



RESEARCH PAPER

Salinity Improves Zinc Resistance in *Kosteletzkya pentacarpos* in Relation to a Modification in Mucilage and Polysaccharides Composition

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Abstract

The halophyte plant species *Kosteletzkya pentacarpos* produces high amounts of mucilage which may play a role in heavy metal tolerance. The amount and composition of mucilage in *K. pentacarpos* exposed to zinc toxicity in the presence or in the absence of 50 mM NaCl was investigated. Exposure to 0.1 mM Zn had a detrimental impact on plants growth and water content. Most of Zn was accumulated in the roots of plants exposed to Zn toxicity both in the absence and in the presence of NaCl. Salinity, however, partially alleviated the toxicity of zinc, increasing the plant biomass and decreasing zinc absorption by the root. Crude mucilage content increased in all plant organs in response to Zn stress. Acid hydrolysis of crude mucilage extract revealed that the main neutral monosaccharide constituents were rhamnose, arabinose, galactose, and glucose. Zinc induced a rise in uronic acids in roots and stems but not in the leaves where a high constitutive proportion of uronic acids was observed. Meanwhile, changes were also observed in neutral monosaccharides component in different plant organs. Zinc stress increased the hemicellulose content, but decreased the lignin and cellulose content in the stem. Salinity slightly increased the cellulose content. We suggest that excessive zinc induces a modification in the composition and structure of polysaccharides and that mucilage may contribute to Zn tolerance through toxic ion sequestration.

Article Highlights

- *Kosteletzkya pentacarpos* is a halophyte plant species producing high amounts of mucilage.
- Salinity improved the resistance of this species to Zn excess.
- Zinc increased hemicellulose content as well as rhamnose and uronic acid in root and stem mucilage.
- Mucilage contributes to Zn tolerance through toxic ion sequestration

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Introduction

Following rapid social and economic development over the past several decades, heavy metal pollution in soil and water became one of the hottest environmental issues in the world. Although small amounts of heavy metals are naturally present in soil, local accumulation results from anthropogenic activities such as agriculture, urbanization, industrialization, and mining (Gao et al. 2017; Men et al. 2018). Zinc is one of the most frequent elements found in heavy metal polluted soil and high Zn amounts are released through waste from ore extraction, coal and fuel combustion, incineration of domestic waste, sewage sludge, and, finally, the use of fertilizer and pesticides containing zinc in agriculture (Rakotondrabe et al. 2018; Wang et al. 2017).

Zinc is an essential micro-element required for plant growth, development, and reproduction (Javed et al. 2017; Park et al. 2017). It is the only metal present in all six enzyme classes (oxidoreductase, transferase, hydrolases, lyases, isomerases, and ligases) (Baltaci et al. 2017). It acts as a catalytic cofactor (Barrameda-Medina et al. 2017) and is also involved in phytohormone regulation (Liu et al. 2017), signal transduction (Lin et al. 2005), and repairing process of PSII complex during photoinhibition (Rizwan et al. 2017). However, high concentration of Zn has deleterious impact on plant, resulting in inhibition of photosynthesis, interference with the absorption of other essential element, synthesis of reactive oxygen species, and subsequent damages to biological membranes, proteins, DNA, and RNA (Leskova et al. 2017; Pramanick et al. 2017; Yu et al. 2015).

Several techniques are available to remove heavy metals from a contaminated substrate or polluted water, but classical approaches are very expensive (Vareda et al. 2019). Phytoremediation which implies the use of heavy metal-resistant plants appears as a cheap attracting alternative (Ali et al. 2013). In areas simultaneously contaminated by salt and heavy metals, halophyte species have been recommended as a promising tool for phytomanagement purposes (Lutts and Lefèvre 2015). One of the most efficient strategies to cope with high amounts of accumulated toxic ions consists to store them in metabolically poorly active or inactive cell compartments. Vacuolar sequestration of heavy metals associated or not with phytochelatins, as well as exclusion to apoplast and cell wall retention are well documented in the literature (Printz et al. 2016; Sharma et al. 2016; Berni et al. 2019). Although less documented, heavy metal retention by mucilage is also a feasible option to limit toxic ion accumulation in cytosol.

Mucilage is composed of water-soluble heteropolysaccharides of high molecular weight comprising a mixture of

L-rhamnose, L-fucose, L-galactose, galacturonic acid, L-arabinose, D-xylose, and D-galactose. It is frequently found at the surface of the root (Morel et al. 1986, 1987; Javed et al. 2013; Lapie et al. 2019), seeds (Elboutachfati et al. 2017; Kaur et al. 2018; Lodhi et al. 2019), and fruits (Ajala et al. 2016). The high water-binding capacity of hydroxyl and carboxyl groups in the polysaccharides allows mucilage to hydrate and thus store huge amounts of water which may offer plants the ability to overcome drought periods (Minjares-Fuentes et al. 2017; Schwartz et al. 2015). Mucilage issued from root exudation may also prevent toxic metal uptake through chelation of the cations by the carboxylic groups of the uronic acids, hydroxyls, and carbonyl functions located on the polysaccharide chains (Morel et al. 1987; Javed et al. 2013; Lodhi et al. 2019; Lapie et al. 2019).

