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Accumulation and *in vivo* tissue distribution of pollutant elements in *Erica andevalensis*

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ABSTRACT

Erica andevalensis is an endemic shrub from an area in the southwest of Spain (Andalucia) characterized by acidic and contaminated soils. Scanning electron microscopy (SEM) of samples after conventional or cryo-fixation preparation protocols was used for morphological and anatomical studies. SEM coupled with EDX-analysis was employed to localise and quantify different elements within plant parts (leaves, stems and roots) in samples collected in the field. Morphological studies revealed that the species has typical adaptive structures to drought-stress such as rolled needle-like leaves, sunken stomata and a thick waxy cuticle on the upper epidermis. Roots were associated with fungi which formed intra and extra-cellular mycelia. The SEM studies showed that Cu was not sequestrated into the root tissues and was uniformly distributed in leaf tissues. Meanwhile, Pb was only localised within epidermal root tissues which indicates that its sequestration in an external matrix might represent a tolerance mechanism in this species. Iron was uniformly distributed throughout the leaves, while in roots it was predominantly retained on the epidermal cell walls. The exclusion and tolerance mechanisms adopted by this species to survive in mining areas indicate that it can be used successfully in the re-vegetation of contaminated areas.

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1. Introduction

Metallophyte plants are species able to colonize metalliferous soils since they have evolved metal tolerance mechanisms. Such species are divided into two main groups: the so-called pseudometallophytes that grow on both contaminated and non-contaminated soils, and the absolute metallophytes that grow only on metal-contaminated and naturally metal-rich soils (Baker, 1987). Mining areas constitute a hostile environment for plant survival since they have high metal concentrations, acidic pH and often nutrient imbalances. Plants that are able to grow in these conditions have developed effective mechanisms to tolerate all these environmental constraints. The mechanisms involved in heavy-metal resistance are species-specific and usually divided into avoidance and tolerance systems (Gratão et al., 2005). Avoidance is expressed as external protection against toxic elements that can be facilitated by mycorrhizae or the active orientation of the roots to the less toxic soil (Tyler et al., 1989). Tolerance can be due to the compartmentalization of the metals in cell walls or vacuoles, complexation with chelating agents such as proteins (phytochelatins, metallothioneins) or other organic compounds (malate, citrate), or by active excretion through roots and leaves (Verkleij and Schat, 1989; Ernst et al., 1992, Bringezu et al., 1999; Heumann, 2002; Yang et al., 2005). Avoidance and tolerance may operate singly or in combination in metal detoxification (Turnau et al., 2007).

The Ericaceae is a big family that includes species that are efficient colonizers (Luteyn, 2002). *E. andevalensis* Cabezudo & Rivera is an edapho-endemic species of the Iberian Pyrite Belt

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where it may be considered a metallophyte species. Although many aspects of this species have been investigated (Soldevilla et al., 1992; Aparicio, 1999; Abreu et al., 2007, Rodríguez et al., 2007; Rossini Oliva et al., in press) there is only one study on the localisation of metals in *E. andevalensis* plants from Portugal (Turnau et al., 2007).

Scanning electron microscopy has often been used to localise metals in plant tissues (Fernando et al., 2006; Turnau et al., 2007; Solís-Domínguez et al., 2007) but sample preparation is the most crucial step in order to obtain reliable data and to provide an accurate representation of the spatial *in vivo* distribution of elements (Heumann, 2002; Kachenko et al., 2008). The crucial point is to find a method that preserves plant tissue/cellular structures avoiding element loss or redistribution during sample preparation. The cryo-method has the advantage of rapidly freezing plant material so that vulnerable biological structures are well preserved, elements are immobilized and during cryo-fracture the material breaks cleanly along weak edges (e.g. membranes) without causing much deformation (Erlandsen et al., 2003; Sriamornsak et al., 2008). Therefore in the

