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2016

http://hdl.handle.net/10539/21657

Zamxaka, Mtutuzeli (2016) Micro analytical observation of elemental distribution in arbuscular mycorrhizal (AM) roots from mining sites in South Africa and identification of their AM fungi, University of Witwatersrand, Johannesburg, <http://wiredspace.wits.ac.za/handle/10539/21657>

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MICRO ANALYTICAL OBSERVATION OF ELEMENTAL DISTRIBUTION IN ARBUSCULAR MYCORRHIZAL (AM) ROOTS FROM MINING SITES IN SOUTH AFRICA AND IDENTIFICATION OF THEIR AM FUNGI

BY

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Submitted: **10 August 2016**

A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg,
in fulfilment of the requirements for the degree of
Doctor of Philosophy
Johannesburg, 2016

Abstract

South Africa, as one of the leaders in mining industry, due to the variety and quantity of minerals produced, has been and is still producing a number of mine tailings which are contaminated by heavy metals. Heavy metals are very harmful to plants and especially to human beings and animals due to their non-biodegradable nature. The problem of environmental metal pollution could be combated by the establishment of Arbuscular Mycorrhiza (AM) vegetation on the surface of mine tailings. Besides the toxicity of the substrate, such areas usually lack essential nutrients (mainly N, P, and K) and organic matter. AM fungi contribute to soil structure by forming micro- and macro- soil aggregates within the net of external hyphae. Their presence may reduce stress caused by lack of nutrients or organic matter and increase plant resistance to pathogens, drought and heavy metals. Therefore, mycorrhizal fungi may become the key factor in successful plant revegetation of heavy-metal-polluted areas by promoting the success of plant establishment and increasing soil fertility and quality.

The aim of this project was to identify AM fungi from a number of heavy metal sites in South Africa using both morphological and molecular techniques, followed by the evaluation of heavy metal distribution and localisation in mycorrhizal roots. Soil samples were collected from three different provinces, namely: Gauteng, Mpumalanga and North West provinces. The sites were selected based on their historical and current heavy metal contamination. Indigenous AM fungal isolates (which are adapted to local soil conditions) can stimulate plant growth better than non-indigenous isolates. AM fungal spores were isolated from 100g of representative soil sample by the wet sieving and decanting method, followed by assessment of spore numbers and infective propagules. The spores of a subset of the pot samples were mounted on microscope slides in polyvinyl lactic acid glycerol and identified by morphological characteristics to the level of genus or species. Most of the spores counted were observed in a 45 µm sieve. These spores were tiny and had different sizes, colours and shapes. The majority of the observed spores were small, brown and oval in shape. For morphological identification, plant roots were stained and hyphae were found to be the most abundant in roots.

For molecular identification, two sets of nested PCR primers, namely NS1 & NS4 coupled with AML1 & AML2, were employed in this study due to their ability to amplify all subgroups of arbuscular mycorrhizal fungi (AM fungal, Glomeromycota), while excluding sequences from other organisms. Through both morphological characteristics and molecular identification, the following fungal genera were identified for the first time in the studied sites in South Africa. The study identified a total of 14 AM fungal genera and 55 AM fungal species, which are: *Glomus (15), Acaulospora (11), Scutellospora (6), Gigaspora (6), Rhizophagus (3), Funneliformis (3), Archaeospora (2), Claroideoglomus (2), Ambispora (2), Sclerocystis (1), Fuscutata (1), Entrophospora (1), Diversispora (1), Paraglomus (1)***.** Both *Glomus* and *Acualospora* have been observed to be the highest occurring genera in the analysed soil samples, followed by *Scutellospora* and *Gigaspora* and others mentioned.

PIXE technique was successful in localising elemental concentration in both plant roots and AM fungal structures, as well as in indicating the large vesicles in root tissue. AM fungal structures in the outer cortex or outer epidermal layer of the root cross-sections were observable, as shown by the more significantly enriched Si in the vesicles and arbuscules. Distinctive elemental maps can be used to localise sites of colonisation and verification of the symbiotic nature of the tissue. This indicates that a range of metals can be sequestered in AM fungal structures above levels in surrounding host root tissue, and demonstrates the potential of Micro-PIXE to determine metal accumulation and elemental distribution in mycorrhizal plant roots and inter-and intracellular AM fungal structures.

This research highlights the potential of AM fungi for inoculation of plants as a prerequisite for successful restoration of heavy metal contaminated soils. It also illustrates the importance of AM fungal diversity in selected high heavy metal (HM) sites in RSA, particularly in the North West and the Gauteng gold mining slime dams. Therefore, phytoremediation of mine tailings by mycorrhizal plants seems to be one of the most promising lines of research on mine tailings contamination by heavy metals. The strategies which evolved during this project have great potential for phytoremediation of toxic mining sites, and thus can help mitigate the environmental problems, especially in the mining waste sites.