Ecotoxicology and Environmental Safety 182 (2019) 109460

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Salinity modifies heavy metals and arsenic absorption by the halophyte plant species *K*osteletzkya pentacarpos and pollutant leaching from a polycontaminated substrate.

Mingxi Zhou, Thibaut Engelmann, Stanley Lutts*

Groupe de Recherche en Physiologie Végétale, Earth and Life Institute – Agronomy (ELI-A), Université Catholique de Louvain, 5 (Bte 7.07.13) Place Croix du Sud, 1348, Louvain-la-Neuve, Belgium

ARTICLE INFO

Keywords:
Arsenic
Cadmium
Lead
Phytoremediation
Seashore mallow
Zinc

ABSTRACT

Phytomanagement of polycontaminated soils is challenging, especially in areas simultaneously affected by salinity. The wetland halophyte plant species *Kosteletzkya pentacarpos* was cultivated in a column device allowing leachate harvest, on a polycontaminated spiked soil containing Cd (6.5 mg kg^{-1} DW), As (75 mg kg^{-1} DW), Zn (200 mg kg^{-1} DW) and Pb (300 mg kg^{-1} DW) and irrigated with salt water (final soil electrical conductivity 5.0 ms cm^{-1}). Salinity increased Cd bioavailability in the soil and Cd accumulation in the shoots while it had an opposite effect on As. Salinity did not modify Pb and Zn bioavailability and accumulation. Cultivating plants on the polluted soil drastically reduced the volume of leachate. In all cases, salinity reduced the total amounts of heavy metals removed by the leachate and significantly increased the proportion of Cd and Zn removed by the plants. Heavy metal contamination induced a decrease in shoot dry weight and an increase in malondialdehyde (an indicator of oxidative stress); both symptoms were alleviated by the additional presence of NaCl but this positive impact was not related to increase in protecting phytochelatins synthesis. It is concluded i) that bioavailability estimated by the 0.01M $CaCl_2$ extraction procedure is not fully relevant from the heavy metal mobility, ii) that salinity decreased heavy metal percolation, especially in soils cultivated with *K. pentacarpos* and iii) that salinity improves plant tolerance to heavy metals in *K. pentacarpos* and that this species is a promising plant material for phytoremediation of polycontaminated soils.

1. Introduction

Mineral nutrition is of paramount importance for optimal plant growth and development. Although a reduced availability in nutrients induces numerous physiological disorders, an excess of elements may also compromise plant growth and survival. Ion toxicity may involve essential or non-essential elements: aluminium, ferrous iron, salinity (mainly NaCl) and heavy metals constitute important constraints for plant (Dufey et al., 2009; Lefèvre et al., 2014; Riaz et al., 2018; Singh et al., 2017). In numerous cases, several elements are simultaneously present in excess, leading to a complex stressing environment. This is especially the case for heavy metals and toxic metalloids. These persistent pollutants may occur as a consequence of geological processes but also result from human activities (Clemente et al., 2012; Wang et al., 2013; Yang et al., 2018; Liu et al., 2019; Wen et al., 2019). Coastal areas are especially prone to heavy metal pollution and wetlands soils act as a sink for numerous pollutants (Bai et al., 2019; Liu et al., 2019; Wen et al., 2019).

As transitional region of land and ocean, coastal areas and sediments are typical zones suffering from the simultaneous presence of salinity and heavy metal in excess (Liu et al., 2019). It has been reported that salinity increases mobility of heavy metals in soils (Acosta et al., 2011; Du Laing et al., 2008; Zhao et al., 2013). Soil parameters controlling heavy metal chemistry and bioavailability are soil mineralogy, organic matter content, pH and electrical conductivity. Salinity implies the competition of salt-derived cations with positively charged heavy metals for sorption sites on the solid phase and complexation capacity of salt-derived anions with heavy metals. Consequences of salinity on heavy metal mobility is well documented for Cd readily forming stable complexes with chloride ligands ($CdCl_n^{2-n}$) which are loosely bound to soil particles (McLaughlin et al., 1997; Filipović et al., 2018). Cd-chlorocomplex are less available than free Cd^{2+} for root absorption but Cd may nevertheless enters the roots directly or dissociating from the chlorocomplex after reaching binding sites at the

* Corresponding author.
E-mail address: stanley.lutts@uclouvain.be (S. Lutts).

https://doi.org/10.1016/j.ecoenv.2019.109460
Received 12 April 2019; Received in revised form 12 July 2019; Accepted 19 July 2019
Available online 23 July 2019
0147-6513/

root surface (Lefèvre et al., 2009; Filipović et al., 2018). Acosta et al. (2011) demonstrated that salinity impact on heavy metal mobility varies depending on the considered heavy metal but also depending on the precise nature of salinity and an increase in ionic strength by any salt has commonly lower impact on Zn and Pb mobilization than on Cd. In some cases, high salinity may even reduce heavy metal mobility as a consequence of Zn-containing (bechererite and hordaite) or Pb-containing (laurionite) precipitates (Tandon et al., 2018). Bai et al. (2019) demonstrated that salinity may significantly increase arsenic mobility in coastal wetland soils even if this element is commonly present in the soil in anionic forms (arsenate or arsenite, depending on the soil redox potential).

Phytoremediation is a biological tool frequently proposed as a promising alternative to expensive chemical and physiological strategies for management of heavy metal contaminated soils. Heavy metal phytoremediation of salt-affected soils is challenging since suitable plant species must be able to cope with both salt and pollutant constraints. Numerous halophyte species have been recommended for this purpose (Clemente et al., 2012; Cheng et al., 2012, 2018a; 2018b, 2019; Lefèvre et al., 2009; Van Ootsen and Maggio, 2015; Lutts and Lefèvre, 2015; Vromman et al., 2016; Li et al., 2019). Indeed, tolerance to different toxic elements may at least partly rely on similar physiological properties in relation to ion sequestration, regulation of the plant water status, management of oxidative stress and protection of cellular structures.

Filipović et al. (2018) recently demonstrated that a salt-induced increase in heavy metal mobility does not necessarily implies an increase in phytoavailability. Salt may induce a particular speciation for pollutant which is less suitable for root absorption by unspecific transporters. Salinity also induces a water stress component leading to stomatal closure and thus a decrease in the transpiration stream which compromises root to shoot translocation (Lutts and Lefèvre, 2015). Cheng et al. (2012) demonstrated that in some mangrove species, salt-treated roots encountered anatomical changes and precocious lignification which strongly reduces Pb, Zn and Cu accumulation. Hence, despite an increase in heavy metal mobility, salinity often leads to a decrease in heavy metal accumulation in plants, even in halophyte species (Cheng et al., 2018b, 2019; Lefèvre et al., 2009; Lutts and Lefèvre, 2015; Van Oosten and Maggio, 2015). Mobilized heavy metals that are not absorbed by the roots constitute a major ecological risk of leaching leading to pollution of drainage water and contamination of aquifers (Wahla and Kirkham, 2008; Zhao et al., 2013).

Kosteletzkya pentacarpos (syn *Kosteletzkya virginica*) is a deep-rooted wetland halophyte plant species commonly found in salt marshes of coastal areas (He et al., 2003; Qin et al., 2015). Previous experiments demonstrated that moderate doses of salinity helps the plant to cope with heavy metals such as Cu, Cd and Zn (Han et al., 2012a, 2012b; 2013a, 2013b). Additional works showed that mixed heavy metal treatment (Cd + Zn) induces a specific physiological constraints at the plant level comparatively to single treatment as assessed by a specific hormonal status (Zhou et al., 2018a), tissular distribution of pollutant (Zhou et al., 2018b) or synthesis of osmoprotectants (Zhou et al., 2019). In order to carefully and precisely control the stress intensities, those studies relied on plant culture in nutrient solution and did not pay attention to the interaction between roots and solid substrate.