Kosteletzkya pentacarpos (L.) Presl. (syn. *Kosteletzkya virginica*) is a perennial halophyte species of Malvaceae family, which is distributed from Louisiana to Florida and north along the Atlantic coast to Delaware and the state of New York (Islam et al. 1982; Zheng et al. 2017). It is recommended as a potential resource for food, feed, biodiesel, as well as health-promoting products (Halchak et al. 2011; Qin et al. 2015; Vaughn et al. 2013). Beside a high level of resistance to salt stress, this species is also able to cope with heavy metals and the concomitant presence of NaCl was reported to improve its resistance to Cd and Zn (Han et al. 2013; Zhou et al. 2018a, b, 2019a, b). This species produces high amounts of mucilage not only at the root level but also in stems and leaves where it is localized primarily within the xylem vessels and in the leaf epidermis (Ghanem et al. 2010). Salinity may impact mucilage content and composition and Ghanem et al. (2010) demonstrated that an important fraction of accumulated Na^+ was retained on mucilage. Lutts et al. (2016) also showed that dry root powder is an efficient material for heavy metal biosorption and that salinity improved the Zn biosorption process in relation to an increase in the amounts of mucilage pectic compounds and hemicellulose. A recent study (Zhou et al. 2019c) demonstrated that for *K. pentacarpos* growing on a polycontaminated soil, salinity reduced the Zn leaching process and increased the proportion of Zn removed by the plant as a possible consequence of a Zn-induced modification of root mucilage content.

These data suggest that root mucilage contributes to heavy metal retention in the roots to limit toxic ions accumulation in the photosynthetic tissues. No data are available, however, regarding the influence of the simultaneous presence of salinity and heavy metals on the mucilage content and composition in the shoot part. The aims of the present work were: (1) to determine the impact of ion toxicities on

mucilage and cell wall polymers (cellulose, hemicellulose, and lignin) content in plants of *K. pentacarpus* exposed to deleterious concentration of Zn in the absence or in the presence of NaCl, and (2) to compare this impact for roots, stems and leaves.

Materials and Methods

Seeds of *Kosteletzkya pentacarpus* [issued from Jinhai Agricultural Experimental Farm of Yancheng (Jiangsu Province, China)] were rinsed with sterile distilled water for three times and then placed in trays containing a mix of perlite and vermiculite (1:3 v/v) regularly moistened with a half-strength modified Hoagland nutrient solution. Seedlings were grown in a phytotron under a 12 h photoperiod [PAR = 150 $\mu\text{moles m}^{-2} \text{s}^{-1}$ provided by Osram Sylvania (Danvers, MA) fluorescent tubes (F36W/133-T8/CW) with 25 °C/23 °C day/night temperature and 70%/50% atmospheric humidity]. Fifteen days after sowing, seedlings were transferred and fixed on polyvinylchloride plates floating on aerated half-strength modified Hoagland nutrient solution in 50 L tanks. The nutrient solution contained the following chemicals (in mM): 2.0 KNO_3 , 1.7 $\text{Ca}(\text{NO}_3)_2$, 1.0 KH_2PO_4 , 0.5 NH_4NO_3 , 0.5 MgSO_4 and (in μM) 17.8 Na_2SO_4 , 11.3 H_3BO_3 , 1.6 MnSO_4 , 1 ZnSO_4 , 0.3 CuSO_4 , 0.03 $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, and 14.5 Fe-EDDHA. The temperatures in the chamber were adjusted to 25 °C and 18 °C in the day and night, respectively. Light was supplemented by Philips lamps (Philips Lighting S.A., Brussels, Belgium) (HPLR 400 W) to maintain a light irradiance of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the canopy.

TF_c = Concentration in the shoot ($\text{mg g}^{-1}\text{DW}$)/concentration in the roots ($\text{mg g}^{-1}\text{DW}$)

TF_a = Total amount in the shoot (mg)/total amount in the roots (mg)

Twenty-five days after sowing (acclimation without stress), NaCl, and ZnCl_2 were added to corresponding containers to induce four treatments: (1) Control; (2) 50 mM NaCl; (3) 0.1 mM ZnCl_2 ; (4) 0.1 mM ZnCl_2 + 50 mM NaCl (Zn + Na). The pH of the solution was readjusted to 5.7 ± 0.02 with KOH every 2 days; the solution was renewed every week and tanks randomly rearranged in the phytotron

BF = Shoot concentration of heavy metal (μM)/concentration in nutrient solution (μM).

at this occasion (Zhou et al. 2018a, b). For each treatment, three replications with 12 plants per replication were used for the measurement of physiological and biochemical parameters. After 4 weeks under stress condition, roots, stems, and leaves were separately harvested for analysis.

Growth Parameters

Roots were quickly rinsed in sterile deionized water for 30 s to remove ions from the free space and gently blotted dry with a paper towel. Roots, stems, and leaves of each plant were separated and weighed for fresh weight determination ($n = 5$), and then incubated in an oven for 72 h at 70 °C for dry weight determination (Dufey et al. 2009; Ghanem et al. 2010). Water content was calculated as [(fresh weight – dry weight)/fresh weight]/100.

Evaluation of Ion Concentration

Dried plant tissues were ground to a fine powder with porcelain mortar and pestle. 50–100 mg powder of each sample was digested in 3 mL 35% HNO_3 and evaporated to dryness at 80 °C on a sand bath under the hood. Three milliliters of a mix of 37% HCl and 68% HNO_3 (3:1) were added and evaporated at 80 °C again. After full evaporation, the obtained minerals were dissolved in HCl 0.1 N. The concentrations of Na and Zn were determined by SOLAAR S4 atomic absorption spectrometry (Thermo Scientific, Cambridge, UK). For each treatment, five separated plants were considered and each analysis was performed on technical triplicates (Han et al. 2012; Lefevre et al. 2014).

