, the water often has deleterious effects to the soil that must be managed (Vance et al., 2008). Produced water's effects on plants are less understood.

Produced water affects two main soil parameters: salinity and sodicity. Sodicity is often expressed with either the exchangeable sodium percentage (ESP) or the sodium adsorption ratio (SAR). The equation for ESP (United States Salinity Laboratory Staff, 1954) measures sodium ion concentrations in relation to all cations present in the soil solution.

$$\text{ESP} = \frac{\text{Exchangeable Na (milliequivalents/100g soil)}}{\text{Cation Exchange Capacity (milliequivalents/100g soil)}} \times 100$$

The equation for SAR (United States Salinity Laboratory Staff, 1954) expresses the ratio of soluble Na in comparison to soluble Ca and Mg only

$$\text{SAR} = \frac{\text{Na}(\text{meql}^{-1})}{\sqrt{\frac{\text{Ca}(\text{meql}^{-1})+\text{Mg}(\text{meql}^{-1})}{2}}}$$

Generally, increased Na levels affect normal plant growth and may decrease biomass yields. Previous studies have been done on both salinity and sodicity individually using Na on a number of plant species. Prasad et al. (2001) studied the effects of Na carbonate in water on soil sodication and yield of lemongrass and palmarosa. The palmarosa essential oil yields increased at 4 meq l^{-1} by 22.6% and decreased thereafter, whereas the lemongrass essential oil yield decreased at each treatment level of Na carbonate compared to the control. Increased soil sodicity decreased oil and biomass yields as well as decreased the content of K, Ca, and N in peanut (*Arachis hypogaea*) plants while increasing the content of Na (Singh and Abrol, 1985).

Qureshi et al. (2005) studied salt and Pb effects on *Artemisia annua* and found that biomass yield decreased with increasing concentration of salts while artemisinin concentration increased at moderate salinity levels. Kim et al. (2012) studied salt effects on germination and growth of two bioenergy candidate species: prairie cordgrass (*Spartina pectinata* Bosc ex Link) and switchgrass (*P. virgatum*). Aboveground biomass from both species was reduced. Treating spearmint and marjoram with 2 M Cl^- was toxic (El-Keltawi and Croteau 1987). Mitigation of this could be largely achieved with foliar application of cytokinin. Essential oil concentration in the plants was largely unchanged, but oil yield was highest in the control group.

Some studies have utilized produced water itself, but the number of species treated with produced water has been few. Mullins and Hajek (1998) treated sorghum-sudangrass in a greenhouse study with varying dilutions of produced water and deionized water up to 2000 mg l^{-1} total dissolved solids based on limits set by Alabama's Department of Environmental Management for saline irrigation water. Plants in both studies were assigned one of two watering schedules: continuous or intermittent. The continuously watered plants in both studies decreased forage yield due to water logging. Vance et al. (2008) studied the soil physical parameters as well as plant diversity on range and crop land being irrigated with produced water. Sulfur burners have been used to decrease the pH of the water; however this did not decrease the amount of total dissolved solids, salts, or Na content of the water. Thus, even after treatment with sulfur, the low quality water still adversely affected the soils. Produced-water applied to spearmint and peppermint at greater than 50% decreased biomass yields (Zheljazkov et al., 2013b). The effect on spearmint essential oil and composition had a positive correlation up to 100% but a negative correlation for peppermint.

The plant species selected for this study were *P. virgatum* (switchgrass) and *A. annua* (sweet wormwood). Switchgrass has been widely studied as a candidate feedstock for cellulosic ethanol (McLaughlin and Kszos, 2005). With yields having 5–10 year averages of 12–19 Mg ha^{-1}, switchgrass has been at the forefront of biofuel research. *A. annua* is not grown for biomass, so minimal data is available on biomass yield, but given its antimalarial and essential oil uses it is a likely candidate for dual-utilization. Data do exist on dry leaf yield and dry stem yield separately. Maximum dry leaf yield of *A. annua* occurs just prior to flowering with a range of 2.6–3.1 Mg ha^{-1} and a maximum dry stem yield at flowering with value ranging from 7.1 to 8.2 Mg ha^{-1} (Jha et al., 2011). These yields combined would make wormwood competitive with switchgrass and possibly give it an economic advantage when essential oil and artemisinin are factored in.Given how little data are in the literature regarding effects of produced water on the plants as well as the contradictory nature of some of the studies, it is paramount that we understand its effects. The objective of this study was to quantify how different levels of produced water would affect plant growth and physiology of *P. virgatum* and two varieties of *A. annua.*

