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Full Length Research Paper

Elements sequestered by arbuscular mycorrhizal spores in riverine soils: A preliminary assessment

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The elemental composition of spore wall of arbuscular mycorrhizal fungi (AMF) was analyzed by energy-dispersive X-ray spectrometry (EDS) in a preliminary assessment. Measurements of AMF spores were made for riparian soils in both urban (rivers subjected to different human activities) and farm sites in southeastern Brazil. Spore populations belonging to four genera: *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* were analyzed. The results suggest that Glomeraceae spores sequester more elements than Acaulosporaceae and Gigasporaceae; however, the presence of nickel was observed in one *Scutellospora* species. These data showed the elements sequestered by AMF spores in riparian sites presenting different conditions of **disturbance.**

Key words: Arbuscular mycorrhizal spores, urban sites, energy-dispersive X-ray spectrometry, riparian forest.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligatory dependent on host-plants for the resources necessary to produce reproductive propagules, the external spores (Read 2003). AMF are affected by disturbs in the ecosystems, like global change (Rillig et al., 2002), or heavy metal pollution (Meharg and Cairney, 2000); however, plant symbiotic mycorrhizal fungi can accumulate metals from soil components (Gadd, 2005). There are several reports of metal-tolerant AMF (Orlowska et al., 2002; Hildebrandt et al., 2007; Soares and Siqueira 2008); however, there is to the best of our knowledge no report of metal-tolerant AMF from riparian soils in Brazil.

Morphological studies of small structures are possible using energy-dispersive spectrometers and wavelength-dispersive spectrometers (WDS) coupled to a scanning electron microscope (SEM); however, these methods do not detect minor and trace elements (Przybylowicz et al., 2004). Not only is the energy-dispersive X-ray spectrometry (EDS) technique limited to the detection of elements with an ordinal number between 10 and 25, such as aluminum (Al), calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P) and sulfur (S) (Leapman and Hunt, 1991), but also peak values of micronutrients and lighter elements, such as nitrogen (N), cannot be clearly distinguished from the background (Bücking et al., 1998). Weiersbye et al. (1999) showed several elements (heavy metals) in AMF spores from uranium mine tailings in South Africa using Micro-Pixe mapping; however they did not identify isolated AMF species. Moreover, Cruz (2004) showed that quantitative light element microanalysis of AMF spores employing EDS is a technique still little explored and which may inform the chemical spectrum of AMF spores and point the differences among species. Within a research project to study the diversity, role and potential of AMF in riparian areas of Velhas river basin in Minas Gerais State, Brazil, different restored sites with native woody species and other presenting native vegetation were evaluated.

The aim of the present study was to investigate the elemental concentration of identified AMF genera or species isolated from riparian soils in southeastern Brazil.

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Figure 1. Location of sampling sites, Southeast Minas Gerais, Brazil. Site codes as in Table 1.

Table 1. Study sites, characteristics and land use in Minas Gerais, Brazil.

Site code	River	Coordinates	Locality	Elevation (m.a.s.l.)	Vegetation	River conditions	AMF spores[#]
P	Gaia stream	19º 52' S 43º 47'W	Sabará (Reserve forest)	735	Native herbaceous and woody species (Atlantic Forest and the Cerrado savannas)	Natural	*Scutellospora aurigloba* *Glomus* sp.
SR	Sabará river	19°53'32"S 43°48'31" W	Sabará (Urban site)	637	Restored with native woody species	Urban	*Acaulospora* sp. *S. gregaria*
SD	Sabará river	19°53'32"S 43°48'31" W	Sabará (Urban site)	637	Herbaceous cover	Urban	*S. fulgida*
VR	Velhas river	43º 51' 58" S 19º 50' 51" W	Sabará (Farm)	662	Restored with native woody species	Disturbed	*S. reticulata*

[#]Spores evaluated for microanalysis.

