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Ecological potential of *Epilobium dodonaei* Vill. for restoration of metalliferous mine wastes

Dragana Ranđelović[a,*], Gordana Gajić[b], Jelena Mutić[c], Pavle Pavlović[b], Nevena Mihailović[d], Slobodan Jovanović[e]

[a] University of Belgrade, Faculty of Mining and Geology, Department for Mineralogy, Crystallography, Petrology, and Geochemistry, Đušina 7, 11000 Belgrade, Serbia
[b] University of Belgrade, Institute for Biological Research "Siniša Stanković", Department of Ecology, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia
[c] University of Belgrade, Faculty of Chemistry, Department for Analytical Chemistry, Studentski Trg 12-16, 11000 Belgrade, Serbia
[d] Institute for the Application of Nuclear Energy (INEP), Banatska 31b, 11080 Zemun, Serbia
[e] University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden Jevremovac, Takovska 43, 11000 Belgrade, Serbia

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ABSTRACT

Metalliferous mine wastes represent one of the major sources of environmental contamination from mining activities. Bor region (Serbia) is one of the largest copper mine basins in Europe where long-term mining caused severe environmental deterioration and created one of the most degraded locations in Serbia and Europe. At the spontaneously colonized metalliferous mine wastes in Bor, plant species *Epilobium dodonaei* dominates in the mine slopes and mine waste surfaces. *Epilobium dodonaei* has the status of endangered and protected species in parts of European range (i. e. plant is included in the Red lists of the countries in the Carpathian mountains region), primarily due to losses of natural gravel habitats. The main focus of this research was physico-chemical characterization of mine waste, assessment of phytoremediation potential and plant metabolic stress response of *Epilobium dodonaei* at the hot spot metalliferous mine site in order to evaluate the possibility for application of endangered species in ecological restoration. The Bor mine wastes are characterized by coarse soil texture, various pH (4.58–8.30), and elevated concentrations of arsenic (44.5–271 mg kg^{-1}) and copper (311–2820 mg kg^{-1}) that exceed the Serbian limiting threshold and remediation values. Oxidation of metal-sulfide minerals on waste surface leads to increased acidity, followed by elevated metal mobility of the mine spoil solution. Content of arsenic, copper, lead and zinc in roots of *E. dodonaei* was correlated with pseudo-total and EDTA-available concentrations in Bor mine spoils. Furthermore, the content of arsenic, copper, lead and zinc in roots (3.98 mg kg^{-1}, 140 mg kg^{-1}, 3.19 mg kg^{-1}, and 72.8 mg kg^{-1}, respectively) and shoots (4.69 mg kg^{-1}, 57.7 mg kg^{-1}, 1.17 mg kg^{-1}, and 59.3 mg kg^{-1}, respectively) of *E. dodonaei* reflected the multi-metal pollution at the investigated site. *Epilobium dodonaei* largely retains copper, lead and zinc in roots than in shoots and has the potential for phytoremediation of mine wastes. *Epilobium dodonaei* at Bor mine spoil had a high content of malondialdehyde in roots and leaves as well as reduced chlorophylls and carotenoids content in leaves, indicating great oxidative stress. However, elevated arsenic and copper content could promote biosynthesis of antioxidants in roots and leaves of *E. dodonaei* at mine spoil. Creation of an endangered species habitat on mine waste rocks of the Bor mining area and similar sites of Carpatho-Balkan metallogenic province could successfully contribute to the preservation of *E. dodonaei*. Development of practical procedures for the selection and application of endangered plant species in reclamation should create stronger link between ecological restoration and conservation biology. Finally, the application of endangered plant species should take a more prominent role in the restoration process and ecosystem design.

1. Introduction

Restoration of ecosystems disturbed by human activities is an important field of ecological engineering that involves design, construction, and operation of new ecosystems (Mitsch and Jorgensen,

* Corresponding author.
E-mail addresses: dragana.randjelovic@rgf.bg.ac.rs, dragana.randjelovic@gmail.com (D. Ranđelović).