2. Materials and methods

2.1. Plant material, treatments

Two varieties of *A. annua* (a Canadian variety and a Bulgarian variety) and *P. virgatum* (switchgrass) were started in the greenhouse and allowed 3 months to establish in 36 plug trays. Greenhouse conditions were maintained at 30 °C during the day and 24 °C at night. Plants were then transplanted into 3 gallon pots utilizing 12 kg of sandy loam topsoil (0–8 inches depth) excavated from the Sheridan Research and Extension Center. Florikan® 18-6-12 slow-release fertilizer was mixed with the soil at 30.0 g/pot. Plugs were transplanted into pots and allowed to establish for one week before initiation of treatments. Plants were then randomly assigned a treatment, with four replications per treatment. Plants were placed along the center of the southern greenhouse wall to maintain a similar microclimate for each pot. Treatments were five different ratios of produced water combined with municipal water: control (0% produced or 100% municipal water), 25% produced, 50% produced, 75% produced, and 100% produced water. Treatments were applied daily or as needed to give the plants sufficient moisture at 600 ml per pot.

2.2. Water properties

The produced water used in this trial was tested by Inter-Mountain Labs of Sheridan, Wyoming. The report shows the following conditions: a pH of 8.4, an EC of 2 dS m^{-1}, total dissolved solids (TDS) at 1390 mg l^{-1}, an SAR of 32.8 $(\text{meq l}^{-1})^{0.5}$, bicarbonate and carbonate levels at a combined 1389 mg l^{-1}, and Na concentrations of 555 mg l^{-1}.

Municipal water from the study was sourced from the city water system of Sheridan, WY. Tests are regularly performed to ensure

the water is safe for human consumption (Tom Manolis, personal communication, 16 July 2014). Values for relevant variables are as follows: an average pH of 7.7, an EC of 0.00094 dS m^{-1}, TDS concentrations ranging from 13 to 70 mg l^{-1}, and bicarbonate concentrations ranging from 11 to 60 mg l^{-1}. SAR is not calculated as Na levels are never high enough to warrant concern in the municipal water.

2.3. Soil properties and laboratory analysis

The soil used in this container study was a Hargreave-Moskee sandy loam. Properties were determined using Web Soil Survey (Soil Survey Staff, 2013). It is characterized as a well-drained alluvium derived from sandstone and sedimentary rocks with moderate (3–9%) slope and neutral pH conditions. Of importance to note is salinity normally does not exceed 2.0 dS m^{-1}.

Analysis of the soils was done by Olsen Agricultural Laboratory, McCook, NE. Methods used for soil analysis were as follows: pH was analyzed using a 1:1 saturated paste according to Watson and Brown (1998); nitrate was analyzed according to Carson (1980); EC and soluble salts were analyzed using the saturated 1:1 paste according to Dahnke and Whitney (1988); SAR was calculated according to procedures defined by United States Salinity Laboratory Staff (1954); P was analyzed using the bicarbonate method (Knudsen and Beegle, 1988); and sulfate, K, Ca, Mg, and Na were analyzed according to Brown and Warncke (1988). Micronutrients (Zn, Fe, Mn, Cu, and B) were analyzed according to Whitney (1988).

2.4. Essential oil extraction

Plants were harvested at flowering of the *A. annua* varieties in order to obtain maximum yield of essential oils (Ćavar et al., 2012; Gouveia and Catilho, 2013). All above ground biomass was collected, weighed, and the samples were steam distilled for 180 min using a 2-L Clevenger type steam distillation unit (Clevenger, 1928; Ferreira et al., 2013; Furnis et al., 1989; Gawde et al., 2009; Zheljazkov et al., 2013a). Due to low plant biomass yields from *A. annua*, all plant material was used in contrast to the study by Ferreira et al. (2013), where only stems with a diameter of <2 mm and leaves were utilized for the distillation. This also led to replicates being pooled together with replicates 1 and 2 being combined and 3 and 4 being combined in order to have sufficient amounts of plant biomass to produce measureable essential oil samples. Bulgarian wormwood oil can only be collected in measureable quantities from leaves, flowers, and small diameter stems (Ferreira et al., 2013) whereas essential oil from Canadian wormwood can be collected from the whole plant. However, insufficient plant material was harvested from Bulgarian wormwood to collect any measureable oil. Essential oil samples from Canadian wormwood were then analyzed on gas chromatography–mass spectroscopy.

