Science of the Total Environment 562 (2016) 480–492

Contents lists available at ScienceDirect

Science of the Total Environment

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

Enhancement of ecosystem services during endophyte-assisted aided phytostabilization of metal contaminated mine soil

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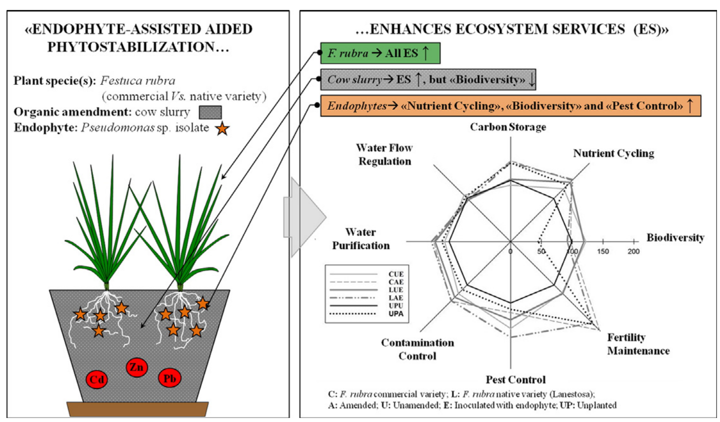
HIGHLIGHTS

- An endophyte-assisted aided phytostabilization study was performed.
- The application of cow slurry had the most pronounced effects.
- Growth of native *F. rubra* reduced soil Cd and Zn bioavailability by 19 and 22%.
- In the field, lower metal concentrations were found in endophyte-inoculated plants.
- The assessment of soil quality at the ecosystem service level has proven useful.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form 11 April 2016
Accepted 11 April 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Pb/Zn soil contamination
Organic amendment
Phytoremediation
Soil health
Soil microbial properties
Soil quality

ABSTRACT

Endophytic plant growth-promoting bacteria (endophytes) were isolated from a variety of (pseudo)metallophytes growing in an abandoned Zn/Pb mine and then characterized according to their plant growth-promoting traits (i.e. ACC deaminase activity, IAA production, siderophore production, phosphate solubilising capacity, metal and salt tolerance and phenotypic characterization). Initially, under growth chamber conditions, an endophyte-assisted aided phytostabilization study was carried out with *Festuca rubra* plants (native vs. commercial variety) inoculated with a *Pseudomonas* sp. isolate and cow slurry as organic amendment. The effect of treatments on soil physicochemical and microbial indicators of soil quality, as well as plant physiological parameters and metal concentrations, was assessed. We performed a complementary interpretation of our data through their grouping within a set of ecosystem services. Although the application of cow slurry had the most pronounced effects on soil quality indicators and ecosystem services, the growth of native *F. rubra* plants reduced soil bioavailability of Cd and Zn by 19 and 22%, respectively, and enhanced several soil microbial parameters. On the other hand, endophyte (*Pseudomonas* sp.) inoculation improved the physiological status of *F. rubra* plants by increasing the content of carotenoids, chlorophylls and Fv/Fm by 69, 65 and 37%, respectively, while also increasing the values of several soil microbial parameters. Finally, a consortium of five endophyte isolates was used for an endophyte-assisted aided phytostabilization field experiment, where lower metal concentrations in native excluder plants were found. Nonetheless, the field inoculation of the endophyte consortium had no effect on the biomass of native plants.

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http://dx.doi.org/10.1016/j.scitotenv.2016.04.080
0048-9697/

1. Introduction

Phytostabilization, i.e. the use of metal tolerant plants to decrease soil metal bioavailability and mobility, can be an effective phytomanagement option for soils highly contaminated with metals such as mine soils (Galende et al., 2014). However, plant growth on mine soil is often compromised due to high metal concentrations and nutrient deficiencies (Mendez and Maier, 2008). Then, the incorporation of amendments to mine soil during phytostabilization (aided phytostabilization) has been frequently used to facilitate plant colonization (Alvarenga et al., 2009a, 2009b; Epelde et al., 2009). Regrettably, the beneficial effect of amendments might be transient (Epelde et al., 2014b). Besides, potential environmental impacts and human health risks derived from the application of amendments, such as groundwater contamination by nitrates or an increase in microbial resistance to antibiotics (Goss et al., 2013; Jorge-Mardomingo et al., 2015), have brought up the necessity to explore alternative possibilities.

Metalliferous environments shelter a unique and highly valuable biodiversity. Thus, pseudometallophytes and metallophytes from mine tailings can be most suitable for phytomanagement of metal contaminated sites (Barrutia et al., 2011). On the other hand, plant growth-promoting rhizobacteria (PGPR) and bacterial endophytes isolated from plants growing in metalliferous environments have successfully demonstrated their potential for phytomanagement, owing to their ability to stimulate plant growth and/or protect plants against metal toxicity through several mechanisms (Babu et al., 2013; Barzanti et al., 2007; Chen et al., 2010; Mastretta et al., 2006; Zhang et al., 2011).