The aim of the present study was to quantify the impact of salinity on the relative proportion of Cd, Zn, Pb and As percolation and plant accumulation by the halophyte wetland plant species *Kosteletzkya pentacarpos* growing on a polycontaminated soil.

2. Material and methods

2.1. Soil sampling and analysis

Soil was collected in the experimental farm of Université catholique de Louvain (Centre Alphonse de Marbaix, Corroy le Grand; Belgium) on a field previously used for Miscanthus production. After removal of the organic layer, soil was collected in the first 20 cm, air-dried, sieved through a 4-mm mesh, homogenized and mixed with washed sand (ratio soil/sand: 4/1). Half of the harvested soil was maintained as a thin layer (3 cm) in a greenhouse and sprayed with heavy-metal containing solution in order to obtain a final concentration of 75, 6.5, 200 and 300 mg kg^{-1} for As, Cd, Zn and Pb, respectively (As was added as $Na_2HAsO_4.7H_2O$; Cd as $CdCl_2$, Zn as $ZnCl_2$ and Pb as $PbNO_3$). Doses were chosen in order to induce a medium toxicity according to the Belgian rules (http://environnement.wallonie.be/legis/solsoussol/sol003.htm). The remaining soil was used as unpolluted control. Spiked polluted soil was maintained for an additional period of one month to reach equilibrium. Soil electrical conductivity (EC) and pH-H_2O were determined using glass electrodes (WTW Multi 350i) in a 1:5 soil/water paste. Exchangeable base concentrations (Ca^{2+}, Mg^{2+}, Na^+ and K^+) were determined using percolation columns and ammonium acetate 1.0 M pH 7.0 (Page et al., 1982) and inductive-coupled plasma atomic emission spectrometry (ICP-AES; Thermo Jarrell Ash Iris Advantage). Cation exchangeable capacity (CEC) was assessed using the same percolation columns and KCl 1.0 M pH 3.0. Total N (NT) and organic C (Corg) were determined according to Kjeldahl and Walkley–Black methods (Page et al., 1982). Heavy metal concentrations were obtained by dissolving 0.5 g of soil in an open Teflon crucible with 3 mL HNO_3 (AnalaR NORMAPUR 65%) and 5 mL HF (AnalaR NORMAPUR 40%). The mixture was gently heated at 130 °C on a hot plate until complete dryness. The residue was redissolved with 2 mL $HClO_4$ (AnalaR NORMAPUR 70%), and the mixture was heated again until complete dryness. The residue was redissolved with aqua regia (HCl AnalaR NORMAPUR 37% and HNO_3 65%) and filtered (Whatman-41). Finally, the solution was diluted to 50 mL with deionized water and analyzed with ICP-AES. Quality of the procedure was controlled by the use of certified samples (BCR CRM 141R and 142; Evisa). The proportions of clay, silt and sand in soil were 25.4 ± 1.5, 51.1 ± 3.4 and 23.5 ± 2.0%, respectively (Table S1; NFX 31–107 sandard)). The values of pH_{water} (NFX31-130 standard) and CEC were 7.34 ± 0.13 and 18.4 ± 1.3 $cmol^+$ kg^{-1}, and EC was 0.83 $mS.cm^{-1}$. Other main characteristics of the used soil were also provided in Table S1.

In order to assess soil heavy metal mobility, a 0.01 M $CaCl_2$ selective extraction was performed according to Houba et al. (2000) and Degryse et al. (2003): 2.5 g of soil and 25 mL of $CaCl_2$ were shaken during 24 h and then centrifuged at 3000 *g* during 15 min (Sigma 3–30K, Germany). The supernatant was filtered (Whatman 2) and analyzed for heavy metal concentration with ICP-AES.

Soil was then introduced in plastic column (72 columns, 10.5 cm of diameter and 33 cms height; each column received 2.5 Kg of soil; 36 column with polluted soil and 36 column with control unpolluted soil). The bottom of the column was filled with 3 cms of gravel layer and a tap was adapted on the system to collect the leachates during the time course of experiment.

2.2. Plant material and growing conditions

Seeds of *Kosteletzkya pentacarpos* were surface-sterilized for 30 s in 75% ethanol (v/v) and germinated on a substrate of perlite/vermiculite (3:1) moistened with Hoagland solution in a phytotron under a 16 h day/light photoperiod, a relative humidity of 51.3%, a mean temperature of 25 °C during the day and 23 °C during the night and a mean light intensity of 150 µmol m^{-2} s^{-1} provided by Sylvania fluorescent tubes (F36W/133-T8/CW; Danvers, MA). Uniform young seedling at the three leaves stage and a mean height of 10.0 ± 1 cm was then transferred to the column (three seedlings per columns), except on 12 columns which remained free of plants and were subsequently used as control.

Columns were randomly distributed in a greenhouse with semi-controlled environmental conditions (16 h daylight photoperiod, natural light supplemented by Phillips lights 'Philips lighting SA, Bruxelles,

HPLR 400W to provide a minimal light intensity of 280 μmol.m^{-2}s^{-1} at the top of the canopy; mean temperature of 24.4 °C; mean relative humidity of 51%). Soil was preliminary watered before seedling transfer with deionized water to field capacity (volumetric soil water content of 27.8%). After 8 days of acclimatization, half of the colmuns were regularly irrigated at 2 days intervals with salt water providing a total amount of 6 gr NaCl in each column. Four treatments were thus considered: 1) controls (no pollution and no salt), 2) polluted, 3) NaCl 4) polluted + NaCl. Each treatment involved 15 columns with 3 plants per column, and for each treatment, 3 columns that did not contain any plant were used as additional control. Plants were cultivated during a total period of 84 days. Columns were irrigated to ensure mean soil humidity close to 80% of the field capacity controlled by a ProCheck Decagon Devices EC-5 soil moisture probe. Leachates at the bottom of the column were collected every 3 days, filtered and stored in the dark at 4 °C until analysis.

Three harvests were performed after 4, 8 and 12 weeks. At each harvest, 5 columns were considered and all the plants from a same column were collected. Stem length, number of leaves, stem and leaf fresh and dry weight (obtained after 72 h of incubation in an oven at 68 °C) were considered. Roots, which cannot be accurately separated from the soil, were not considered. Soil was collected after harvest from columns at two different depths (10 and 20 cm) and pooled to constitute samples for subsequent assessments of soil heavy metal concentration and bioavailability.

2.3. Plant ion concentration

For mineral analysis, c.a. 50 mg dry material were ground to a fine powder using a porcelain mortar and a pestle, digested in 35% HNO_3 and evaporated to dryness on a sand bath at 80 °C. The remaining minerals were diluted in a mix of 37% HCl and 68% HNO_3 (3/1); the mixture was slightly evaporated and dissolved in HCl 0.1N. The samples used for Pb quantification were preliminary submitted to a progressive rise of temperature until 450 °C, and maintained during 24 h at this temperature to obtain ashes. Final metal concentrations in the sample were assessed by plasma emission spectrometry ICP-OES (Thermo Jarrell As Iris Advantage). Quality control was based on the use of certified samples (NIST: National Institute of Standard and Technology, Gaithersburg, Md; SRM 1573 (tomato leaves), SRM 1547 (peach leaves) and BCR 679 (white cabbage, trace elements)). For each samples, all measurements were performed in technical triplicates. Mineral concentration was expressed on a dry weight basis.