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1989; Kangas, 2005). Metalliferous mine wastes are unfavorable for plant development due to nutrient-poor conditions and elevated content of chemical elements. Environmental impacts may be significant, especially when sulfides or pyrite are present (Lottermoser, 2010). Incomplete technical reclamation of mine wastes often results in the construction of steep slopes that are devoid of vegetation and prone to erosion (Gentili et al., 2010). The amelioration of hostile environment is necessary for ecological restoration of mine waste site. Since plants pose capability to self-engineer or exert limited control over the rhizosphere and local biogeochemistry, selection and application of suitable plant species for the restoration of mining sites is one of the primary tools of ecological engineering design (McCutcheon and Schnoor, 2003; Simmons et al., 2007; Jørgensen, 2009). Use of plants to reduce the volume, mobility, or toxicity of contaminants in soil and water is operationalized through phytoremediation (USEPA, 2000). Phytoremediation is the cost-effective biotechnology for managing industrial wastes that represents an important part of the field of ecological engineering (McCutcheon and Schnoor, 2003). Phytostabilization is phytoremediation technique that stabilizes wastes and prevents exposure pathways via wind and water erosion, providing metal(loid) immobilization by adsorption or accumulation within the rhizosphere zone (Prasad and Freitas, 2003; Mendez and Maier, 2008). Successful establishment of the vegetation cover on mine spoil leads to pollutant control, slope stabilization, biodiversity, and aesthetic improvement (Wong, 2003).

Generaly, many authors recommended conservation of rare or endemic metallophytes as a unique biological resource (Brooks et al., 1985; Dobson et al., 1997; Jordan et al., 1998; Young, 2000; Whiting et al. 2004; Boogert et al., 2006; Baker et al., 2010), but only few papers actually show that endangered or protected pioneering plants can be used to reclaim or stabilize mining and industrial sites (Brofas et al., 2007; Boisson et al., 2015). In this sense, Whiting et al. (2004) suggested systematic screening of plants on metalliferous sites (particularly those likely to be the focus of future mining) in order to identify priority candidates for conservation, implementing ecological restoration of mine sites, and the development of "green" technologies for removing metals from the soil.

Plants that grow on mine wastes are exposed to toxicity of chemical elements, such as copper, lead, arsenic, and zinc (Conesa et al., 2006; Haque et al., 2008; Santos et al., 2009; Nadgórska-Socha et al., 2013). Heavy metals and metalloids (pollutants) can cause oxidative stress generating reactive oxygen species which are known to damage membrane lipids, proteins, pigments, and nucleic acids, and disturb the basic physiological processes, such as photosynthesis, respiration, and mineralization (Mittler, 2002; Sharma and Dietz, 2009; Gajić et al., 2009; Hossain et al., 2012). Copper as an essential redox-active transition metal when present in excess can catalyze the production of hydroxyl radicals ($OH^{\bullet}$) from superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) via the Haber-Wiess and Fenton reaction in plants (Halliwel, 2006). Arsenic leads to the generation of reactive oxygen species through the conversion of arsenate to arsenite in plants, induces lipid peroxidation, and enhances membrane permeability (Talukdar, 2013). Lead and zinc induce oxidative stress in plants causing lipid peroxidation of membranes (Pandey et al., 2009; Rossato et al., 2012). According to Weber et al. (2004) malondialdehyde is one of the final products of peroxidation of unsaturated fatty acids in membrane phospholipids, and is an indicator of the reactive oxygen species. Plants have different defense strategies to cope with toxicity of metals. Plant responses to pollutants that contribute to resistance and tolerance include exclusion (active process that prevents or reduces uptake of toxins), amelioration (active metabolic and detoxification processes that segregate or transform toxins to protect organs, tissues, and metabolic function), avoidance (restriction of the uptake of metals within root tissue by immobilizing metals through root exudates or binding metal in the cell wall), and evasion (removal of plant from the stressful environments) (McCutcheon and Mitsch, 1994; Medina et al., 2003). Plants have antioxidant mechanisms that control and scavenge the reactive oxygen species using non–enzymatic and enzymatic antioxidants (Smirnoff, 2005; Sharma et al., 2012; Hossain et al., 2012; Singh et al., 2016). In addition, phenolics are secondary metabolites which can directly scavenge reactive oxygen species, inhibit lipid peroxidation and chelate metal ions (Michalak, 2006; Sharma et al., 2012). Phenolics accumulation in plant leaves and roots occur under copper, lead, zinc, and arsenic stress (Gajić et al., 2009; Janas et al., 2010; Gajić et al., 2016). The alleviation of oxidative damage and increased resistance to metal stress is related to an effective antioxidant system, and plants with high antioxidant capacity show less sensitivity to metal toxicity (Hossain et al., 2012; Singh et al., 2016). According to Medina et al. (2003) understanding plant responses to pollution stress defines whether a plant has the potential to transform and tolerate a wide range of pollutants, which would enhance phytoremediation.

