Science of the Total Environment 637–638 (2018) 625–635

Contents lists available at ScienceDirect

Science of the Total Environment

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

Seasonal ionomic and metabolic changes in Aleppo pines growing on mine tailings under Mediterranean semi-arid climate

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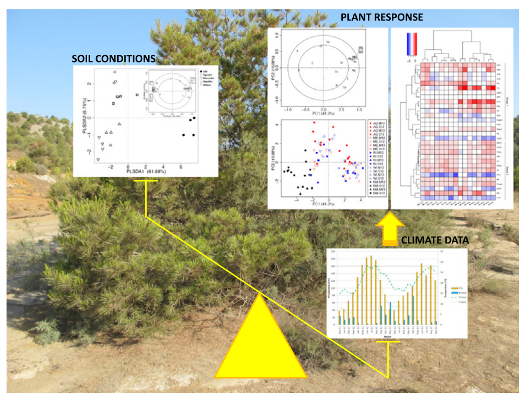
HIGHLIGHTS

- Seasonal factors influenced needle metabolism more markedly than soil factors did.
- Higher GSH and phenol antioxidants let needles to cope with higher HMMs in spring.
- RWC was maintained in summer needles mainly by accumulation of monovalent ions.
- Lower chlorophylls and higher carotenoids increased photoprotection in summer needles.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 January 2018
Received in revised form 28 April 2018
Accepted 4 May 2018
Available online xxxx

Editor: Charlotte Poschenrieder

Keywords:
Antioxidative/oxidative profiles
Ionomic profiles
Mine tailings piles
Stress combinations
Mediterranean climate

ABSTRACT

Aleppo pine is the most abundant conifer species in Mediterranean basin. Knowledge of adaptive mechanisms to cope with different environmental stresses simultaneously is necessary to improve its resilience to the predicted climatic changes and anthropogenic stressors, such as heavy metal/metal(loid)s (HMMs) pollution. Here, one year-old needles and rhizosphere soil samples from five mining and non-mining (NM) populations of Aleppo pines grown spontaneously in SE Spain were sampled in two consecutive years during spring and summer. Quantitative determination of a wide suite of edaphic, biochemical, and physiological parameters was performed, including soil physicochemical properties, ionome profile, foliar redox components, primary and secondary metabolites. Mining rhizosphere soils were characterized by elevated contents of HMMs, particularly lead and zinc, and low carbon, nitrogen and potassium levels. Multivariate data analysis based on needle ionome and antioxidative/oxidative parameters revealed a clear distinction between seasons irrespective of the population considered. Spring needles were characterized by higher levels of HMMs, sulfur, glutathione (GSH), proanthocyanidins (PAs), and soluble phenols (TPC), whereas reduced chlorophylls and increased levels of carotenoids, relative water content and K^+, Na^+ and Cl^- typified summer needles. In general mining populations had higher levels of ascorbate, and TPC, and exhibited higher antioxidant activities than the NM population. This could contribute to prevent oxidative injury induced by HMMs. Taken together, results suggest that seasonal factors have a more marked effect on the metabolism of the Aleppo pine populations studied than that exerted by soil conditions. This effect could be mediated by water availability in surface soil layers. If this conclusion is right, predicted rainfall reduction and temperature increase in the Mediterranean basin associated to global climate

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https://doi.org/10.1016/j.scitotenv.2018.05.049

change would lead to pine needle metabolism to express the summer pattern for more prolonged periods. This, in turn, could negatively affect the performance of Aleppo pine populations.

1. Introduction

Aleppo pine (*Pinus halepensis* Mill.) is the most widely distributed conifer species in the Mediterranean region (Maseyk et al., 2008; Querejeta et al., 2008). In this area the species exhibits a bimodal growth pattern, with two optimal growth periods one in spring and a second in fall associated with favorable growing conditions (i.e., mild temperatures and adequate soil-water availability) (López-Serrano et al., 2005; Pacheco et al., 2017). Although this species is noted for its high resilience to heat and drought stress (Maseyk et al., 2008; Wellburn et al., 1996), climate warming is expected to have negative effects on tree survival and development, according to the Intergovernmental Panel on Climate Change 2014 (IPCC, 2014; http://ipcc.ch/). Moreover, growth rate reductions of forest trees can be aggravated as a consequence of increased concentrations of hazardous pollutants such as heavy metal and metal(loid)s (HMMs), which are particularly prevalent in areas subjected to intense mining activity (Panagos et al., 2013). The ecological consequences of the simultaneous occurrence of natural and anthropogenic stressors are difficult to predict because they cannot be inferred from individual stress studies, especially if the stress combinations result in antagonistic or conflicting responses (Choudhury et al., 2017; Suzuki et al., 2014).