2.4. Malondialdehyde (MDA) content and total antioxidant activities

The level of lipid peroxidation in the control and ash-treated plants was assessed from the concentration of malondialdehyde (MDA) as determined by the thiobarbituric acid (TBA) reaction (Heath and Packer, 1968). Ground fresh shoots and roots (0.25 g) were homogenized in 5 mL of 5% (w/v) trichloroacetic acid (TCA) containing 1.25% glycerol. The homogenate was centrifuged (Sigma 3–30K, Germany) at 12,000 *g* for 10 min at 4 °C and filtered on Wathman No. 1 filter paper. 2 mL of TBA (0.67%) was added to 2 mL of supernatant and the mixture was heated at 100 °C for 30 min. The reaction was stopped by placing the reaction tubes in an ice bath. The samples were centrifuged at 12,000 *g* for 1 min and their absorbance was measured at 532 nm (UV-1800 Shimadzu, Belgium). The results were corrected by subtracting the non-specific absorbance component as measured at 600 nm. The concentration of MDA (nmol g^{-1} DW) was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

To estimate the total global antioxidant activity, ferric reducing ability of plasma (FRAP) was assayed according to Benzie and Strain (1996) considering the ability of plant extract to reduce ferric to ferrous ion at low pH and to produce a colored ferrous-tripyridyltriazine complex which was spectrophotometrically detected at 593 nm: 1 g FW was frozen in liquid nitrogen, ground in the presence of 10 mL methanol, incubated during 12 h at 4 °C, and then centrifuged during 20 min at 10,000 *g* at 4 °C (Sigma 3–30K, Germany). Supernatant contains the hydrophilic fraction (AOAM) and was stored at −20 °C until analysis. Pellets were dissolved in 10 mL dichloromethane, homogenized and incubated at 4 °C during 12 h, and centrifuged again at 10,000 *g* during 20 min (Sigma 3–30K, Germany). The obtained supernatant corresponds to the hydrophobic fraction (AOAD) and was stored at −20 °C. For final analysis, 150 μL of each fractions were separately added to 300 μL of freshly prepared FRAP reagent (25 mL acetate buffer pH 3.6, 2.5 mL of 2,4,6-tripyridyl-s-triazine and 2.5 mL $FeCl_3.6H_2O$ 20 mM). Standard curve was established with Trolox (50–800 μM).

2.5. Glutathione and total non-protein thiols

For reduced (GSH) and total (GSHt) glutathione quantification, 200 mg of frozen samples were extracted and derivatized by orthophthalaldehyde (OPA) according to Cereser et al. (2001). GSHt was quantified after a reduction step of oxidized glutathione (GSSG) by dithiotreitol. Extracts were filtered through 0.45 μm microfilters (Chromafil PES-45/15, Macherey-Nagel) prior to injection and OPA derivatives were separated on a reversed-phase HPLC column with an acetonitrile-sodium acetate gradient system and detected fluorimetrically. Five μL of sample were injected into a Shimadzu HPLC system (Shimadzu, 's-Hertogenbosch, The Netherlands) equipped with a Nucleodur C18 Pyramid column (125 × 4.6 mm internal diameter; 5 μm particle size) (Macherey-Nagel, Düren, Germany). Derivatives were eluted in acetonitril gradient in a 50 mM sodium acetate buffer pH 6.2 at 30 °C at a flow rate of 0.7 mL min^{-1}. Fluorimetric detection was performed with a spectra system Shimadzu RF-20A fluorescence detector at 420 nm after excitation at 340 nm. GSH was quantified using nine-point calibration curves with custom-made external standard solutions ranging from 0.0625 to 50 μM and every ten injections, a check standard solution was used to confirm the calibration of the system. The recovery was determined using GSH as an internal standard.

The total non-protein thiol (NPT) concentration was determined according to De Vos et al. (1992): 200 mg FW of tissue was ground in 2 mL of 5% (*w/v*) sulfosalicylic acid plus 6.3 mM diethylenetriaminepentaacetic acid (pH < 1) at 0 °C with quartz sand in a mortar. The homogenate was centrifuged at 10,000 *g* for 10 min at 4 °C (Sigma 3–30K, Germany). The supernatants were collected and used for the determination of thiols using Ellman's reagent. Three hundred microliters of supernatant were mixed with 630 μL of 0.5 M KH_2PO_4 and 25 μL of 10 mM 5,5-dithiobis 2-nitrobenzoic acid (final pH 7.0). The absorbance at 412 nm was recorded after 2 min, and the NPT concentration was estimated using an extinction coefficient of 13,600 M^{-1} cm^{-1}. Phytochelatins content was evaluated as difference between NPT and GSH levels (Schäfer et al., 1997). Glutathione and NPT were quantified in the leaves of plants exposed to 8 and 12 weeks of treatment.

2.6. Statistical treatment of the data

Normality of the data was checked by the Shapiro-Wilk tests and homosedasticity was verified using the Levene test. Data were transformed ($log10(x+1)$ or $asin(x/\Sigma x)$) when required to ensure normal distribution. Variance analysis (ANOVA I) was then performed at each harvest using the R software to determine the impact of the treatment on the recorded parameters. Post hoc analyses were performed using the Tukey test.

