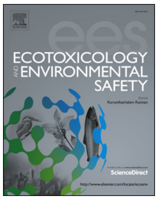
Ecotoxicology and Environmental Safety 164 (2018) 69–74

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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



Leaf shape variation as a potential biomarker of soil pollution

Maria de la Paz Pollicelli[a], Yanina L. Idaszkin[a,b], Rolando Gonzalez-José[c], Federico Márquez[b,d,*]

[a] Instituto Patagónico para el Estudio de los Ecosistemas Continentales (IPEEC- CONICET), Boulevard Brown 2915, U9120ACD Puerto Madryn, Chubut, Argentina
[b] Universidad Nacional de la Patagonia San Juan Bosco, Boulevard Brown 3051, U9120ACD Puerto Madryn, Chubut, Argentina
[c] Instituto Patagónico de Ciencias Sociales y Humanas (IPCSH - CONICET), Boulevard Brown 2915, U9120ACD Puerto Madryn, Chubut, Argentina
[d] Instituto de Biología de Organismos Marinos (IBIOMAR - CONICET), Boulevard Brown 2915, U9120ACD Puerto Madryn, Chubut, Argentina

ARTICLE INFO

Keywords:
Cressa truxillensis
Geometric morphometrics
Heavy metals
Salt marsh
Patagonia

ABSTRACT

Halophytic plants play a fundamental role in salt marshes, influencing their structure, dynamics, and cycling of nutrients and minerals. These plants have the ability to retain metals in the soil, or absorb and retain them in underground structures, or transport them to their aerial structures. Here we aim to study shape variation in the leaves of *Cressa truxillensis* inhabiting the salt marsh of San Antonio Oeste, according to their proximity to a source of metals in the soil. A gradient of bioavailability of metal was observed in the soil, decreasing from the site closest to the source to the most distant point, where Zn was the most abundant metal followed by Pb and Cu. We used landmark-based geometric morphometric tools to study leaf shape variation. We observed more oval leaf growth on the farthest point of the pollutant's source, and lanceolate shape close to it. No significant among-site size differences were found. Collectively, these results suggest that the stress conditions associated with the soil metals' concentration generate changes in the leaf shape of *Cressa truxilensis*. Considering that this species has not been extensively analyzed, this study establishes a baseline and supports the use of the leaf as an early biomarker of stress by contamination in plants associated with marshes.

1. Introduction

In salt marshes affected by pollution, soils and plants play an important role due to their ability to hold the pollutants, hence altering their dynamics in the environment (Almeida et al., 2011; Duarte et al., 2010; Hung and Chmura, 2007). In this regard, plants interact with these elements modifying their bioavailability, absorbing pollutants from the soil and distributing them in their tissues, both accumulating them in the roots and rhizomes or in stems and leaves (Burke et al., 2000; Duarte et al., 2010; Idaszkin et al., 2014, 2017c; Reboreda and Caçador, 2007b). Like other halophytes, plants inhabiting in salt marshes are adapted to extreme conditions of flooding, and soil anoxia and high salinities. Furthermore, occupation of contaminated soils has been reported as well (Duarte et al., 2010; Idaszkin et al., 2011, 2015, 2017c; Redondo-Gómez, 2013). However, when pollutants such as heavy metals are in excess they could be toxic for the plants, having deleterious effects. In this sense, plants could display diverse strategies to counteract the soil metal excess, either limiting the uptake or transporting of the metal, or through internal tolerance mechanisms (Ashraf et al., 2010). Some of the most common signs that exhibit plants facing metal toxicity are decrease in growth and biomass production, alterations of the metabolism, activation of the antioxidant system, senescence, and morphological changes (in root, stem and/or leaves) (Kabata-Pendias, 2011; Nagajyoti et al., 2010).