The translocation factor (TF) was calculated in two ways: on the basis of concentration (TF_c) and on the basis of the total amount (TF_a) of translocated zinc, respectively. TF_c allowed us to quantify the physiological capacity of *K. pentacarpus* for Zn accumulation in relation to xylem loading, while TF_a provides information regarding the capacity of the plant to perform phytoextraction by removing a quantity of Zn from a contaminated substrate

Besides, as an indicator of the plant's ability to accumulate the heavy metal in harvestable organs, the bioaccumulation factor (BF) was also calculated. Since a nutrient solution was used in the present experiment, we expressed shoot Zn concentration on a tissue water-content basis for BF calculation according to:

Mucilage Analysis

The harvested roots, stems, and leaves of *K. pentacarpus* were oven-dried at 40 °C for 1 week. Dried material was then ground into a fine power and passed through a

0.315 mm mesh sieve. The crude mucilage in root, stem, and leaves was determined as described in Classen and Blaschek (1998). Briefly, 500 mL water was added to 5 g homogenized plant material; samples were stirred in warm water (40 °C) for 40 h, and then centrifuged at 20,000g and 4 °C for 5 min. The supernatant was collected and the pellet was re-extracted once more using the same procedure. All the aqueous supernatants were combined and concentrated to 150 mL by evaporation. 600 mL of a mixture of 96% ethanol and 1% acetic acid were then added and the obtained precipitate was washed with cold ethanol and freeze-dried. The dry weight of the lyophilized precipitate as crude mucilage was determined.

Quantification of neutral monosaccharides in the mucilage of *K. pentacarpos* tissues was performed by acetylation analysis followed by gas liquid chromatography (GLC method) (Blakeney et al. 1983): 10 mg lyophilized crude mucilage were added to 1 mL 2 M trifluoroacetic acid as well as 50 µL Inositol-Standard in leak proof Wheaton vials. After heating for 1 h at 121 °C, the samples were transferred into small conical evaporator flask to evaporate until dryness. Dry samples were then reduced with 1 mL NaBH₄ in dimethyl sulfoxide. The acetylation step was then performed with 1-methylimidazole and acetic anhydride. Afterward, the sample was extracted by adding dichloromethane. The lower phase was transferred into a GC vial to analyze by gas–liquid chromatography (HP 6890 Plus Series; Hewlett Packard) with a flame ionization detector. In addition, quantification of uronic acids has been performed by silylation analyses and GLC following the protocol of Bleton et al. (1996). The obtained chromatograms for neutral monosaccharides and for uronic acids are provided in Figs. S1 and S2, respectively (supplemental data).

Structural Polysaccharide Analysis

Concentration of lignin and structural polysaccharide (cellulose and hemicellulose) were determined according to Van Soest et al. (1991). Dry matter was crushed and exposed successively to a neutral detergent solution during 1 h at 100 °C to obtain by filtration the NDF (neutral detergent fibers), then to an acid detergent solution during 1 h to get the ADF (acid detergent fraction), and then to sulfuric acid 72% during 3 h to obtain the ADL fraction (acid detergent lignin fraction). The ADL fraction was incinerated at 550 °C during 3 h, and the mass loss allowed us to calculate the lignin percentage. Cellulose and hemicellulose were obtained according to the following equations and expressed in g per 100 g of organic matter (OM):

Hemicellulose content (g 100g⁻¹ OM) = NDF – ADF

Cellulose content (g 100g⁻¹ OM) = ADF – ADL

Lignin content (g 100g⁻¹ OM) = ADL.

Statistical Analysis

For each treatment, three 50 L tanks containing 12 plants each were used in a complete randomized block design. Tissue materials of 5 *K. pentacarpos* were dried in 70 °C oven, which were used to analyze for growth parameters and ionic determination. The remaining plant materials were dried in 40 °C oven for mucilage analysis and structural polysaccharides analysis. All parameter data were subjected to an analysis of variance, one-way ANOVA, using the SPSS software, with the treatment considered as the main factor. The statistical significance of the results was analyzed by Turkey test at 5% level ($P < 0.05$).

Results and Discussion

Plant Growth

All plants remained alive until the end of the treatment and salinity alone had no impact on plant dry weight and water content (Fig. 1). In halophyte plant species, moderate salinity may lead to plant growth stimulation allowing dilution of the ion content within the shoot (Lutts and Lefèvre 2015). Such a growth stimulation has been reported for *K. pentacarpos* (syn. *K. virginica*) at 100 mM NaCl (Ghanem et al. 2010), but our results corroborate the data of Han et al. (2012, 2013) who reported that a lower dose of 50 mM NaCl did not stimulate growth but nevertheless modified the physiological status of the plant in such a way that it more adequately responds to heavy metal toxicity.

Plants exposed to 0.1 mM Zn presented some symptoms of chlorosis and necrosis after already one week of treatment. Zinc excess had a detrimental impact on plant growth with a decrease of 60%, 52%, and 56%, for roots, stems, and leaves, respectively, after 4 weeks (Fig. 1a–c). Addition of NaCl to the Zn-containing solution, however, significantly improved the growth of all organs when compared to plants exposed to Zn in the absence of NaCl, the recorded improvement being higher for roots and leaves than for stem. As shown in Fig. 1d–f, salinity did not affect water content in plant. In contrast, Zn excess led to a significant water loss in root, stem, and leaves. However, the presence of NaCl was able to limit the deleterious impact of Zn on WC, especially in roots and stems.