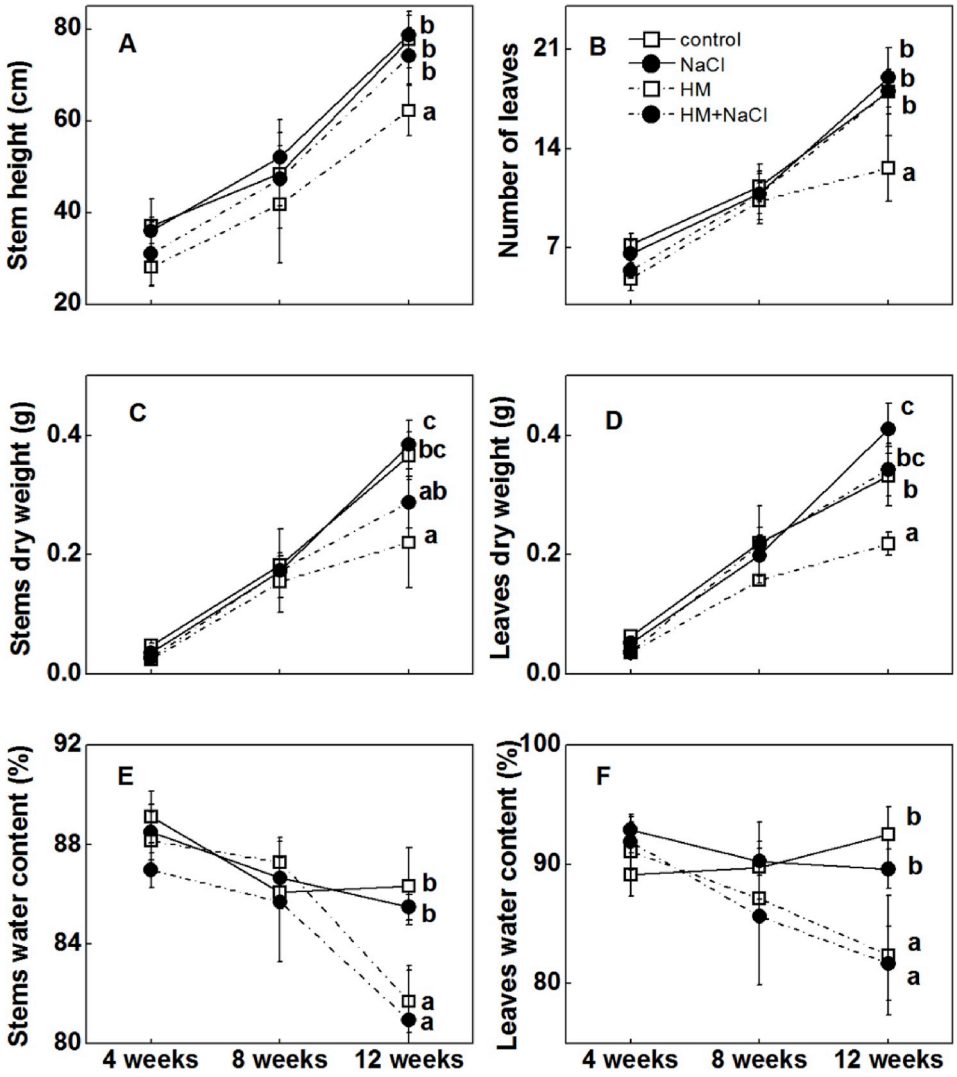

Fig. 1. Stem height (A), number of leaves (B), stem dry weight (C), leaf dry weight (D), stem water content (E) and leaf water content (F) in plants of *Kosteletzkya pentacarpos* cultivated during 4, 8 and 12 weeks on a non-polluted soil (control), the same soil added with NaCl (Na), with a mixture of heavy metals (HM) or with heavy metals and NaCl (HM + Na). Each value is the mean of 5 replicates and vertical bars are standard errors. Mean sharing a common letter are not significantly different according to the Tukey test ($P > 0.05$).

3. Results

3.1. Plant-related parameters

Plant growth-related parameters are presented in Fig. 1. Stem height remained similar in control and in NaCl-treated plants (Fig. 1A) but decreased as a consequence of exposure to HM. However, the addition of NaCl to HM-treated plants allowed to significantly reduce the deleterious impact of pollution on stem elongation. A similar trend was observed for the number of leaves which drastically decreased in HM-treated plants after 8 weeks of treatment (Fig. 1B) but still increased in a similar way in plants exposed to the other treatments. The stem (Fig. 1C) and the leaves (Fig. 1D) dry weights displayed similar trends: they remained similar for all treatments up to 8 weeks but then started to differ and exhibited the lowest value for plants exposed to heavy metals and the highest value for controls (stem) or NaCl-treated plants (leaves). Once again, the presence of NaCl on HM-contaminated soil reduced the deleterious impact of those pollutants on plant growth. Heavy metals reduced water content in both organs (Fig. 1E and F) and to a similar extent in HM- and HM + NaCl-treated plants.

During the time course of the experiment, As was not detected in control and in NaCl-treated plants. Salinity reduced As accumulation in the stems after 4 and 12 weeks (Fig. 2A) and in the leaves after 12 weeks (Fig. 2B). Salinity had an opposite impact on Cd content: it increased it in both stems (Fig. 2C) and leaves (Fig. 2D) of plants maintained on contaminated soil. Lead was detected in low amounts in control and in NaCl-treated plants but it obviously increased in plants exposed to the contaminated substrate. It remained constant during the experiment in the stem and NaCl had no impact on Pb accumulation in plants (Fig. 2E and F). Zinc accumulation it the stem (Fig. 2G) significantly decreased with the duration of the treatment in plants exposed to the contaminated substrate while it remained constant in plants growing on uncontaminated substrate: at the end of the experiments, similar values were recorded for Zn stem concentration in all plants. A similar trend was not observed in the leaves (Fig. 2H). Moreover, as previously mentioned for Pb, NaCl had no impact on Zn accumulation in stems and leaves.

Table 1 indicated that salinity induced a decrease in the leaf K content and an increase in leaf Na which was higher for plants exposed to NaCl in the presence of HM. Heavy metals reduced P, Fe and Mn content and in all cases, the deleterious impact of HM was reduced by additional NaCl. Conversely, HM increased the S and Cu concentrations in the leaves in the absence but not in the presence of NaCl.

