19th International Conference on Environmental Science and Technology

Kos, Greece, 3 to 6 September 2025

Complementary approaches to characterize rhizospheric microbial diversity of *Pistacia lentiscus* in a highly contaminated mine tailing dump

MANDARESU M.[1,3], VITALI F.[2], MOCALI S.[2], CAPPAI G.[3], TAMBURINI E.[1*]

[1]Departnent of Biomedical Sciences, University of Cagliari, Cittadella Universitaria Sp.8, 09042 Monserrato, CA, Italy
[2]Research Centre for Agriculture and Environment, Council for Agricultural Research and Economics, Via Di Lanciola 12/A, 50125, Florence, Italy
[3]Department of Civil- Environmental Engineering and Architecture, University of Cagliari, Piazza d'Armi, 09123 Cagliari, Italy

*corresponding author
e-mail: etamburini@unica.it

Abstract

Among phytoremediation strategies, phytostabilization takes place at the root-substrate interface, where excluder-type metallophytes and their root-associated microorganisms reduce metal mobility and bioavailability. The effectiveness of this process largely depends on the structure and function of the rhizospheric microbiome. To better understand the factors influencing phytostabilization under real field conditions, a multifactorial approach is essential. In this study, we conducted an integrative analysis of the microbial diversity and enzymatic activity in the rhizosphere of *Pistacia lentiscus*, an excluder-type metallophyte naturally growing in a highly contaminated (Zn, Pb and Cd) mine tailing dump and in surrounding area. Our findings provide insights into the ecological role of root-associated microbiomes in metal-contaminated environments and support their relevance for site-specific remediation strategies.

Keywords: ribosomal genes, BIOLOG, mining area, phytoremediation, bacteria, fungi

1. Introduction

In recent years, more than ever, we are realizing how crucial it is to gain knowledge about microbial communities to manage heavy-metal contaminated soils, both for the environment and for the communities living nearby. Phytoremediation is an efficient technique to manage polluted environments. Research shows that data about the microbiome in the rhizosphere and roots of native plant species are a key component in a successful remediation strategy. The environmental context must be analysed with a multifactorial approach to understand the most suitable application of phytoremediation in real field conditions. A comprehensive insight into soil communities can be achieved by using diverse techniques. In this study we analysed: i) physico-chemical properties of mine substrates, ii) microbial activity by the dehydrogenase assay, iii) functional diversity patterns of microbial community with the BIOLOG system, iv) bacterial and fungal communities by high-throughput sequencing of ribosomal genes. In the literature, the Biolog EcoPlate method has been employed to study the functional diversity of complex ecosystems (Acin-Albiac et al., 2020). Dehydrogenases play a role in the redox systems of microorganisms and these enzymes have been used to evaluate the oxidative-reductive functionalities of soil microbes in mine degraded lands (Bandyopadhyay & Maiti, 2021). In addition, ribosomal genes have been analysed by high-throughput sequencing (Hong et al. 2015) to study how microbial communities were affected by mine activities. Therefore, each of these different methodologies should provide complementary information about the soil communities living in the highly contaminated environment under study.

2. Materials and methods

2.1. Site description and sampling

Different specimens of the autochthonous plant species *Pistacia lentiscus* were collected at the Campo Pisano tailing dump (SW Sardinia, Italy), based on their position relative to the mining dump: i) outside, ii) at the border, and iii) inside the tailing dump. Substrates in contact with roots were sampled, transferred into sterile polyethylene bags and transported to the laboratory under refrigerated conditions at 4°C.

2.2. Physico-chemical analysis

Mine substrates were processed according to official Italian analytical methods (D.M. 13/09/1999). The determinations were carried out in triplicate. Moisture was evaluated through oven drying at 105 °C for 24 h, as a percentage of wet-weight loss. The pH was measured in a 1:2.5 substrate to water solution (KCl 1 M), after 2 h

mixing and subsequent settling. Total and bioavailable concentrations of Zn, Pb, and Cd in the mine substrates were evaluated.

2.3. Dehydrogenase assay

According to Mandaresu et al. (2023), the dehydrogenase activity was assayed by using iodonitrotetrazolium violet (INT) solution as a substrate. The test was performed overnight and stopped by the addition of acetonitrile. The INT-F concentration was determined from the linear least squares best-fit line of a standard curve prepared with the INT-F solutions (5–100 mg L−1) in acetonitrile.

