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Characterization of the Chemical Composition of the Halophyte *Salicornia bigelovii* under Cultivation

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ABSTRACT: Straw of the halophyte *Salicornia bigelovii* was chemically analyzed for lignocellulosic components, extractives, and ash in relation to varying cultivation conditions (namely, irrigating water salinity and fertilizer grade). Irrigation water contained 10−50 ppt salt, and fertilizer application varied between 1 and 2 gN/m^2. Composition of the biomass was comparable to traditional lignocellulosic biomasses, containing glucan (up to 27 g/100 g total solids (TS)), xylan (up to 23 g/100 g TS), and lignin (24 g/100 g TS), but also high amounts of ash (up to 53 g/100 g TS) and water−ethanol soluble extractives (up to 25 g/100 g TS). As most of the ash is extractable (up to 90%), a simple water wash is sufficient to bring the ash content down to a typical value found in the lignocellulosic materials. It was found that increasing water salinity used for the plant irrigation decreases lignocellulosic components content, increases ash content, and does not affect extractives content. The fertilizer application rate was not found to influence any of the responses, except for ash composition (lowering mineral content) and its amount in the flowering spike fraction. Stem and spike fractions were found to be significantly different in composition, with stems being closer to a typical lignocellulosic material.

1. INTRODUCTION

Second generation biofuels represent a dynamically developing research area, using a wide variety of lignocellulosic feedstocks. Because of their complex structure, lignocellulosics are an abundant group of materials with a large potential for replacing most of the petroleum-based products via the biorefinery concept.[1−5] These include fuels, chemicals, polymers, and pharmaceuticals. Most commonly used feedstocks include energy crops (e.g., prairie grasses) and agricultural residues (e.g., corn stover, wheat straw).[6−9] However, these materials are not readily available in the arid lands of Middle East or North Africa.

Halophytes, salt-tolerant plants, have interesting properties, including medicinal and nutritional applications.[10−16] These plants managed to adapt to extremely difficult environments including salinized arid lands of the Middle East, South America, and Africa. The main advantage of these plants over others is their ability to tolerate high salt concentrations (i.e., mainly sodium chloride) in the soil and lack of nutrients.[17,18] An ongoing search for alternative feedstocks for biorefinery processing continuously leads the researchers to explore new, more efficient materials that do not require arable land or fresh water. This is especially important for arid regions of the world (such as Middle East), where food production is already limited by the scarcity of freshwater resources. Halophytes can be grown in salinized soils and using marginal water sources and can actually thrive in conditions that are lethal for most conventional glycophyte plants. Growing halophytes in high-salinity soils can remediate and prepare it for use in conventional crops cultivation.[19] Halophytes have been discovered to adjust to the high salinity in the irrigation water with osmotic pressure adjustment by producing glycine betaine and accumulating it as an osmoticum in the cytoplasm or by taking up salt.[20,21] Salinity of the soil has been reported to influence the growth yield of shoots of halophyte species native to Pakistan and United Arab Emirates coastline (e.g., *Suaeda fruticosa*, also known as *Suaeda vermiculata*), and the optimal growth salinity was found between 10 and 20 ppt.[20]

Table 1. Experimental Design for *S. bigelovii* Grown at Different Conditions

sample code	salinity [ppt]	fertilizer [gN/m^2]	fraction [stems/pods]
S1 F1 (S)	10.0	1.0	stems
S1 F1 (P)	10.0	1.0	pods
S1 F1.5 (S)	10.0	1.5	stems
S1 F1.5 (P)	10.0	1.5	pods
S1 F2 (S)	10.0	2.0	stems
S1 F2 (P)	10.0	2.0	pods
S2 F1 (S)	20.0	1.0	stems
S2 F1 (P)	20.0	1.0	pods
S2 F1.5 (S)	20.0	1.5	stems
S2 F1.5 (P)	20.0	1.5	pods
S2 F2 (S)	20.0	2.0	stems
S2 F2 (P)	20.0	2.0	pods
S3 F1 (S)	30.0	1.0	stems
S3 F1 (P)	30.0	1.0	pods
S3 F1.5 (S)	30.0	1.5	stems
S3 F1.5 (P)	30.0	1.5	Pods
S3 F2 (S)	30.0	2.0	stems
S3 F2 (P)	30.0	2.0	pods
S5 F1 (S)	50.0	1.0	stems
S5 F1 (P)	50.0	1.0	pods
S5 F1.5 (S)	50.0	1.5	stems
S5 F1.5 (P)	50.0	1.5	pods
S5 F2 (S)	50.0	2.0	stems
S5 F2 (P)	50.0	2.0	pods

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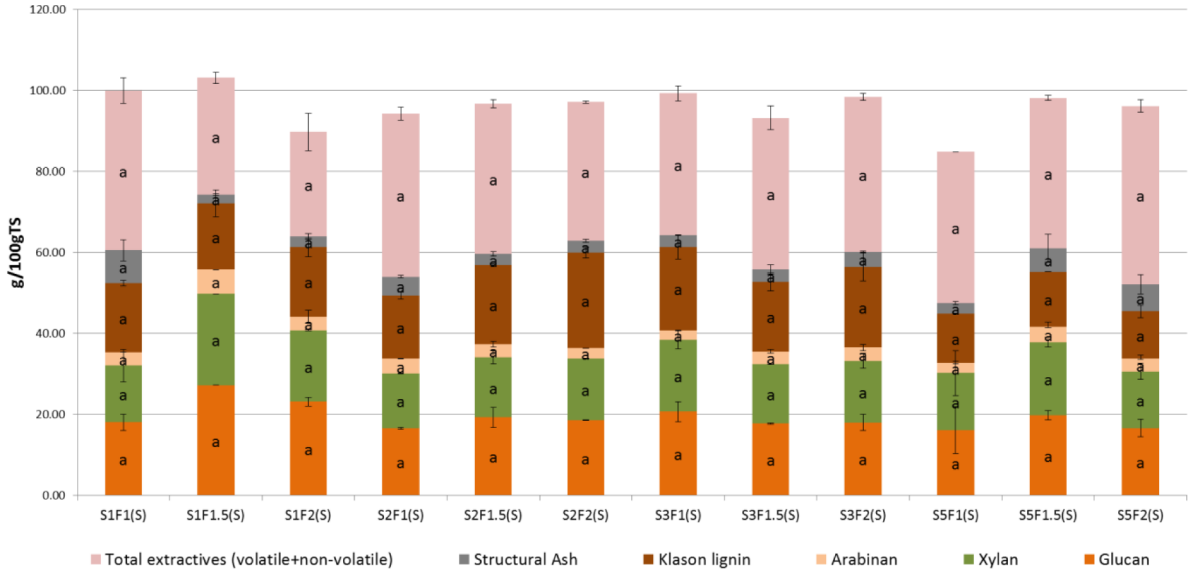

Figure 1. Chemical composition of the *S. bigelovii* samples (stem fraction) cultivated at different conditions (letters denote significant differences among treatments based on Tukey's HSD).

