

Occurrence and Quantification of Vesicular-Arbuscular Mycorrhizal (VAM) **Fungi in Industrial Polluted Soils**

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Abstract A survey for vesicular-arbuscular mycorrhizae (VAM) status was undertaken in three different industrially polluted sites at Uyyakondan channel of Senthanneerpuram area in Trichy, India. The soils and the effluents were acidic, and contained higher Zn (621 to 711 ppm) than the other heavy metals, such as Cu, Pb, and Ni. Eighteen plant species were collected from the rhizosphere soils, and 13 species were positive for VAM colonization. Fifteen VAM fungal species were isolated from the plant species. The number of VAM fungal spores from the soils ranged from 45 to 640 per 100 g of soil. There was a significant correlation observed between the number of spores and percentage root colonization, as exemplified by Acalypha indica (45 and 20%, respectively) and Paspalum vaginatum (640 and 98%, respectively). Hostspecific and site-specific associations were observed in site 2; particular VAM species, Gigaspora gigantea and Glomus fasciculatum, were specific to particular host plants, Phyllanthus maderaspatensis and A. indica, respectively, even though Eclipta prostrata and Physalis minima were maximally associated with 8 VAM species. G. fasciculatum was found in 11 plant species and predominant VAM species. These results led us to conclude that VAM fungi are associated with a majority of the plants in the industrial polluted sites and support the plants to survive in the acidic soils, polluted with heavy metals of the industrial effluents.

Key words: Mycorrhizal plants, VAM fungi, colonization, industrial effluents

Mycorrhizal symbiosis formed between plant roots and vesicular-arbuscular mycorrhizal (VAM) fungi is of great

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interest in environmental protection because of its potential influence on ecosystem processes, role in determining plant diversity in natural communities, ability to induce a wide variety of growth responses in coexisting plant species, and also its ability to increase tolerances in the plant communities to adverse environmental conditions [2, 19].

The major part of the beneficial effects of VAM fungi is attributed to their role in the uptake and translocation of both immobile elements including P, Zn, and Cu, and also more mobile elements like K, S, Mg, Ca, Na, Fe, and Mn [9, 39]. Since the hyphae are narrower, longer, and more versatile in their direction of growth, they should be more efficient than root hairs. The hyphae are also able to reach soluble nutrients, otherwise inaccessible to the roots or its hairs. The fungi produce enzymes, auxins, vitamins, cytokinins, and other compounds that increase rootlet size and longevity [28]. In general, most of the vascular plants require mycorrhizae to survive. The VAM association is geographically ubiquitous, occurring in plants from arctic to tropical regions, over a broad ecological range from aquatic to desert environments [30].

In recent decades, rapid industrialization, application of pesticides in crop protection, and release of effluents followed by their accumulation have created more problems of health hazards as a result of pollution. Earlier report [3] revealed the imbalances and disturbances of microbial ecosystem in polluted soils. The role of mycorrhizae in plant ecology is based on the widespread occurrence in natural ecosystem. However, it is still worthwhile to survey the wide diversity of habitats in search of local VAM endophytes [14].

During the past two decades, research on VAM technology in India has been impressive. The occurrence of all the VAM genera in Indian soils has recently been reported [33], even though only a few literature is available on the occurrence of VAM fungi in polluted habitats [18].

Little attempts have been made to examine the impact of the physicochemical factors of the soil in relation to the quantitative and qualitative assessment of VAM fungi in the soil polluted with industrial effluents. Hence, the present work was carried out in the following aspects: to study the status of VAM fungi in root-zone soils in the plant species occurring at heavy metal polluted sites, to study the physico-chemical characteristics of soil and industrial effluents from three different sites, and to search the dominant effluent-tolerant strain of VAM fungi in the three different sites.

MATERIALS AND METHODS

Selection of Study Sites

Three different sites were selected at Uyyakondan channel of Senthanneerpuram area in Trichy, Tamil Nadu, India (Fig. 1). The Uyyakondan channel, which originates as a rivulet from Akandakaveri near Pettavaithalai is located 30 km from Trichy junction, and runs through Trichy suburb and city, and is the main artery where all the industrial effluents of Trichy city and suburb area are dumped rather than to the river Kaveri. Site 1 is located at Trichy Distillaries and Chemicals Ltd. Effluents coming out from

the industry pass through a small channel and eventually confluents in Uyyakondan channel. Site 2 is located at Aruna Sugarcane farm in between the Trichy Steel Rolling Mills and Kaveri Engineering Industries Ltd. Site 3 is located at Ariyamangalam (5 km from Trichy junction) wherein industrial effluents from tanneries are mingled with the Uyyakondan channel. At each site, a 3-cm² area was chosen for sampling.

