Restoring the soil while preserving functions: a winning approach by exploiting microbial biodiversity

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Abstract summary

The mining activity often causes drastic changes in the soil profile, altering the equilibrium among the factors that influence the self-regulation capacity and resulting in a loss of soil biodiversity, soil composition and negatively affects ecosystem functionality. The contamination is reflected in the surrounding territories, contributing to their degradation and the limitation of their use for human activities and agricultural use. The remediation of these areas is a priority. Phytoremediation is a sustainable approach to contain and reduce environmental damages, since it respects and improves over time the soil biodiversity and soil functions. The effectiveness of phytoremediation can be enhanced by plant growth promoting bacteria (PGPB). The experience at the mining site of Ingurtosu (Sardinia, Italy) is here reviewed. The abandoned mine was chosen as experimental field trial to test an assisted phytoremediation, designed by creating a toolbox with native plants and bacteria. Aim was to improve soil functions needed to stabilise metals leaching in soil. The toolbox was selected on an ecological basis, associating the endemic pioneer plant *Euphorbia pithyusa L.* with an autochthonous PGP bacterial consortium. The positive effects on the plots treated with microbial bioaugmentation were evident over time, improving soil microbial biodiversity, soil functions and triggering a spontaneous revegetation. The field trial has been monitored over 7 years (2011-2018) and is still under observation.

Keywords: Microbial biodiversity, Bioremediation, Bioaugmentation, Soil recovery

Introduction, scope and main objectives

Soil degradation has assumed great importance in recent decades and the agri-food system is strongly affected by its impact. The growing demand for food collides with the conditions of severe soil degradation. The persistence of inadequate agricultural and forestry practices, industrial activities and ongoing climate change, suggest that soil degradation is destined to grow. Pollution contributes to a good extent to this phenomenon and requires to be faced through an assessment on ecosystem scale. It is known that pollution spreads from the areas of origin to the surrounding territories, in particular those downstream, which are often destined for agricultural use.

The maintenance of the available agricultural areas depends on the measures we manage to mitigate degradation of contaminated areas.

An interesting case is represented by abandoned mining areas, where environmental contamination chronically occurs, by release of metals through leaching, air dispersion or erosion of metals contained in the mine wastes or in contaminated sediments. In the European Union, the problem of soil degradation, estimated at around 38 billion euros per year, is moreover intensified due to the high population density. The remediation of these areas is therefore a strategic goal for European policies.

The remediation measures must be compatible with the conservation of the soil functions.

Chemical-physical technologies return, in the majority of cases, a substantially sterile soil, after treatment. Soil is therefore deprived of its functions of recycling elements in nature, primary for the survival of life on the Planet and peculiarly performed by microorganisms. To date, although it is not yet fully mature, bioremediation is the only technology capable of restoring the soil while preserving its functions.

Microbial biodiversity plays a key role in bioremediation technologies. The question is: how to use this biodiversity? The implementation of a "knowledge-based" functional bioaugmentation is based on a rational selection of the inoculum, identifying the autochthonous (or allochthonous) functional groups suitable for enhancing the metabolic functions necessary for remediation.

The 7-year-long experience at the mining site of Ingurtosu (Sardinia, Italy) is reviewed. The case study concerns an abandoned mine of sphalerite and galena, in Sardinia, where a field trial was set up in October 2011 (PF7 UMBRELLA project), continued within the regional project S.ME.RI. (2014-2015) and is still under observation.

Methodology

Ingurtosu mine site (Sardinia, IT) was one of the largest and most productive of sphalerite and galena mines in Sardinia, today it is part of the Geological and Mining Park and in 1997 it became one of the UNESCO networks of Geo-parks. The mine is inserted in a highly natural environmental and landscape context. The peak of productivity was the period between the 19th and 20th centuries, and the plants closed in 1969.

The scarce prevention during the extraction activity and following closure led to a considerable environmental impact. Under the European Project UMBRELLA, in September 2011 a field trial was set up in the abandoned mine (Figure1) in order to design a phytoremediation process, assisted by plant growth promoting bacteria (PGPB).