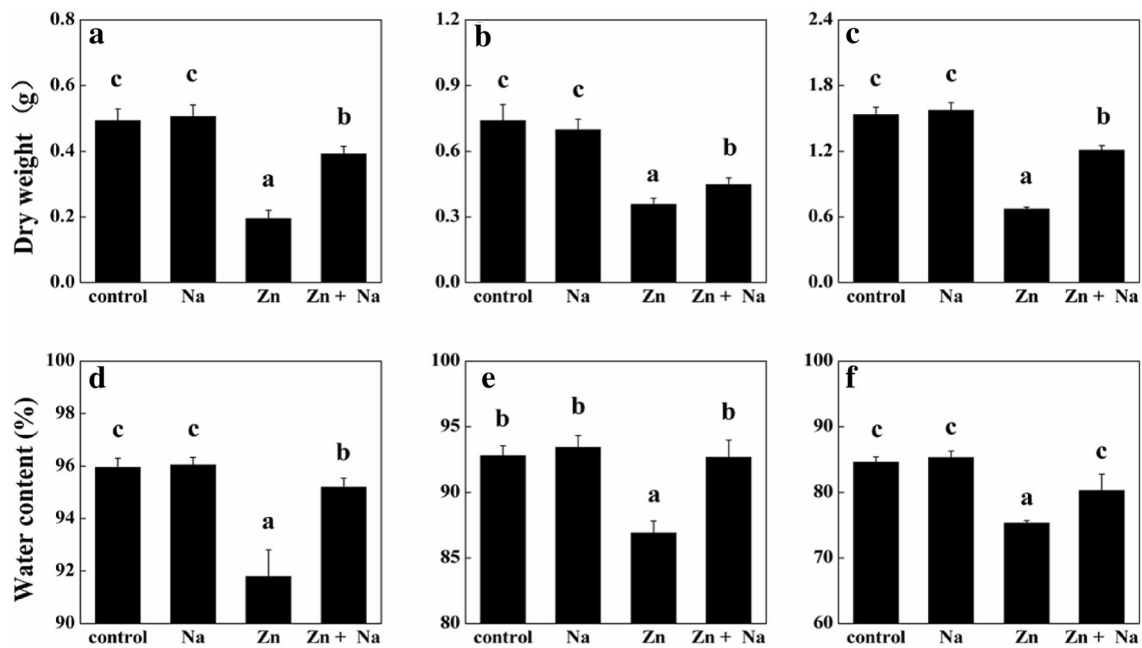


Fig. 1 Dry weight of root (a), stem (b), and leaves (c) and water content of root (d), stem (e) and leaves (f) in *Kosteletzkya pentacarpos* exposed during 4 weeks to 0.1 mM ZnCl_2 in the presence or in the

absence of 50 mM NaCl. Each value is the mean of five replicates and vertical bars are S.E. Values exhibiting different letters are significantly different at $P < 0.05$ according to Tukey's test

Salt stress induces both an ionic component related to the presence of Na^+ and Cl^- , and an osmotic component related to the decrease in the external osmotic potential (Ψ_s) of the soil solution (Ghanem et al. 2010). Heavy metals such as Zn also affect the plant water status, but this impact should not be regarded as the consequence of a decrease in external Ψ_s , since only low concentration are commonly used. Zhou et al. (2019b) demonstrated that 50 mM NaCl did not lead to osmotic adjustment in *K. pentacarpos*, while 200 μM ZnCl_2 induced a drop in Ψ_s which could be partly related to proline accumulation. According to this study, such an accumulation was reinforced by the concomitant presence of salt which could explain that, in the present study, NaCl partly alleviated the deleterious impact of Zn excess on organ water content.

Biomass production is directly related to the efficiency of the photosynthetic process which is sensitive to Zn excess in *K. pentacarpos* (Han et al. 2013). Zinc was reported to have a negative effect on the PSII chemistry by an interaction with the donor side of PSII, leading to inhibition of the Hill reaction (Tang et al. 2013). If Zn-induced inhibition of photosynthesis was the underlying cause of dry weight decrease, the fact that NaCl mitigated the deleterious impact of Zn suggests that Na^+ and/or Cl^- may positively act on photosynthesis, or that NaCl induces a decrease in Zn absorption and/or translocation. Although photosynthesis was not measured in the present study, it clearly appears from our data that NaCl itself did not improve plant growth in the absence

of Zn, and that salt-induced improvement was also observed in non-photosynthetic organs such as roots and stems.

Ion Content

As expected, control plants absorbed and accumulated physiological concentrations of Zn required for normal plant metabolism (Fig. 2). Salinity did not affect Zn concentration in plants exposed to non-contaminated solution (Fig. 2a–c). In plants exposed to 0.1 mM Zn, most of Zn accumulated in the roots in the absence as well as in the presence of NaCl (69% and 67%, respectively). Salinity, however, significantly reduced Zn accumulation in roots and leaves, while it had no significant impact on Zn concentration in the stems. These observations reinforced our hypothesis that NaCl-induced improvement of plant growth in Zn-treated plants may be due, at least partly, to a decrease in Zn accumulation in the leaves.