Collection of Samples

From each study site, 3-6 vigorous appearing mature plants per species were selected for collection of their roots and root-zone soil samples [24]. Soil samples from the root-zone area of the host plants were collected at 0-30 cm soil depth, a region in which the mycorrhizal incidence is maximal [8]. All the soil samples were placed in separate plastic bags, sealed, and kept at 5-10°C in the laboratory [24]. From that sample, 100 g of soil were used to estimate VAM spore numbers. Effluents were also separately collected in plastic bags from the three sites.

Analysis of Soil and Effluent Samples

The soil samples collected at the three sites were studied for the general soil characteristics. The soil samples of each site were analyzed for pH, total nitrogen, available phosphorus,

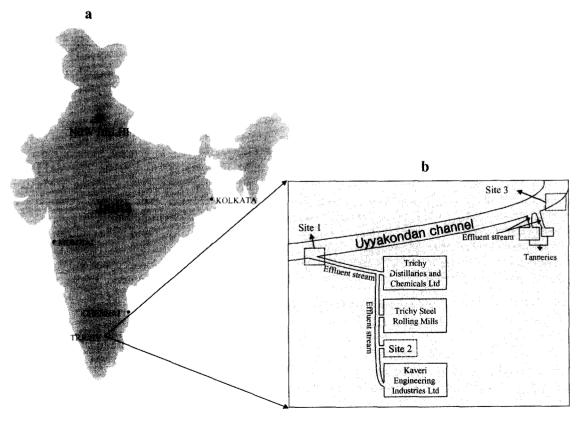


Fig. 1. Location of the sampling sites. a, Location of Trichy in India; b, an enlarged view of the sites.

Table 1. Physicochemical characteristics and heavy metal levels in the soils taken from the sites polluted with industrial effluents.

Site	Soil type	pН	Macronutrients (mg/kg) ^b			Micronutrients (ppm)		Heavy metals (ppm)			
			N	P	K	Mn	Fe	Cu	Ni	Pb	Zn
Site 1	Sandy loam	6.4±1.2	45.8±10.2	15.3±11.0	128±12.4	5.6±2.4	2.3±1.4	147±1.2	20±1.4	51±0.8	621±18.4
Site 2	Sandy loam	6.9 ± 1.4	35.8±10.8	19.4±12.2	124±14.8	4.8 ± 1.8	2.1 ± 1.2	199±1.2	31±1.6	79±0.8	628±17.4
Site 3	Sandy loam	5.8±1.0	42.0±17.5	30.2±10.4	148±10.8	4.6±1.4	2.2±1.2	231±1.4	24±1.4	61±1.2	711±18.0

Site 1, Trichy Distillaries and Chemicals Ltd; Site 2, Aruna Sugarcane Farm; Site 3, Ariyamangalam. Values are mean of 10 replicates.

potassium, and also microelements (Mn and Fe) by following the standard procedures [35]. Another portion of the earmarked soil samples was analyzed for heavy metals (Cu, Ni, Pb, and Zn), using standard procedures [15, 17]. Ten g of soil sample were taken and washed with lithium metaborate. Organic substances in the extracts were destroyed by heating them at 105°C to dryness with 25 ml of concentrated HNO₃, and the residue was taken up in 5 ml of trace elements-free 1 M sodium acetate. After extraction, heavy metal amounts were determined by atomic absorption spectrophotometry. The industrial effluents were analyzed for physicochemical characteristics and heavy metal concentrations by following standard procedures [6].

Estimation of Mycorrhizal Colonization

To check the mycorrhizal status of the sampled plant species, feeder root samples were collected, washed of all the attached soil particles, cut into small (1 cm) fragments, fixed in FAA [6 ml of formalin (40% formaldehyde), 1 ml of glacial acetic acid, 20 ml of ethanol (96%), and 40 ml of distilled water], and analyzed for the incidence of VAM structures in the root tissues [31]. The percentage of mycorrhizal colonization in the root was estimated by the method of Giovannetti and Mosse [13]. The roots were cleared and stained by the method of Phillips and Hayman [31].

