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Seasonal changes in antioxidative/oxidative profile of mining and non-mining populations of Syrian beancaper as determined by soil conditions

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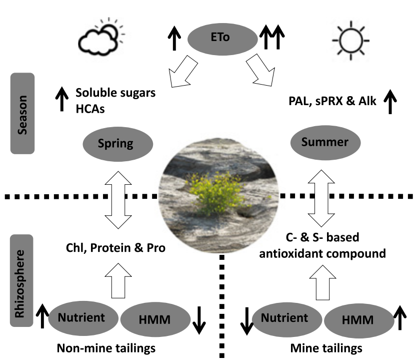
HIGHLIGHTS

- Seasonal redox changes in *Z. fabago* populations are determined by soil conditions.
- Higher C- and S- and lower N-based antioxidant levels were found in mining plants.
- The highest antioxidant capacity was found in mining populations at the end of summer.
- Proline or sugars, depending on soil N content, help maintain water balance in plants.
- Sugars and phenols in spring and alkaloid, PAL and PRX in summer typify populations.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil pollution by heavy metals/metalloids (HMMs) is a problem worldwide. To prevent dispersion of contaminated particles by erosion, the maintenance of a vegetative cover is needed. Successful plant establishment in multi-polluted soils can be hampered not only by HMM toxicities, but also by soil nutrient deficiencies and the co-occurrence of abiotic stresses. Some plant species are able to thrive under these multi-stress scenarios often linked to marked fluctuations in environmental factors. This study aimed to investigate the metabolic adjustments involved in *Zygophyllum fabago* acclimative responses to conditions prevailing in HMM-enriched mine-tailings piles, during Mediterranean spring and summer. To this end, fully expanded leaves, and rhizosphere soil, of three contrasting mining and non-mining populations of *Z. fabago* grown spontaneously in south-eastern Spain were sampled in two consecutive years. Approximately 50 biochemical, physiological and edaphic parameters were examined, including leaf redox components, primary and secondary metabolites, endogenous levels of salicylic acid, and physicochemical properties of soil (fertility parameters and total concentration of HMMs). Multivariate data analysis showed a clear distinction in antioxidative/oxidative profiles between and within the populations studied. Levels of chlorophylls, proteins and proline characterized control plants whereas antioxidant capacity and C- and S-based antioxidant compounds were biomarkers of mining plants. Seasonal variations were characterized by higher levels of alkaloids and PAL and soluble peroxidase activities in summer, and by soluble sugars and hydroxycinnamic acids in spring irrespective of the population considered. Although the antioxidant systems are subjected to seasonal variations, the way and the intensity with which every population changes its antioxidative/oxidative profile seem to be determined by soil conditions. In short, *Z. fabago* displays

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a high physiological plasticity that allow it to successfully shift its metabolism to withstand the multiple stresses that plants must cope with in mine tailings piles under Mediterranean climatic conditions.

1. Introduction

Soil pollution by heavy metals/metalloids (HMMs) is a problem worldwide, affecting not only terrestrial and aquatic ecosystems, but also wildlife and human health (Ali et al., 2013). In the European Union, ~340,000 polluted sites, most commonly contaminated by HMMs, have been identified (Panagos et al., 2013). Worldwide, it is estimated that about 22 million ha of land are contaminated by HMMs, and some of them are unsuitable and/or prohibited for food production (Nsanganwimana et al., 2014). Metalliferous mine tailings are considered the major source of pollution from mining activity due to their high metal content, and the high incidence of wind- and water-driven erosion events that promotes distribution to the surrounding areas (Ali et al., 2013; Mendez and Maier, 2008). A cost-effective tool for minimizing the erosion and dissemination of the contaminants in HMM-polluted areas is phytomanagement, which implies the establishment of a self-sustainable vegetal cover (Parraga-Aguado et al., 2013; Tordoff et al., 2000). However, in mine tailings the limited supply of essential nutrients, associated with high HMM content, and low water holding capacity may become a serious constraint for plant growth and survival. In semi-arid regions plant establishment on mine tailings is further hampered by heat and drought (Tordoff et al., 2000; Parraga-Aguado et al., 2014).

For the last 30 years, >450 hyperaccumulator plants with a strong HMM removal potential and high tolerance have been identified (Barceló and Poschenrieder, 2003; Maestri et al., 2010). However, in practice, there is a continuous interest in searching for native tolerant plants that are adapted to the local ecological environment, as a useful strategy to reach acceptable levels of plant cover in land reclamation processes (Mendez and Maier, 2008; Parraga-Aguado et al., 2014; Tordoff et al., 2000). In this regard, the Syrian beancaper *Zygophyllum fabago* (L.) is a succulent perennial shrub native from the desert of Syria, although nowadays it can be found in all Mediterranean countries and in the Southwest United States (Lefèvre et al., 2016). *Z. fabago* is considered a promising species for restoring HMM polluted soils in semi-arid regions, due to its outstanding pioneer characteristic that makes this species capable to colonize and persist in disturbed areas, including metalliferous mine tailings highly polluted by a broad range of HMMs like As, Pb, and Zn (Boojar and Tavakkoli, 2011; Conesa et al., 2006; Martínez-Sánchez et al., 2012).