A preliminary characterization of the field was carried out through a hydro-geo-chemistry survey, a bioprospection for microbiology (Fig 2) and botany, heavy metal content and mobility analysis.

Following a greenhouse experiment (Wernitznig *et al.,* 2014) a toolbox was established by the endemic pioneer plant *Euphorbia pithyusa L.* associated with a bacterial consortium (UI), composed by ten selected native strains, metal-tolerant and good PGP (Figure 2; Table 1).

The field experiment considered 9 conditions tested in triplicate on the 27 subplots it was divided (Figure1). The different treatments were applied singly or in combination: bioaugmentation with bacterial consortia, mycorrhizae and the commercial mineral amendment Viromine™, a by-product of the bauxite industry which is widely used for environmental remediation processes due to its metal-trapping capacity. About three years later (December 2014), a second bioaugmentation was performed with a modified consortium (USMI), obtained with new PGP strains isolated from the microbial community that had spontaneously evolved in the meantime (Figure 3). In addition, the native species *Juncus maritimus Lam.* was added to the association.

The field management was minimal, minimal irrigation and bacteria dispersion were performed with the water taken from the Rio Naracauli, which flows under the experimental site, and which carries a load of 40 kg of zinc per day.

Sporadic inspections have been carried out from 2015 to the 2018,

Survival of plants in relation to soil metabolic activity, microbial biodiversity (Sprocati *et al.,* 2014b) and other biochemical and microscopic parameters (Medas *et al.,* 2015) were the parameters observed.

Microbial community was monitored over the time through biochemical (EcoPlate, BIOLOG System™) and molecular (PCR-DGGE, Denaturing Gradient Gel Electrophoresis) profiling.

The cultivable bacterial fraction was isolated from the rhizosphere, identified by sequencing of rDNA16S and characterized for PGP functions: auxin production, N_2 fixation, PO_4 mobilization and production of siderophores.

Results and discussion

An early assessment carried out 5 months after the first inoculum proved that subplots, which underwent bioaugmentation, retained the best metabolic activity with a high functional diversity, supporting the decision to proceed with the field trial (Sprocati *et al.,* 2014a).

In the first two years, a general gradual decrease in the plants survival was observed. In the plots treated with bioaugmentation the plant survival was 20 percent higher than controls. Later on, as a result of a very dry season, without any irrigation, the control plants survival fell close to 100 percent, while 20 percent of the plants assisted by bacteria survived and recovered over time, unlike the controls (Figure4). These differences in the survival and resilience of plants were reflected in the physiological soil profile -at the community level- presenting a much higher metabolic activity, measurable over time, in the plots treated with bioaugmentation (Sprocati, 2015).

The results of molecular and biochemical profiles in soil samples collected from June 2014 to March 2016 indicated that the bioaugmentation contributed to the development of a more specialized community, consisting of few species better adapted to the extreme conditions.

At a distance of 7 years from the beginning of the trial, many of the introduced plants *E. pithyusa* and *Juncus maritimus* are established and other new spontaneous plants have settled (mainly *Rumex* sp. and *Helichrysum* sp.).

Conclusions

The phytoremediation approach aimed at enhancing the growth-promoting functions of plants in the soil of the mine, through bioaugmentation with autochthonous PGP bacteria. The results obtained with only two bioaugmentation applications, show that a positive process was triggered, improving the soil metabolic activity, favouring the creation of a stable soil-plant-bacteria system, the survival of introduced plants and a spontaneous revegetation with endemic plants. The experience developed during Umbrella project (2009-2012) in the experimental field of Sardinia, together with the other experimental fields across Europe (Wells, Germany, Poland, Romania, Sweed) had led to the definition of a first attempt of guideline for the application of bioaugmentation for assisted phytoremediation. The continuation of the experience that took place in Sardinia up now, provides a useful indication for defining better guidelines.

