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DIVERSITY OF MYCORRHIZAL FUNGI ARBUSCULAR AT PHOSPHATES SLUDGE, KHOURIBGA REGION (MOROCCO)

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Abstract

Arbuscular mycorrhizal fungi to have been sought in the sludge of phosphates and various plant species of the region of Khouribga (Morocco).

The density of spores of mycorrhizal fungi in phosphates sludge is low, ranging from 43 and 4 spores / 100 g soil. Some species are specific, case of *Glomus aureum* encountered at the level of the 1.5 basin; while others are common, case of *Glomus intraradices* present in all studied sites.

The preliminary morphological identification of the isolated species have allowed to highlight the presence of 31 species belonging to 4 genera (*Glomus*: 16 species, *Acaulospora*: 9 species, *Entrophospora*: 3 species, *Scutellospora*: 3 species), 4 families (Glomaceae, Acaulosporaceae, Scutellosporaceae, and Entrophosporaceae), and 2 Orders (Glomerales, and Diversisporales).

This study demonstrated the wealth of sludge in arbuscular mycorrhizal fungi. This wealth could be exploited in the production of a phospho-compost based on a composite endomycorrhizal inoculum, phosphate waste and organic residues.

Key words : Phosphate sludge, rhizosphere, arbuscular mycorrhizal fungi (AMC), rhizosphere, diversity.

Introduction

In Morocco, the mining industry generates huge quantities of mining waste such as waste rocks, concentrating residues and sludge (1). So, one of the big problems today, from an economic and environmental point of view, is the treatment of the large volumes of waste produced continuously by these industrial activities (Ouakibi *et al.,* 2013).

Washing the phosphate, for example, generates significant amounts of sludge are usually evacuated by pumping towards spreading basins, which allows you to store and retrieve the supernatant water. The sludge which is stored in the tanks has a residence time to reach a minimum humidity before being sloped (Loutou, 2015). According to this author, the large quantities of these discharges, stored generally in the open air, alter the landscape and arable land.

Exploitation and valorization of phosphates sludge may play an important role in the country's economy. One of the initiatives to develop these sludges is to use them in the agricultural sector in the form of phospho-compost to solve the problems of soil phosphorus deficiencies and improve agricultural production. In this sense, ancient works have been conducted to propose

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methods of manufacturing composts enriched with natural phosphates mainly from straws of sorghum and rice straw (Lompo, 1989). These methods have led to the production of organic fertilizers which not only maintain the organic fraction of the soil but also partially fill the needs of plant nutrients (Chaibou, 2013). They offer the possibility of practicing an organic farming (Agridoc, 2003).

Phosphorus is one of the most limiting nutrients in agricultural production (Sedogo, 1981; Pieri, 1989; Compaoré *et al.,* 2001; Lompo, 2009). The concentration of phosphate ions in the soil solution is low and does not allow adequate phosphate nutrition of crops (Compaoré *et al.,* 2001). Mycorrhizal bacteria and fungi with phosphate solubilizing arbuscules, common in soils and rhizospheres of plant species, can be used in the correction of this phosphorus deficiency (Singh et Kapoor, 1999). Indeed, these microorganisms, the best ecological candidates for plant nutrition by phosphorus (Sharma *et al.,* 2007), have phosphate solubilizing ability and the ability to convert insoluble phosphate compounds present in the soil in soluble forms utilized by plants (Pradhan and Sukla, 2006).

Thus, the composting of phosphate sludge can be done with organic matter (Lompo *et al.,* 1995) and the effect of phospho-compost can be improved by using phosphate-solubilizing microorganisms. Mycorrhizal fungi with arbuscules, for example, are an important component of soil microorganisms and the rhizosphere of plant species (Schreiner *et al.,* 1997; Kim *et al.,* 1997; Zhu *et al.,* 2001; Ruotsalainen *et al.,* 2002). They solubilize natural phosphates and promote the absorption of nutrients, such as phosphorus (Bolan, 1991; Gulden et Vessey, 2000; Vessey and Heisinger, 2001). Endomycorrhizae also stimulate the growth and productivity of host plants (Chen *et al.,* 2002; Hodge, 2000; Villegas and Fortin, 2001).