Biochemical, physiological and morphological responses produced in organisms growing under stressful conditions, such as soil metal excess, can be considered biomarkers of stress. Currently, as opposed to the quantification of accumulated pollutants in soils and tissues, biomakers are considered more useful in environmental studies, being that they provide information on the potential effect of these substances on the health of organisms. Furthermore, these have the advantage of evidencing early symptoms of the damages caused by the contaminants, and therefore they can be used as an early sign of the presence of contaminants in the environment. Within the more common biomarkers used as a response in plants exposed to heavy metals are variations in the content of photosynthetic pigments, phytochelatins and non-protein thiols, free proline, phenolic acids and antioxidant enzyme activities (Ferrat et al., 2003; Keltjens and van Beusichem, 1998; Kirbag Zengin and Kirbag, 2007; Monni et al., 2001). Also, the use of these techniques could enable rapid, continuous, and low-cost monitoring protocols of the pollution's deleterious effects on communities, being the search of an overcoming method to be applicable in environmental quality researches a fundamental challenge.

On the other hand, an alternative way to evaluate the pollution

---

[*] Corresponding author.
E-mail address: fede@cenpat-conicet.gob.ar (F. Márquez).

https://doi.org/10.1016/j.ecoenv.2018.08.003
Received 16 April 2018; Received in revised form 26 July 2018; Accepted 1 August 2018
0147-6513/

effects in the organism shape is through the application of geometric morphometric methods (GMM), which allows the detection of subtle morphological variations (Márquez et al., 2017). This method allows the study of shape and size variations with a high level of detail (Adams et al., 2004; Bookstein, 1991). GMM, unlike the conventional linear-distance based morphometrics, enables a proper separation between size and shape variation (Bookstein, 1997). Another advantage is the preservation of the geometric information throughout the statistical analyses, which allow the detailed and graphic visualization of both the magnitude and the direction of the morphometric changes (Zelditch et al., 2012). In the last two decades the use of GMM has increased in zoological and botanical studies (Klingenberg et al., 2012; Viscosi et al., 2009; Viscosi and Cardini, 2011). Some of these assess the relationship between variations in shape of some structure and the pollution of their environment, with most of the previous research being performed on animals (Márquez et al., 2011, 2017; Primost et al., 2016). However, main plant studies using GMM are focused on determining patterns of variation in form at the inter- or intra-specific level, on structures such as seeds (Chemisquy et al., 2009), floral organs (Shipunov and Bateman, 2005), and leaves (Iwata et al., 2002; Jensen et al., 2002; Vieira et al., 2014). For example, GMM was used to classify and discriminate varieties of Orchids, based on variations in shape of floral pieces (sepals, petals and labellum) (Dalayap et al., 2011). Regarding pollution studies, Vujić et al. (2015) evaluated pollution impact on the petal shape of *Iris pumila* flowers using GMM, finding that plants growing on a site exposed to polluted air present a smaller and more rounded shape than plants from a site without contamination.

Previous studies evidence a gradient in soil metals content within the salt marsh surrounding the San Antonio Bay (Patagonia, Argentina) (Idaszkin et al., 2015, 2017a, 2017b, 2017c). This salt marsh is inhabited by the cord grasses of the genus *Spartina* and other halophytes such as *Sarcocornia perennis, Limonium brasiliense,* and *Cressa truxillensis*. In particular, *C. truxillensis* Kunth (Convolvulaceae) is a native perennial halophyte widely distributed in the American continent. It has small pubescent leaves (3–12 * 1.5–4 mm) with an elliptic to lanceolate form. It was precisely the shape of its leaves which motivated its selection as the subject of this study, considering that their size and their arrangement in a two-dimensional plane allow the application of geometric morphometrics techniques in 2D. Therefore, the main goal of this study was to evaluate the relationship between shape variations on leaves of *C. truxillensis* plants and the metal bioavailables in soils in the San Antonio Oeste salt marsh, which will allow postulating the use of a potential response as a biomarker of soil stress pollution.