MATERIALS AND METHODS

Study sites

The study sites are located in the southeast of Brazil, in Mina Gerais State. The sites are: riparian forests at Sabará and Velhas rivers, belonging to the São Francisco basin, in the south of Minas Gerais State. Spores were isolated from restored sites at urban or farming areas, presenting sandy soils, and from a preserved forest upstream of the urban areas (Figure 1 and Table 1), presenting a vegetation dominated by trees, which is a transition between the Atlantic Forest and the Cerrado savannas (Rizzini, 1997). The climate is tropical (Aw) with temperatures between 22 and 23°C, and mean annual rainfall is 1.400 mm.

Sample collection

Soil samples (500 g) were collected to a depth of 25 cm from the soil surface of the four studied sites in 2007 and 2008 during visits at the riparian sites in Minas Gerais. Soils were air-dried and stored until processed. AMF spores were recovered from 100 g soil

Table 2. Semi quantitative analyses by EDS of some AMF species (spores) in riparian soils, Minas Gerais, Brazil.

AMF species	Elements												
	C	O	Na	Al	Si	P	S	Cl	Ca	Fe	K	Ni	Mg
Acaulosporaceae													
Acaulospora sp.	84.9b	4.8ab	0.04ab	0.27a	4.15a	ND	0.58c	1.32bc	1.35a	0.69ab	0.02ns	ND	1.6ns
Gigasporaceae													
Scutellospora aurigloba	93.1* a	3.2b	0.01b	<0.01b	0.3b	0.04	0.5c	2.5b	ND	ND	ND	ND	ND
S. fulgida	93.5a	5.15ab	0.01b	0.01b	0.56b	ND	0.6c	ND	0.1c	ND	<0.01	ND	ND
S. gregaria	89.75ab	5.6a	<0.01b	0.12ab	1.39ab	ND	1ab	0.15c	0.43bc	0.37ab	0.01	ND	0.85
S. reticulata	88.7ab	3.07b	ND	0.26ab	2.38ab	ND	1.15a	0.28c	1.72a	0.99a	0.02	1.28	ND
Glomeraceae													
Glomus sp.	82.6b	4.2b	0.08a	0.13ab	3.5a	<0.01	0.68bc	5.13a	1.27ab	0.25b	0.02	ND	2.04

*Spore wall atomic %. Data are means of five replicate entire spores. 10 kV potential, electron beam 20 nA, 200 s. ND = not detected. Different letters indicate significant differences as determined by Tukey's test ($p < 0.05$). Ns = non-significant

samples, separated by wet sieving (Gerdemann and Nicolson 1963), decanting, sucrose centrifugation and flotation (Walker et al., 1982) and were collected on 0.5, 0.062 and 0.037 mm sieves. Distilled water was used during the sieving process to avoid impurities on the spore surfaces. Samples were mounted in microscopic slides and were dried.

Microanalyses

AMF spores were analyzed in this work by EDS to examine the sequestration of elements by these structures. The chemical composition of the AMF spores (five replicates), for the assigned elements (Table 2) in the periodical table, was measured by electron probe microanalysis with energy-dispersive X-ray and the sample composition was determined by the ZAF method for semi quantitative analysis, as described by Goldstein et al. (1992). EDS measurements were made on a JXA-8900RL, JEOL WD/ED COMBINED MICROANALYZER, following Goldstein et al. (1992), at the Electron Microscopy and Microanalysis Laboratory, Physics Department, Federal University of Minas Gerais, Brazil.

Samples were mounted in microscopic slides and were dried on a support membrane prior to carbon coating. The entire slide was coated. Selected areas of spore wall (100 μm^2 in size) were used for microanalyses. Data were subjected to one-way ANOVA using Statistica 7.0, Statsoft, and means were compared by Tukey's test ($p < 0.05$). Average concentrations of elements in the spores were used in the analysis. A control spectrum taken from an area of adjacent support membrane close to the spore (carbon coated) was observed. The elemental mapping was done by data extracted from arbitrarily selected micro areas of the selected spore.