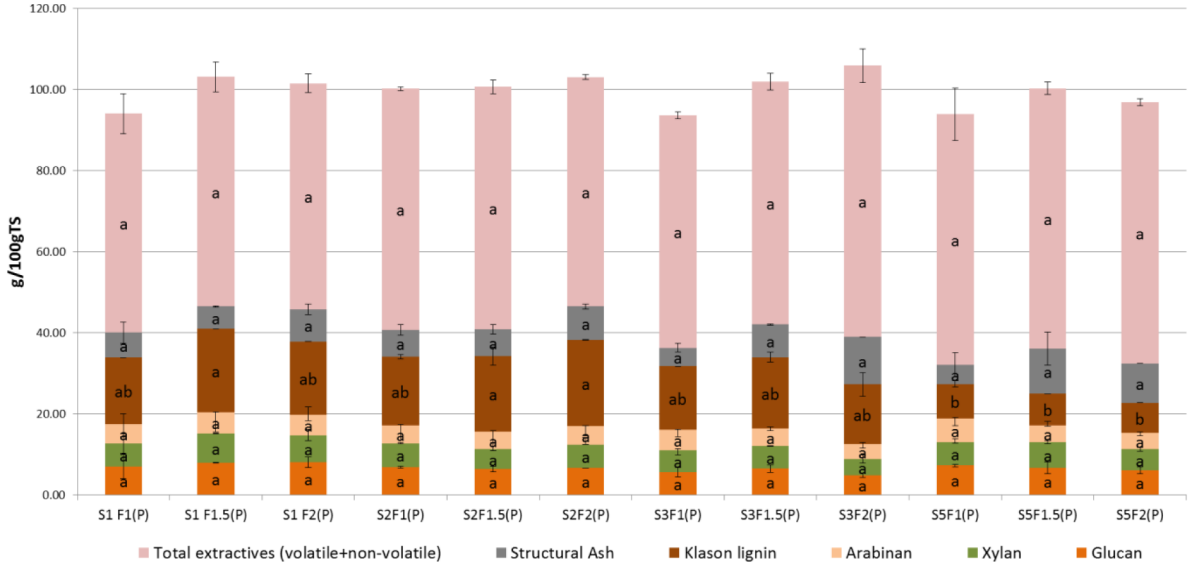

Figure 2. Chemical composition of the *S. bigelovii* samples (seed spike fraction) cultivated at different conditions (letters denote significant differences among treatments based on Tukey's HSD).

Table 2. Comparison of the Chemical Composition of Stems and Pods (Seed Spikes)

component [g/100 g TS]	stems	pods
glucan	16.02–27.12	4.73–8.03
xylan	13.51–22.63	4.00–7.27
arabinan	2.29–5.93	3.82–5.24
klason	11.61–23.63	7.44–21.31
structural ash	2.18–8.11	4.60–11.76
total extractives	25.82–44.04	54.04–66.97

Table 3. Significance of the Cultivation Factors Based on the ANOVA Performed for Lignocellulosic Components and Extractives Response Variables[a]

response variable	fraction [stems/pods]	salinity [ppt]	fertilizer [gN/m^2]
total extractives [g/100 g TS]	$p < 0.001$**	$p < 0.001$**	$p = 0.4467$
glucan [g/100 g TS]	$p < 0.001$**	$p = 0.0016$*	$p = 0.7467$
xylan [g/100 g TS]	$p < 0.001$**	$p = 0.0889$	$p = 0.9376$
arabinan [g/100 g TS]	$p < 0.001$**	$p = 0.0645$	$p = 0.0649$
lignin [g/100 g TS]	$p = 0.0019$*	$p < 0.001$**	$p = 0.1390$

[a]Asterisks indicate: * - model significant at $p < 0.01$; ** - model significant at $p < 0.001$.

Higher salinity (20 ppt and higher) was observed to negatively influence the amount of water in the plant tissues (succulence).[20] A study performed on *Salicornia bigelovii* in Mexico concluded that optimal conditions for cultivating this plant are approximately 10 ppt of sodium chloride concentration in the soil.[22,23]

Varying nitrogen fertilizer concentration (between 4 and 12 gN/m^2) used in a study on five halophyte species (*Spartina sp.*, *Distichlis palmeri*, *Paspalum vaginatum*, *Juncus roemarianus*, *Batis maritima*) grown at saline conditions (22 ppt NaCl) did not show a consistent influence on the biomass yield. Some species responded to the increasing fertilization producing more biomass,

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while others did not show a significant increase in the growth yield.[24] A study on *S. bigelovii* grown in China revealed that increasing the nitrogen content in the fertilizer used will increase the dry weight yield of the plant, but only in the case when it is in the form of nitrate. Ammonium fertilizer was concluded not to influence *S. bigelovii* growth at optimal salinity conditions (10 ppt).[25] Therefore, the nitrogen concentration in the fertilizer influences the halophyte growth in a way that is species-specific.

According to Ramawat (2010) variations in salinity of water in the soil influence carbohydrate and protein content in the halophyte species. Salinity of the irrigation water affects the plant metabolism resulting in changes in its chemical composition.[26] It has been reported that three halophyte species: *Salsola dendroides*, *Salsola richten*, and *Salsola orientalis* grown at high salinity accumulate more carbohydrates and proline as a mechanism of osmotic regulation. Positive close-linear correlations between the salinity (applied at 0−20 ppt) and soluble sugar and proline content have been observed.[27] Total sugar content in different halophytic plants grown in high salinity environment (20 ppt NaCl) has been reported to be between 10 and 39 mg/g dry weight.[28] Thus, salinity has been proven to influence the biomass yield and chemical composition of the halophytes.

No studies on the full chemical composition of *S. bigelovii* related to cultivation conditions have been presented to date. This work presents the composition variation of *S. bigelovii* straw in relation to varying salinity of the irrigation water and nitrogen content in the fertilizer used. The components of interest in this study include carbohydrates, lignin, extractives, and ash, as *S. bigelovii* straw is a potential feedstock for bioenergy production.

2. MATERIALS AND METHODS

2.1. *Salicornia bigelovii*. *S. bigelovii* is an annual salt marsh succulent native to North America and the Caribbean. It has been investigated as a possible candidate oilseed crop for seawater irrigation.[29,30] Wild collected seed from the U.S. state of Texas were provided to the International Center for Biosaline Agriculture (ICBA) in Dubai, United Arab Emirates, to cultivate. The effort described here was an initial trial of a larger project to develop an integrated seawater agriculture/aquaculture operation to produce aviation biofuels from halophytes.[31] Cultivation was performed using saltwater at four salinity levels (10, 20, 30, and 50 ppt of NaCl concentration) and ammonium nitrate as a fertilizer at three fertilization levels (1.0, 1.5, and 2.0 gN/m^2). Seeds were sown in early February into 4 m^2 field plots with sandy soil via two methods. In one case, seed was sown directly into plots. In the other method seed was sown into Jiffy-7 pellets, and Jiffy-7 pellets were transplanted into the field. The seedlings established in the Jiffy-7 pellets were transplanted at 30 cm intervals each, near the drippers, which were placed at 30 cm within and between the irrigation lines. Survival was much higher for plants grown from Jiffy-7 pellets. Plants were irrigated three times a day with drip irrigation adjusted to the water requirement plus a 20% leaching fraction to reduce soil salinity. The straw was separated from the oil seeds after harvesting. The resulting biomass (stems, seedless inflorescences, and branches) was dried and then separated into stems and seedless seed spikes (for brevity, seed spikes are called pods in all figures). Separated fractions were milled using a knife mill (IKA, 10 MF Basic) to achieve a particle size of <1 mm. Weight fraction of stems and seed spikes in relation to the whole plant were estimated gravimetrically.

2.2. Chemical Composition Analysis. Complete biomass compositional analysis was applied to the milled samples of *S. bigelovii*. The analysis followed standard analytical protocols developed by National Renewable Energy Laboratory (NREL)[32,33] and was performed in two steps: determination of the extractives and lignocellulosic components (carbohydrates and lignin). Additionally, the mixed biomass (including stems and seed spikes) was analyzed for the inorganics (mineral) content using inductively coupled plasma optical emission spectrometry (ICP-OES).