Malondialdehyde (MDA) concentration significantly increased in

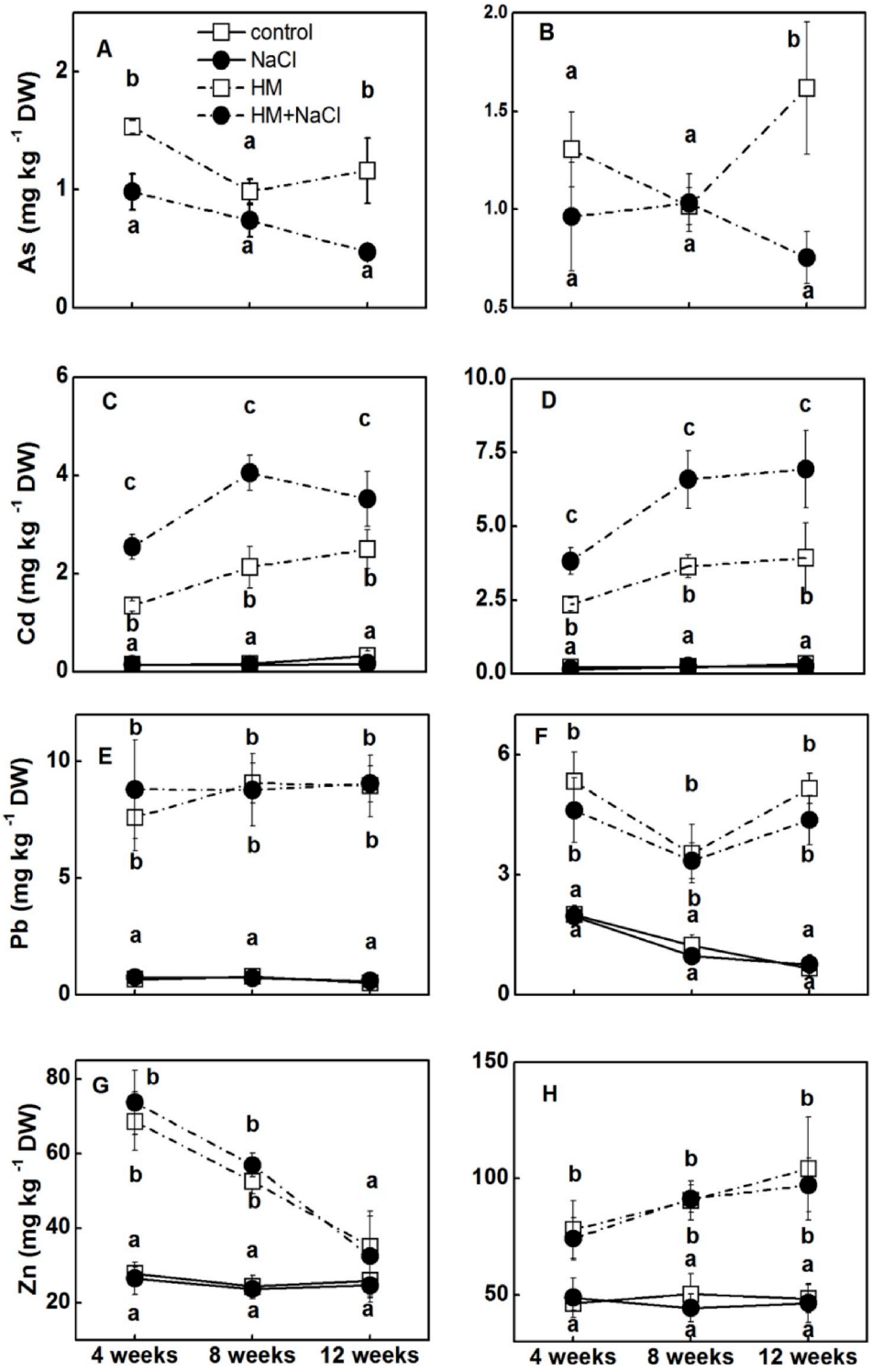

Fig. 2. Stem (A, C, E, G), and leaf (B, D, F, H) concentration in arsenic (A, B), cadmium (C, D), lead (E, F) and zinc (G, H) in plants of *Kosteletzkya pentacarpos* cultivated during 4, 8 and 12 weeks on a non-polluted soil (control), the same soil added with NaCl (Na), with a mixture of heavy metals (HM) or with heavy metals and NaCl (HM + Na). Each value is the mean of 5 replicates and vertical bars are standard errors. Mean sharing a common letter are not significantly different according to the Tukey test ($P > 0.05$).

plants exposed to HM stress (Fig. 3A). Salinity in the absence of HM had no impact on the leaf MDA content and it abolished to HM-induced increase in leaf MDA. Leaves of plants exposed to HM + NaCl treatment for 12 weeks even exhibited lower MDA concentration than control plants. Data for total antioxidant capacity are provided by Fig. 3B and C for the hydrophilic (AOAM) and hydrophobic (AOAD) fractions, respectively. After 8 weeks of treatment, AOAM was reduced in plants exposed to HM but remained unchanged in other treatments. After 12 weeks of treatment, the leaf AOAM fraction was slightly higher in plants exposed to HM + NaCl treatment. After 8 weeks, The AOAD fraction was significantly increased in plants exposed to NaCl, both in presence and absence of HM. After 12 weeks, the highest FRAP-AOAD values were recorded for plants exposed to HM + NaCl treatments.

After 8 weeks of treatment, reduced glutathione (GSH; Fig. 3D)

Table 1
Leaf concentration of essential elements (Ca, Mg, K, P and S estimated in mg g^{-1} DW; Fe, Mn and Cu estimated in mg kg^{-1} DW) and Na (in mg g^{-1} DW) in plants of *Kosteletzkya pentacarpos* maintained during 12 weeks on a soil substrate containing contaminated by arsenic and heavy metals (HM), NaCl (6 g kg^{-1}) or both treatment (HM + NaCl). Each value is the mean of 5 replicates ± S.E. Means followed by the same letter are not statistically different according to the Tukey test ($P > 0.05$).

	Ca (mg g^{-1})	Mg (mg g^{-1})	K (mg g^{-1})	Na (mg g^{-1})	P (mg g^{-1})	S (mg g^{-1})	Fe (mg g^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
Control	5.3 ± 0.4 a	2.9 ± 0.1 a	26 ± 2 a	0.48 ± 0.14 a	3.0 ± 0.4 b	9.5 ± 1.3 a	137 ± 17 b	80 ± 6 c	11.8 ± 1.1 a
NaCl	5.5 ± 0.7 a	2.7 ± 0.2 a	14 ± 3 b	4.20 ± 0.99 b	2.7 ± 0.2 ab	9.0 ± 1.3 a	150 ± 17 b	81 ± 7 c	13.5 ± 1.1 a
HM	5.0 ± 0.4 a	3.1 ± 0.3 a	24 ± 3 a	0.71 ± 0.11 a	2.2 ± 0.3 a	13.4 ± 1.6 b	74 ± 7 a	52 ± 4 a	19.5 ± 4 b
HM + NaCl	5.1 ± 0.2 a	2.9 ± 0.3 a	10 ± 1 c	6.2 ± 1.0 c	2.5 ± 0.3 ab	8.5 ± 1.1 a	160 ± 11 b	68 ± 7 b	10.8 ± 0.8 a