This hypothesis is also supported by the fact that the presence of NaCl slightly reduced BF values (Table 1), which implies that although it increases Zn resistance, the presence of NaCl could reduce the efficiency of *K. pentacarpos* for phytoextraction purposes. In contrast, there was no significant difference of TF_c and TF_a between Zn treatment and Zn + NaCl treatment (Table 1). This suggests that Zn concentrations were reduced to similar extent in shoots and roots, and that NaCl-induced decrease in shoot Zn content was the consequence of a global decrease in Zn absorption,

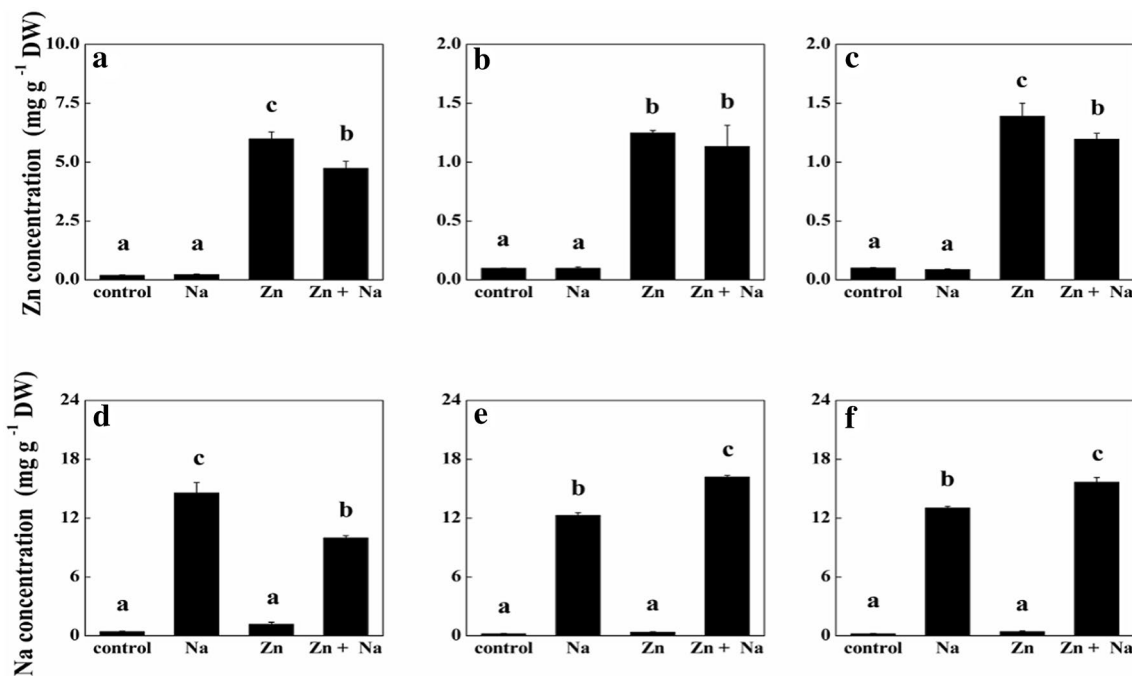


Fig. 2 Zn accumulation (mg kg^{-1} dry weight) in root (a), stem (b), as well as leaves (c) and Na concentration (mg kg^{-1} dry weight) in root (d), stem (e), and leaves (f) in seedlings of *Kosteletzkya pentacarpus* exposed during 4 weeks to 0.1 mM ZnCl_2 in the presence or in the

absence of 50 mM NaCl. Each value is the mean of three replicates and vertical bars are S.E. Values exhibiting different letters are significantly different at $P < 0.05$ according to Tukey's test

Table 1 Bioaccumulation factor (BF) and translocation factor (TF_c and TF_a) of Zn in *Kosteletzkya pentacarpus* exposed during 4 weeks to 0.1 mM ZnCl_2 in the presence or in the absence of 50 mM NaCl

	BF	TF_c	TF_a
Control	3.9 ± 0.15 b	1.0 ± 0.04 c	2.3 ± 0.10 c
50 mM Na	3.9 ± 0.67 b	0.81 ± 0.08 b	1.8 ± 0.10 b
0.1 mM Zn	3.2 ± 0.45 ab	0.43 ± 0.01 a	1.2 ± 0.17 a
Zn + Na	2.5 ± 0.34 a	0.49 ± 0.04 a	1.1 ± 0.08 a

Each value is the mean of five biological replicates. Values exhibiting different letters are significantly different at $P < 0.05$ according to Tukey's test

rather than an inhibition of Zn translocation. Zinc absorption and long-distance transport are under the control of a huge number of transporters, which differ in terms of precise location and affinities (Gupta et al. 2016). Some of them are involved in cellular absorption, while others are involved in xylem loading in the roots and unloading in the leaves. It may be hypothesized that those transporters may be affected by the presence of 50 mM NaCl. On the other hand, the rise in the solution electrical conductivity could have modified Zn speciation in the solution, and the presence of chloro-complex (ZnCl^+) may reduce Zn absorption as previously suggested for Cd (Lefèvre et al. 2009). In another saltmarsh species *Juncus acutus*, Mateos-Naranjo

et al. (2018) reported that besides a NaCl-induced decrease in Zn absorption, salinity also clearly helped to maintain photosynthesis in Zn-treated plants mainly through an overall protecting effect of the photosynthetic machinery and maintenance of Rubisco activities despite the presence of a Zn excess. Glycinebetaine is a well-known osmoprotecting compound, mainly accumulating in chloroplasts, especially in plants belonging to the family of Malvaceae and which helps to stabilize the photosynthetic apparatus in salt stress conditions. Although glycinebetaine was indeed reported in *K. pentacarpus*, Zhou et al. (2019b) recently demonstrated that glycinebetaine did not accumulate in response to 50 mM NaCl and could thus not explain the protection afforded by salinity to Zn-treated plants. According to Han et al. (2013), salinity may also induce a modification in the tissular distribution of accumulated Zn in reproductive organs of *K. pentacarpus* and this prompted us to consider mucilage synthesis in vegetative organs and the impact of NaCl on mucilage content and composition.

