Microbial activity in mine tailing dump in South-West Sardinia (Italy)

MANDARESU M.[1,2,*], CAPPAI G.[2], TAMBURINI E.[1]

[1]Departnent of Biomedical Sciences, University of Cagliari, Cittadella Universitaria Sp.8, 09042 Monserrato, CA, Italy
[2]Department of Civil- Environmental Engineering and Architecture, University of Cagliari, Piazza d'Armi, 09123 Cagliari, Italy

*corresponding author
e-mail: melinda.mandaresu@unica.it

Abstract Abandoned mines represent a major environmental concern worldwide, posing serious risks to human health and ecosystems. In this study, microbial abundance and dehydrogenase activity were evaluated in surface substrates collected from a highly contaminated (Zn, Pb and Cd) tailing dump and in the surrounding areas. The heterotrophic community inside the studied site was found to be severely impaired as compared to control sectors located at the border and outside the tailing dump. The analysis of the microbial community composition by sequencing of the ribosomal genes is currently in progress. Overall, the study will allow us to identify the microbial components most severely affected by the extreme environmental conditions present in the mine tailings.

Keywords: mine tailings, metals, microorganism, toxicity, NGS

1. Introduction

Mine tailings, which are the principal byproduct of ore processing for metal extraction, represent a severe issue for the environmental pollution caused by mining activity. Composed mainly of silt- or sand-sized grains, tailings are low in nutrients (N, P, K) and contain minimal organic matter. The primary causes of toxicity in mining residues are their acidic pH and high metal concentration. Consequently, most tailing disposal sites are characterised by the absence of vegetation and a compromised heterotrophic microbial community (Mendez & Maier, 2008). Microorganisms native to mine tailings, having been exposed to metals for extended periods, have acquired the ability to tolerate significant contaminant levels through various detoxification mechanisms. Moreover, the level of contamination at these sites has been found to alter the structure of microbial communities and reduce their biodiversity.

The area of Sulcis-Iglesiente (South-West Sardinia, Italy) was for centuries one of the most important mining areas in Europe. The purpose of our study was to characterize the microbial community in mine tailings of the abandoned Campo Pisano tailing dump, chosen as a case study site for the high metal levels (Zn, Pb and Cd) in mine tailings. Dehydrogenase activity and microbial abundance were determined and the spatial and temporal variations in the parameters evaluated. Moreover, we are carrying out the analysis of microbial community by high-throughput sequencing of the ribosomal genes.

2. Materials and methods

2.1. Site description and sampling

Campo Pisano is a tailing dump in the Iglesiente district (SW Sardinia, Italy), one of the most important Pb-Zn extraction areas for centuries. The study area was divided into three sectors (Tab.1) based on their position relative to the mining dump: i) outside (out), ii) at the border (start), and iii) inside the tailing dump (in). Four sampling campaigns were conducted at the site during summer (2023), autumn (2023), winter (2024), and spring (2024). In each of the three previously established sectors, surface substrate samples were collected. The samples were transferred into a sterile polyethylene bag and were transported to the laboratory under refrigeration at 4°C.

Table 1. Coordinates of sampling points in Campo Pisano

Sector	Coordinates
out	N 39°17.520' E 008°32.598'
out	N 39°17.447' E 008°32.653'
start	N 39°17.699' E 008°32.283'
start	N 39°17.777' E 008°32.222'
start	N 39°17.686' E 008°32.018'
in	N 39°17.731' E 008°32.267'
in	N 39°17.771' E 008°32.239'
in	N 39°17.694' E 008°32.242'
in	N 39°17.721' E 008°32.019'
in	N 39°17.710' E 008°31.976'
in	N 39°17.635' E 008°32.120'
in	N 39°17.717' E 008°32.105'

2.2. Dehydrogenase assay

The dehydrogenase activity was measured using INT as substrate (Kumar et al., 2013; Mandaresu et al., 2023). The assays were incubated overnight in an orbital shaker at 28°C and 180 rpm and then stopped by adding 3 mL of acetonitrile (Mosher et al., 2003). The insoluble INT-F was extracted by shaking at 180 rpm at 28°C for 30 min, and the absorbance was measured at 490 nm. The INT-F concentration was determined from the linear least squares best-fit line from a standard curve of the INT-F solutions (5–100 mg L−1) in acetonitrile. All the determinations were performed in triplicate.