2.5. Gas chromatography–mass spectroscopy essential oil quantitative analysis

A total of 28 constituents were identified and quantified in *A. annua* essential oil. Oil samples were analyzed by gas chromatography with flame ionization detector (GC–FID) on a Agilent 5975C inert XL MSD with triple axis detector/7890A GC system equipped with an Agilent 7693 Autosampler, a DB-5 fused silica capillary column (30 m × 0.25 mm, with a film thickness of 0.25 μm) operated using the following conditions: injector temperature, 240 °C; column temperature, 60–120 at 3 °C/min, then held at 240 °C at 20 °C/min for 5 min; carrier gas, He; injection volume, 1 μl (split on FID, split ratio 50:1); FID temperature was 300 °C.

The following compounds were identified in oil samples by Kovat analysis and comparison of mass spectra with those reported in the National Institute of Standards and Technology mass spectra database or by direct comparison with commercially available standards (Adams, 2007): santolina triene, α-pinene, camphene, sabinene, α-phellandrene, β-pinene, D-limonene, 1,8-cineole, artemisia ketone, artemisia triene, β-thujone, α-thujone, isothujol, myrtenyl acetate, camphor, menthone, pinocarvone, isoborneol, D-carvone, 3-carene, α-copaene, β-myrecene, e-caryophyllene, β-farnesene, germacrene D, β-selinene, bicyclogermacrene, and elemene. Compounds were quantified by performing area percentage calculations based on the total combined FID area. For example, the area for each reported peak was divided by total integrated area from the FID chromatogram from all reported peaks and multiplied by 100 to arrive at a percentage. The percentage of a peak is a percentage relative to all other constituents integrated in the FID chromatogram.

2.6. Statistical analysis

Statistical analysis of the soil response variables was done using a two-factorial set in a completely randomized design. Using this analysis we tested the null hypothesis that average responses were the same among the three crops versus the alternate that at least two were different. We also tested the null hypothesis that the average responses were the same among the five water treatments versus the alternate that at least two were different. Finally, we tested the null hypothesis that there was no crop by water treatment interaction versus the alternate that there was ($\alpha = 0.05$). Computations for the analyses of variance were facilitated by using the GLM procedure of SAS (version 9.4) and computations for mean separations were facilitated by using the LSMEANS statement (SAS Institute Inc., Cary, NC). Homoscedasticity of variances was tested using Hartley's test (Hartley, 1950) and normality of residuals were assessed by using the Shapiro–Wilk test, assessing stem and leaf plots, and assessing the normal probability plots. Computations and development of plots for assessing normality were facilitated by using the UNIVARIATE procedure of SAS; whenever heteroscedasticity or non-normality of residuals occurred, values for the response variable were transformed to and analyzed in the $y^{0.5}$ scale.

Regression analysis of the essential oil response variables for Canadian wormwood was done using simple linear regression for all but β-pinene, which was analyzed using a 2nd order polynomial. For the simple linear regressions, we tested the null hypothesis that the slope was equal to zero versus the alternate it was not using *t* tests ($\alpha = 0.05$). For the quadratic regression, we tested the null hypotheses for the linear and quadratic slopes that they were equal to zero versus the alternate that they were not, again using *t* tests. Diagnostic statistics such as studentized residuals and leverage values were assessed to identify potential 'outliers' and plots of regular residuals were assessed to ensure the variances were homoscedastic and that the correct model was fitted. Statistical computations were facilitated by using the REG procedure in SAS (version 9.4).

3. Results

3.1. Harvest weight

There were significant interactions among harvest weight and water treatments. The interaction among the three crops shows a sharp decline in the Bulgarian wormwood's harvest weight from 118 g at the 75% treatment level down to nearly half that at 63 g/pot at the 100% level. This response is inconsistent with the response seen from either Canadian wormwood or switchgrass which both

Table 1
Interaction among biomass yields. Significant interaction between water treatments and wormwood populations occurred. Bulgarian *A. annua* had higher yields under low produced water concentrations but Canadian *A. annua* biomass production was more tolerant to increased concentrations of produced water in comparison.