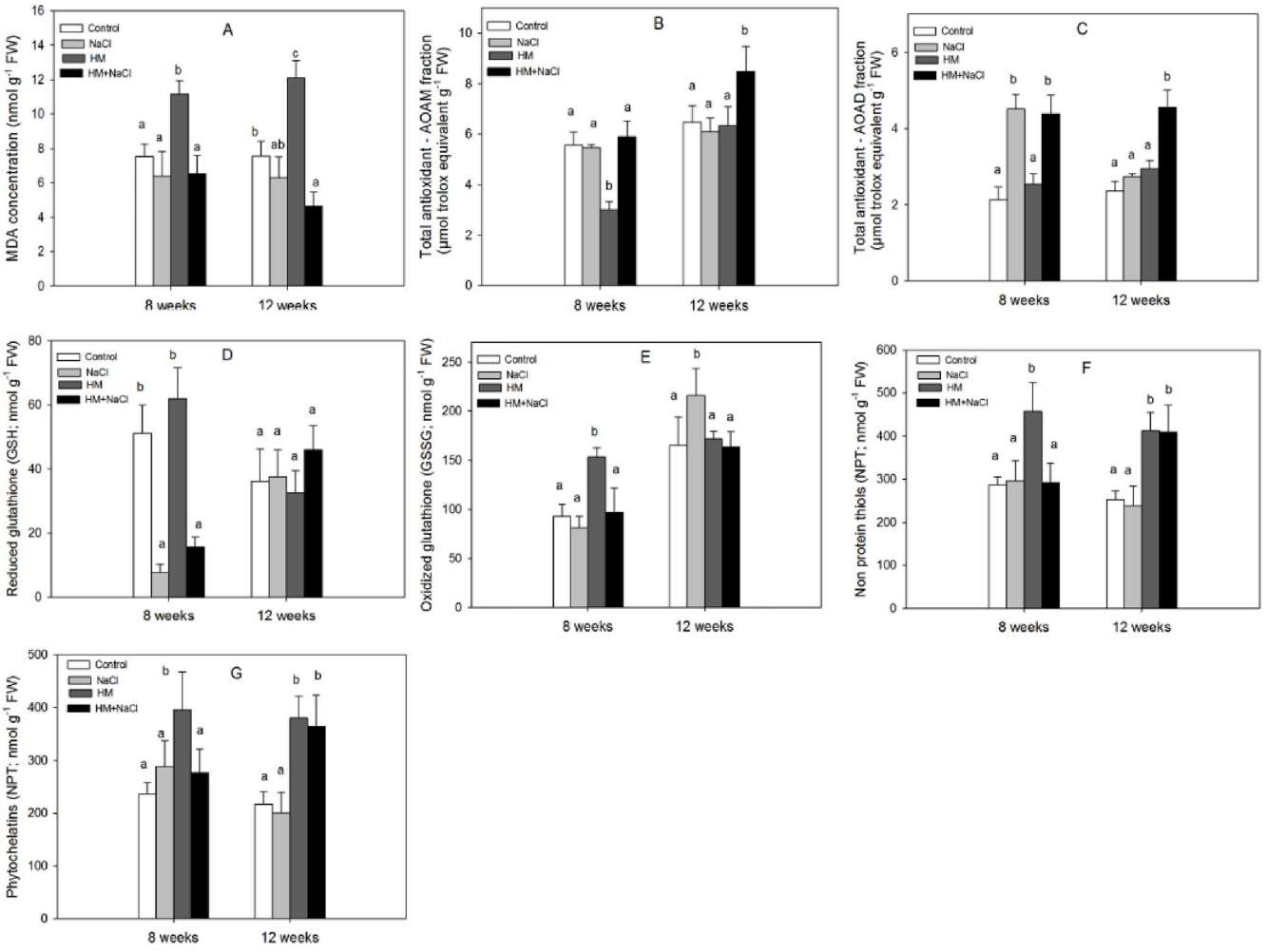

Fig. 3. Leaf malondialdehyde (MDA) concentration (A), total antioxidant activity (FRAP; hydrophilic AOAM (B) and lipophilic AOAD (C) faction), reduced glutathione (GSH; D) oxidized glutathione (GSSG; E), total non-protein thiols (F) and phytochelatins (G) in plants of *Kosteletzkya pentacarpos* cultivated during 8 and 12 weeks on a non-polluted soil (control), the same soil added with NaCl (Na), with a mixture of heavy metals (HM) or with heavy metals and NaCl (HM + Na). Each value is the mean of 5 replicates and vertical bars are standard errors. Mean sharing a common letter are not significantly different according to the Tukey test ($P > 0.05$).

dropped in leaves of plants exposed to NaCl while heavy metal treatment had no significant impact on this parameter. After 12 weeks of treatment no difference was recorded for GSH between treatments. Oxidized glutathione (GSSG; Fig. 3E) was significantly higher in HM-treated plants after 8 weeks and in NaCl-treated ones after 12 weeks. Heavy metal also increased non-protein thiols (NPT; Fig. 3F) and phytochelatin content (PC; Fig. 3G) after 8 weeks but no increase was recorded at this time for plants exposed to HM + NaCl treatment. After 12 weeks, however, a similar increase of NPT and PC was recorded in HM- and in HM + NaCl-treated plants.

3.2. Soil and leachate-related parameters

Samples from contaminated soils were collected at each harvest to assess the pollutant bioavailability. Data are presented in Table 2 for 4 and 12 weeks of treatment which also presents the data for soil issued from column maintained in the absence of plants. Addition of NaCl did not impact pH values after 4 weeks and the presence of plants had no effect on this parameter. The mean EC increased as a consequence of salinization but remained the same in the presence and in the absence of plants. Pollutant bioavailability was the lowest for Pb and the highest for Cd. Salinity significantly reduced As bioavailability but increased Cd bioavailability. Cadmium bioavailability increased from 4 to 12 weeks

Table 2
The pH, electrical conductivity (EC) and pollutant bioavailability in polluted soils cultivated in column with *Kosteletzkya pentacarpos* during 4 or 12 weeks. Contaminated soils were exposed or not to additional NaCl (6 g kg^{-1}). Soil issued for the same duration in a column free of plants was used as control. Bioavailability was estimated through selective extraction with $CaCl_2$ 0.01 M and was expressed as a percentage of corresponding elements estimated by total analysis through acid attack (see material and Methods). For a given parameter and a given period of treatment, values followed by the same letters are not significantly different according to the Tukey test ($P > 0.05$).

	Column with plants		Column without plants	
	0 NaCl	+NaCl	0 NaCl	+NaCl
4 Weeks				
pH	7.07 a	7.12 a	7.24 a	6.99 a
EC (mS cm^{-1})	0.78 a	5.04 b	0.91 a	5.22 b
As (%)	0.37 c	0.21 a	0.29 b	0.23 a
Cd (%)	1.23 a	2.77 c	1.25 a	2.01 b
Pb (%)	0.002 a	0.003 a	0.002 a	0.005 a
Zn (%)	0.66 a	0.70 b	0.81 c	0.75 bc
12 Weeks				
pH	6.92 a	7.0 a	6.98 a	7.13 b
EC (mS cm^{-1})	0.81 a	4.99 b	0.83 a	5.05 b
As (%)	0.30 a	0.28 a	0.34 ab	0.37 b
Cd (%)	2.89 b	3.26 c	2.74 b	2.53 a
Pb (%)	0.004 a	0.004 a	0.002 a	0.003 a
Zn (%)	0.34 a	0.39 a	0.78 b	0.83 b

Table 3
Total amounts (in µg) of As, Cd, Zn and Pb removed from the columns systems. Contaminated soils were cultivated or not by the halophyte plant species *Kosteletzkya pentacarpos* during 12 weeks while some column remained free of plants. Half of the columns were irrigated by NaCl solution. The relative proportion of the pollutant removal by the plants with subsequent storage in the shoot part on the one hand, and the proportion of pollutants removed by leaching on the other hand are given as percentages.

	Total amount removed (in µg)	% removed by plant	% removed by leaching
Plant + 0 NaCl			
As	24.16 ± 2.13	8.94	91.06
Cd	7.62 ± 0.84	44.88	55.12
Zn	117.4 ± 9.8	65.93	34.07
Pb	11.8 ± 1.4	94.07	5.93
Plant + NaCl			
As	12.17 ± 0.95	9.6	90.4
Cd	10.28 ± 1.02	95.14	4.86
Zn	126.7 ± 15.1	97.32	2.68
Pb	15.05 ± 2.31	95.35	4.65
No Plant + 0 NaCl			
As	35.91 ± 0.98	0	100
Cd	22.94 ± 2.06	0	100
Zn	164.95 ± 1.45	0	100
Pb	15.72 ± 1.13	0	100
No Plant + NaCl			
As	27.68 ± 2.03	0	100
Cd	33.56 ± 2.56	0	100
Zn	159.4 ± 13.2	0	100
Pb	18.37 ± 3.0	0	100

of treatment and was still the highest in soil treated with NaCl in the presence of *K. pentacarpos*. Such an impact was however not recorded in the absence of *K. pentacarpos*. Zinc bioavailability also decreased in columns cultivated with *K. pentacarpos* after 12 weeks comparatively to 4 weeks.

Cumulative values for leakage volume are provided for the four treatments in Fig. 4A. It is interesting to notice that the total volume of leachate collected only slightly increased between week 8 and week 12. Nevertheless, the leakages were highly concentrated in pollutants comparatively to those collected at the beginning of the experiment: as a consequence, the total amount of pollutant recovered in the leachates mainly increased at the end of the experiment as indicated in Fig. 4B for As, Fig. 4C for Cd and Fig. 4D for Zn. Considering its low mobility, only small amounts of Pb were quantified in the leachate and data are not presented. In all cases, the presence of NaCl strongly reduced leaching of pollutant, salt impact being especially important at the end of the experiment.

