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# Molecular characterization and glomalin production of arbuscular mycorrhizal fungi colonizing a heavy metal polluted ash disposal island, downtown Venice

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ABSTRACT

In this work the arbuscular mycorrhizal (AM) fungal communities colonizing a polluted ash dump island, downtown Venice, were studied by using a multimodal approach. The island, Sacca San Biagio, was covered with a thick layer of municipal solid waste residues produced by an incinerator operating from 1973, to 1984. Such residues contained high levels of heavy metals (Cu, Pb and Zn). We characterized the AMF communities present in soils on Sacca San Biagio island by using molecular methods. Nine AM fungal sequence types were detected in the roots of three plant species, representative of the dominant flora, by using partial SSU ribosomal RNA genes. The most abundant sequence types corresponded to *Glomus intraradices*/*Glomus fasciculatum*, and to Glo18, a sequence detected so far only *in planta*. Two sequences were new to science. Glomalin-related soil protein (GRSP), extracted from rhizosphere soil of dominant plant species, ranged from 1.6 to 2.3 mg $g^{-1}$. The occurrence of an active AM fungal community able to live in such harsh environment was evinced by the correlation between mycorrhizal colonization and GRSP content.



## 1. Introduction

Incineration is a major approach, throughout the world, for managing the increasing production of municipal solid wastes (MSW) (Klein et al., 2001). Although incineration reduces greatly the volume and the mass (about 90% and 75%, respectively) of MSW (Chimenos et al., 1999), it generates huge quantities of ashes (about 17 Mt per year worldwide) as fly ashes (5% of initial weight of wastes) and bottom ashes (up to 30% of initial weight of wastes) (Lapa et al., 2002), usually disposed in dumps (Chimenos et al., 1999; Klein et al., 2001). Although in some European countries significant quantities (40–60%) of bottom ashes from MSW are utilized for road construction and similar purposes, landfilling represents the main management strategy for disposing of such residues (Hjelmar, 1996).

Bottom ashes contain high levels of toxic compounds and heavy metals, whose concentration during the incineration process represents a risk for the environment (Gau and Jeng, 1998). Besides, ashes can be easily transported to the surrounding areas by the action of wind and water (Rigo et al., 2009).

Sacca San Biagio is an ash disposal island located downtown Venice (Italy). The island, of about 4.5 ha, hosted a MSW furnace operating from 1973 to 1984, and producing bottom ashes that were disposed all over the island area. Since 1984 no significant activities were carried out on the island and in 2003 the furnace was demolished. In the last 25 years the highly polluted soil of the island has been naturally colonized by spontaneous vegetation. Sacca San Biagio ash dump is a unique site since, even if concrete embankments had been erected all around the island, they did not prevent water dispersion of ashes and leachates into the water of the Venice Lagoon. Such ashes, characterized by the presence of heavy metals and toxic compounds, are considered ecotoxic, genotoxic and immunotoxic (Lapa et al., 2002; Feng et al., 2007, Chibuisi et al., 2009). Nevertheless, they represent habitats which could be colonized by plants and soil microorganisms able to survive in harsh environmental conditions.

An important group of soil microorganisms, arbuscular mycorrhizal (AM) fungi (Phylum Glomeromycota), play an important role in soil fertility, plant nutrition and aboveground plant community composition (van der Heijden et al., 1998; Smith and Read, 2008). AM fungi (AMF) establish mutualistic symbiosis with the roots of most terrestrial plant species, acting as a living bridge between soil and plants. AMF are critical in the establishment and fitness of plants in severely disturbed sites (Miller and Jastrow, 1990), including those contaminated by heavy metals

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(Turnau, 1988; Vallino et al., 2006). They affect also the physico-chemical characteristics of the substrate and contribute to the formation and maintenance of soil structure, enmeshing soil particles by means of extraradical hyphae and their exudates and residues (Tisdall and Oades, 1982; Miller and Jastrow, 1990). Moreover, AMF produce glomalin, a protein extracted from the soil as glomalin-related soil protein (GRSP) (Wright and Upadhyaya, 1996; Rillig, 2004), which plays a key role in soil stability (Wright and Upadhyaya, 1998; Rillig et al., 2002; Bedini et al., 2009a).

The characterization and identification of AMF adapted to harsh conditions of degraded areas can be fundamental for their utilization in successful managements of re-vegetation programs. Although AM fungal identification is usually performed on the basis of spore morphological characteristics (Walker, 1983; Morton, 1988), the identification *in planta* by using PCR-based techniques, mainly targeted to the ribosomal DNA gene regions containing sequences of different variability, represents a reliable method to detect active AM fungal communities living in the roots (Helgason et al., 1998).

With the aim of characterizing the AMF communities of the abandoned ash dump island Sacca San Biagio, downtown Venice, we assessed the mycorrhizal status of spontaneous plant species, GRSP content of rhizospheric soil, the occurrence of AM fungal spores, and identified, by molecular methods, the AMF colonizing plant roots.