Figure 1: the Ingurtosu Mine site (Sardinia, Italy) and the in situ experimental camp

The field experiment considered 9 conditions tested in triplicate on the 27 subplots.

Microbial load	9*10^6 CFU/g soil^{-1}
Colony morphotypes	41
N_2fixer	90 %
PO_4 mobilizer	44 %
Siderophore producer	63 %
Auxine producer	32 %
Soil extract (metal resistant)	54 %

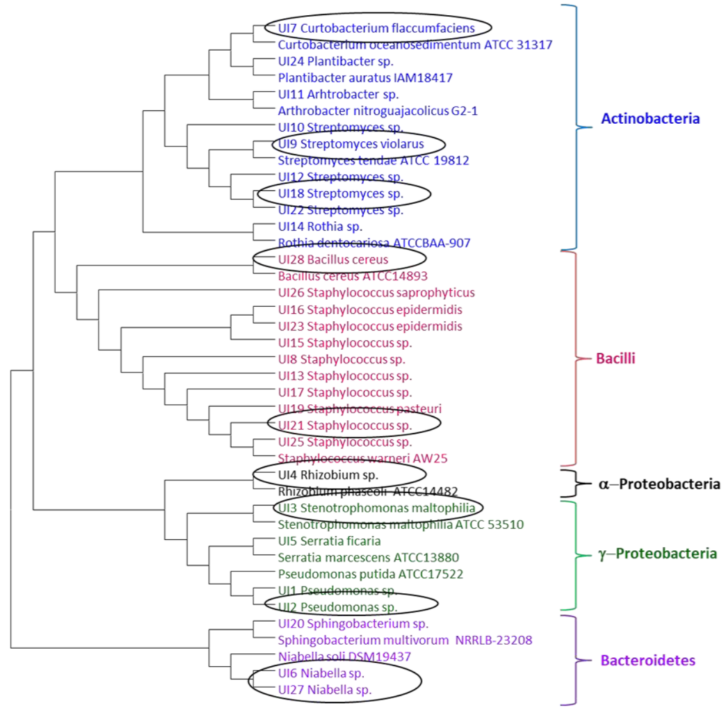
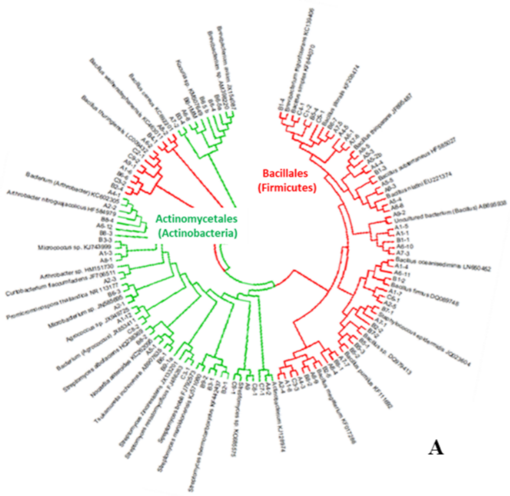


Figure 2: Phylogenetic three of the bacterial strains isolated from Ingurtosu mine site

In the table the composition of PGPB.

Table 1: Bacterial consortium UI, composed by ten selected native strains, metal-tolerant and good PGP, used for the bioaugmentation inoculum in the 2011

Bacterial Strain	N_2 fixation	PO_4 mobilisation	Siderophore production	Auxine production
Pseudomonas sp.	+	+	+	+
Stenotrophomonas maltophilia	+	–	+	+/–
Rhizobium sp.	+	–	+	++
Niabella sp.	+	–	+	+
Curtobacterium flaccumfaciens	+	+	+	+/–
Strept. violarus	+	+/–	+	+
Streptomyces sp.	+	+	+	–
Plantibacter sp.	+	+	+	+
Niabella sp.	+	–	+	+
Bacillus cereus	+	+	++	+



A

Colony morphotypes	**94**
N_2fixer	82 %
PO_4 mobilizer	35 %
Siderophore producer	93 %
Auxine producer	21%