Under natural conditions, plants have evolved a complex network of signal transduction pathways to cope with multiple environmental stresses occurring simultaneously. Whether a plant is able to acclimate to these challenging environmental conditions or not is going to be, ultimately, determined by the appropriate signaling and coordination of plant responses (Harfouche et al., 2014; Urano et al., 2010; You and Chan, 2015). Extensive evidence now strongly supports that reactive oxygen species (ROS) are key signal transduction molecules in plant stress signaling (Mittler, 2017), although elevated ROS levels, above a physiological threshold, can cause oxidative damage to biomolecules and cellular structures (De Gara et al., 2010). Increased production of ROS in plant cells has been widely shown under abiotic stress conditions, including HMM exposure (Gill and Tuteja, 2010; Schützendübel and Polle, 2002; You and Chan, 2015), as well as in different stress combinations (Choudhury et al., 2017; Suzuki et al., 2014). To keep ROS steady-state concentrations low, plants possess a particularly complex and redundant ROS-scavenging system, in which enzymes and metabolites are linked in a network of reactions (De Gara et al., 2010). Recent "omics" studies have highlighted that antioxidant defense machinery can play an important role not only in plant HMM-tolerance mechanisms (Dalcorso et al., 2013; Hossain and Komatsu, 2012; Singh et al., 2015) but also in the response of plants to stress combinations (Suzuki et al., 2014; Zandalinas et al., 2017). Indeed, the induction of ROS-scavenging enzymes, as well as a high content of both primary antioxidants, i.e., ascorbate (AA) and glutathione (GSH), and secondary antioxidants, such as carotenoids, proline (Choudhury et al., 2017), and different phenolic compounds (Martinez et al., 2016), were found to have a key role in plant acclimation to stress combinations. In fact, the diversity and plasticity of phenolic compounds are considered to play a key role in plant defense mechanisms towards biotic and abiotic stresses (Agati et al., 2012; Brunetti et al., 2015; Pourcel et al., 2007). Phenylalanine ammonia-lyase (PAL), the key enzyme in controlling phenolic biosynthesis, and the large family of secreted class III plant peroxidases (PRX), which catalyze the oxidation of a wide variety of phenolic compounds using hydrogen peroxide as the electron acceptor, have also been reported to be stimulated by infection and environmental stress (Almagro et al., 2009; Dixon and Paiva, 1995). In addition, ionomics approaches have revealed that the study of shoot ionome, which represents the mineral nutrient and trace element composition of a plant (Salt et al., 2008), could potentially be used as a tool to detect specific physiological responses to environmental variation, or nutritional statuses (Baxter et al., 2008).

Metalliferous mining wastes represent very stringent conditions for plant growth because of nutrient deficiencies, high HMM content and salinity (Tordoff et al., 2000). Nevertheless, several studies have described the spontaneous colonization of HMM-enriched mine tailings by Aleppo pine in semi-arid areas (Parraga-Aguado et al., 2014, and refs. herein). Recently, woody and tree species have gained increasing interest in mine reclamation programs because of their massive and deep root systems (Luo et al., 2016). Although recent achievements in the study of the molecular responses to single stresses have been reported (Harfouche et al., 2014), the physiological and molecular mechanisms underlying the adaptation to HMMs under semi-arid Mediterranean conditions in woody plants are not clearly understood.

With this background, the overarching aim of the current work is to evaluate metabolic adjustments in response to the harsh conditions of mine tailings during both a favorable and a less-favorable growing season in Aleppo pines. To address this aim, a comparison of the antioxidative/oxidative profile, needle ionomics, physiological and edaphic parameters were carried out among five Aleppo pine populations growing either in a non-mining site (NM), or in multi-metal(loid) polluted mining tailings, located in the Cartagena-La Unión Mining District (SE Spain) during late spring (May) and late summer (September) in two consecutive years (2012 and 2013). Moreover, different dimensionality reduction and classification statistical methods were performed to identify inter-correlations among the different physiological and antioxidative/oxidative parameters evaluated, as well as possible associations between plant markers, concentrations of nutrients/metal(loid)s and soil parameters.

This work is framed within a larger study devoted to examine the oxidative stress signatures and the metabolic adjustments in response to the adverse conditions of mine-tailings under semi-arid Mediterranean conditions in different pioneer plant species, including both herbaceous (López-Orenes et al., 2018, 2017) and woody plants.

2. Materials and methods

2.1. Plant and soil sampling

Aleppo pine needles were obtained from mature trees growing spontaneously in the Cartagena-La Union Mining District (SE of the Iberian Peninsula) in four different tailings piles known as Agustin (37°36′20″N, 0°50′15″W), Mercader (37°36′15″N, 0°50′04″W), Ripolles (37°36′18″N, 0°50′10″W), and Wikon (37°36′15″N, 0°50′08″W), and in a non mining-impacted forest (37°35′47″N, 0°49′23″W) located about 1.5 km away from these mining sites (Supplemental Fig. S1). These tailings are located at a natural park which includes forests of Aleppo pine and endemic xerophytic thickets (Parraga-Aguado et al., 2014). This mining area contains one of the largest Pb and Zn content in the SW of Europe. Average annual rainfall of the area was around 210 mm and 220 mm during 2012 and 2013, respectively (Supplemental Fig. S2), and potential evapotranspiration (ETo) exceeded rainfall by sixfold (ETo was 1312 and 1258 mm yr^{-1} during 2012 and 2013, respectively [Supplemental Fig. S2]). In these years the sampling date corresponding to September 2012 was that one in which the greatest