RESULTS

The results revealed differences in elemental composition between spores isolated from riparian areas (Table 2). Figure 2 shows micrographs and their EDS-measured composition spectra of the analyzed spores from the riparian site. The spore wall mainly contained Carbon (C), and varying proportions of oxygen (O), silicon (Si), Al and Ca. Spectra from spores showed a major C peak and smaller peaks of Si and Al. The control spectrum from the Adjacent support membrane did not have any significant significant characteristic peaks.

With respect to microanalyses of spores, relative high values for C (Table 2) were obtained in this work for three *Scutellospora* species. On the other hand, *Scutellospora fulgida* and *Scutellospora aurigloba* showed lower amounts of all the elements, except for C.

Significant differences in the elemental concentration of spore wall between *Glomus* and *Scutellospora* were found. In general, some species of *Scutellospora* were characterized by higher concentration of S than spores of *Glomus*. The distribution of Ca and Cl in spores was related to the AMF species, and the concentration of P was low in all samples. Nickel was detected in only one spore type (*Scutellospora reticulata*) isolated from restored riparian site of Velhas River (Table 1), which is presented in Figure 3, showing an elemental map distribution of some selected elements. The distribution of C was high in the interconnecting ridges that form a reticulum and in the spines. Al, S and Si showed lower intensity than C. Ni, P and Ca were relatively homogeneous distributed.

DISCUSSION

The presence of C observed in the spores in our study is in line with the report by Cruz (2004). As expected, C was detected in high proportions in six AMF species, since it is the main element in the organic compounds of the spore structures.

Moreover, in the present study, the microanalysis of the spores showed four elements (K, Ca, Ni and Fe) in common with spores analyzed for uranium mine tailings (Weiersbye et al., 1999). The presence of Ni, which at