2.3. Selective Enumeration of Microorganisms by the Most Probable Number

The selective enumeration of heterotrophic microorganisms was performed by most probable number (MPN) procedure with five replicates (five-tube MPN) (Mandaresu et al., 2023). The samples were left to settle for 15 min and the selective enumeration was carried out in 96-well microtiter plates in a total culture volume of 200 µL per well. Inoculation was performed by adding 20 µL of each 10-fold dilution (up to 10^{-10}) to five wells per plate. For each plate, five wells were used as sterile controls, omitting the sample inoculation. The microtiter plates were wrapped in plastic bags and incubated at 28±1°C for 21 days. Culture setup was completed within 24 hours of the sampling. At the end of the incubation period, positive wells were visually inspected. The viable titer (MPN g^{-1} wet w) was calculated according to Alexander (1982).

2.4. Statistical Analysis

The Kruskal–Wallis rank non-parametric ANOVA was performed by PAST software package v4.03 to compare the median of values measured.

3. Results

The selected case study was the abandoned Campo Pisano tailing dump. Located along the Rio San Giorgio valley, the site is a basin where the mine wastes from the flotation process were settled for decades. At this site, metal concentrations (Zn, Pb, Cd) in the tailings have been found well above the contamination thresholds set by the Italian law for industrial use of soil (Lgs. D. 152/2006), although a certain degree of heterogeneity in metal content across the site has also been detected (Tamburini et al. 2023). As shown in Figure 1, a clear and statistically significant decreasing gradient in microbial activity was highlighted from the outside to the inside of the tailing dump. Moreover, the abundances of heterotrophic microorganisms was significantly lower in mine tailings as compared to the surface substrates collected at the border and outside the mine tailing dump.

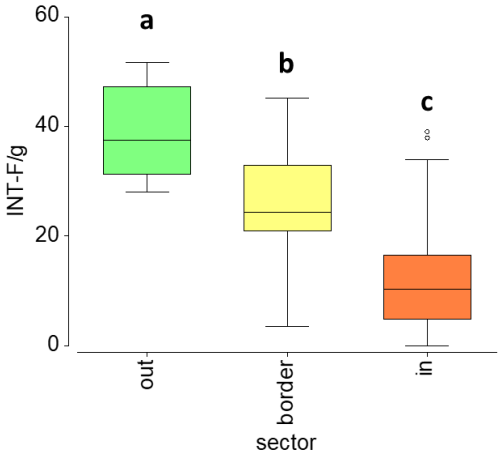

Figure 1. Dehydrogenase activity in top substrates. Significant differences ($p < 0.05$) are represented by different letters.

The analysis of the microbial community in the superficial substrates is currently in progress, which will allow us to identify the microbial taxa more severely impaired and those more tolerant to the stressful conditions in the mine tailings.

References

Kumar, S.; Chaudhuri, S.; Maiti, S.K. (2013) Soil Dehydrogenase Enzyme Activity in Natural and Mine Soil—A Review. Middle East J. Sci. Res., 13, 898–906.

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.

Mendez MO, Maier RM (2008) Phytoremediation of mine tailings in temperate and arid environments. Rev Environ Sci Biotechnol 7:47–59.

Mosher, J.J.; Levison, B.S.; Johnston, C.G. (2003) A Simplified Dehydrogenase Enzyme Assay in Contaminated Sediment Using 2-(p-Iodophenyl)-3(p-Nitrophenyl)-5-Phenyl Tetrazolium Chloride. J. Microbiol. Methods, 53, 411–415.

Tamburini, E., Mandaresu, M., Lussu, R., Sergi, S., Vitali, F., Carucci, A., Cappai, G., (2023) Metal phytostabilization by mastic shrub (Pistacia lentiscus L.) and its root-associated bacteria in different habitats of Sardinian abandoned mining areas (Italy). Environ. Sci. Pollut. Res. Int. 30, 122107–122120.

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).