2. Materials and methods

2.1. Sampling

We worked in San Antonio salt marsh, located surrounding the San Antonio Bay (40°44′S, 54°68′W), in a Natural Protected Area (Río Negro, Argentina, Fig. 1). Sampling sites comprised three sites within the salt marsh adjacent to the main tidal channel (sites called “A”, “B” and “C”), site A is located in the topographically higher area of the salt marsh and receives the surface runoff from the mining deposits drainage. Site B is located near the above site but in a lower topographic sector of the salt marsh, whereas site C is located in the same channel as sites 1 and 2, but in an external sector of the salt marsh with more marked tidal influence (Fig. 1).

Table 1
Number of leaves collected at each site (n), their length (between 1 and 2 landmarks; mean (SD)) and width (between semilandmarks 6–11; mean (SD)) per site.

Sites	n	Length (mm)	Width (mm)
A	133	6.45 (0.90)	3.10 (1.07)
B	116	6.65 (0.89)	3.34 (1.16)
C	97	6.60 (0.73)	3.44 (1.10)

In order to study the size and shape attributes on leaves of *Cressa truxillensis*, plant samples were collected during December 2015 within each sample site. From these plants, branches with flowers (Table 1) from which the fully deployed leaf was separated immediately below the last flower (bracts) were randomly collected. A total of 346 leaves were obtained. In order to avoid the loss of turgor and consequently any type of modification of the leaf shape, each sheet was digitized in situ using a conventional Epson perfection v37 scanner, obtaining the image of the adaxial side of the leaf.

2.2. Soil bioavailable metals

In order to determine the soil bioavailable metal concentrations, at these three sites of the salt marsh, thirty soil samples (five per site) 15-cm-diameter and 15-cm-depth were collected at low tide. The soil samples were dried at 80 °C until constant weight and sieved through a 2 mm mesh to remove large stones and dead plant material. Then, to extract the labile or potentially bioavailable metals, 1 g of dried and sieved soil was used to make a cold extraction with 25 ml of 0.5 N HCl (Agemian and Chau, 1976). Copper (Cu), lead (Pb), and zinc (Zn), in both matrixes were then measured by inductively coupled plasma (ICP-AES) spectroscopy (Shimadzu 9000). In all cases, the average uncertainty of metal ion determination was < 2%. All extractions were carried out in duplicate and blanks were processed as the samples. Results were reported on a dry weight. Reagents of analytical grade were used for the blanks and for calibration curves. Quality assurance of soils was done through analysis of standard reference freshwater sediment CNS392-050. The recovery in soil was > 87% for all measured metals.

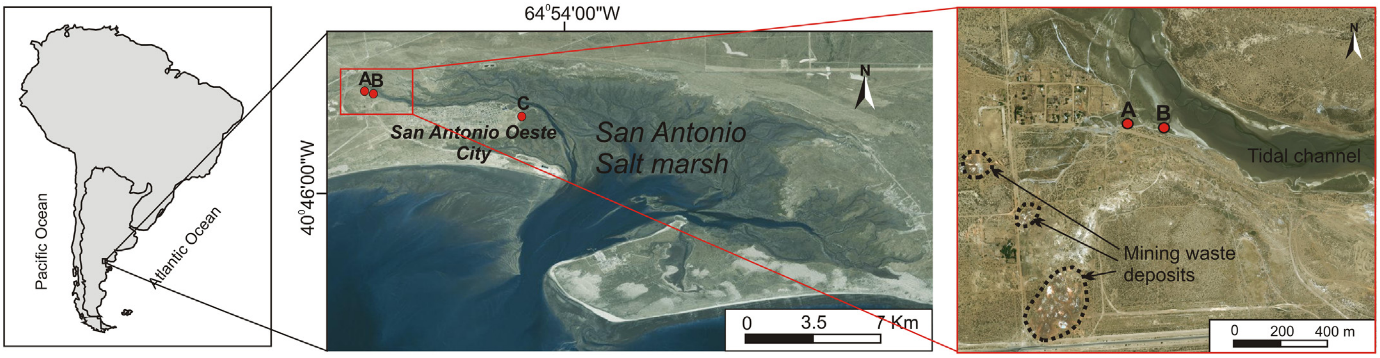


Fig. 1. Location map showing the study area and the sampling sites.