Produced water (%)	Bulgarian *Artemisia annua* (g)	Canadian *Artemisia annua* (g)	*Panicum virgatum* (g)
0	131a[a]	100bcd	21f
25	111abc	107bcd	31f
50	107bcd	103bcd	22f
75	118ab	93cd	28f
100	63e	86d	19f

[a] Treatment means in columns followed by different letters differ significantly (Fisher's protected LSD, $p \leq 0.05$).

only slightly decreased from the 75% treatment level 93 g/pot and 28 g/pot respectively down to 86 g/pot and 19 g/pot respectively (Table 1).

3.2. Effect of treatments on Soil parameters

All soil parameters except nitrate and K were affected by the produced water (Table 2). All the parameters relating to Na content of the soil (i.e. soil exchangeable Na percentage (ESP), soil Na, soil soluble Na, and SAR) increased as produced water concentrations increased. The ESP among the water treatments was highest in the 100% produced water treatment; whereas the control and 25% treatments had lower soil ESP values (Table 2).

The highest average Na content was in the 100% treatment, at 1160 mg kg^{-1} with each subsequent treatment level statistically separated from the next. The control treatment had an average soil Na content of 56 mg kg^{-1}. Soil soluble Na was highest among the 100% and 75% treatments. The 50% treatment was intermediate and the 25% and control treatments had the lowest average soil soluble Na (Table 2).

Soil SAR had similar means separation to that of ESP, with the 100% produced water treatment having the highest SAR. The 75% treatment was next followed by the 50% treatment. The 25% and control treatments had the lowest SAR averages (Table 2). Calcium and Mg (i.e., soil Ca, soil Mg, soil soluble Ca, and soil soluble Mg) levels also differed among water treatments. Soil Ca and soluble Ca had similar separation of means with the control and 25% treatments having the highest averages followed by the 50% treatment. The 75% treatment was intermediate to the 100% and 50% treatments. The 100% treatment had the lowest averages for soil Ca and soil soluble Ca (Table 2).

The concentration of Mg in the soil was the highest in the control with the 50% treatment being statistically intermediate. The 25%, 75%, and 100% treatments were all statistically similar (Table 2). Soil soluble Mg followed a negative linear pattern with the control averaging the highest. The 100% treatment had the lowest soluble Mg. Each treatment level was statistically separated from the others (Table 2).

Zinc (Zn) and boron (B) concentrations in the soil were affected by the water treatments. Boron concentrations were separated into 3 distinct groups, with the 100% treatment having the highest average followed by the 75% and 50% treatments. The 25% and control treatments were statistically similar and had the lowest averages. Zinc did not respond predictably to increasing concentrations of produced water. The 100% treatments had the highest average soil Zn; however the control treatment was next with an intermediate average. Following were the 50%, 75%, and 25% treatments (Table 2).

Average soil pH in the control treatment across species was a moderately neutral 7.2 whereas the average soil pH under 100% and 75% produced water was a slightly alkaline 8.3 and 8.2 respectively. The 50% and 25% treatment levels were intermediate averaging 7.9 and 7.8 respectively (Table 2). Soil sulfate was impacted by produced water. Water treatments were separated into two groups, with the 100% and 75% averaging the highest. The control, 25%, and 50% treatments averaged lowest (Table 2). EC was different among water treatments with the 100%, 75%, and 50% concentrations being statistically similar. The 25% and the control were statistically similar (Table 2).

There were differences among crops across soil parameters as well (Table 3). The Canadian variety of wormwood containers showed the highest average soil nitrate. The Bulgarian variety and switchgrass were statistically similar. Soil ESP and SAR were different among crops. Soil under switchgrass had the highest average ESP and highest average SAR. The soils for Bulgarian and Canadian wormwoods were statistically similar for both ESP and SAR (Table 3).

Soil Ca and soil soluble Ca was also different among crops. Soil Ca had all three crops separated into different groupings with Canadian wormwood having the highest average followed by Bulgarian wormwood and then switchgrass. Soil soluble Ca had the wormwoods statistically similar and higher than that of switchgrass (Table 3). Soil B was different between switchgrass and the wormwoods. Switchgrass containers averaged the highest soil B. Bulgarian and Canadian wormwood were similar (Table 3). Potassium was different among crops. Canadian wormwood averaged the highest soil K while Bulgarian wormwood was intermediate. Switchgrass averaged the lowest soil K (Table 3). Soil sulfate was different among crops. For the crop term, the soils under the wormwoods were statistically similar. Soils under switchgrass had the lowest average (Table 3). Electrical conductivity was different among crops. Canadian wormwood had a higher EC than Bulgarian wormwood or switchgrass (Table 3).