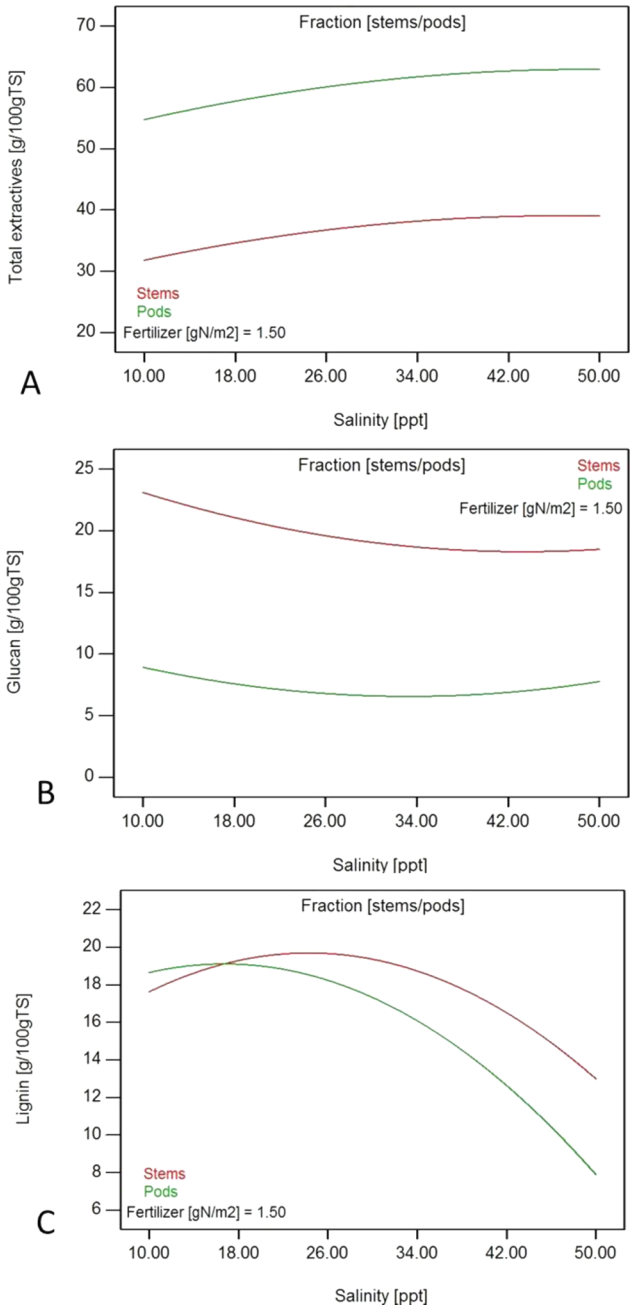

Figure 3. Interactions between the factors at the 1.5 gN/m^2 treatment (regression of the data presented in Figure 2). (A) Total extractives content [g/100 g TS], (B) Glucan content [g/100 g TS], (C) Lignin content [g/100 g TS].

2.3. Determination of Water- and Ethanol-Soluble Extractives. The procedure for determining the extractives content was composed of three steps—measuring the weight loss of the extracted solid and analyzing solids in the water extract and ethanol extract.[33] Dried and finely ground samples were subjected to water extraction followed by ethanol extraction using a Soxhlet apparatus. The biomass (5 g) was loaded into a cellulose thimble and subjected to 7 h of extraction with 200 g of water (with 3−4 siphon cycles per hour) and 7 h extraction with 200 g of ethanol (5−6 siphon cycles per hour). After the extraction, the solid was removed from the thimble, dried in the drying oven (at 105 °C) overnight, and weighed to determine the total extractives amount (including volatile and nonvolatile extractives) (eq 1).

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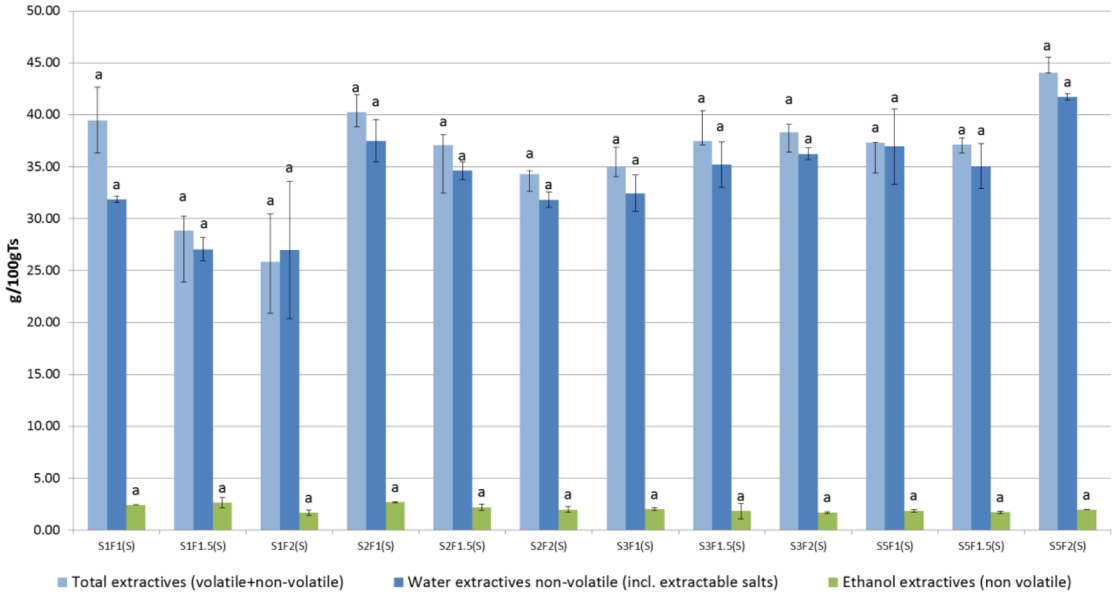

Figure 4. Water and ethanol extractives content in the *S. bigelovii* samples (stem fraction) cultivated at different conditions (letters denote significant differences among treatments based on Tukey's HSD).

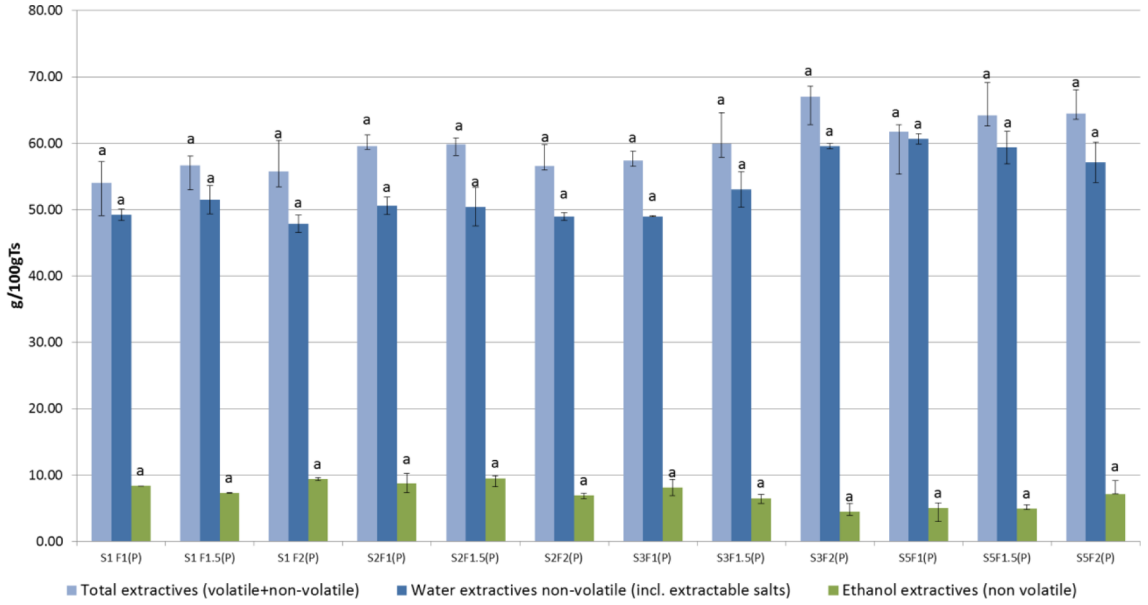

Figure 5. Water and ethanol extractives content in the *S. bigelovii* samples (pod fraction) cultivated at different conditions (letters denote significant differences among treatments based on Tukey's HSD).

$$\text{total extractives (TE)}\left(\frac{\text{g}}{100\ \text{g TS}}\right) = \frac{\text{TS} - W_{\text{dried extracted biomass}}}{\text{TS}} * 100 \quad (1)$$

where TS – total solids of the original biomass [g]; $W_{\text{dried extracted biomass}}$ – weight of the extractives-free biomass removed from the thimble and dried [g].