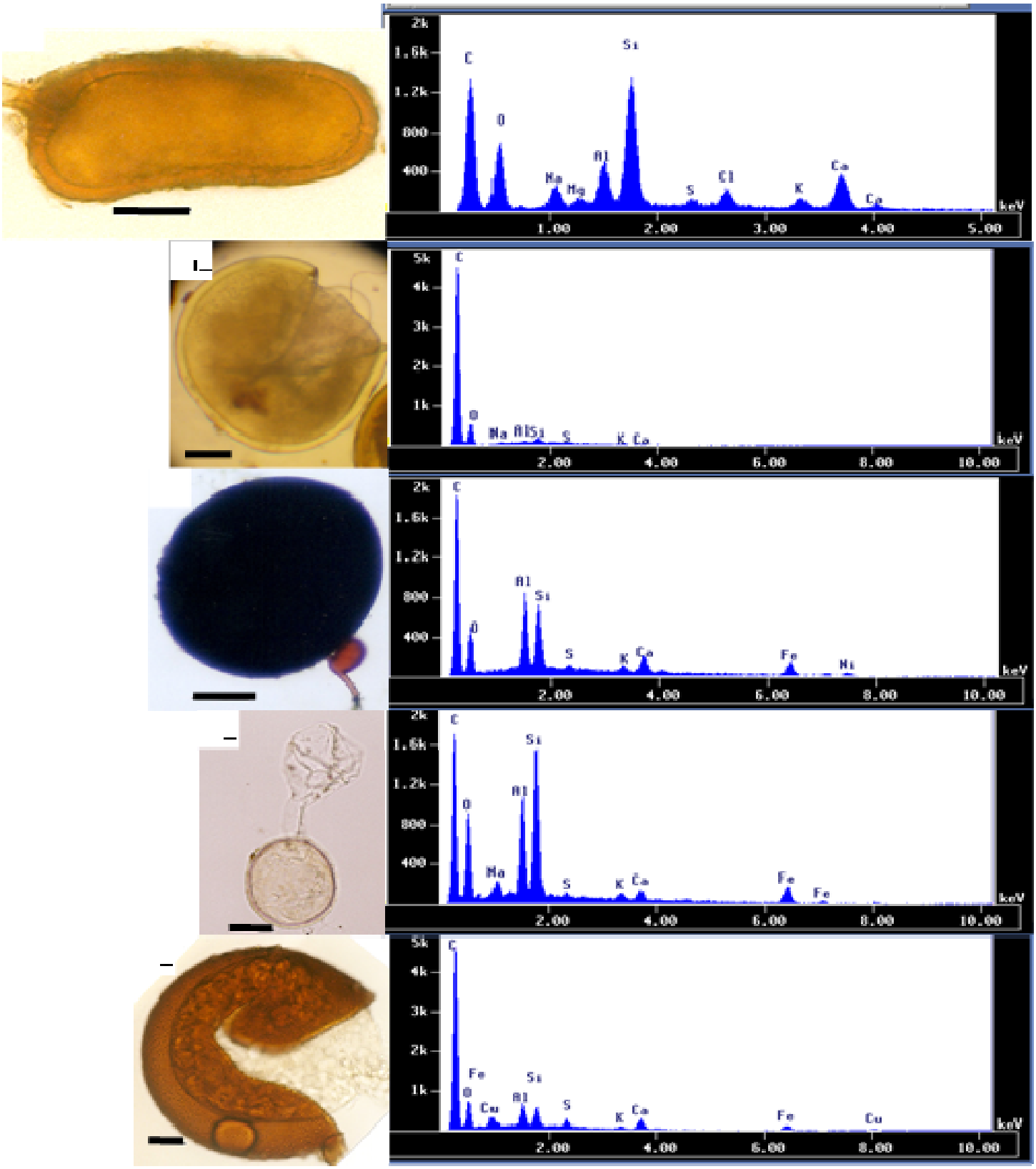

Figure 2. Photomicrographs of some AM spores found at riparian areas, Brazil and spectra of X-ray microanalysis (EDS) of spore wall. a = *Glomus* sp.; b = *S. aurigloba*; c = *S. reticulata*; d = *Acaulospora* sp. 1 and e = *S. gregaria*. The peaks correspond to carbon (C), oxygen (O); iron (Fe); sodium (Na); magnesium (Mg); copper (Cu), aluminum (Al); silicon (Si), phosphorus (P), sulfur (S); chloride (Cl); potassium (K); calcium (Ca) and nickel (Ni). (Bar, a = 25; b, e = 50 µm; d, c = 100 µm).

