Ecological Indicators 64 (2016) 297–308

Contents lists available at ScienceDirect

Ecological Indicators

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

# Assessing of tolerance to metallic and saline stresses in the halophyte *Suaeda fruticosa*: The indicator role of antioxidative enzymes

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ARTICLE INFO

*Article history:*
Received 10 September 2015
Received in revised form
24 December 2015
Accepted 12 January 2016
Available online 29 January 2016

*Keywords:*
*Suaeda fruticosa*
Halophyte
Tolerance
Pb and Zn accumulation
Antioxidant enzymes

ABSTRACT

Many areas are simultaneously affected by high concentrations of salts and trace metal elements (TME), the latter constituting a serious threat to human health. In the present study, we determined the combined effect of high salinity and toxic levels of trace elements on physiological behavior of the halophytic species *Suaeda fruticosa*. Plants were cultivated for three months with an irrigation solution supplemented separately with different concentrations of $Pb^{2+}$ and $Zn^{2+}$ (0, 200, 400 and 600 μM) with and without 200 mM NaCl. Growth, total chlorophyll, water status and ion nutrition were quantified and antioxidant enzyme activities [ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and catalase (CAT)] were studied. Our results revealed that *S. fruticosa* has a strong ability to tolerate lead and zinc. This halophyte accumulated higher concentrations of TME in their roots. Growth parameters of *S. fruticosa* were not significantly affected by TME. An enhancement of $Ca^{2+}$ concentration accompanied by a decrease of $Mg^{2+}$ content was observed under $Pb^{2+}$ or $Zn^{2+}$ treatments whereas $K^+$ content was not affected by TME. Of the antioxidant enzymes, the activity of CAT and APX was increased by metal stress. However, the activity of GPX was diminished by increasing TME concentrations. It was concluded that NaCl 200 mM had a positive impact on the response of *S. fruticosa* to $Zn^{2+}$ toxicity, acting through a decrease in Zn absorption.



## 1. Introduction

Salt marsh plants have evolved and naturally adapted to the abiotic constraints of environment, particularly to the inherent land salinization. Adding to the saline stress, these plants also showed the adaptation to trace metal elements (TME) in the contaminated salinized land (Kholodova et al., 2010), highly prevalent in salt marshes (Han et al., 2013a).

Halophytes are tolerant species to highly salinity conditions, having developed different strategies to survive and complete their life cycles in such a harsh environment (Eid, 2011). These salt marsh plants are also known to tolerate and accumulate TME at levels that would be widely phytotoxic for most other species (Matthews et al., 2005). The tolerance of these plants to NaCl and TME may, partly, rely on common physiological mechanisms (Manousaki and Kalogerakis, 2011) such as the controlled uptake and compartmentalization of $Na^+$, $Cl^-$ and TME, the induction of antioxidant systems, and the synthesis of organic solutes (Martinez et al., 2005).

Salinity can influence TME uptake by plants (Fitzgerald et al., 2003; Kadukova and Kalogerakis, 2007), e.g. by altering the absorption capacity at the roots or even the translocation of TME to the shoots (López-Chucken and Young, 2005; Lefèvre et al., 2009). The interaction between TME and salinity may vary according to the plant species under study (Comino et al., 2005). For example, Bankaji et al. (2014) signaled that the addition of 200 mM NaCl to the irrigation solution significantly reduced the endogenous $Cd^{2+}$ and $Cu^{2+}$ concentration in different tissues of *Suaeda fruticosa* and *Atriplex halimus*.

Zinc (Zn) and lead (Pb) are widely distributed in the salt marsh environment and are considered important pollutants (Barak and Helmke, 1993; Kosobrukhov et al., 2004). Zn has great mobility and availability to plants (Morillo et al., 2004), and is an essential micronutrient with an important role in plant metabolism (Kabata-Pendias and Pendias, 2001). However, in high concentrations is responsible for toxic effects (Chaney, 1993), affecting, for example, the Calvin cycle or photosystem activity (Van Assche and Clijsters, 1986). Pb is considered as one of the most abundant metal in the earth's crust (Tong et al., 2000). Unlike Zn, Pb has no essential role in plant metabolism, being very toxic to plants (Kosobrukhov et al., 2004). Several interferences in plant metabolism have been described for Pb: it affects photosynthesis at the stomata level,

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http://dx.doi.org/10.1016/j.ecolind.2016.01.020
1470-160X/

mesophyll cells, pigment content and light and dark reactions, disturbs ion distribution within the plant (Trivedi and Erdei, 1992) and inhibits growth (Malkowski et al., 2002).