3.3. Essential oil analysis

Low biomass production led to no essential oil being collected from the Bulgarian *A. annua* variety. However, sufficient oil was collected from pooled replicates of the Canadian variety. Total essential oil yield was negatively correlated to increased concentrations of produced water, with an R^2 of 0.43. Analysis of β-pinene indicated a yield curve ($y = 0.00038977x^2 - 0.05136x + 2.21571$), with constituent concentration decreasing to the 50% treatment and leveling off as produced water concentrations increased (Fig. 2).

All other response variables had no such yield curve evident in the data and were thus only analyzed using a simple linear regression. The following constituents showed a slightly positive correlation to increased produced water treatment: E-caryophyllene, α-thujone, pinocarvone, camphor, α-copaene, β-farnesene, β-selinene, and elemene. D-Carvone and β-thujone were not analyzed due to insufficient data. All other response variables had negative correlation to increased produced water concentrations. The weakest R^2 value was 0.02 for Artemisia ketone and the strongest value was 0.43 for elemene (Table 4).

Essential oil analysis revealed varying concentrations of each constituent in the oil. Artemisia ketone averaged the highest with 44.07% of the chemical composition of the essential oil with β-selinene averaging second with an average concentration of 9.55%. Camphor, 1,8-cineole, and germacrene D were next with average concentrations of 8.29%, 7.74%, and 7.29% respectively. Other minor constituents were (in order of average concentration) E-caryophyllene, α-copaene, artemisia triene, β-farnesene, sabinene, myrtenyl acetate, β-pinene, α-pinene, menthone, pinocarvone, camphene, isoborneol, bicyclogermacrene, β-thujone, elemene, α-thujone, and D-carvone.

Table 2
Soil response variables with significant effects to produced water treatment.

Soil response variable		Concentration of produced water in irrigation treatment					
		0%	25%	50%	75%	100%	*P*-value
B	mg kg^{-1}	0.34c[a]	0.37c	0.44b	0.46b	0.54a	<0.0001
Ca	mg kg^{-1}	2911a	2823a	2614b	2521bc	2443c	<0.0001
Soluble Ca	mgeq l^{-1}	21.3a	19.3a	12.9b	8.9bc	6.8c	<0.0001
Electrical conductivity (EC)	dS m^{-1}	2.4b	2.5b	3.1a	3.2a	3.4a	0.0001
Exchangeable sodium percentage	%	3d	5d	13c	23b	31a	<0.0001
K	mg kg^{-1}	239	219	252	244	261	
Mg	mg kg^{-1}	410a	360b	365ab	318b	321b	0.004
Soluble Mg	meq l^{-1}	6.1a	5.3ab	3.8bc	2.2cd	1.6d	<0.0001
Nitrate	mg kg^{-1}	119	115	148	127	128	
Na	mg kg^{-1}	57e	369d	631c	905b	1160a	<0.0001
Soluble Na	meq l^{-1}	7.3c	11.5c	26.6b	35.6a	39.3a	<0.0001
pH	N/A	7.2c	7.8b	7.9b	8.2a	8.3a	<0.0001
Sodium adsorption ratio	(meq l^{-1})$^{0.5}$	2d	4d	10c	16b	21a	<0.0001
Sulfate	mg kg^{-1}	37b	42b	47b	75a	63a	<0.0001
Zn	mg kg^{-1}	0.31ab	0.26c	0.30abc	0.28bc	0.34a	0.005

[a] Treatment means followed by different letters differ significantly (Fisher's protected LSD, $P \leq 0.05$).

4. Discussion

Given the above soil characteristics, it is first necessary to address the excess concentration of Na in the soil through application of amendments (usually amendments containing Ca and/or Mg). Gypsum is commonly used in the Rocky Mountain Region. Addressing the Na concentrations first is necessary because if excess irrigation is used to first leach out salts, then soil structure and tilth will be adversely affected due to higher ratios of Na in the soil as other salts are being leached away leading to soil dispersion (McCauley and Jones, 2005).