In the literature, little space has been reserved for the collection and knowledge of arbuscular mycorrhizal fungi from phosphate mining sites. In this work, importance has been given to the diversity of these endomycorrhizal fungi that may be present in the phosphate laundering sludg.

Materials and Methods

Sampling

Phosphate sludge samples (sludge from 1998, 1994, 2000, 2002-98, 1094-98, Ponds 1, 5, Mixed soil) were collected in 2017 (April-May). These samples were taken at a depth of 0-20 cm and a composite sample of sludge was made per site. Samples were placed in plastic bags and labeled.

Extraction of spores

The wet sieving method described by Gerdemann and Nicholson (1963) was adopted to extract spores. A quantity of 100 g of soil was poured into a beaker then diluted in 0.5 L of tap water and stirred for 1 min with a spatula. After a few seconds of decantation, the supernatant was passed through four superposed sieves of decreasing mesh diameter (500, 200, 80 and 50 µm). The same soil was again submerged, stirred, and the wet sieving was repeated 3 times. The solution retained by the sieves of 200, 80 and 50 µm was divided into two tubes and centrifuged for 4 minutes at 9000 rpm. The supernatant was discarded and a viscosity gradient was created by adding 20 mL of a 40% sucrose solution to each centrifuge tube (Walker *et al.,* 1982). The supernatant was discarded and a viscosity gradient is created by adding 20 mL of a 40% sucrose solution to each centrifuge tube. The supernatant containing the spores was passed through the 50 µm sieve, and the pellet was discarded. The recovered spores were rinsed with distilled water to eliminate sucrose. The spores were then recovered with a little distilled water in an Erlenmeyer flask. The estimation of the number of spores in the soil was made by counting the spores contained in 1 mL of supernatant and by extrapolation to the total volume (100 mL). If no spore was observed, all the supernatant was reduced to 1 mL and observed again.

The characteristic structures (color, shape, size and number of separation membranes, etc.) of the spores were demonstrated by mounting between slide and slide of 0.1 mL of supernatant.

Identification of spores

Spores were observed under an optical microscope and identified morphologically based on spore color, shape, size, surface ornamentation, spore contents and wall structures, sporulous saccule, germination shield, bulb and suspensor (Bethenfalvay and Yoder, 1981; Schenck and Perez, 1987; Goto, 2009). Slides of each different spore morphotype were prepared using either polyvinyl-alcohol alone or mixed with Melzer's solution (Goto, 2009; Azcón-Aguilar *et al.,* 2003). The identification of spores was made based on the criteria proposed by Schenk and Smith (1982), Hall (1984), Schenck and Perez (1987), Morton and Benny (1990), (INVAM, 2017) following the classification of Redecker *et al.,* (2013).

Results

The average spore density varies from one site to another (Fig. 1), that of the 2000 sludge is the highest (43 spores / 100 g of soil), followed by those of sludge 98-94 (34 spores / 100 g soil) and 98 (30 spores/100 g soil). The average spore density varies from one site to another, that of the 2000 sludge is the highest (43 spores / 100 g of soil), followed by those of sludge 98-94 (34 spores/ 100 g soil) and 98 (30 spores/100 g soil) (Fig. 1). The spores isolated from the studied sites are small Table 1, it varies between 98 and 34 µm.

Morphological identification of isolated spores revealed the presence of 31 species belonging to 4 genera: *Glomus* (16 species), *Acaulospora* (9 species), *Entrophospora* (3 species), *Scutellospora* (3 species) and to à familles (*Glomaceae, Gigasporaceae, Acaulosporaceae, Scutellosporaceae* and *Entrophosporaceae*) and 3 orders (Glomerales, Gigasporales, Diversisporales).