The nonvolatile water- and ethanol-soluble extractives were measured by evaporating the extracts to dryness and thus determining the solids content gravimetrically (eq 2).

$$\text{nonvolatile extractives (NE)}\left(\frac{\text{g}}{100\ \text{g TS}}\right) = \frac{W_{\text{dried water or ethanol extract}}}{\text{TS}} * 100 \quad (2)$$

where $W_{\text{dried water or ethanol extract}}$ – weight of the extract (evaporated to dryness) [g].

Water extracts were additionally analyzed for free sugars (glucose, xylose, and arabinose) and organic acids (acetic, lactic, and formic acids) using high performance liquid chromatography Agilent 1260 Infinity Bio-inert Binary LC). The Hi Plex-H column (Agilent) and refractive index detector (RID) were used to determine the concentrations of glucose, xylose, and arabinose at 65 °C using 0.005 M H_2SO_4 as the mobile phase (eluent) with a flow rate of 0.6 mL/min. The content of sugars and organic acids was calculated per total solids of the original biomass sample to estimate the amount of these components that have been released during extraction (eq 3).

$$\text{sugar/acid}_{\text{extract}}\left(\frac{\text{g}}{100\ \text{g TS}}\right) = \frac{C_{\text{extract}} V_{\text{extract}} \frac{1\ \text{g}}{1000\ \text{mg}}}{\text{TS}} * 100 \quad (3)$$

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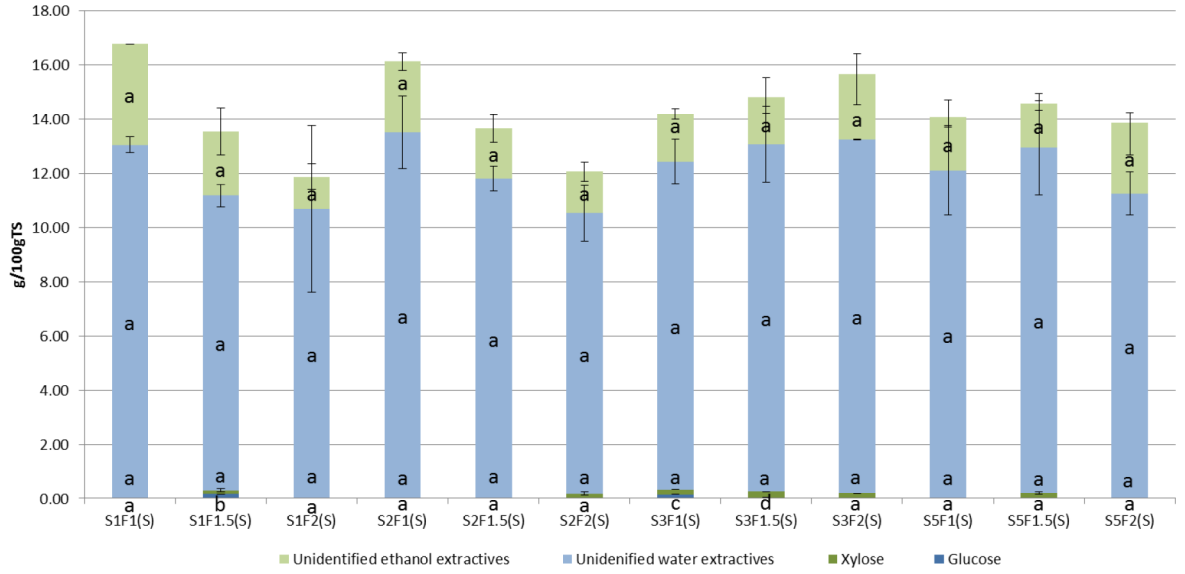

Figure 6. Composition of the extractives obtained from *S. bigelovii* samples (stem fraction) cultivated at different conditions (letters denote significant differences among treatments based on Tukey's HSD).

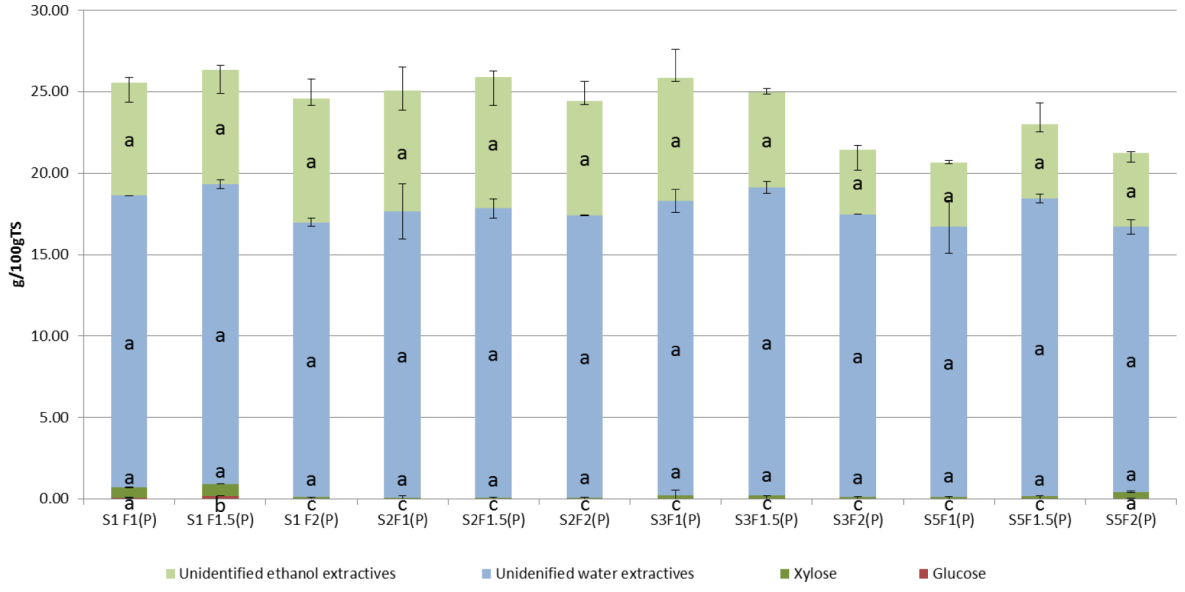

Figure 7. Composition of the extractives obtained from *S. bigelovii* samples (pod fraction) cultivated at different conditions (letters denote significant differences among treatments based on Tukey's HSD).

Table 4. Significance of the Cultivation Factors Based on the ANOVA Performed for Water and Ethanol Extractives (Ash-Free) Content Response Variables[a]

response variable	fraction [stems/pods]	salinity [ppt]	fertilizer [gN/m^2]
water extractives ash-free [g/100 g TS]	$p < 0.001$**	$p = 0.9969$	$p = 0.0759$
ethanol extractives ash-free [g/100 g TS]	$p < 0.001$**	$p < 0.001$**	$p = 0.1103$

[a]Asterisks indicate model significant at $p < 0.001$.

where $C_{extract}$ – concentration of a measured component (sugar or organic acid) analyzed by the HPLC [mg/mL]; $V_{extract}$ – volume of the extract [mL].

2.4. Determination of Lignocellulosic Components (Carbohydrates and Lignin). After the extraction with water and ethanol, the dry solid (referred to as "extractives-free") was subjected to a strong acid hydrolysis, following the NREL protocol.[32] The biomass (0.3 g) was digested with 3.0 mL of 72% sulfuric acid for 1 h at 30 °C, and then 84 mL of deionized water was added to quench the reaction by diluting the acid to 4%. The mixture was then autoclaved at 121 °C for 1 h. After the digestion, the hydrolyzate was filtered through a fritted ceramic funnel, and the filtrated was analyzed for carbohydrates and digestion byproducts using the HPLC at conditions as described above. Klason lignin was quantified as the acid insoluble residue weight on the fritted ceramic funnel.