The results in this study are consistent with previous data, showing, among other things, Na concentrations in soil becoming very high at higher application rates of produced water. This study illustrated possible utilization solutions for produced water dependent upon the crop species being grown and the application rate of produced water. The variability of produced water among various wells may influence management practices depending on water chemistry. Wells within the Powder River Basin show variability depending on parent formation and watershed, affecting whether the produced water will be re-injected into wells or deemed safe for surface disposal (McBeth et al., 2003; Plumlee et al., 2014). The chemical composition of the produced water used in this study was within the 'typical' range for the produced water in the Powder River Basin, as asserted by the company that is involved in the management of produced water in this region.

4.1. Soil nutrient concentrations under the five water treatments

Soils with an SAR greater than 12 are likely to reduce water uptake by plants due to soil dispersion leading to limited water infiltration (Munshower, 1994). The SAR in the 75% treatment averaged 16 although no differences were seen among plant biomass yield at this level compared to lower concentrations. However, at the 100% treatment level, SAR was above 17, leading to a negative impact on plant biomass yield (Table 1).

When the exchangeable Na percentage (ESP) rises above ~15%, a soil is considered sodic (United States Salinity Laboratory Staff, 1954). This threshold only gives insight into soil conditions and not plant tolerance as some species can tolerate much greater ESP values while others are sensitive below this threshold (Gupta and Sharma, 1990). However, it does give insight into the present experiment and possible impacts to soil health. Under the 100% produced water treatment, ESP was more than double this threshold, with an average of 31%. The 75% produced treatment was still well above the cited threshold with 23% ESP. The 50% produced treatment was just below the USDA defined threshold, although depending on crop species grown on the soil this could still be considered sodic. Such high amounts of Na in the soil could invariably lead to soil dispersion if Ca and Mg salts are not present in high enough quantities (McCauley and Jones, 2005).

Electrical conductivity was elevated at significant levels for the 50, 75, and 100% produced treatments relative to the control, but

Table 3
Soil response variables with significant effects from crops.

Soil response variable		Canadian *Artemisia annua*	Bulgarian *Artemisia annua*	*Panicum virgatum*	*P*-value
B	mg kg^{-1}	0.40b[a]	0.41b	0.49a	<0.0001
Ca	mg kg^{-1}	2775a	2661b	2551c	0.001
Soluble Ca	meq l^{-1}	16a	15a	10b	0.001
Electrical conductivity (EC)	dS m^{-1}	2.8b	3.3a	2.6b	0.003
Exchangeable sodium percentage	%	13.4b	14.2b	17.1a	0.005
K	mg kg^{-1}	266a	238ab	225b	0.03
Mg	mg kg^{-1}	358	374	332	
Soluble Mg	meq l^{-1}	5	4	3	
Nitrate	mg kg^{-1}	165a	112b	106b	<0.0001
Na	mg kg^{-1}	607	616	643	
Soluble Na	meq l^{-1}	28	22	22	
pH	N/A	7.8	7.9	8.0	
Sodium adsorption ratio	(meq l^{-1})$^{0.5}$	9.7b	10.1b	12.2a	0.01
Sulfate	mg kg^{-1}	55a	62a	42b	<0.0001
Zn	mg kg^{-1}	0.29	0.29	0.31	

[a] Treatment means followed by different letters differ significantly (Fisher's protected LSD, $P \leq 0.05$).

Table 4
Regression analysis parameter estimates for the Canadian *Artemisia annua* essential oil analysis. All constituents were reported on a percentage basis while essential oil yield was reported on a g per 500 g plant sample basis.

Essential oil response variable[a]		y-Intercept	Linear slope	R^2	Essential oil response variable		y-Intercept	Linear slope	Quadratic slope	R^2
Total yield	g	0.66 (<0.01)**	−0.005 (0.04)	0.43	Menthone	%	1.07 (<0.01)	−0.004 (0.18)	–	0.22
Artemisia ketone	%	47.64 (<0.01)	−0.071 (0.71)	0.02	Myrtenyl acetate	%	1.54 (<0.01)	−0.004 (0.34)	–	0.11
Artemisia triene (9)	%	2.52 (<0.01)	−0.012 (0.07)	0.40	Pinocarvone (9)	%	0.35 (0.02)	0.002 (0.26)	–	0.18
Bicyclogermacrene (9)	%	0.70 (0.01)	−0.004 (0.29)	0.16	Sabinene	%	2.14 (<0.01)	−0.011 (0.06)	–	0.38
Camphene	%	0.97 (0.01)	−0.005 (0.32)	0.12	α-Copaene	%	1.23 (0.34)	0.028 (0.20)	–	0.19
Camphor (9)	%	4.66 (0.35)	0.065 (0.39)	0.11	α-Pinene (9)	%	0.33 (<0.01)	−0.002 (0.19)	–	0.23
1,8-Cineole	%	9.62 (<0.01)	−0.037 (0.15)	0.25	α-Thujone	%	0.06 (0.21)	0.001 (0.21)	–	0.19
e-Caryophyllene	%	6.16 (0.01)	0.015 (0.64)	0.03	β-Farnesene (9)	%	1.27 (0.02)	0.011 (0.14)	–	0.28
Elemene (7)	%	0.04 (0.59)	0.002 (0.11)	0.43	β-Pinene	%	2.22 (<0.01)	−0.051 (0.01)	0.0004 (0.04)	0.67
Germacrene D	%	9.73 (<0.01)	−0.049 (0.19)	0.20	β-Selinene	%	4.77 (0.35)	0.096 (0.26)	–	0.16
Isoborneol (8)	%	1.30 (0.11)	−0.009 (0.40)	0.12						