Table 1 : Endomycorrhizal fungi isolated from the laundered sludge of the studied phosphates.

Number	Name	Form size	Color surface	Medium	Spore	length of hypha	Number of walls
1	*Acaulospora scrobiculata*	Globular	Yellow brown	85	Smooth	17	2
2	*Glomus intraradices*	Globular	Yellow brown	79	Smooth	_	2
3	*Glomus versiforme*	Globular	Yellow brown	82	Granular	_	2
4	*Glomus deserticola*	Globular	White yellow	70	Smooth	34	2
5	*Acaulospora* sp1.	Oval	Dark brown	59	Smooth	40	2
6	*Acaulospora excavate*	Globular	Light yellow	61	Smooth	_	2
7	*Glomus intraradices*	Oval	Black brown	69	Granular	88	2
8	*Entrophospora kentinensis*	Globular	Pale yellow	56	Smooth	-	2
9	*Acaulospora laevis*	Oval	Beige	74	Smooth	_	2
10	*Acaulospora scrobiculata*	Globular	Yellow brown	60	Granular	-	2
11	*Entrophospora infrequens*	Globular	Grey White	40	Smooth	_	2
12	*Entrophospora schenckii*	Oval	White yellow	51	Granular	_	2
13	*Glomus deserticola*	Oval	Yellow	46	Smooth	_	1
14	*Scutellospora nigra*	Globular	Black	50	Smooth	_	2
15	*Scutellospora biornata*	Oval	White blanc	34	Granular	_	2
16	*Glomus deserticola*	Globular	Brown	68.5	Smooth	15	2
17	*Acaulospora lacunose*	Oval	Brown yellow	44	Smooth	21	2
18	*Glomus etunicatum*	Globular	Yellow brown	79.2	Smooth	-	2
19	*Acaulospora gedanensis*	Globular	White yellow	71.5	Smooth	_	2
20	*Acaulospora* sp2.	Oval	Brown yellow	88	Smooth	_	2
21	*Acaulospora morrowiae*	Oval	Yellow black	52	Smooth	_	2
22	*Glomus aggregatum*	Globular	Orange	70	Smooth	_	2
23	*Glomus caesaris*	Globular	Brown orange	98	Smooth	-	2
24	*Acaulospora colossica*	Oval	Yellow	47	Smooth	_	2
25	*Acaulospora* sp3.	Globular	Black Yellow	59.5	Smooth	_	2
26	*Glomus caesaris*	Globular	Light orange	32.7	Granular	_	2
27	*Glomus aureum*	Oval	Light brown	49	Smooth	_	2
28	*Glomus macrocarpum*	Oval	Light brown	62.8	Smooth	_	2
29	*Glomus mossae*	Oval	Light brown	53	Smooth	_	2
30	*Glomus claroideum*	Oval	Pale yellow	76.4	Smooth	_	2
31	*Glomus globiferum*	Globular	Light brown	77	Granular	_	2
32	*Acaulospora scrobiculata*	Globular	Yellow	46	Smooth	_	2
33	*Glomus claroideum*	Oval	Yellow	32	Smooth	_	2
34	*Glomus intraradices*	Globular	Yellow	61.7	Smooth	_	2
35	*Glomus fasciculatum*	Globular	Yellow	55.40	Smooth		2
36	*Glomus versiforme*	Globular	Brown	77.34	Smooth		4
37	*Glomus* sp.	Oval	Beige	50.7	Smooth	60	2

Table 2 : Appearance of endomycorrhizal fungal species at different sludge of studied phosphates.