The overall purpose of this article is to assess the tolerance of *S. fruticosa* under anthropogenic stresses induced by TME ($Zn^{2+}$ or $Pb^{2+}$) alone or combined with NaCl. To achieve this aim, we will (1) test the survival, growth and physiological response of *S. fruticosa* to different metal concentrations, in the presence and absence of salt; (2) quantify the capacity of this species for accumulating Zn and Pb; and (3) determine antioxidant enzyme activities, as specific biochemical biomarkers, to investigate the defensive responses of the plant.

## 2. Material and methods

### 2.1. Plant material and culture

Young plants of *S. fruticosa* were obtained by cutting propagation (Bankaji et al., 2014). In these conditions, rooting occurred one month after planting. Young rooted cuttings were irrigated three times a week with Hewitt nutritive solution (1966) enriched with iron as complex EDTA-K-Fe and micronutrients as mixture of salts: $MnCl_2$; $CuSO_4{\cdot}5H_2O$; $ZnSO_4{\cdot}7H_2O$; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$; and $H_3BO_3$. The electrical conductivity of the nutritive solution was 1.7 dS $m^{-1}$. After four weeks of pretreatment, plants were divided into 14 groups of twenty plants that were stressed for three months. Control plants were regularly (three times a week) irrigated with the same nutritive solution and the rest of groups used the same solution supplemented with 1) $PbSO_4$ 200 μM; 2) $PbSO_4$ 400 μM; 3) $PbSO_4$ 600 μM; 4) $ZnSO_4$ 200 μM; 5) $ZnSO_4$ 400 μM; 6) $ZnSO_4$ 600 μM; 7) NaCl 200 mM; 8) $PbSO_4$ 200 μM + 200 mM NaCl; 9) $PbSO_4$ 400 μM + 200 mM NaCl; 10) $PbSO_4$ 600 μM + 200 mM NaCl; 11) $ZnSO_4$ 200 μM + 200 mM NaCl; 12) $ZnSO_4$ 400 μM + 200 mM NaCl; 13) $ZnSO_4$ 600 μM + 200 mM NaCl. Plants were grown in a greenhouse with a natural photoperiod, mean temperature (night-day) of 15–25 °C and relative humidity between 60 and 90%.

Two harvests were made, at the beginning of treatment and 90 days later. At harvest, plants were randomly separated into groups of five plants for further analysis. In the first group, leaves and shoots were separated from roots, washed, frozen in liquid nitrogen and kept at −80 °C respectively for chlorophyll and enzymatic activities assays. The remaining plants (groups of 10 plants), for the cation assays, were divided into shoots and roots, three times successively rinsed in cold water and blotted with filter paper. Treated roots were dipped in a cold solution of $CaCl_2$ during 5 min (Stolt et al., 2003) to eliminate trace elements adsorbed to the root surface and then rinsed three times with cold distilled water. Fresh weight (FW) was immediately determined and dry weight (DW) was measured after plant material desiccation in an oven at 60 °C until constant weight.

The relative growth rate (RGR) based on shoots and roots fresh weight production, was calculated as: $RGR = (\ln (W_2) - \ln (W_1))/(t_2 - t_1)$, where W is the fresh matter at the beginning of treatment ($W_1$) and at the end ($W_2$), and ($t_2 - t_1$) is the duration of this period (Hunt, 1990).

### 2.2. Chlorophyll assay

Leaves were washed with distilled water and homogenized with 10 ml of 80% ethanol solution to extract chlorophyll. The mixture was placed in a water bath at 80 °C for 20 min. The absorbance of the chlorophyll solution was measured at 654 nm using a spectrophotometer (Thermo Spectronic Genesis UV10, USA). The chlorophyll content was estimated using the formula of Arnon (1949).