Epilobium dodonaei Vill. [syn. *Chamaenerion angustissimum* (Weber) D. Sosn.; *Ch. dodonaei* (Vill.) Schur; *Ch. palustre* auct. mult., non (L.) Scop.; *E. rosmarinifolium* Haenke] is a herbaceous perrenial hemicryptophyte belonging to the Onagraceae family (Raven, 2001). The range of *E. dodonaei* is disjunct and confined to the Alpine areas and marginal of Europe, Asia Minor, and the Caucasus. Primarily, this species belongs to the vegetation class of mountain screes (Thlaspietea rotundifolii). In the central European temperate zone *E. dodonaei* often appears as a subthermophilous demontane species descending along the gravel alluvia of water courses, as well within plant communities developed in the warm and open habitats of gravel banks of the European (usually Alpine) streams (Valachovič et al., 1997). Slavik (1986) notes the presence of this species in the both Czech Republic and Slovakia in river basins growing on diverse geological substrates: limestones, shales, calcareous sandstones and phyllite, diabase, and granite. Besides the primary occurrence of *E. dodonaei* in gravel bed habitats, apophytization has been taking place over the past hundred years in those industrial areas whose properties are closely related to the original habitats (Slavik, 1986; Smejkal, 1997). Thus, *E. dodonaei* has often been found on anthropogenic formations such as quarries, sandpits, gravel-pits, and mine waste dumps in various parts of Europe (Koutecká and Koutecký, 2006; Brofas et al., 2007; Himmler, 2008; Ranđelović et al., 2014). The dispersal to suitable artificial habitats and extinction from the original habitats (due to natural loss or anthropogenic disturbance) changed the boundaries of species distribution. In some parts of Europe *E. dodonaei* has the status of endangered and protected species, primarily due to losses of natural habitats. This species is included in the Red List of the vascular plants of Luxembourg (Colling, 2005), Germany – Bavaria province (Scheuerer and Ahlmer, 2003); Poland (Mirek and Piekos-Mirkowa, 1992), Slovakia (Májeková et al., 2014), Czech Republic (Procházka, 2001), Romanian (Daraban, 2007), Hungary (Official Gazette, 128) and Red List of flora of the Serbian Republic within Bosnia and Herzegovina (Official Gazette, 124/12). Additionally, *E. dodonaei* is very rare on natural habitats in Serbia (Diklić, 1973). Slavik (1986) recommends *Epilobium dodonaei* as an ideal model species for the study of the recent migration in time and space. Therefore, this species can be a good example for linking active conservation procedures with ecological restoration.

The locations of *E. dodonaei* in areas with greater concentrations of metals have not been well mapped. The species has been investigated in the serpentine soils of Gjegjan in Albania, with a limited accumulation of nickel, zinc, cobalt and cromium in biomass (Shallari et al., 1998). Brofas et al. (2007) have been establish germination protocol for establishment of *E. dodonaei* in rehabilitation of calcareous mine spoils at bauxite mining region in Greece.