Endomycorrhizal species	Sludge 98	Sludge 94	Sludge 2000	Sludge 98-2002	Basin 1.5	Mixture	Sludge 94-98
Acaulospora gedanensis	-	-	-	-	-	+	+
Acaulospora lacunose	-	-	-	-	-	+	-
Acaulospora laevis	-	-	+	-	-	-	+
Acaulospora morrowiae	-	-	-	-	-	-	+
Acaulospora scrobiculata	+	-	+	-	-	-	-
Acaulospora sp 1	-	-	+	-	-	-	+
Acaulospora sp3	-	-	-	+	-	-	-
Entrophospora infrequens	-	-	+	-	-	-	-
Entrophospora schenckii	-	-	+	-	-	-	-
Glomus aggregatum	-	-	-	-	-	-	+
Glomus aureum	-	-	-	-	+	-	-
Glomus claroideum	-	+	+	-	-	-	+
Glomus deserticola	+	-	+	-	-	-	-
Glomus etunicatum	-	+	-	-	-	-	-
Glomus intraradices	+	+	+	+	+	+	+
Glomus mossae	-	-	-	-	+	-	+
Glomus pansihalos	-	+	-	-	-	-	-
Glomus manihoti	-	-	-	+	-	-	-
Glomus sp.	-	+	-	+	-	-	-
Glomus versiforme	+	-	-	-	-	+	-
Scutellospora biornata	-	-	+	-	-	-	+
Scutellospora nigra	-	-	+	-	-	-	-
Scutellespora castenea	-	+	-	-	-	-	-

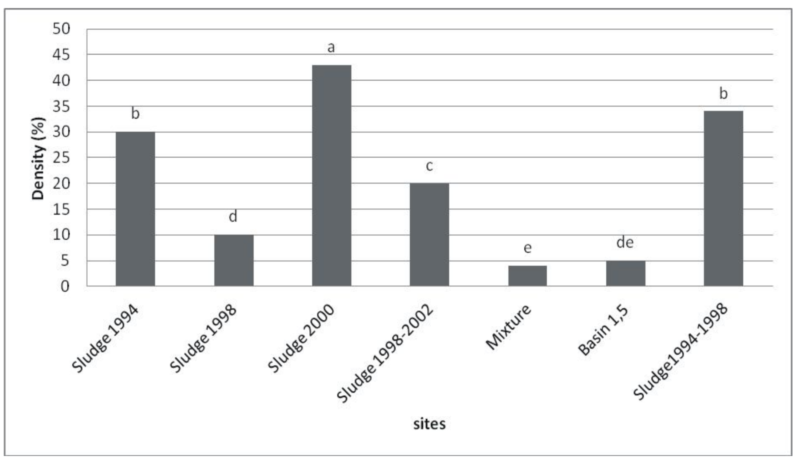

Fig.1: Average density of spores / 100 g soil of endomycorrhizal fungi in different studied phosphate sludges.

The encountered endomycorrhizal species (Table 1 and Fig.s 2 and 3) are *Acaulospora scrobiculata, Acaulospora laevis, Acaulospora colossica, Acaulospora gedanensis, Acaulospora morrowiae, Acaulospora excavate, Acaulospora* sp1, *Acaulospora* sp2, *Acaulospora* sp3, *Entrophospora schenckii, Entrophospora kentinensis, Entrophospora infrequens, Glomus intraradices, Glomus deserticola, Glomus etunicatum, Glomus caesaris, Glomus aggregatum, Glomus aureum, Glomus macrocarpum, Glomus mossae, Glomus claroideum, Glomus globiferum, Glomus fasciculatum, Glomus versiforme, Glomus pansihalos, Glomus manihoti, Glomus verruculosum, Glomus* sp*, Scutellospora nigra, Scutellospora biornata, Scutellespora castenea.*

The appearance of these endomycorrhizal species table 2 varies from one site to another. *Glomus intraradices* is present in all the studied sites while *Acaulospora morrowiae* and *Glomus pansihalos* are respectively confined to slimes 94-98 and 94. *Entrophospora infrequens* and *Entrophospora schenckii* were only found at the level of sludge sludge 2000. *Acaulospora lacunose,* and *Scutellospora biornata* were noted at the level of the Mixture. However, *Glomus aureum* has been observed only at basin 1.5.