Sodium concentration increased from 0.45 ± 0.02 to 14.6 ± 1.0 mg g^{-1} DW in root of control and NaCl-treated plants, respectively (Fig. 2d–f). The presence of Zn in the solution significantly decreased the root Na concentration to 10.0 ± 0.18 mg g^{-1} DW, while it significantly increased Na concentration in stem and leaf. This indicates that the excess

of Zn increased Na translocation from the root to the shoot even if NaCl conversely had no impact on Zn translocation.

Mucilage and Polysaccharide Analysis

The total amount of Zn accumulated within a given organ is not fully relevant from its real physiological impact, since Zn may be accumulated in specific compartments where it has no or low physiological impact (Lefèvre et al. 2014). This is especially the case for mucilage-producing plant species, since divalent cations are known to bind more or less strongly to mucilage polysaccharides (Morel et al. 1986, 1987; Lapie et al. 2019; Lodhi et al. 2019). Mucilage deposit occurs in specific areas within the plant. Although mucilage at the root surface is reported in a wide range of species as a result of root exudation (Schwartz et al. 2015; Cai et al. 2013), wetland plant species (and especially halophytes) also produce high amounts of mucilage in the shoot part including leaves where mucilage may be involved in water absorption from the atmosphere (Jones et al. 2016). The present work demonstrates that crude mucilage content was the highest for leaves (Fig. 3), and that in plants that were not exposed to Zn toxicity, mucilage content was similar in roots and stems. It is noteworthy that zinc toxicity obviously increased crude mucilage content in all organs, and that in this case, crude mucilage content was slightly higher in stems than in roots. In the shoots of *K. pentacarpus*, mucilage mainly accumulated within the xylem vessels and as deposit in the leaf epidermis (Ghanem et al. 2010). It may thus be hypothesized that increase in stem mucilage content was an attempt to retain Zn excess during the translocation process to reduce its accumulation in photosynthetic leaves,

while Zn fixation on epidermal mucilage contributes to protect chloroplast in the mesophyll, as reported for the other halophytes (Lefèvre et al. 2014).

It has also to be mentioned, however, that NaCl improved Zn resistance, but did not modify the crude mucilage content comparatively to Zn-treated plants, whatever the considered organ. This contrasts with the data provided by Ghanem et al. (2010) for *K. pentacarpus* exposed to higher salinity (100 mM NaCl), and by Golkar et al. (2017) who reported a salt-induced increase in mucilage content in *Plantago ovata*. It may, nevertheless, be argued that a smaller amount of Zn, as recorded in NaCl-treated plants exposed to Zn toxicity, may be more efficiently retained by a similar amount of mucilage. Moreover, Zn binding is not only a matter of mucilage amounts, but also depends on mucilage composition directly, as demonstrated by mucilage-Zn²⁺-pectinate controlled-release matrices used in the pharmaceutical industry (Guru et al. 2018). A modification in the mucilage composition was also observed in response to non-ionic constraints such as water deficit inducing decreases in water content similar to those recorded in the present study (Minjares-Fuentes et al. 2017; Elboutachfaiti et al. 2017).

Acid hydrolysis of crude mucilage extract in *K. pentacarpus* revealed that the main neutral monosaccharides constituents were rhamnose, arabinose, galactose, and glucose (Table 2). Zinc and salinity had different effects on the proportion of monosaccharides depending on the considered organs.

When plants were exposed to 0.1 mM Zn, the percentage of rhamnose in root decreased, while the percentage of glucose and uronic acid (Fig. 4) increased, compared to control. NaCl increased the percentage of arabinose and galactose but decreased the percentage of glucose. The highest value of glucose was recorded in the roots of plants exposed to Zn excess in the absence of NaCl. Furthermore, plant had a relatively high content of glucose especially in the root: hot water used in the present experiment for mucilage extraction might also extract limited part of intracellular starch (Ratnayake and Jackson 2006), leading to the relatively high values recorded for glucose concentration.

In stem, the percentage of rhamnose, glucose (Table 2), and uronic acid (Fig. 4), increased while the percentage of arabinose decreased in Zn treatment compared to control. Salt increased the percentage of galactose in stems. In both roots and stems of plants exposed to Zn toxicity, the percentage of uronic acids was much higher than control (Fig. 4). Uronic acids are thought to play a key role in heavy metal sequestration through electrostatic interactions between carboxyl groups and positive charges of divalent cations, and an increase in the proportion of uronic acids may contribute to an improved Zn retention independently of the total mucilage amount (Jones et al. 2016; Lodhi et al. 2019). In the root powder issued from *K. pentacarpus* and used for biosorption