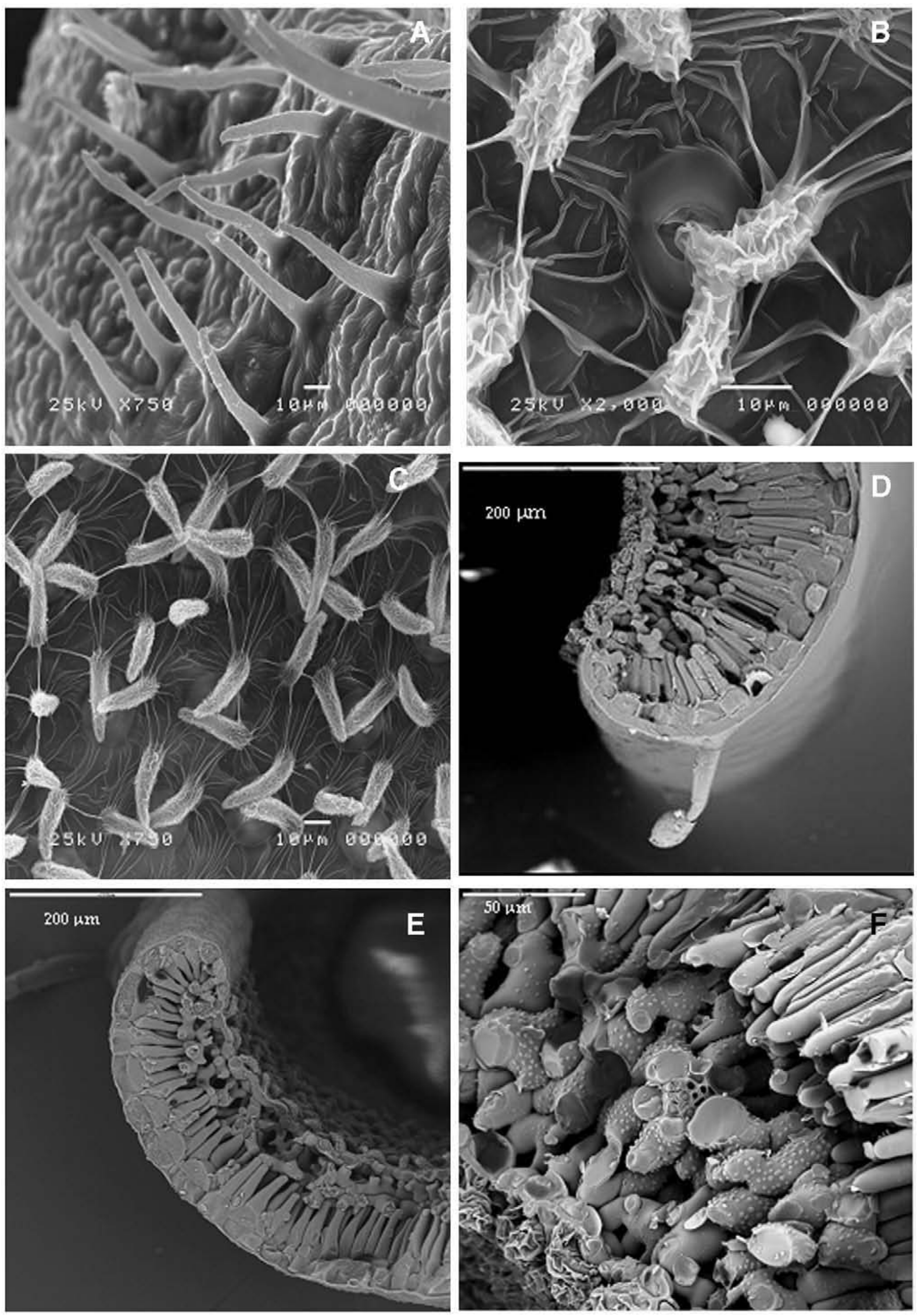

Fig. 1 – Leaves of *Erica andevalensis*. A–C: SEM micrographs of adaxial (A) and abaxial surface, with details of stomata (B) and papillae (B–C). D–F: details of palisade and spongy mesophyll viewed by cryo-SEM.