Discussion and Conclusion

Isolated spores from phosphate sludge of Khouribga region are morphotypes of 31 species belonging to four

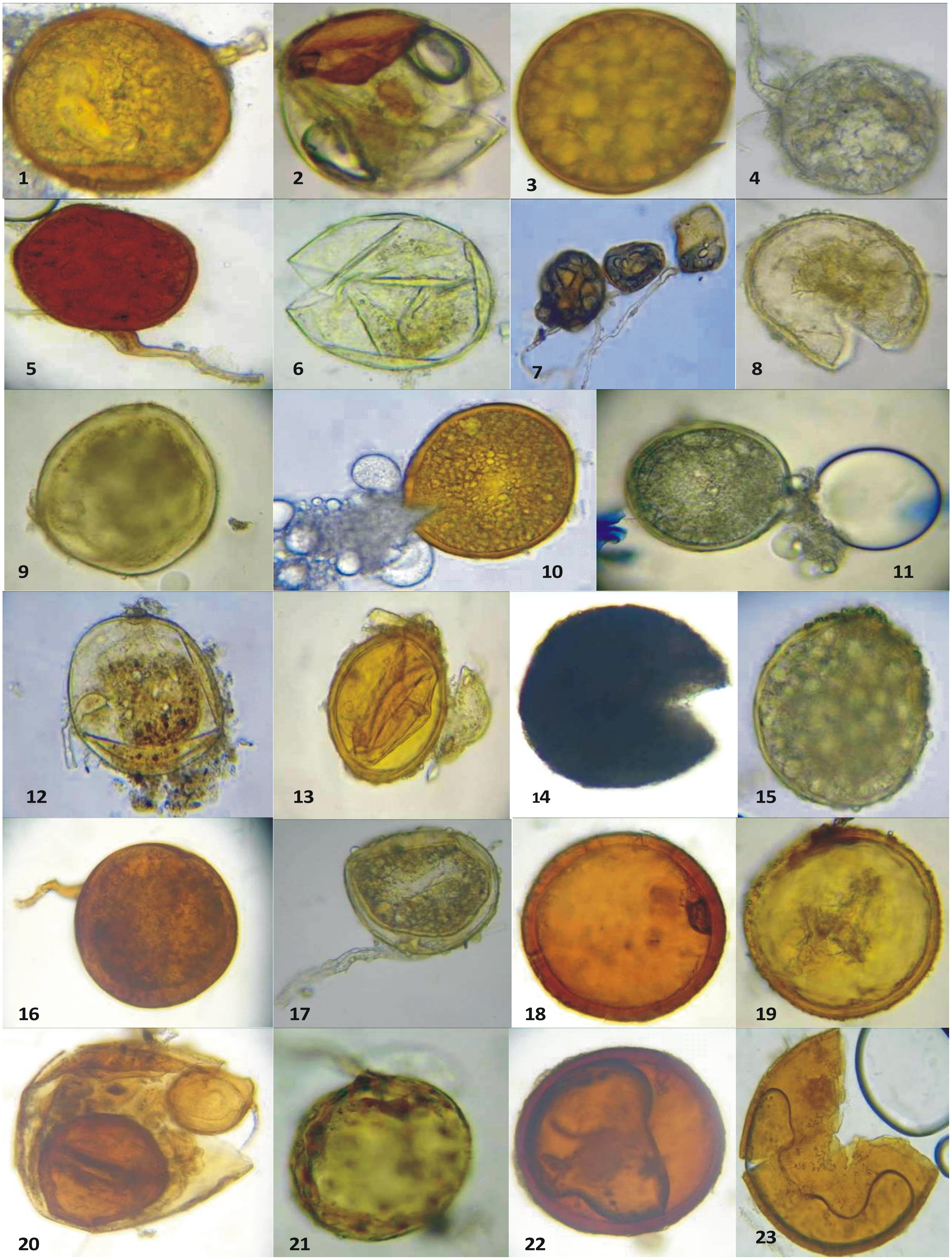

Fig. 2 continued....