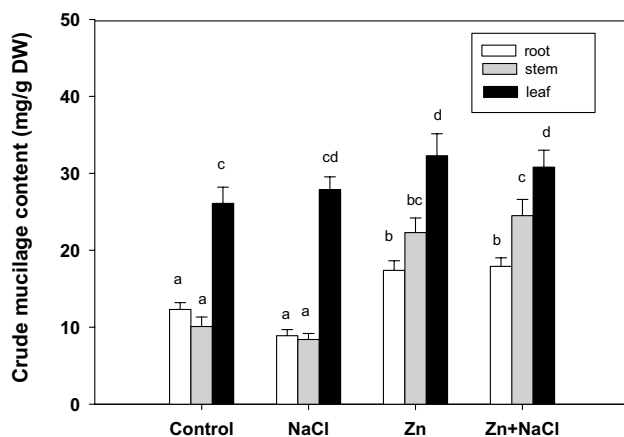


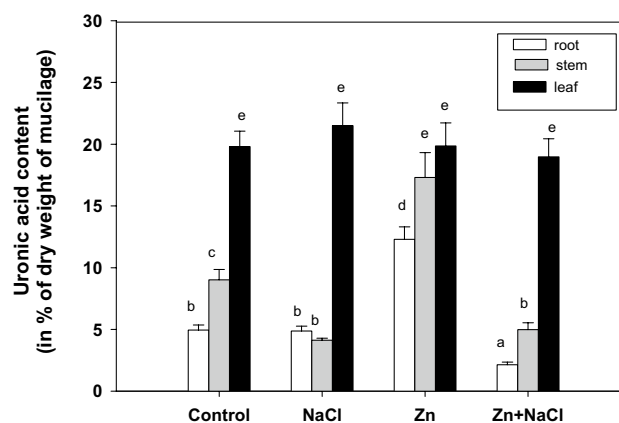
Fig. 3 Crude mucilage content (mg g⁻¹ dry weight) in root, stem, and leaves in *Kostelitzkya pentacarpus* exposed during 4 weeks to 0.1 mM ZnCl₂ in the presence or in the absence of 50 mM NaCl. Each value is the mean of three replicates and vertical bars are S.E. Values exhibiting different letters are significantly different at $P < 0.05$ according to Tukey's test

Table 2 Neutral monosaccharide composition (in mol% of total neutral monosaccharides) mucilage extracted from *K. pentacarpus* exposed to 0.1 mM ZnCl₂ in the absence or in the presence of 50 mM NaCl for 4 weeks

Monosaccharide constituent	Root			Stem			Leaf		
	Control	50 mM Na	0.1 mM Zn	Na+Zn	Control	50 mM Na	0.1 mM Zn	Na+Zn	Control
Rha	25.3±1.6 c	27±3.1 c	19.3±0.90 b	15.5±0.32 a	29.7±0.15 a	30.6±0.25 a	42.6±1.1 b	29.2±5.1 a	17.6±1.9 ab
Fuc	5.4±0.21 c	0	4.0±0.67 b	2.6±0.15 a	4.1±0.26 b	0	3.1±0.32 a	0	0.8±1.2 a
Ara	12.3±0.85 ab	17.6±2.1 bc	10.7±0.64 a	18.7±3.3 c	20.2±0.55 bc	23±2.6 c	14.3±0.50 a	17.8±2.3 ab	30.7±2.0 a
Xyl	5.5±0.92 a	4.5±1.1 a	6.1±0.45 b	3.9±0.67 a	9.2±0.91 c	5.2±0.64 b	3.6±0.15 a	3.7±1.3 a	2.0±0.36 a
Man	7.4±2.20 a	6.6±1.3 a	10.4±0.59 b	7.5±1.4 a	7.7±0.31 c	7.4±0.25 c	4.0±0.32 a	6.5±0.20 b	5.7±0.15 b
Gal	24.1±2.1 a	30.1±2.9 b	25.2±0.50 a	33.3±0.81 b	18.9±0.10 ab	17.3±0.87 a	19.8±0.21 b	28.7±1.0 c	33.7±2.4 a
Glc	20±0.45 b	14.5±0.51 a	24.3±1.3 c	18.6±0.1 b	10.1±0.87 a	16.6±1.0 c	12.6±1.5 b	14.2±0.64 bc	9.5±1.1 a

Each value is the mean of three replicates. For a given organ and a given compound, values exhibiting different letters are significantly different at $P < 0.05$ according to Tukey's test

Rha rhamnose, Fuc fucose, Ara arabinose, Xyl xylose, Man mannose, Gal galactose, Glc glucose

**Fig. 4** Uronic acid content (in % of dry weight of mucilage, Gal A+Glc A) of mucilage extracted from *K. pentacarpus* (roots, stems, and leaves) exposed to 0.1 mM ZnCl₂ in the absence or in the presence of 50 mM NaCl for 4 weeks. Each value is the mean of three replicates and vertical bars are S.E. Values exhibiting different letters are significantly different at $P < 0.05$ according to Tukey's test

process, Lutts et al. (2016) demonstrated that root powder issued from NaCl-treated plants was by far more efficient for Zn retention than powder issued from control plants, suggesting once again a modification in the mucilage composition. In the present case, however, NaCl added to Zn-treated plants induced an obvious drop in uronic acids in roots and stems which were even lower than in control plants.