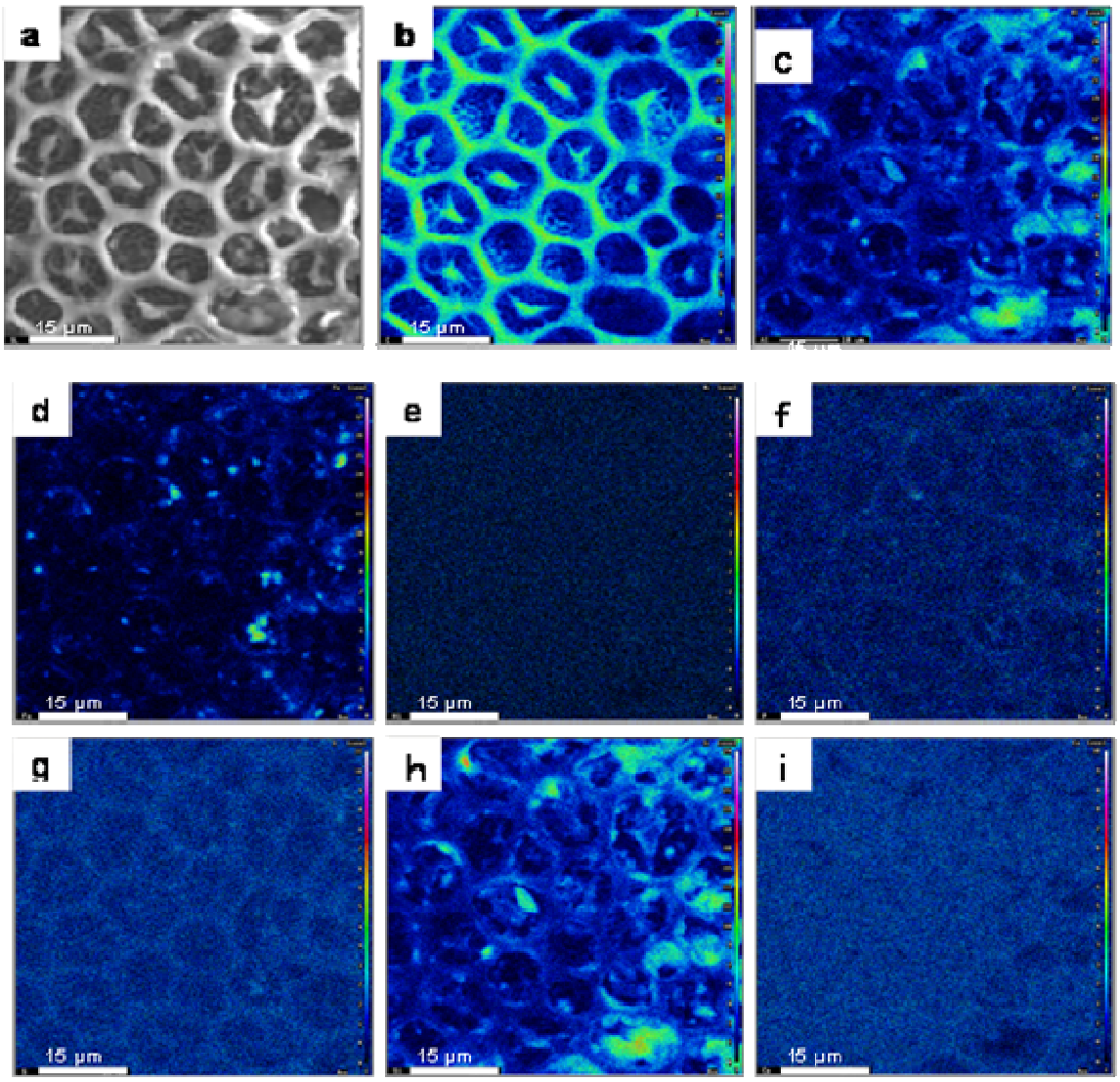

Figure 3. WDS elemental map showing the distribution of some elements in *S. reticulata*, isolated from soil of the riparian vegetation of Velhas River. Resolution: 900 × 900 pixels points. (a) Micrograph of the spore, showing the wall ornamentation; (b) map showing the distribution of C; (c) Al; (d) Fe; (e) Ni; (f) P; (g) S; (h) Si and (i) Ca. Bar, 10 µm.

higher concentrations can lead to poisoning (heavy metal), detected in only one AMF spore (*S. reticulata*) isolated from restored riparian forest at the farm site (Velhas River), may be related to riverine soil pollution. Orlowska et al., (2008) showed that external AM hyphae can bind Ni, influencing the uptake of heavy metals by plants. The presence of chemical elements in AMF spores is important to clarify their structure, since it allows characterizing these elements as a part of the components in the spores, and distinguishing them by their nutrient composition, which is useful for spore survival. Furthermore, the sequestered elements may reflect a polluted environmental condition.

The chemical composition of suggested indicator genus or species for disturbed (*Acaulospora*, *S. gregaria* and *S. reticulata*) or for more pristine sites (*S. aurigloba*) in the riparian forest showed that spores from the disturbed site could present more nutrient richness in their wall than spores from natural sites. The spores of *S. aurigloba* isolated from the preserved riparian site contained less chemical elements. On the other hand, the results suggest that other AMF species present a rich element composition.

This study is, to the best of our knowledge, the first report of information on metals sequestered among the AMF species or genera from urban and farm sites. The results suggest that *Glomus*, a dominant genus in the sites (Pagano, MC unpublished), has high nutrient richness in the spore wall, and that the presence of the heavy metal Ni in spores can be investigated by EDS.

Finally, this technique also allows the seasonal variation of nutrient amounts in AMF spores to be investigated in the riparian vegetation of Minas Gerais's Rivers subject to different land uses.

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