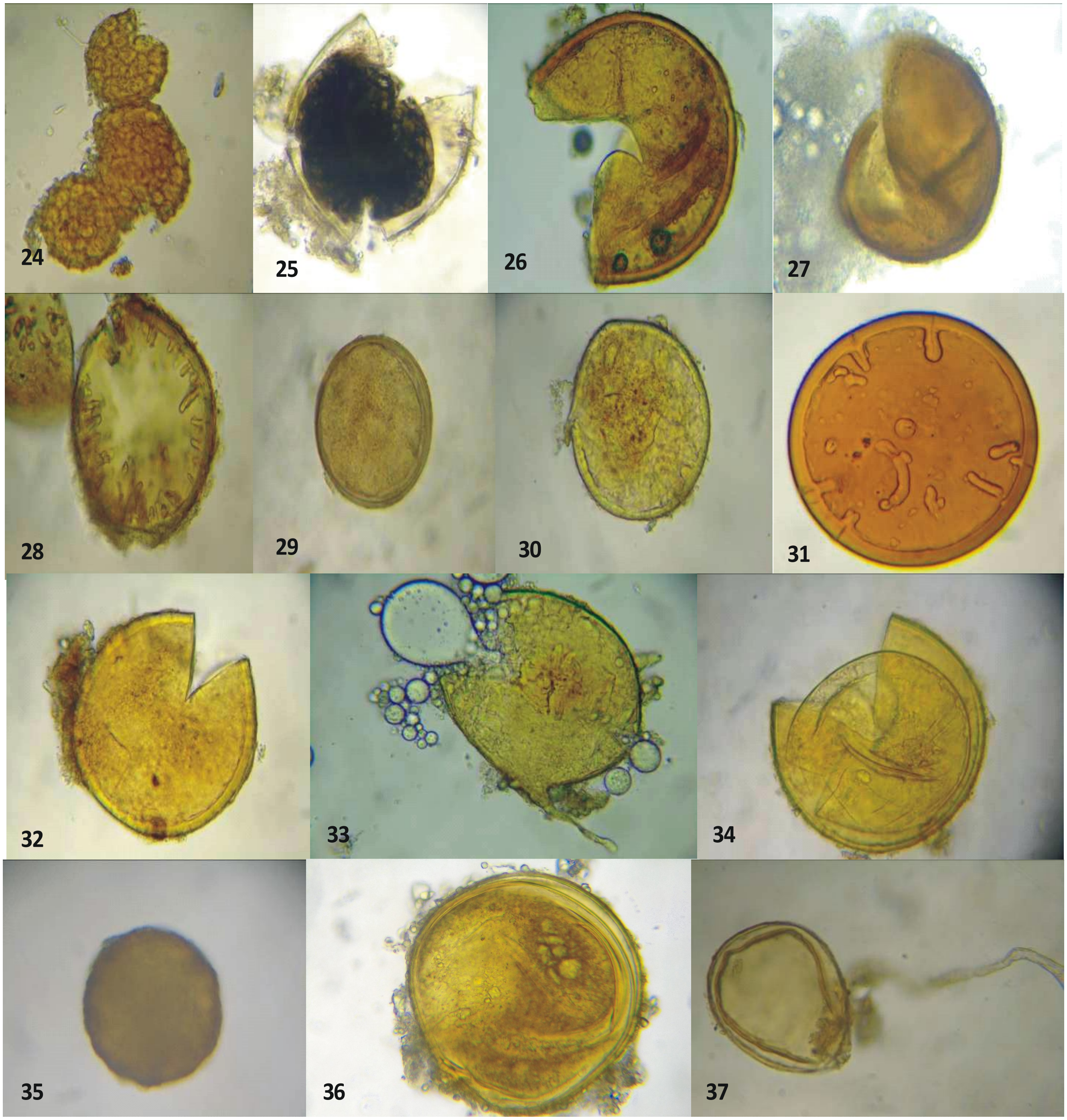

Fig. 2: Appearance of mycorrhizal species at different sludge launderings of studied phosphates.

genera *Glomus* (16 species), *Acaulospora* (9 species), *Entrophospora* (3 species), *Scutellospora* (three species), and 5 families (*Glomaceae, Gigasporaceae, Acaulosporaceae, Scutellosporaceae, Entrophosporaceae*) and 2 orders (Glomerales, Diversisporales).