Carbohydrate content in the extractives-free biomass was calculated as presented by eq 4.

$$\text{sugar}_{\text{extractives free}}\left(\frac{\text{g}}{100\ \text{g TS}}\right) = \frac{C_{\text{anhydro}} V_{\text{hydrolysate}} \frac{1\ \text{g}}{1000\ \text{mg}}}{\text{TS}_{\text{extractives-free}}} * 100 \quad (4)$$

where C_{anhydro} – concentration of a measured sugar converted to its polymeric form, additionally corrected for degradation during the dilute-acid step of the hydrolysis (using a recovery factor measured by spiking

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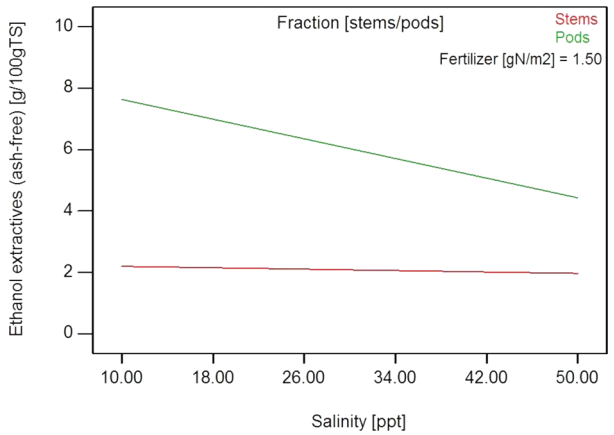

Figure 8. Interactions between the factors for ethanol extractives response variable (regression of the data presented in Figures 6 and 7).

sample replicates with a standard) [g/L]; $V_{\text{hydrolyzate}}$ – volume of the hydrolyzate [mL].

Carbohydrate content in the extractives-free biomass does not reflect its content in the original plant; thus the carbohydrate content on the "as received" basis was additionally calculated to reflect the original plant composition (eq 5).

$$\text{sugar}_{\text{as received}}\left(\frac{\text{g}}{100\ \text{g TS}}\right) = \text{sugar}_{\text{extractives free}}\frac{(100 - \text{extractives})}{100} \tag{5}$$

Klason lignin (acid insoluble lignin) was calculated based on the weight measurement of the dry residue remaining after the acid digestion (eq 6). Lignin content in the original plant was then calculated following the principle of eq 5.

$$\text{AIL}_{\text{extractives free}}\left(\frac{\text{g}}{100\ \text{g TS}}\right) = \frac{(W_{\text{AIL}})}{\text{TS}_{\text{extractives free}}}*100\% \tag{6}$$

where AIL – acid insoluble lignin [g/100 g TS]; W_{AIL} – weight of AIL after drying at 105 °C [g].

2.5. Determination of Ash (Structural, Extractable and Total). Mineral salt content (measured as ash) plays an important role in the *S. bigelovii* characterization, as it represents up to 50% of the plant composition. Part of the salts is deposited on the plant surface, making it easy to remove by simple fresh water extraction or wash. Therefore, it is crucial for the processing of this plant to measure the content of the ash that is incorporated in the plant matrix (structural ash) as well as the ash that can be easily extracted (extractable ash). Total ash was measured in the original plant following the NREL protocol[34] (eq 7). Extractable ash was analyzed by measuring the total solids and then ash content in the water extracts, and then relating it to the dry matter raw sample (eq 8). Ash content measured in the extractives-free material (after extractable ash removal) was characterized as structural ash (eq 9).

$$\text{TA}\left(\frac{\text{g}}{100\ \text{g TS}}\right) = \frac{\text{ash weight in raw [g]}}{\text{TS [g]}}*100\% \tag{7}$$

$$\text{EA}\left(\frac{\text{g}}{100\ \text{g TS}}\right) = \frac{\text{ash weight in the extract [g]}}{\text{TS [g]}}*100\% \tag{8}$$

$$\text{SA}\left(\frac{\text{g}}{100\ \text{g TS}}\right) = \frac{\text{ash weight in extractives} - \text{free [g]}}{\text{TS [g]}}*100\% \tag{9}$$

2.6. Mineral Analysis (ICP-OES). Dry *S. bigelovii* samples (10 g) were ashed at 450 °C for 12 h. Leaching of the resulting ash was performed with aqua regia (concentrated nitric and hydrochloric acid mixed in a volume ratio of 1:3). The mixture was centrifuged and the supernatant was collected while the residue was treated with hot concentrated hydrofluoric-nitric acid mixture until dissolution. The resulting solution was then mixed with the collected supernatant and analyzed using Varian vista ICP-OES instrument for aluminum (Al), barium (Ba), calcium (Ca), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorus (P), rubidium (Rb), scandium (Sc), strontium (Sr), vanadium (V), yttrium (Y), zinc (Zn). Only elements detected in amounts above 100 ppm of total dry matter were reported and calculated based on the ash content.

2.7. Statistical Analysis. Experimental design used in this study was a full factorial with three factors: one categorical factor (fraction: stems or seed spikes) and two numeric factors (salinity of the water used for irrigation and fertilizer concentration). The categorical factor was analyzed on two levels, while salinity and fertilizer factors were analyzed on five and three levels, respectively. The experimental design was summarized in Table 1, along with coding used to simplify each sample's characteristics.

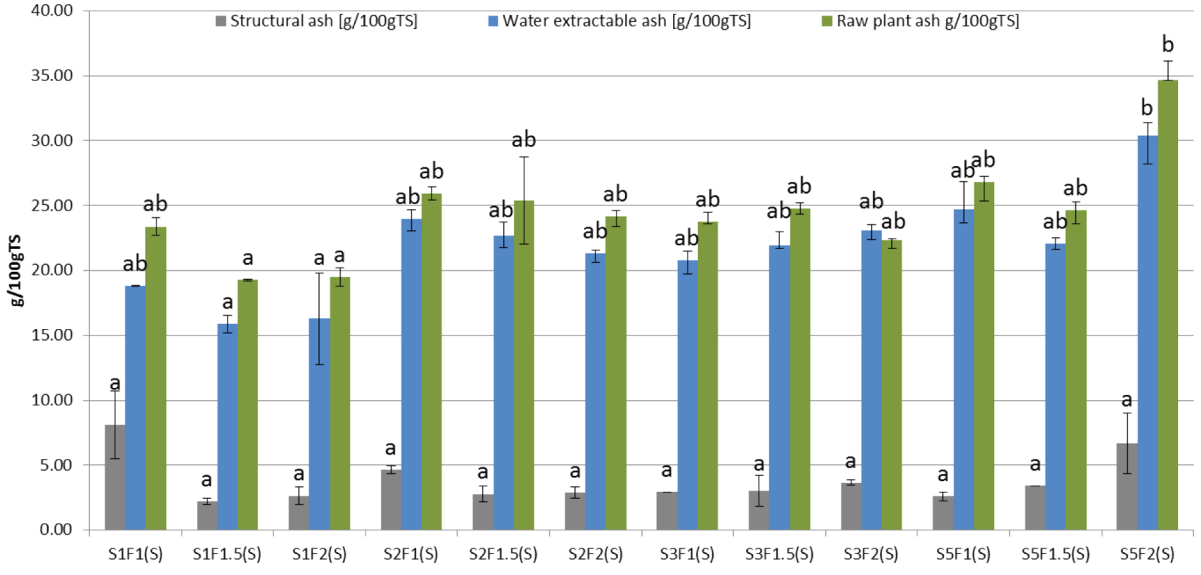

Figure 9. Ash distribution in *S. bigelovii* samples (stem fraction) cultivated at different conditions (letters denote significant differences among treatments based on Tukey's HSD).