2.3. BIOLOG for community profiling

Functional diversity pattern of microbial community in mine substrate samples was analysed using Biolog EcoPlate™ (Biolog Inc., CA, USA). EcoPlates contain 3 repeated sets of 31 carbon sources and employ a tetrazolium redox dye as an indicator of microbial metabolism. In order to obtain the microbial suspensions, 2 g of soil was mixed with 20 mL of a sterile saline solution (0.9%) and shaken for 1 h at 200 rpm. Soil suspension was centrifuged at 3000 g and the supernatant was applied to Biolog EcoPlate™. The plates were incubated at 30°C. The absorbances were measured at 590 nm for 3 days. Absorbance data were used to calculate several indices: Average Well Colour Development (AWCD), Shannon-Weaver index and Richness.

2.4 DNA extraction and Illumina sequencing

DNA was extracted using DNeasy PowerSoil Pro Kit (QIAGEN) according to manufacturer's instructions. The sequences of V3/V4 hypervariable regions of 16S rRNA gene and the Internal Transcribed Spacer 2 (ITS2) sub-regions were amplified to analyse bacterial and fungal communities, respectively. The PCR amplification products were then sequenced using an Illumina MiSeq platform.

3. Results

The selected case study was the abandoned Campo Pisano tailing dump where native plant species of *Pistacia lentiscus* were present. At this site, metal concentrations (Zn, Pb, Cd) in the tailings showed a certain degree of spatial heterogeneity.

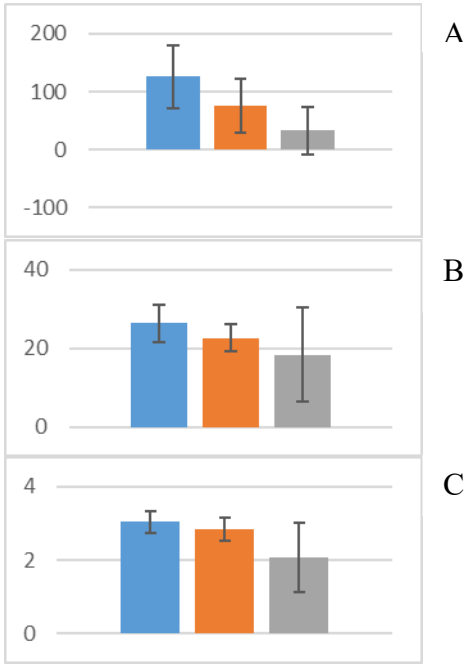

Figure 1. Average well-colour development (AWCD) (A), Richness (B) and Shannon–Weaver index (C) of metabolized substrates in Biolog EcoPlate. Legend: blue=outside, orange=border, grey=inside.

Community level physiological profiles indicated differences in the functional diversity of the rhizospheric microbial communities from the three different investigated areas. Indeed, the mine substrate collected outside the taling dump showed higher values of AWCD, Richness and Shannon–Weaver indices (Figure 1). Moreover, a marked and statistically significant decrease in the microbial activity was observed from the outside to the inside of the tailing dump. Finally, the analysis of bacterial and fungal communities by high-throughput sequencing of ribosomal genes is still in progress.

References

Acin-Albiac M, Filannino P, Gobbetti M, Di Cagno R (2020) Microbial high throughput phenomics: The potential of an irreplaceable omics. Comput Struct Biotechnol J 18:2290–2299.

Bandyopadhyay S, Maiti SK (2021) Different Soil Factors Influencing Dehydrogenase Activity in Mine Degraded Lands—State-of-Art Review. Water Air Soil Pollut 232:.

Hong C, Si Y, Xing Y, Li Y (2015) Illumina MiSeq sequencing investigation on the contrasting soil bacterial community structures in different iron mining areas. Environ Sci Pollut Res 22:10788–10799.

Mandaresu, M., Dessì, L., Lallai, A., Porceddu, M., Boi, M.E., Bacchetta, G., Pivetta, T., Lussu, R., Ardu, R., Pinna, M., Meloni, F., Sanjust, E., Tamburini, E., (2023). Helichrysum microphyllum subsp. tyrrhenicum, Its Root-Associated Microorganisms, and Wood Chips Represent an Integrated Green Technology for the Remediation of Petroleum Hydrocarbon-Contaminated Soils. Agronomy 13, 812.

Acknowledgment

National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.5 - Call for tender No.3277 published on December 30, 2021 by the Italian Ministry of University and Research (MUR) funded by the European Union – NextGeneration EU. Project Code ECS0000038 – Project Title eINS Ecosystem of Innovation for Next Generation Sardinia – CUP F53C22000430001- Grant Assignment Decree No. 1056 adopted on June 23, 2022 by the Italian Ministry of Ministry of University and Research (MUR).