B

Bacterial Strain	N_2 fixation	PO_4 mobilisation	Siderophore production	Auxine production
Bacillus aryabhattai	+	+	+	+
Micrococcus sp.	+	+	+	+
Streptomyces thermocarboxydus	-	+	+	-
Bacillus safensis	+	+	+	-
Streptomyces resistomycificus	+	+	+	+/-
Streptomyces bobili	-	+	+	-
Kocuria rhizophila	+	+	+	+
Streptomyces sp.	+	+	+	+/-

C

Figure 3: Phylogenetic tree of the bacterial strains isolated from Ingurtosu mine site

At the right the microbial community composition of PGPB and in the table the Bacterial consortium USMI of selected PGP bacteria used for a second bioaugmentation inoculum in 2014.

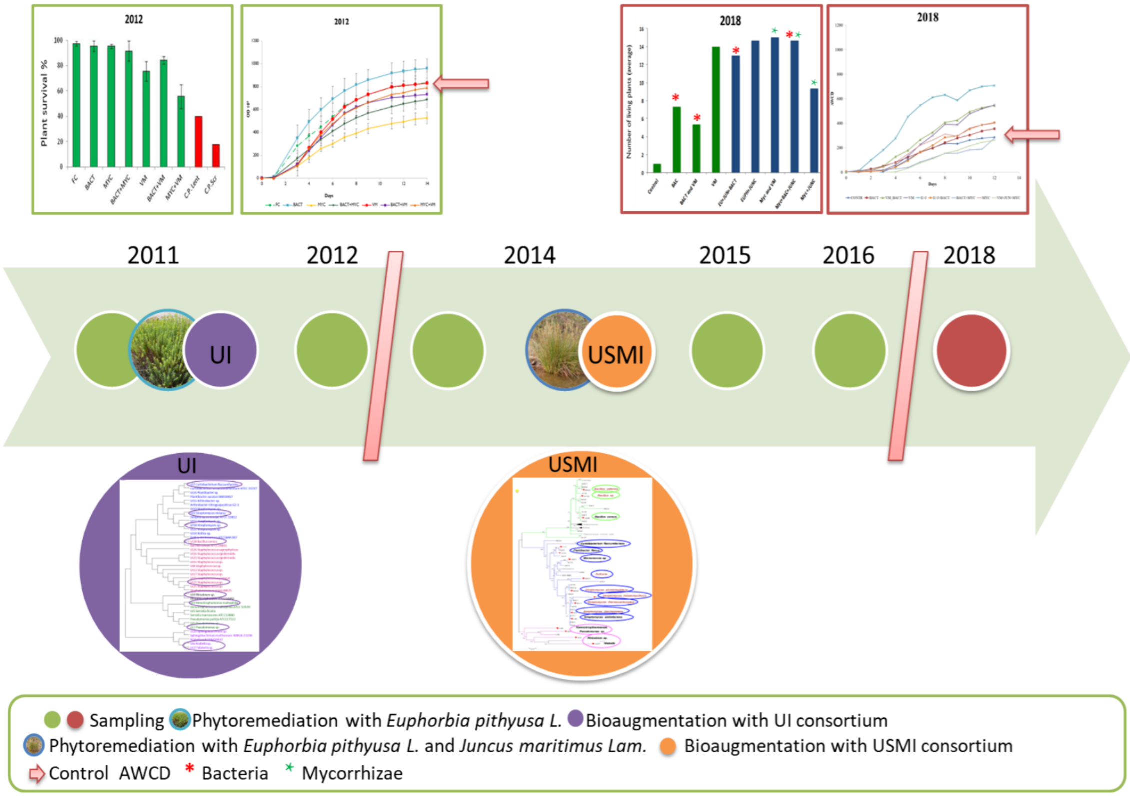


Figure 4: Timeline of interventions and monitoring done from 2011 to the present in Ingurtosu field experimental camp.

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