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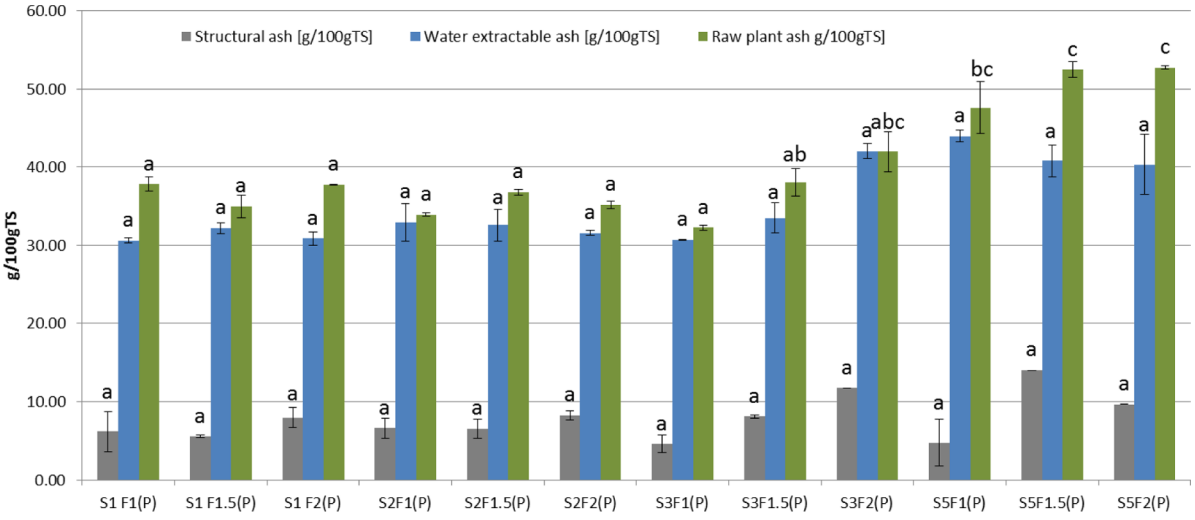

Figure 10. Ash distribution in *S. bigelovii* samples (pod fraction) cultivated at different conditions (letters denote significant differences among treatments based on Tukey's HSD).

Table 5. Significance of the Cultivation Factors Based on the ANOVA Performed for Total, Extractable and Structural Ash Content Response Variables[a]

response variable	fraction [stems/pods]	salinity [ppt]	fertilizer [gN/m^2]
total ash [g/100 g TS]	$p < 0.001$**	$p < 0.001$**	$p = 0.1088$
extractable ash [g/100 g TS]	$p < 0.001$**	$p < 0.001$**	$p = 0.1928$
structural ash [g/100 g TS]	$p < 0.001$**	$p = 0.0383$*	$p = 0.0047$**

[a]Asterisks indicate: * - model significant at $p < 0.01$; ** - model significant at $p < 0.001$.

Analysis of variance was performed along with generating the regression models for the following response variables: glucose [g/100 g TS], xylose [g/100 g TS], arabinose [g/100 g TS], lignin [g/100 g TS], total extractives (including extractable ash) [g/100 g TS], water and ethanol extractives (ash-free) [g/100 g TS], total, structural and extractable ash [g/100 g TS] as well as composition of the ash (including aluminum, calcium, iron, potassium, magnesium, phosphorus, and sodium) [g/100 g ash]. Ash composition was tested only for the influence of two factors—salinity of the irrigation water and fertilizer concentration. Both factors were tested in the same ranges as presented in Table 1. Analyses were conducted using Design Expert 8.0.7.1 software. Additionally, mean comparisons over all treatments have been performed using Tukey's honestly significant difference (HSD) method calculated with a critical q-value at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Determination of Extractives and Lignocellulosic Components. On the basis of the gravimetric measurements, stems were found to represent 25.30 ± 0.42 g/100 g TS, and seedless seed spikes were found to represent 74.40 ± 0.42 g/100 g TS of the whole plant. Results of the full compositional analysis of *S. bigelovii* are presented in Figures 1 and 2. A substantial part of *S. bigelovii* composition was represented by the extractives (from 25.82 ± 4.64 g/100 g TS to 66.97 ± 4.16 g/100 g TS), which included water- and ethanol-soluble extractives and extractable ash. The highest extractives content was found in the seedless seed spikes fraction (spanning between 54.04 ± 4.94 g/100 g TS to 66.97 ± 4.16 g/100 g TS), which was almost double the extractives content in the stems fraction (25.82 ± 4.64 g/100 g TS to 44.04 ± 1.54 g/100 g TS). Glucan (cellulose) content was more than 2−3 times higher in the stems fraction (from 16.02 ± 5.78 g/100 g TS to 27.12 ± 0.01 g/100 g TS) when compared to the seed spikes fraction (4.73 ± 0.53 g/100 g TS to 8.03 ± 1.26 g/100 g TS). Xylan was a second carbohydrate found in significant amounts, which also differed between stems and seed spikes fractions (13.51 ± 0.14 g/100 g TS to 22.63 ± 0.01 g/100 g TS for stems and 4.00 ± 0.44 g/100 g TS to 7.27 ± 0.10 g/100 g TS for seed spikes). Arabinan was detected in relatively low amounts in both stems and seed spikes across various growing conditions (2.29 ± 0.04 g/100 g TS to 5.85 ± 0.23 g/100 g TS). Lignin was found in amounts of 7.44 ± 0.01 g/100 g TS to 23.63 ± 1.32 g/100 g TS depending on the salinity and fraction analyzed. High salinity was observed to affect lignin content negatively, especially in the seed spike fraction. The mass balance was completed for most of the samples, showing that the analysis has identified all the components of *S. bigelovii*.

As already observed, seedless seed spikes and stems are very different in their composition, regardless of the cultivation conditions. Table 2 summarizes composition of both fractions, showing that stems have significantly more lignocellulosic components, whereas the seed spikes fraction contains more extractives and structural ash.

Statistical analyses (regression models and ANOVA) revealed that the lignocellulosic components and extractives response variables all strongly depend on the analyzed plant fraction (Table 3). Salinity significantly influenced the total extractives, glucan content, and lignin content in the samples analyzed. Fertilizer concentration used during the plant growth did not have a significant influence on any of these responses. Significant interactions of the factors were illustrated in Figure 3.

Salinity was observed to positively influence total extractives content, both for seed spikes and stems (Figure 3A). This trend could be connected to the extractable ash content, which will be discussed further in this section. A clear difference between the fractions can be also seen, showing that seed spikes contain significantly higher extractives content. Glucan content in the stems was slightly negatively affected by the increasing salinity, while seed spikes remained affected to a lower extent (Figure 3B). Stems were statistically confirmed to contain significantly higher amounts of glucan when compared to the seed spikes. A negative influence of the increasing salinity on the lignin content of both stems and seed spikes was observed (Figure 3C). The stems were found to contain significantly higher lignin content than the seed

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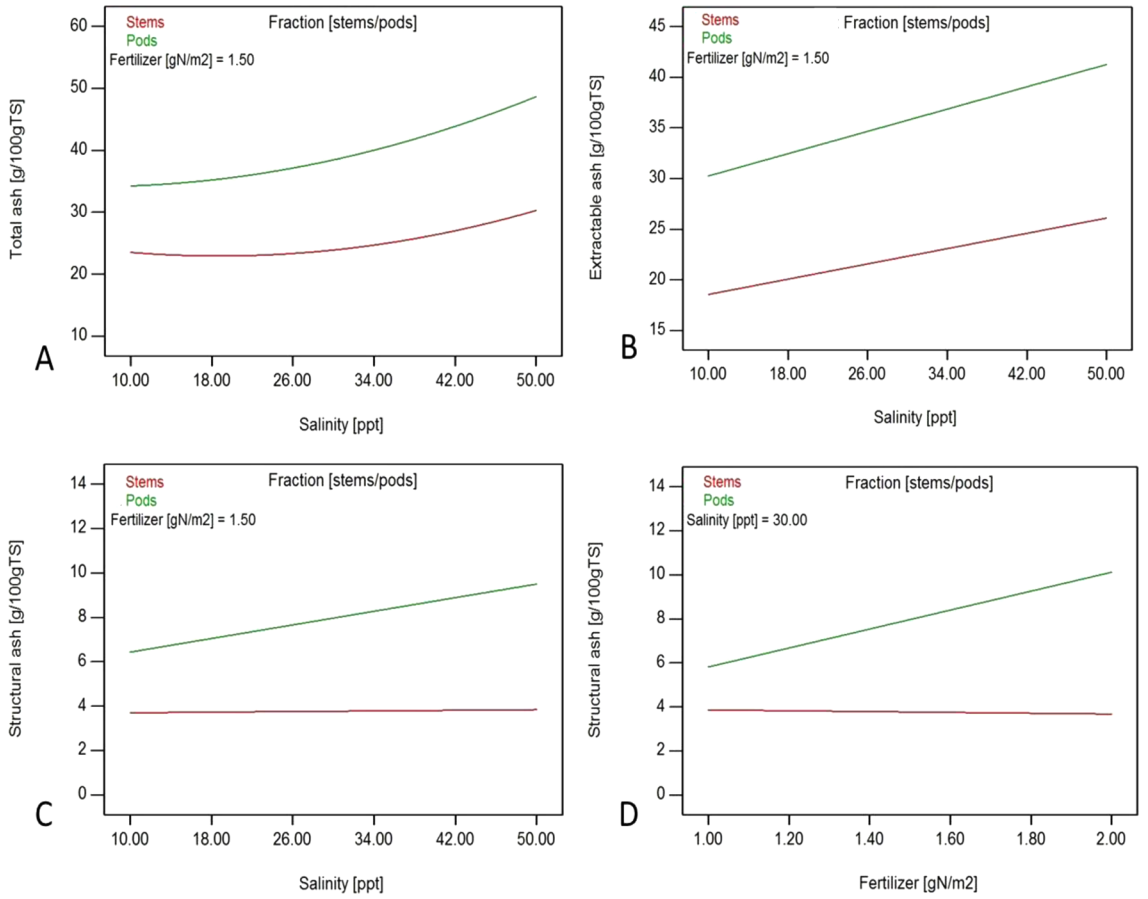