The ultimate goal of any soil metal remediation method must be not only to remove the metals from the soil or to render them harmless, but also to restore soil quality (Epelde et al., 2009). Soil microbial parameters have great potential as biological indicators of the effectiveness of phytomanagement (Epelde et al., 2009). But despite their proven value as biological indicators of soil quality, microbial parameters are highly-context dependent and usually difficult to interpret. Consequently, we proposed to group soil microbial parameters in higher-level categories such as "ecological attributes" or "ecosystem services" in order to facilitate interpretation and, most importantly, to provide long-term phytostabilization monitoring programs with the required stability through time against changes in techniques, methods, interests, etc. (Epelde et al., 2014a; Garbisu et al., 2011). Considering that the concept of "ecosystem services" is gaining traction as a way of bridging the scientific-economic-policy making divide (Millennium Ecosystem Assessment, 2005), the abovementioned monitoring programs might be based on ecosystem services, thereby providing the best information for decision-making (Chapman, 2012).

The objective of this study was to assess the effectiveness of endophyte-assisted aided phytostabilization for the phytomanagement of metal contaminated mine soil, with special emphasis on the enhancement of ecosystem services. To this purpose, the following steps were undertaken: (i) isolation of bacterial endophytes from five native (pseudo)metallophyte species growing in an abandoned Pb/Zn mine; (ii) selection of the bacterial endophyte strains with the best plant growth-promoting traits; (iii) an endophyte-assisted aided phytostabilization study was carried out under growth chamber conditions (with a *Pseudomonas* sp. endophyte strain isolated from native *Festuca rubra* and cow slurry as organic amendment) using native *F. rubra* plants versus plants of a commercial variety of *F. rubra*; and (iv) an endophyte-assisted phytostabilization field study was carried out in the abovementioned Pb/Zn mine.

2. Materials and methods

2.1. Site description and plant sampling

The study was carried out in an abandoned Pb/Zn mine located in the province of Biscay (northern Spain, 43°43′N, 3°26′W), with a temperate Atlantic climate (mean annual rainfall: 1400 mm $year^{-1}$; mean annual temperature: 11–15 °C). Lead and zinc extraction by open cast mining ceased in the late 1970s. The mining area includes open pits, overburden surfaces, tailing dams and degraded zones where the soil contains high levels of Cd, Pb and Zn; for a detailed site description, see Barrutia et al. (2011). Healthy plants of *Festuca rubra* L., *Noccaea caerulescens* J. & C. Presl. and *Rumex acetosa* L., together with plantlets of *Betula alba* Ehrh. and *Salix atrocinerea* Brot., were collected at random from this mining site. Plants were washed extensively to remove any adhering soil, first in tap water and then in 0.01 M EDTA, followed by three rinses with deionized water, and then separated into roots, stems and leaves.

2.2. Isolation, characterization and identification of endophytes

Bacterial endophytes were isolated separately from roots, stems and leaves following Surette et al. (2003). Based on their colony morphotype (size, shape and colour), a total of 78 bacterial endophyte strains were isolated. Individual colonies were sub-cultured three times on respective growth medium plates (Surette et al., 2003) and then −80 °C culture stocks supplemented with 20% (v/v) glycerol were prepared.

Initially, all these 78 strains were tested for their 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity following Penrose and Glick (2003), since the ability of bacteria to promote plant growth has been reported to be closely related to their ACC deaminase activity (Glick, 2014); accordingly, only ACC deaminase activity-containing strains were tested for other plant growth-promoting traits, i.e. indole-3-acetic acid (IAA) production (Becerra-Castro et al., 2011), siderophore production (Schwyn and Neilands, 1987), and phosphate solubilising activity (Nautiyal, 1999). The tolerance of each isolate to Cd, Pb and Zn was evaluated as described by Long et al. (2011). Maximal tolerable concentrations (MTC) were recorded as the highest metal concentration in which each isolate grew. Salt tolerance was tested using Luria-Bertani (LB) (Sigma-Aldrich, USA) agar plates supplemented with increasing concentrations of NaCl (Rashid et al., 2012). A phenotypic characterization of the isolates was performed using GEN III MicroPlates™ (Biolog Inc., Hayward, USA).

The isolates were identified using 16S rRNA gene sequencing. Total genomic DNA was extracted from each isolate using E.Z.N.A.™ bacterial DNA extraction kit (Omega, USA). Amplification of the 16S rRNA gene sequence was performed with the bacterial universal primers 938F and 1378R (Heuer et al., 1997) as described in Epelde et al. (2014b). Subsequently, amplification products were purified with the MultiScreen HTS PCR 96 kit (Merck MilliPore, USA). Sequencing was carried out by using an automatic sequencer ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, CA, USA) at the Genomic Core Facility SGIker (UPV/EHU, Spain). DNA sequences were analysed with basic sequence alignment BLAST program, run against the database from National Center for Biotechnology Information (NCBI).

2.3. Growth chamber study

2.3.1. Experimental design

An endophyte-assisted aided phytostabilization experiment with *F. rubra* was performed, under controlled growth chamber conditions, in pots containing soil from the abovementioned mine. The soil was collected from the top layer (0–20 cm) in March 2014 and immediately transported to the laboratory where all visible roots were removed. The soil was air-dried, thoroughly mixed and sieved to <4 mm. Subsamples were sieved to <2 mm for physicochemical characterization according to standard methods (MAPA, 1994). The soil was a sandy-loam with the following physicochemical properties: pH = 6.7; OM (%) = 5.3; total N (%) = 0.23; extractable P (mg kg^{-1} dry weight-DW soil) = 2.2; exchangeable K^{+} (mEq 100 g^{-1}) = 1.22; exchangeable Ca^{2+} (mEq 100 g^{-1}) = 2.94; exchangeable Mg^{2+} (mEq 100 g^{-1}) =