In column maintained in the absence of plants, leakage volume was 118 mL, 395 mL and 420 mL after 4, 8 and 12 weeks respectively. The analysis of leachate in terms of pollutant concentration allowed us to compare pollutant leaching in the absence and in the presence of plants and to quantify for each element, the percentage of mobilized pollutant

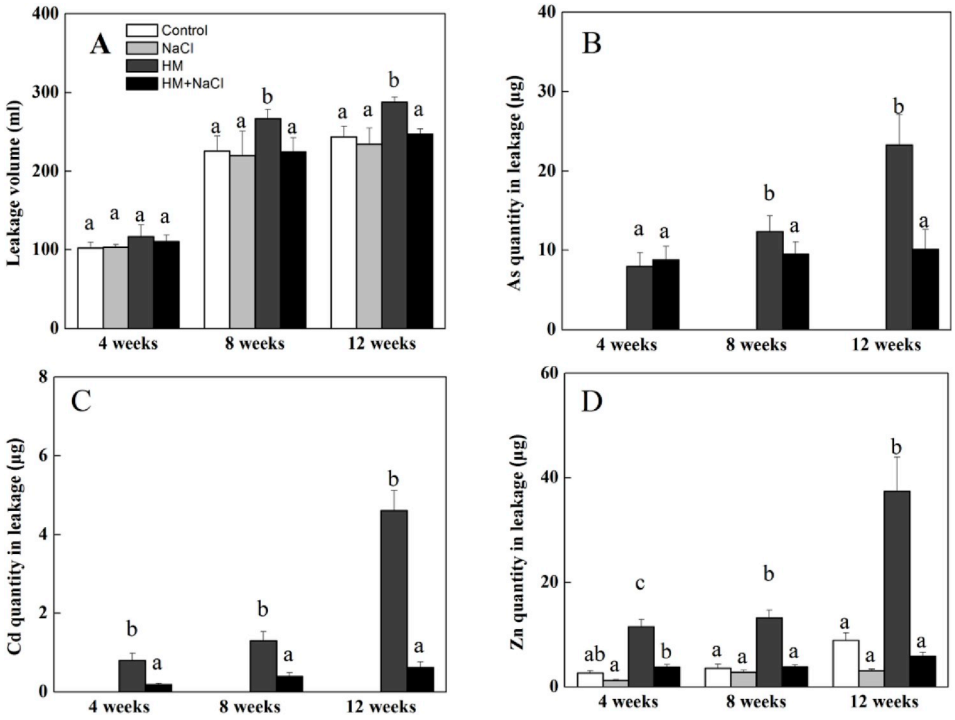

Fig. 4. Cumulative volume of leachate (A) and amounts of arsenic (B), cadmium (C) and zinc (D) recovered at the bottom parts of the columns after 4, 8 and 12 weeks of culture of *Kosteletzkya pentacarpos* cultivated on a non-polluted soil (control), the same soil added with NaCl (Na), with a mixture of heavy metals (HM) or with heavy metals and NaCl (MH + Na). Each value is the mean of 5 replicates and vertical bars are standard errors. Mean sharing a common letter are not significantly different according to the Tukey test ($P > 0.05$).

which accumulated in the plant shoot of *K. pentacarpos* (Table 3). In columns cultivated with *K. pentacarpos*, salinity reduced the total amount of As removed from the system, while a slight increase was recorded for Cd, Zn and Pb. From a relative point of view, removal of Pb was especially low. In the presence or in the absence of NaCl, most of the extracted As was removed by the leaching process (> 90%). The addition of NaCl however strongly increased the proportion of Cd and Zn taken up by the plants and drastically reduced the proportion of pollutant removed by the leachates. Only a slight proportion of removed Pb was obtained in the leachates. In the specific cases of columns maintained in the absence of plants, the total amounts of pollutants removed by the system were higher for all considered elements. Salinity had a minor impact on HM leaching, except for Cd which significantly increased. For columns maintained in the absence of plants, all removed pollutants were of course recovered in the leachates. Plants exposed to NaCl and to NaCl + HM removed 10.71 and 12.78 mg of Na in the shoot part (detailed data not shown) but for the two situations, Na content removed by leachate was close to 250 mg and constituted more than 95% of the removed Na.

4. Discussion

Halophyte plant species have been recommended as promising material for phytoremediation of heavy-metal contaminated sites (Lutts and Lefèvre, 2015; Van Oosten and Magio, 2015). However, most studies dealing with heavy metal resistance in halophyte are performed in nutrient solution under hydroponic culture and do not consider the impact of plant on pollutant mobility and availability. The present study demonstrates that the use of the wetland halophyte plant species *Kosteletzkya pentacarpos* allows to extract toxic elements and to reduce pollutant leaching, and that the extent of these properties depends on the nature of the considered element and the presence of NaCl. Salinity indeed increased Cd bioavailability and decreased As bioavailability while it had a limited (Zn) or no (Pb) impacts on other elements bioavailability. Nevertheless, salinity increased the proportion of mobilized Cd, Zn and Pb removed by the plant and strongly decreased the proportion of those elements removed by leaching process. A salt-induced increase in Cd mobility has been reported in other studies and may lead to ground water contamination (Du Laing et al., 2008; Wahla and Kirkham, 2008; Zhao et al., 2013). In our experimental system, cultivating *K. pentacarpos* allows to reduce considerably from 33.56 to less than 1 µg the amounts of Cd removed by the leachate. A decrease was also recorded for Pb and Zn, although NaCl had no or very low impact on these elements bioavailability which suggest that NaCl was acting on the plant absorbing capacities rather than on element availability in the substrate.

The 0.01 M $CaCl_2$ extraction provided information about exchangeable metal pools and has also been used to study the impact of soil ageing on bioavailability (Schreck et al., 2011). The spiked soil used in this study was allowed to equilibrate for a limited duration and was therefore not fully relevant from the behavior of a soil contaminated since a long time. This could, at least partly, explain the high bioavailability recorded in this study (Lambrechts et al., 2011a, 2011b). The advantage of the system is that a control soil with similar physical properties may be used and allow comparison. In all treatments, *K. pentacarpos* reduced the volume of leachate comparatively to column maintained in the absence of plants. This contrast with the data obtained by Lambrechts et al. (2011b) who demonstrated that cultivating the grass species *Lolium perenne* on a contaminated soil increased percolation and lead to a higher leachate volume in relation to an increase in infiltration capacity caused by the root development. Those discrepancies may be related to the contrasting morphophysiological properties on the Malvaceae *K. pentacarpos* comparatively to the Gramineae L. *perenne*. Indeed, *K. pentacarpos* is a wetland species exhibiting a high transpiration rate (He et al., 2003; Qin et al., 2015), which implies an important flow of solution to the roots and to the shoot. The process should increase with the plant development in relation to an increase in the total leaf area, which may thus reduce the volume of leachate recovered from a fixed volume of soil, even if this soil is regularly moistened to 80% of field capacity. In contrast to *L. perenne*, *K. pentacarpos* also develops a strong taproot system and a dense network of fibrous roots which facilitate nutrient absorption. Some previous reports clearly demonstrated that *K. pentacarpos* is not a hyperaccumulating plant and thus sequester high amounts of pollutant in the root system (Han et al., 2012a, 2012b).

Beside its quantitative importance, root system of *K. pentacarpos* also produces high amounts of mucilage that forms a gel-like structure (Ghanem et al., 2010). Pectin is a major constituent of mucilage and this ionic polysaccharide exhibits a high metal binding capacity. Lutts et al. (2016) demonstrated that salinity increased mucilage accumulation at the root surface and strongly increased Zn and, to a lesser extent, Cd binding capacities. Binding of pollutant on the root surface of salt-treated plants may thus explain a decrease of HM in the leachate. In the present study, however, amounts of As, Cd and Zn recovered in the leachate were especially important between 8 and 12 weeks for plants not exposed to NaCl, while during this period, the volume of leakage was rather low. A possible explanation could be that after 8 weeks, binding sites of root mucilage were saturated on plants cultivated in the absence of salt but those plants still absorbed important volume of water for transpiration, the overall consequence being a low volume of percolates with a high concentration of pollutant. Since roots were not analyzed in the present study considering the difficulties to accurately separate roots from adhering soil particles, additional work is required to test this hypothesis.