Figure 11. Interaction of the factors significant for the ash response variables (regression of the data presented in Figures 9 and 10). (A) Influence of irrigation water salinity on the total ash content [g/100 g TS], (B) influence of irrigation water salinity on extractable ash content [g/100 g TS], (C) influence of irrigation water salinity on structural ash content [g/100 g TS], (D) influence of fertilizer grade on the structural ash content [g/100 g TS].

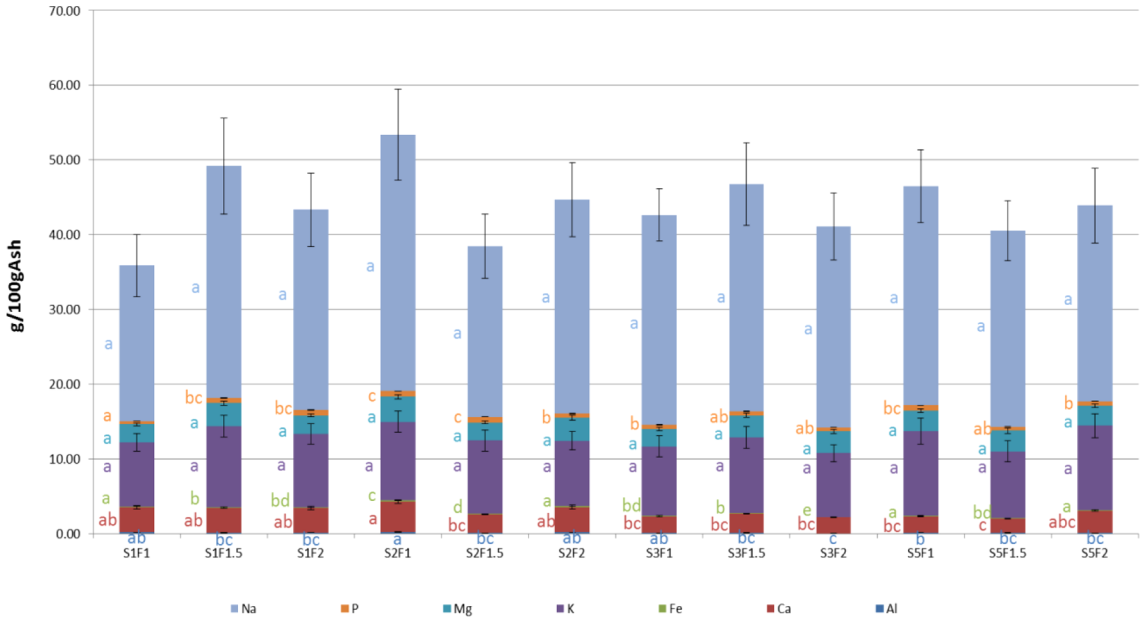

Figure 12. Mineral composition of ash obtained from *S. bigelovii* (whole plant) cultivated at different conditions (letters denote significant differences among treatments based on Tukey's HSD).

spikes at higher salinities. This fact, together with higher glucan content, makes the stems more typical lignocellulosic material than the seed spikes.[7,35]

3.2. Composition of the Extractives. As mentioned above, extractives represent a substantial part of the *S. bigelovii* composition. The majority of the total (volatile and nonvolatile)

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Table 6. Significance of the Cultivation Factors Based on the ANOVA Performed for Ash Composition Response Variables[a]

response variable	salinity [ppt]	fertilizer [gN/m^2]
aluminum [g/100 g ash]	$p = 0.0180$*	$p = 0.0040$**
calcium [g/100 g ash]	$p < 0.001$**	$p = 0.7361$
iron [g/100 g ash]	$p = 0.0489$*	$p = 0.0003$**
potassium [g/100 g ash]	NSM[b]	NSM
magnesium [g/100 g ash]	NSM	NSM
phosphorus [g/100 g ash]	NSM	NSM
sodium [g/100 g ash]	NSM	NSM

[a]Asterisks indicate: * - model significant at $p < 0.01$; ** - model significant at $p < 0.001$. [b]NSM = nonsignificant model.

extractives is represented by nonvolatile water extractives (approximately 80% in all samples) (Figures 4 and 5). The remaining part is mainly composed of nonvolatile ethanol extractives. Again, seed spikes were observed to contain almost twice as much of both water and ethanol extractives than the stems.

Since halophytes have been found to contain valuable active components in their various extracts, including organic acids (e.g., chlorogenic acid),[36] sterols (e.g., stigmasterol or β-sitosterol),[37] flavonoids (e.g., quercetin),[38] triterpenoid saponins (e.g., oleanolic acid glucoside),[39] and betaine[40] found in *Salicornia spp.*, the amount of the ash-free extractives was calculated (by removing extractable ash), in order to estimate the "true" extractives content. Analyses of the extracts for free sugars and simple organic acids (acetic, lactic, formic, propionic acids) failed to identify most of their components, detecting only small amounts of glucose (0.00−1.53 g/100 g TS) and xylose (0.00−3.78 g/100 g TS). Therefore, most parts of both extractives fractions remain unidentified (Figures 6 and 7).

ANOVA and regression models revealed that the fertilizer amount used during the plant cultivation did not affect either water- or ethanol-soluble extractives (ash-free) content in the harvested plant (Table 4). Salinity was found to have a significant influence on the ethanol extractives content, but did not affect the water extractives. Extractives content was significantly different for seed spikes and stems, being higher in the seed spikes fraction. Thus, water extractives content was found to depend only on the plant fraction, while ethanol extractives content was affected by both plant fraction and salinity, as illustrated in Figure 8. Interestingly, a significant linear negative effect of the salinity factor was only detected for the seed spikes fraction of the plant. This could be caused by the lower oil production at the high salinity, assuming most of the extractives in the seed spikes are represented by fatty acids (ICBA, 2011).