### 2.3. Water content

The tissue water content was determined as: WC = (FW − DW)/DW. This parameter (WC) is expressed in ml of $H_2O$ $g^{-1}$ DW).

### 2.4. Cation assays

Dry plant material was digested using a mixture of $HNO_3$:$HClO_4$ acids (4:1, v/v) (Mediouni et al., 2006). Mineralization was conducted gradually from 100 to 350 °C for 2 h. After that, the samples were taken into 50 ml of $HNO_3$ 0.5%. The extracts were filtered through a filter paper (Whatman N° 1) and concentrations of $Pb^{2+}$, $Zn^{2+}$, $K^+$, $Na^+$, $Ca^{2+}$and $Mg^{2+}$ in plants were determined by atomic absorption spectrometry (Perkin Elmer PinAAcle 900T, USA). The blanks were processed as described above. It is used to adjust the zero in the atomic absorption spectrometer and to assess contamination during the process.

### 2.5. Enzyme assays

The frozen shoots were ground in a mortar and pestle with a small amount of inert sand, in a solution (2.0 ml/0.4 g FW) containing 50 mM $KH_2PO_4$/$K_2HPO_4$ (pH 7.0), 5 mM Na-ascorbate and 0.2 mM EDTA. After filtration through four layers of Miracloth, the homogenate was centrifuged at 6000 rpm for 15 min. All operations were performed at 4 °C and the samples were prepared for CAT, APX and GPX analysis. Enzyme activities were immediately determined in the supernatant, by a UV/vis spectrophotometer (TOMOS, UV-1200 Spectrophotometer). Results of the kinetic study of enzymes showed that the reaction took place during the second minute. Indeed, 60 s after the addition of the protein extract, there was a sudden variation in the optical density after which the values remained constant.

Catalase activity (CAT, EC1.11.1.6) was determined by monitoring the loss of $H_2O_2$ and by measuring the decrease in absorbance at 240 nm (molar extinction coefficient: $\varepsilon = 0.036$ $mM^{-1}$ $cm^{-1}$) of a reaction mixture containing 25 mM K-phosphate buffer (pH 7.0), 10 mM $H_2O_2$ and 2 ml of enzyme extract (Aebi, 1984).

Guaiacol peroxidase (GPX, EC1.11.1.7) was assayed according to Fielding and Hall (1978). The reaction mixture consisted of 25 mM K-phosphate buffer (pH 7.0), 10 mM $H_2O_2$, 9 mM guaiacol and 2 ml enzyme extract. The enzyme activity was measured by monitoring the increase in absorbance at 470 nm (molar extinction coefficient: $\varepsilon = 26.6$ $mM^{-1}$ $cm^{-1}$) during polymerization of guaiacol.

Ascorbate peroxidase (APX, EC1.11.1.11) was determined by the decrease in absorbance at 290 nm (absorbance coefficient of 2.8 $mM^{-1}$ $cm^{-1}$) as ascorbate was oxidized according to Nakano and Asada (1981). The reaction mixture contained 25 mM K-phosphate buffer (pH 7.0), 0.5 mM ascorbate, 2 mM $H_2O_2$, 0.1 mM EDTA and 2 ml of enzyme extract.

Each of the above assays was only used in a range in which the rate of reaction was proportional to the amount of extract added. The reactions were started by adding the enzyme extracts to substrates and the absorbance was rapidly recorded after this addition. Control assays were done as described above in the absence of extract. All enzyme activities were expressed per gram fresh weight. One unit of enzyme was defined as the amount necessary to decompose 1 μmol $min^{-1}$ of each substrate at 25 °C in the case of CAT and APX. For GPX activity, one unit was defined as amount of enzyme producing 1 μmol $min^{-1}$ of tetraguaiacol at 25 °C.

### 2.6. Statistical analysis

All samples were analyzed at least in five replicates and the mean values along with the standard error (±) are shown in bars in