Spore counts of mycorrhizal fungi showed a predominance of *Glomus* and *Acaulospora* genera. The *Glomus* genus also dominates the rhizosphere of certain plant species: *Eryngium maritimum* (Hibilik *et al.,* 2016), *Lycium europaeum* (Touati *et al.,* 2015), Sugar cane (Selmaoui *et al.,* 2017), Carob tree (Taleb *et al.,* 2015), Date palm (Sghir *et al.,* 2014), *Argania spinosa* (Sellal *et al.,* 2016), Olive tree (Chliyeh *et al.,* 2014 et 2015), different species of the *Citrus* genus (Artib *et al.,* 2016).

The spore densities observed in the various phosphate sludge vary between 43 to 4 spores per 100 g of soil, the highest AM fungus richness was registered in the 2000 sludge (43 spores/100 g), and the lowest number of species was registered in the 1.5 basin site (4 spores/100 g).

The number of spores encountered is in function of the age of the sludge and the plant species developing there. The average spore density results show that the sludge of 2000 has the highest spore count (43 spores/100 g soil), followed by 1998-1994 sludge (34 spores/100 g soil) and 1998 (30 spores / 100 g soil).

The majority of spores are smaller than 98 µm. The spores of the species *Glomus claroideum* are small (32 µm) and those of *Glomus caesaris* are large (98 µm). Smith and Read (1997) reported that *Glomus* are generally small and abundant in different ecosystems, particularly in tropical forests (Husband *et al.,* 2002), which suggests that most of the extracted spores might be those of the *Glomus*. However, these findings need to be confirmed by further taxonomic analyzes, especially those related to molecular biology (Zézé *et al.,* 1996; Helgalson *et al.,* 1999 ; Husband *et al.,* 2002).

The obtained results showed that *Glomus intraradices* is the most common species in the different types of studied phosphate sludge. This species has a large distribution in the world and dominates almost in all agricultural soils (Mathimaran *et al.,* 2005). In China, it has been found in rhizosphere soils of several plant species (Gai *et al.,* 2006), in Poland in the rhizosphere of *Fargaria versca* L. (Turneau *et al.,* 2001), in Brazil in the rhizosphere of *Araucaria angustifolia* (Moreira *et al.,* 2007), in Morocco in the rhizosphere of *Ceratonia siliqua* (Talbi *et al.,* 2015). Schenck and Smith (1981, 1982) reported in the United States that *Glomus intraradices* is the most common and most encountered species in Florida in the rhizosphere of a large number of plant species. It has also been found in soils in other parts of the United States, California (Bethlenfalvay *et al.,* 1984; Koske et Halvorson 1981), Kentucky (An *et al.,* 1993), Texas (Stutz et Morton, 1996) and Hawaii (Koske et Gemma, 1996). *Glomus intraradices* has been reported in Canada (Dalpé 1989, Klironomos *et al.,* 2001), Switzerland (Jansa *et al.,* 2002, 2005), Africa (Stutz *et al.,* 2000), China (Gai *et al.,* 2006), and in India (Mohankumar *et al.,* 1988). Species of arbuscular mycorrhizal fungi encountered in laundries sludge phosphates can be propagated and exploited as composite endomycorrhizal inoculum which will be incorporated in phospho-compost to enhance phosphate solubilization. Thus, the use of a functional inoculum based on native AM fungi as a biotechnological technique will probably allow better exploitation of nutrients from growing media in order to obtain a better improvement in plant growth.

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