In leaves, there was no major change in uronic acid proportion in response to the various treatment (Fig. 4), but the content of acidic polysaccharides was very high in leaves already in the control plants and an additional increase in uronic acids would not necessarily afford an improvement in Zn sequestration. Interaction between uronic acids and toxic elements may be complex. It was also determined that uronic acids were significantly enhanced by 33% under arsenic stressed conditions (Deepika et al. 2016) and Fox et al. (2012) confirmed that several functional groups present in mucilage could be involved in interaction with negatively charged ions such as arsenate.

Some authors reported that treatment of mucilage with sodium chloride after extraction from the plants could have a positive impact on functional groups and may increase binding activity with metal ions (Chubar et al. 2004; Jones et al. 2016). A similar impact of NaCl could have been expected as a result of salt exposure during plant growth. Our present work, nevertheless, suggests that as far as the shoot part is concerned, such improvement was not due to a salt-induced increase in uronic acid, although it has to be mentioned that the NaCl concentration used in vivo during plant growth was by far lower than NaCl used in vitro for harvested mucilage treatment.

Table 3 Contents of lignin, hemicellulose, and cellulose (in g 100 g⁻¹ of organic matter (OM)) in *K. pentacarpos* stem when plants were exposed to 0.1 mM ZnCl₂ in the absence or in the presence of 50 mM NaCl for 4 weeks, analyzed by the detergent fiber method (Van Soest method)

Treatment	Stem		
	Lignin (g 100 g ⁻¹ OM)	Hemicellulose (g 100 g ⁻¹ OM)	Cellulose (g 100 g ⁻¹ OM)
Control	13.3 ± 1.2 c	7.3 ± 0.16 a	46 ± 0.53 c
50 mM Na	14.1 ± 0.86 c	7.4 ± 0.28 a	48 ± 2.7 c
0.1 mM Zn	10.2 ± 0.58 b	8.3 ± 0.52 b	33 ± 0.90 a
Zn + Na	7.4 ± 0.67 a	8.6 ± 0.71 c	41 ± 0.62 b

Each value is the mean of three biological replicates. For a given class of compound (column), values exhibiting different letters are significantly different at $P < 0.05$ according to Tukey's test

Mucilage is mainly a mixture of pectic polysaccharides. A high amount of pectic rhamnogalacturonans may also have important function in metal-binding capacity. It has been reported by Astier et al. (2014) that an increase in pectin contents of Douglas fir trees was observed in response to increasing soil cadmium concentration in polluted soil. A concurrent reduction in methyl-esterification of pectin suggested that the structure of this major binding site could be modified as a reaction to cadmium contamination and a demethylation process occurring as a result of activation in pectin methylesterase could be regarded as an attempt to increase heavy metal binding (Krzyszowska 2011).

Van Soest sequential fiber solubilization method is widely used to assess the cellulose, hemicellulose, and lignin contents of plants, and to predict the nutritive value of fodder. Table 3 presents the observed concentrations of these polymers in the stem of *K. pentacarpos*: 50 mM NaCl treatment in the absence of Zn had no impact on the contents of lignin, hemicellulose, and cellulose. Zn 0.1 mmol in the absence of NaCl significantly decreased the content of lignin as well as cellulose by 23% and 28%, respectively ($P < 0.05$), while significantly increased hemicellulose content ($P < 0.05$).

When plants were exposed to Zn stress, the additional NaCl significantly increased content of both hemicellulose and cellulose ($P < 0.05$), compared to Zn alone. The cell wall is mainly composed of cellulose and matrix polysaccharides, including pectins and hemicelluloses. In contrast to cellulose, which is generally regarded as an unbranched polymer unable to bind cations, it has been widely reported that plant hemicellulose has potential ability to bind with heavy metals (Wang et al. 2012; Hu et al. 2010). Yang et al. (2011) indicated that 75% of cell wall aluminum (Al) accumulated in the hemicellulose 1 (HC 1) fraction after *Arabidopsis thaliana* was treated by Al stress for 24 h. Meanwhile, a time-dependent kinetic study

showed that only when the HCl fraction was removed, the amount of Al adsorbed decreased sharply. In our results, when plants were exposed to Zn stress in the presence or absence of NaCl, the content of hemicellulose increased in the stem, which indicates that Zn induced more hemicellulose production to fix Zn in cell wall in stem. Lutts et al. (2016) also reported a same trend for roots where Zn, even in the presence of NaCl, significantly increased the hemicellulose content, leading us to hypothesize that Zn retention may occur both at the mucilage and within cell wall, although, according to a recent study (Zhou et al. 2018a), cell wall retention was more efficient for Cd than for Zn in the shoot of *K. pentacarpos*.

Conclusion

Kosteletzkyia pentacarpos is a wetland halophyte plant species able to cope with 0.1 mM Zn in nutrient solution. The presence of 50 mM NaCl improved plant resistance to exogenous Zn in terms of plant growth, but decreased Zn accumulation in roots and leaves but not in the stem. Salinity had no impact on translocation factor from the root to the shoot part, but decreased the bioaccumulation factor, thus mitigating the interest of *K. pentacarpos* for phytoextraction of Zn in salt-affected areas. Crude mucilage content was the highest in leaves than in roots and stems, and zinc toxicity obviously increased crude mucilage content in all organs. An increase in stem mucilage content might be an attempt to retain Zn excess during the translocation process to reduce accumulation of this toxic elements in photosynthetic leaves. An increase in the uronic acid content of the stem mucilage in Zn-treated plants supports this hypothesis, while leaves always constitutively exhibited a high uronic acid content. An increase in stem hemicellulose in Zn-exposed plants may also be involved in Zn fixation.

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