3.3. Distribution and Composition of Ash. As discussed above, *S. bigelovii* is a halophyte and thrives in high-salinity environments. However, salt content present in the irrigation water is generally not taken up by the plant to be built into its internal structure. Most of the salt present in the raw plant, which was found in amounts between 19.26 ± 0.07 g/100 g TS and 52.71 ± 0.21 g/100 g TS (Figures 9 and 10), is in the form of salt deposits on the plant surface. Thus, a large fraction of the initial ash content in the raw plant is in the form of "extractable ash" (representing 80−95% of the total ash), which can be removed by a simple fresh water wash. Structural ash, which represents the salts incorporated in the plant structure, was found in amounts of 2.18 ± 0.24 g/100 g TS to 8.11 ± 2.60 g/100 g TS for stems and 4.60 ± 1.10 g/100 g TS to 14.04 ± 0.01 g/100 g TS for seed spikes. As it can be observed from Figures 9 and 10, stems contain less ash both in their structure and on the surface when compared to the seed spikes. Structural ash content of the

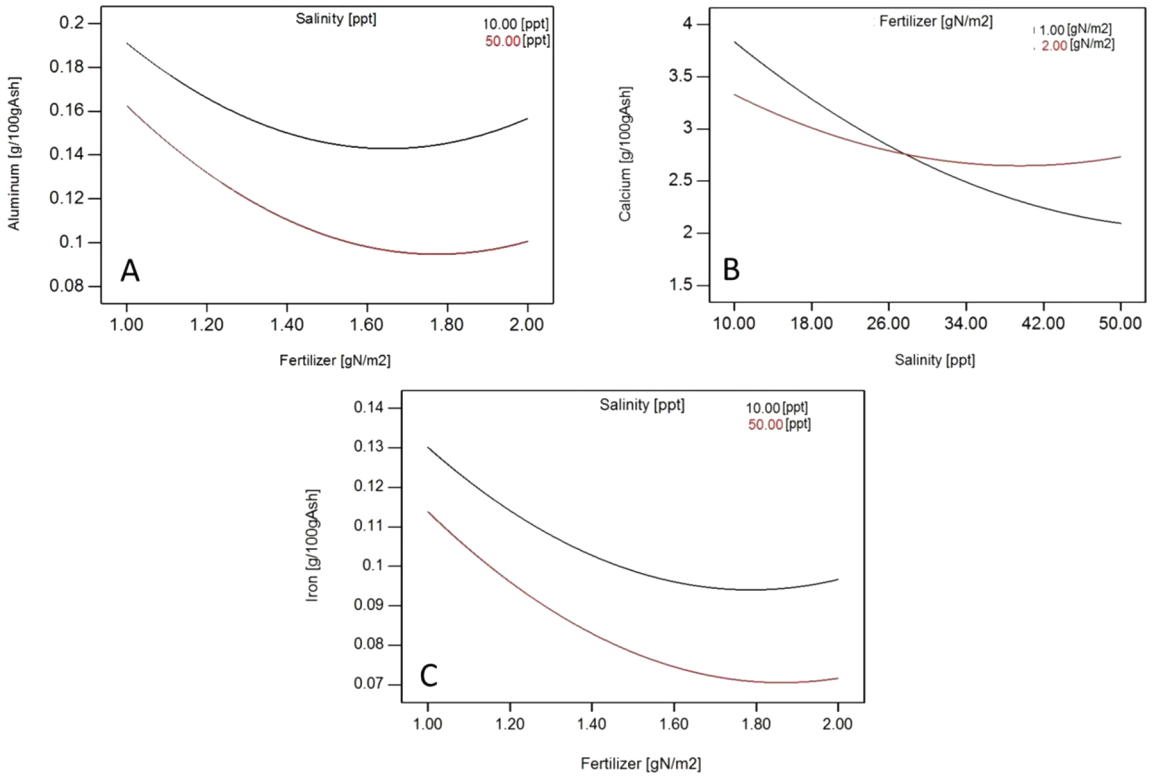

Figure 13. Interaction of the factors found significant for the ash composition response variables (aluminum (A), calcium (B), and iron content (C) [g/100 g ash] (regression of the data presented in Figure 12).

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S. bigelovii stems is similar to that of most of the lignocellulosic biofuel feedstocks, such as prairie grasses or corn stover.[4]

Statistical analysis of the factor influence on the response variables revealed that fraction of the plant had a dominant influence on all three responses analyzed (Table 5), and seed spikes were observed to have a significantly higher content of total, extractable, and structural ash. As expected, salinity was a significant factor for all three responses, and fertilizer influenced only the structural ash.

As can be observed from Figure 11A, total ash was positively influenced by salinity, especially for the seed spikes fraction. A similar correlation was previously found in the case of another halophyte species (*Suaeda fruticosa*).[20] Postitive linear correlation between the extractable ash and salinity of the irrigation water was observed for both fractions analyzed (Figure 11B). This trend can be expected, as with increasing salinity of the water used for irrigation, higher amounts of salt could be found precipitated on the surface of the plant. Structural ash content in the seed spikes fraction was positively affected by both salinity of the irrigation water and fertilizer content used during cultivation of the plant, while no significant correlation with these factors was found for the stems (Figure 11C,D). This suggests that seed spikes fraction contains ash added by the water salinity and fertilizer in its internal structure, which cannot be removed by water extraction. Stems fraction was found free of the added ash, as no correlation was detected between structural ash and cultivation conditions.

As the main component of the irrigation water was sea salt, sodium chloride was expected to be a dominant salt in the ash of *S. bigelovii*. Results presented in Figure 12 include only the metals found in amounts higher than 100 ppm calculated per total solids of the samples analyzed. As anticipated, sodium was the most abundant mineral found in the plant ash, representing 20.81 ± 4.19 g/100 g ash to 34.28 ± 6.11 g/100 g ash. Second mineral found in relatively high amounts was potassium (8.53 ± 1.18 g/100 g ash to 11.31 ± 1.61 g/100 g ash). Other minerals were found in the plant ash in lower amounts (spanning between 0.07 and 4.07 g/100 g ash) and included calcium, magnesium, phosphorus, iron, and aluminum.

Significant regression models were found only for aluminum, calcium, and iron content in *S. bigelovii* ash. This means that no significant influence of the cultivation factors (salinity of the irrigation water and fertilizer amount applied) was observed for potassium, magnesium, phosphorus, or sodium content in the plant ash. Aluminum content was found to decrease significantly with increasing fertilizer amount and with increasing salinity of the irrigation water (Table 6 and Figure 13A). Calcium content in the ash was observed to be significantly influenced only by the salinity (Table 6), and the correlation was negative (Figure 13B). A similar trend was observed in a previous study, stating that excessive salinities can lead to calcium deficiencies in this plant.[23] Another succulent halophyte was also found to follow this correlation (*S. fruticosa*).[20] Iron content, similar to aluminum, decreased significantly with increasing fertilizer amount and salinity (Table 6 and Figure 13C).

4. CONCLUSIONS

S. bigelovii straw biomass was found to have a typical lignocellulosic composition, especially the stems fraction of the plant. The main difference in processing this biomass when compared to a typical lignocellulosic material is the need for a freshwater wash, due to high ash content resulting from salt deposits on the plant surface (up to 53 g/100 g TS). However, recycled water extraction was very efficient in salt removal, extracting up to 90% of the total ash. Extractives were found to represent a substantial component of the plant structure (up to 25g/100 g TS), especially in the pod fraction. Analyses of extractives showed low concentrations of sugars and simple organic acids, and thus further study is needed to quantify the unidentified components.

The analysis of variance of the samples tested showed that the composition of the plant can be in part manipulated by the cultivation conditions. Stem and seedless spike fractions of *S. bigelovii* were found to be significantly different in their composition. Lignocellulosic components were observed to decrease with the increasing salinity for both stems and seed spikes, while the fertilizer concentration did not influence these responses. Extractable ash was found to strongly depend on the salinity of the irrigation water for both fractions, which is to be expected since this form of ash is made up of the salt deposits on the plant surface. However, structural ash of the seed spikes was observed to be affected by both water salinity and fertilizer concentration, which means that both of those factors influence its internal structure composition. Ash mineral composition was partly affected by both factors, specifically in the case of aluminum, calcium, and iron content. In all cases higher salinity and/or fertilizer grade had a decreasing effect.

As a lignocellulosic material, *S. bigelovii* straw is a potential feedstock for bioethanol production in arid regions of the world, such as Middle East or North Africa. However, due to a lower glucan content than typical second generation feedstocks, an additional source of coproducts should be explored. Extractives represent an interesting and not-yet-analyzed group of components of the *S. bigelovii* straw and have been found to contain valuable active components in other halophyte species.[36,38]

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Notes

The authors declare no competing financial interest.

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