BRAZILIAN JOURNAL OF MICROBIOLOGY 47 (2016) 853–862

SBM SOCIEDADE BRASILEIRA DE MICROBIOLOGIA

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Environmental Microbiology

Enrichment of arbuscular mycorrhizal fungi in a contaminated soil after rehabilitation

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ARTICLE INFO

Article history:
Received 11 November 2014
Accepted 11 January 2016
Available online 24 June 2016

Associate Editor: Jerri Édson Zilli

Keywords:
Glomeromycota
Heavy metals
Soil contamination
Mycorrhiza
Revegetation

ABSTRACT

Spore counts, species composition and richness of arbuscular mycorrhizal fungi, and soil glomalin contents were evaluated in a soil contaminated with Zn, Cu, Cd and Pb after rehabilitation by partial replacement of the contaminated soil with non-contaminated soil, and by *Eucalyptus camaldulensis* planting with and without *Brachiaria decumbens* sowing. These rehabilitation procedures were compared with soils from contaminated non-rehabilitated area and non-contaminated adjacent soils. Arbuscular mycorrhizal fungi communities attributes were assessed by direct field sampling, trap culture technique, and by glomalin contents estimate. Arbuscular mycorrhizal fungi was markedly favored by rehabilitation, and a total of 15 arbuscular mycorrhizal fungi morphotypes were detected in the studied area. Species from the *Glomus* and *Acaulospora* genera were the most common mycorrhizal fungi. Number of spores was increased by as much as 300-fold, and species richness almost doubled in areas rehabilitated by planting *Eucalyptus* in rows and sowing *B. decumbens* in inter-rows. Contents of heavy metals in the soil were negatively correlated with both species richness and glomalin contents. Introduction of *B. decumbens* together with *Eucalyptus* causes enrichment of arbuscular mycorrhizal fungi species and a more balanced community of arbuscular mycorrhizal fungi spores in contaminated soil.

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http://dx.doi.org/10.1016/j.bjm.2016.06.001

Introduction

Arbuscular mycorrhizal fungi (AMF – Phylum Glomeromycota) are important components of the soil microbiota that contribute to the diversification and stability of natural ecosystems.[1] AMF are obligate mutualistic symbionts that colonize the roots of more than 80% of plant families, and which establish an association known as arbuscular mycorrhiza.[2] Several studies have reported on the abundance and occurrence of AMF in contaminated soils,[3–6] and have highlighted the high resistance of AMF isolates to several types of stress, including water stress, soil acidification, disaggregation, and absence or scarcity of vegetation cover.

Mining activities produce wastes which may contain heavy metals as contaminants. These residues are generally deposited on the ground, and often occupy large extensions. In order to prevent chemical elements contained in these residues to be exposed to leaching processes, or to the action of the winds, rehabilitation programs are usually employed in these areas through the establishment of vegetation cover. However, plants often have very limited development in contaminated areas. Due to their beneficial effects on plant growth under stressed conditions, AMF have been used to enhance rehabilitation of contaminated soils through phytoremediation.[7] Besides, mycorrhizal plants and their associated microbiota can regulate absorption, transformation and removal of soil pollutants.[8] The contribution of AMF to plant growth in soils contaminated with heavy metals is related to several factors, including diversity, abundance, persistence, and efficiency of AMF populations, which might vary between different locations, and are related to the environmental variables and to the presence of vegetation.[9] The insoluble glycoprotein glomalin, which is abundantly produced by the hyphae of some AMF, is known for accumulating in the soil where it can retain large amounts of heavy metals.[10] It has been suggested that soil glomalin content is related to changes associated with land use and rehabilitation of degraded soils.[11] These changes in vegetation cover may directly influence AMF communities and the quantity and quality of compounds produced by them, such as glomalin.[4] Therefore, abundance and richness of AMF or glomalin content may be useful indicators of rehabilitation. Studies showed that these attributes of AMF are inversely related to concentration of heavy metals in the soil.[3] Therefore, improved knowledge on the dynamics of these fungi in contaminated rehabilitated soils is highly relevant, considering that AMF may contribute to revegetation and phytostabilization.[12] The aim of this study was to evaluate the occurrence and AMF community composition in soils from areas contaminated with zinc (Zn), cadmium (Cd), copper (Cu), and lead (Pb), and subject to rehabilitation.

Materials and methods

Study areas and soil sampling

Soil samples were collected in the summer of 2009 (February) from contaminated sites under rehabilitation, in a Zn smelter unit of the Metal Mining Company (Companhia Mineira de Metais – CMM), currently known as Votorantim Metal Company (CMV), which is located in the municipality of Três Marias (Minas Gerais – MG – Brazil). Soil in the area was classified as anthropogenic, and the regional climate corresponds to type Aw of the Köppen classification system, namely savanna, with dry winter.[13] The annual average temperature varies between 19 °C and 22 °C, and the annual average rainfall varies between 1100 and 1420 mm. The areas are located at an average elevation of 539 m in a flat relief with original cerrado vegetation.

By the year of 2001/2002, two sites in the dumping area designated as industrial waste deposit (System 1 – 18°11′25.70″ S and 45°14′10.90″ W) and ustulation (System 2 – 18°11′10.50″ S and 45°14′8.00″ W), both contaminated by waste disposal, were sampled, and revealed high content of heavy metals: Zn = 17,167 and 1844 mg kg^{-1}; Cu = 882 and 145 mg kg^{-1}; Cd = 584 and 29 mg kg^{-1}; and Pb = 573 and 278 mg kg^{-1}, respectively. Two pilot rehabilitation programs were set up by the research team from the Department of Soil Science of the Federal University of Lavras (UFLA) and by the CMV staff. Rehabilitation strategy project included excavation of the contaminated soil and its replacement with non-contaminated soil. *Eucalyptus camaldulensis* seedlings were planted in rows in 1 m × 1 m × 10 m trenches, where non-contaminated soil replaced the contaminated one. Space inter rows received a 20 cm layer of non-contaminated soil, in which *Brachiaria decumbens* were planted or not (Fig. 1). The soil used to replace (row/trenches) or cover (inter rows) the contaminated one was taken from a Cerrado area adjacent to CMV. Before replacement, soil was subjected to liming, and received organic matter (cow manure) and fertilizers, in order to ensure adequate plant growth.[14] For the assessment of AMF, 10 composite soil samples (5 sub-samples each) were taken at 0–20 cm depth (one composite sample per sampling point. The distance between sampling points was 200 m), from rows and inter-rows of both rehabilitation programs. For comparison, non-contaminated samples were also taken from Cerrado (C – 18°10′55.80″ S and 45°13′38.50″ W) and grass pasture (P – 18°11′28.30″ S and 45°13′55.20″ W), as well as from an adjacent non-rehabilitated area (NR – 18°11′09.50″ S and 45°14′34.80″ W) (Table 1). Five composite samples were taken from the reference areas (C, P and NR).

Analysis of heavy metals in soils

Chemical analyses of soil samples were carried out in the Laboratory of Soil Science of UFLA. Heavy metal contents, including Zn, Cu, Cd, and Pb, were extracted by aqua regia solution, as recommended by the State Council on Environmental Policy (Conselho Estadual de Politica Ambiental – COPAM).[15] Total heavy metal content was determined using the USEPA 3051 method, and soluble heavy metal content was determined by the Mehlich-1 method.

Occurrence and richness of arbuscular mycorrhizal fungi (AMF)

The occurrence of AMF was assessed by direct extraction of spores in 50 mL of soil, using the wet sieving technique,[16] followed by centrifugation in 50% sucrose solution. Spores were counted and mounted on slides