** *P*-values assessed at $\alpha = 0.05$.
[a] Each regression performed had 10 data points except where indicated by asterisk (# of data points in parentheses) due to missing values or to ensure normality of residuals. D-Carvone and β-thujone not analyzed due to insufficient data.

Fig. 1. Pots treated with saline–sodic (produced) water showing signs of soil sodicity (soil crusting and water logging). Note the pooling water and crusting on the surface of the pots in the foreground. Pots in the foreground were treated with 100% produced water.

Fig. 2. Regression analyses of β-pinene: yield curve.

this only tells us the water being used and the soil in question has a high ion concentration. EC was relatively low across water treatments and crops, likely attributable to the higher silt and sand content of the soil used in the experiment (Rhoades and Loveday, 1990). EC does not give insight into the composition and concentration of the ions present in the water.

In this study, we observed a negative effect of increased soil Na concentration on the concentration of other ions in the soil, particularly soil Ca and Mg. Higher concentrations of produced water may limit the availability of soil Mg and Ca. As competition between ions in the soil solution increases, Na will displace Ca and Mg leading to soil dispersion and further decreasing of water infiltration (illustrated in Fig. 1) (Jalali et al., 2008).

Soil pH was also higher in the 100% and 75% concentrations and pH averages for the 25% and 50% were still above preferred soil pH levels for most cash crops. Increased soil pH can be attributed to high pH of the produced water being utilized (water used in the study had an average pH of 8.4).

4.2. Soil nutrient concentrations under the three crops

Similar to the water treatment effects were the differences seen between the three crops. Significant differences were found for soil sulfate, SAR, nitrate, potassium, ESP, soluble Ca, B, and plant

biomass yield. Differences found for SAR and ESP are likely linked to the differences seen for soil Ca and soil soluble Ca as higher Na concentrations displace Ca and Mg. The other parameters that tested significant can be attributed to differences in nutrient requirements, such as different N, K, and S requirements. The differences between the biomass yields are species specific, showing which might be good candidates for high biomass production for uses such as forage or biofuel production, however further study is necessary in order to obtain plant nutritive value and ethanol yield data.

4.3. Essential oil analyses

There was an impact on essential oil yield (g essential oil per 500 g plant sample) and composition after irrigating with produced water. The small data set (10 or fewer data points per response variable) likely lead to the low R^2 values observed. A larger data set would greatly increase the confidence of the regression. Nearly all response variables had a linear correlation to treatment with produced water except for β-pinene, for which a quadratic yield curve was present, indicating a steep decline and leveling off of β-pinene production at high concentrations of produced water (Fig. 2). Our results from analysis did corroborate major constituents in the oil (Ćavar et al., 2012; Zheljazkov et al., 2013a).

5. Conclusions

Implications of this research indicate that produced water can be utilized chemically untreated if diluted with high quality water. Mixing ratios of 25% produced water could be utilized, though management of treated areas may still need to be performed in order to avoid excess salts or Na from accumulating in the root zone. Higher ratios of produced water may lead to impaired yield of the biomass and the essential oil, as well as changing the composition of the essential oil itself.

Feasibility of this recommendation could very well be determined by water availability. However, it is important to repeat this study under actual field conditions to ensure no deleterious effects have been overlooked. Further study into management practices would be of great use to producers utilizing produced water for irrigation in order for them to better plan field activities and mitigation practices.

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