## 2. Materials and methods

### 2.1. Study area

The study area is located in Sacca San Biagio Island (45°25′36″N, 12°18′34″E) in the central lagoon of Venice (Fig. 1). Sacca San Biagio is a tideland which was elevated by the deposition of inert construction waste. The island surface is covered by a layer of MSW incineration residues produced by an incinerator operating since 1973, up to 1984 (Fig. 1). The ashes layer, reaching the depth of about 3 m in the central part of the island, is estimated to occupy a volume of about 60,000 $m^3$.

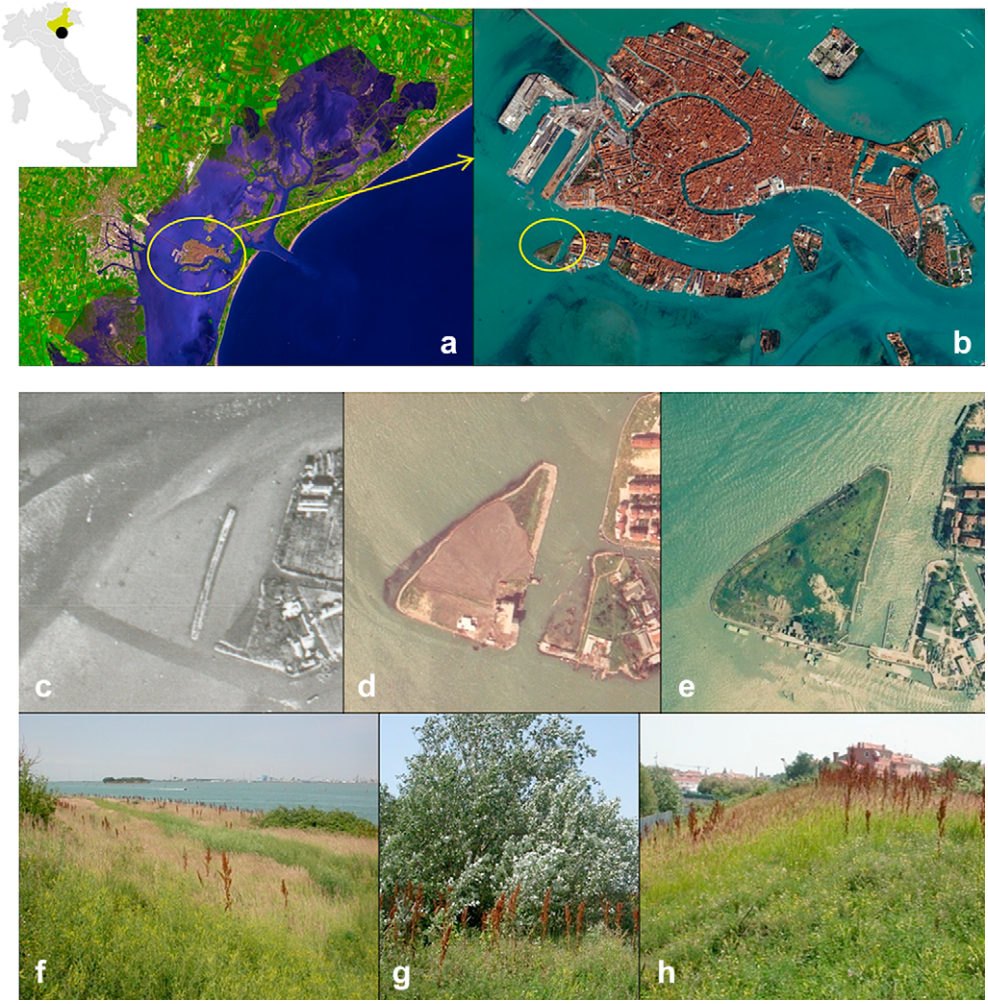


**Fig. 1.** Sacca San Biagio island in the Lagoon of Venice, Italy. (a) Map of Italy focusing on Veneto Region and satellite image of the city of Venice in the lagoon (courtesy of NASA/JPL-Caltech); (b) City of Venice with Sacca San Biagio island highlighted (courtesy of Image Science and Analysis Laboratory, NASA-Johnson Space Center); (c) Year 1955: aerial photo of the tideland, before the elevation by deposition of inert construction waste (courtesy of Istituto Geografico Militare, Italy); (d) Year 1974: aerial photo of Sacca San Biagio island mostly covered by ashes during the incinerator's activity (courtesy of Alifoto Montedison); (e) Year 2005: aerial photo of Sacca San Biagio island, showing the perimetral embankments, the incinerator's demolition debris and the island vegetation coverage (courtesy of Regione Veneto); (f–h) views of the vegetation in Sacca San Biagio island at the sampling time: (f) eastern side with Porto Marghera in the background; (g) *Populus alba* in the most elevated central part of the island; (h) western part of the island with Venice's living quarters in the background.