In order to efficiently contribute to phytomanagement of polluted soils, *K. pentacarpos* needs also to display tolerance mechanisms at the shoot level. Heavy metals reduced stem and leaf dry weight, confirming that accumulated elements may, to some extent, affect plant growth. It is however noteworthy that Cd accumulation increased in response to NaCl (Fig. 2) but that NaCl paradoxically suppressed the heavy metal-induced growth inhibition. This suggests that NaCl may efficiently trigger heavy metal tolerance processes in this plant or that growth inhibition recorded in the present experiment may be due to other accumulated elements, such as As whose absorption was reduced by the presence of NaCl. The Cd concentration recorded in the present study was by far lower than values recorded for Cd accumulation in *K. pentacarpos* (syn. *K. virginica*) using nutrient solution in hydroponic culture. Zhou et al. (2018b) reported values close to 300 $mg.Kg^{-1}$ DW in plants exposed to 10 µM $CdCl_2$ while Han et al. (2013a) mentioned a leaf Cd concentration of 160 $mg.Kg^{-1}$ DW in plants exposed to 5 µM $CdCl_2$. Although growth was more drastically affected in those studies than in this one, most plants remained alive at these high doses and this leads us to consider that in the present study, Cd did not reach a lethal level. It is also interesting to notice that in nutrient solution, NaCl was reducing the Cd accumulation (Han et al., 2012b; Zhou et al., 2018a, 2018b) while NaCl increased Cd accumulation the shoots of plants maintained on a contaminated solid substrate (see Fig. 2). It might be argued that in nutrient solution, formation of chlorocomplexes (especially $CdCl^+$) hampered Cd absorption while on a solid substrate, the recorded NaCl-induced increase in Cd solubility (see Table 2) may be sufficient to lead to an increased Cd accumulation. According to Filipović et al. (2018), formation of $CdCl_n^{2-n}$ chlorocomplexes may also occur in the soil solution, but a higher shoot Cd concentration observed in our study suggests that for *K. pentacarpos*, an increase in Cd solubility compensates for a decrease in availability resulting from a modified speciation.

Zinc was also reported to accumulate in *K. pentacarpos* cultivated in nutrient solution under Zn excess (Han et al., 2012b, 2013b; Zhou et al., 2018a) and, as previously reported for Cd, Zn concentration in plants cultivated on polluted solid substrate (Fig. 2) was lower than values reported for hydroponic culture. Salinity had a limited impact on Zn bioavailability and had no influence on Zn accumulation by the shoots.

Moreover, bioavailability decreased with the duration of the experiment which could partly explain the recorded decrease in stem Zn content (Fig. 2G). Stem was reported to act as an accumulating organ, especially for those elements bound to organic acids (Lutts et al., 2004).

To the best of our knowledge, As accumulation in *K. pentacarpos* has never been considered until now. The present study demonstrates i) that NaCl decreased As bioavailability on a short term basis ii) that NaCl reduced As accumulation in the shoot iii) that NaCl reduced the proportion of As removed by the plant and iv) that the amount of As removed from the columns decreased with NaCl. Vromman et al. (2016) reported that salinity decreased As absorption in the roots of the halophyte plant species *Atriplex atacamensis* but that it also increased As translocation from the roots to the shoots in this species. In the present study, As was the only element whose mobility was reduced by NaCl both in the absence and in the presence of NaCl (Table 3). This specific behavior might be related to the fact that it is present in the soil mainly as anionic form (arsenate (As(V)) in aerobic conditions and arsenite (As (III)) in anaerobic soils). Arsenate is absorbed through phosphate transporters and a decrease in P induced by HM treatment was recorded in the present study, although the concentration of P was 1000 order of magnitude higher than the endogenous accumulated As which hardly explain P decrease as a result of direct As competition for P transporters.

Lead was the less mobile element in our experimental conditions and salinity had no impact on Pb bioavailability, as previously reported by Zhao et al. (2013) for multicontaminated estuaries sediments. Despite its low mobility, the major portion of removed lead was absorbed by the plants and Pb content in plant tissues was higher than in the leachates. Li et al. (2019) recently confirmed that in the halophyte *Suaeda salsa*, salinity reduces phytotoxicity induced by low doses of Pb but, according to these authors, the involved process may be related to an hormesis effect leading to Pb dilution occurring as a consequence of NaCl-induced growth stimulation. In our experiment, 50 mM NaCl however did not stimulate plant growth in *K. pentacarpos*.

Remediation of multiple heavy metal-contaminated soils remains difficult and it is thus interesting to pay attention to physiological properties which are reported to be affected in a similar way by various pollutants, as it is the case for management of plant antioxidative status. The present study shows that *K. pentacarpos* exposed to a polycontaminated substrate encountered oxidative stress (as indicated by an increase in MDA content) but salinity completely abolished this oxidative stress (Fig. 3A). After 12 weeks, MDA content was even lower in HM + NaCl-treated plants than in controls. This reinforces the hypothesis that salinity helped the plants to cope with heavy metals even if NaCl had no effect (Zn and Pb) or even increased (Cd) toxic element accumulation in the shoot part. MDA accumulation after 8 weeks in HM-treated plants may be correlated with a global decrease in total antioxidant activity since both AOAM (Fig. 3B) and AOAD (Fig. 3C) fractions decreased. This, however, was not observed after 12 weeks. Since HM concentration did not strongly vary between 8 and 12 weeks, this suggests that the physiological consequences of toxic element accumulation and the strategy adopted by the plant to cope with them vary not only with endogenous stress intensity, but also with the duration of the stress exposure, as reported elsewhere (Quinet et al., 2012; Vromman et al., 2016).

Glutathione assumes dual functions in HM-stressed plants: it first acts as a major antioxidant but it is also the precursor of phytochelatins involved in HM complexation and vacuolar sequestration (Yadav, 2010). As a major antioxidant, GSH should be recovered in the AOAM fraction and it is therefore puzzling that salinity reduced GSH after 8 weeks with no impact on the total AOAM antioxidant activity. A similar trend was however reported at each repetition of the experiment, and this suggests that other antioxidant such as ascorbate may compensate the GSH decrease (Han et al., 2013a). Similarly, a salt-induced increase of the AOAM fraction may be related to lipophilic antioxidant such as α-tocopherol which was already showed to play an important function in *K. pentacarpos* exposed to Cd (Han et al., 2013a). Phytochelatins are known to efficiently bind Cd and As (Yadav, 2010) but in the present study, the NaCl-induced increase in Cd accumulation did not lead to higher PC content in HM + NaCl-treated plants, and PC content was even lower after 8 weeks than in plants exposed to heavy metals in the absence of NaCl (Fig. 3G), suggesting that other strategies may be adopted by the plant to cope with a higher Cd content. Cell wall sequestration may be an alternative strategy to maintain Cd away from the cytosolic metabolic activities but Zhou et al. (2018a) recently reported that in *K. pentacarpos*, NaCl reduces the proportion of Cd bound to the cell wall and increased the proportion of intracellular Cd.

5. Conclusions

Kosteletzkya pentacarpos appears as a suitable halophyte plant species for phytoremediation of polycontaminated salt-affected soils. It drastically reduced Pb, Cd, Zn and As percolation but also displayed tolerance mechanisms to accumulated pollutants. Salinity increased the proportion of Cd and Zn removed by the plants, improved plant tolerance to heavy metals in *K. pentacarpos* and contributed to reduce heavy metal percolation in polluted soils cultivated with this species. Additional work is however required to precisely identify the physiological properties involved in heavy metal resistance in *K. pentacarpos*.

Acknowledgements

Mingxi ZHOU is grateful to the CSC (China scholarship council) for the award of a research fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109460.

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