In higher plants, a common consequence to environmental stresses, including HMM exposure, is an increased production of reactive oxygen species (ROS) in cells (Schützendübel and Polle, 2002). ROS in conjunction with redox active molecules set the redox environment of cells and tissues, which in turn results in signals that control the plant growth and development as well as initiate the appropriate acclimation responses to stress stimuli (Potters et al., 2010; Suzuki et al., 2012). Although the degree and mechanism of tolerance to HMMs can vary significantly among plant species (Clemens, 2006; Maestri et al., 2010), an overwhelming body of evidence shows that the reinforcement of antioxidant defense system would be essential for restoring redox-homeostasis and normal metabolism under stress (Gill and Tuteja, 2010). In this sense, higher transcript and enzymatic activity levels of several ROS-scavenging enzymes such as superoxide dismutases, peroxidases and catalases as well as greater ability to synthesize ascorbate (AA), glutathione (GSH) and phenolic compounds have been widely reported in different HMM-tolerant plants when compared with HMM-sensitive counterparts (Gill and Tuteja, 2010; Schützendübel and Polle, 2002; Sharma and Dietz, 2009). Apart from being essential compounds in the defense against ROS, GSH and phenolics have been shown to detoxify HMM by chelation, followed by subsequent sequestration into vacuoles and cell walls (for review, see Singh et al., 2015). Several authors have also described that aside from phenolics (Díaz et al., 2001; Kováčik et al., 2011; Martínez-Alcalá et al., 2013; Michalak, 2006), there are other secondary metabolites such as alkaloids and/or saponins and the proteinogenic amino acid proline (Pro) which accumulated in plant cells under HMM stress (López-Orenes et al., 2014; Sharma and Dietz, 2006; Zhao et al., 2016). Secondary metabolites contribute to all aspects of plant responses to biotic and abiotic stimuli, and guarantee flexible adaptations of plants to ever-changing environmental conditions (Akula and Ravishankar, 2011; Hartmann, 2007). However, most studies dealing with the effect of HMMs exposure on plants have been carried out under hydroponics, in a fully nutrient-rich medium, and under laboratory-controlled conditions. Currently, there exists little information regarding HMM-stress-related markers in plants growing in their natural environment, where they are exposed simultaneously to other environmental constraints. Stress combination can have deleterious effect on plant growth, and prolonged exposure to abiotic stresses can result in the weakening of plant defenses (Rizhsky et al., 2004; Suzuki et al., 2014).

Since the cellular antioxidative/oxidative status plays a pivotal role in the capability of plants to cope with oxidative stress induced by environmental factors, the aim of the current work was (i) to compare the antioxidative/oxidative profile along with key growth- and HMM stress-related parameters on three contrasting populations of *Z. fabago* grown spontaneously on two metalliferous mine tailings (Agustin and Mercader) and on a non-mining site (control) during late spring and late summer seasons in two consecutive years; (ii) to carry out an edaphic characterization of the rhizosphere soil (fertility parameters and total concentrations of As, Cd, Cu, Mn, Pb, and Zn) associated with *Z. fabago* roots on the three selected sites, and (iii) to identify inter-correlations among the different physiological and antioxidative/oxidative parameters evaluated in both seasons as well as associations between plant markers and environmental factors using both unsupervised and supervised multivariate statistical methods.

We hypothesized that (1) the low soil fertility conditions in the mine tailings together with their high content of hazardous HMMs would provoke important changes in the accumulation of N-containing metabolites and would enhance the accumulation of metal-chelating compounds, and (2) during summer the combination of nutrient deficiencies, HMM toxicities and high temperature and drought would drastically impact on both photosynthetic performance and the strengthening of the antioxidant network. The results obtained could contribute to improve our understanding of the acclimative responses of plants to stress combinations under natural conditions. Taking into account the potential of this species for thriving under extreme environmental conditions, use seems plausible in land reclamation programs.

2. Materials and methods

2.1. Plant sampling and soil analysis

Samples of *Z. fabago* L. leaves were obtained from plants growing spontaneously in the Cartagena-La Union Mining District (37°37′20″ N, 0°50′55″ W–37°40′03″ N, 0°48′12″ W), specifically in two mine tailings (Agustin and Mercader) and in a non-mining area located about 1.5 km away from the tailings piles (Supplemental Fig. S1). Therefore, it was assumed that all plants from the three populations were exposed to similar weather conditions. The two mine tailings are located in a natural park which includes forests of *Pinus halepensis* and endemic