Isolation and Identification of VAM Fungal Spores

For the isolation of VAM spores, a wet-sieving and decanting technique [10] was followed. The isolated spore was identified by the synoptic keys of Walker and Trappe [42]. Total spore number was counted by following the MPN technique [32].

RESULTS AND DISCUSSION

Characteristics of Soils and Effluents

In this study, the soils and effluent samples were collected from three sites polluted with industrial effluents. Soils of all three sites were acidic, pH 5.8 to 6.9, and sandy loam type (Table 1). The acidic nature of the industrially polluted habitats has already been reported [4, 7]. The industrial sources are considered important, since they are estimated to contribute 90 percent of the world's particulate pollutants [20]. Furthermore, it is worth to mention that the soils at a depth of 0-30 cm was collected both for the analyses of their physicochemical characteristics and the occurrence of VAM spores, and that particular depth was considered to be not only a region of maximum mycorrhizal incidence but also rich in heavy metals [8, 26]. Macronutrients such as total nitrogen, available phosphorus, and available potassium ranged from 35.8 to 45.8, 15.3 to 30.2, and 124 to 148 mg/kg, respectively (Table 1). Earlier report [36] on fertility of Indian soil revealed that N, P, and K levels in the soil were low, 33.5 to 50.1, 16.7 to 44.5, and 114 to 155 mg/kg, respectively, indicating that the soil could act only as a low-fertility soil. The estimated microelements, such as Mn and Fe, in the soils showed only minor variations (Table 1). The heavy metals, Cu, Ni, Pb, and Zn, in the soils were analyzed, because these were not only the most commonly encountered ones but also provide a direct link to the mycorrhizal fungi between soil and roots, therefore, can be of great importance in heavy metal availability and toxicity to plants [27]. The soils contained higher amount of Zn (621) to 711 ppm) than the other heavy metals, Cu, Pb, and Ni (Table 1).

Table 2. Physicochemical characteristics and heavy metal levels of industrial effluents from the three sites.

Cita		Ions (g/l)				Heavy metals (ppm)				
Site*	pН	HCO ₃	Cl	Ca ²⁺	Mg ²⁺	Na⁺	Cu	Ni	Pb	Zn
Site 1	5.4±1.2	10.0±1.2	8.0±0.2	8.0±0.4	10.0±0.2	2.0±0.2	160±1.5	25±1.1	58±0.7	725±15.6
Site 2	6.0 ± 1.0	11.0±0.4	9.0 ± 0.2	6.0 ± 0.4	8.0 ± 0.8	2.5 ± 0.2	215±1.3	39±1.2	95±0.9	730±12.3
Site 3	5.2 ± 1.2	10.0 ± 0.2	12.0 ± 0.8	7.0 ± 0.2	8.0 ± 1.0	4.0 ± 0.2	245±1.2	30 ± 1.0	72 ± 0.7	816±16.2

"Abbeviations as in Table 1.

The effluents were also acidic, pH 5.4 to 6.0 (Table 2). The concentrations of HCO₃, Cl⁻, Ca²⁺, and Mg²⁺ ions ranged from 6.0 to 12.0 g/l, and that of Na⁺ ion from 2.0 to 4.0 g/l (Table 2). The effluents also contained higher amount of Zn (725 to 816 ppm) than the other heavy metals. Previous report [5] revealed that Zn and Cu at concentrations higher than 140 and 36 ppm, respectively, are toxic to biological and biological nonessentials systems in soil or effluent.

Incidence of VAM Fungi

A total of 18 herbaceous plants were selected from three different industrial polluted sites (Table 3). The 18 plants belonged to 8 different families of angiosperms. Among the 18 plants, 10 were common for all three sites and the remaining 8 were present only at specific sites. The site specificity could be attributed to the differences in the edaphic and climatic factors of the sites [23].

Table 3. Percent root colonization, number of VAM spores, and VAM species associated in the root-zone soils of the sites.

Serial No.	Family and plant species	Sites	Percent root colonization	Number of VAM spores/100 g of soil	Name of associated VAM species ^b
I.	NON-MYCORRHIZAL PLANTS	3			
	AIZOACEAE				
1.	Gisekia pharnaceoides	1, 2, 3	0	0	NA
	AMARANTACEAE				
2.	Digera muricata D.C.	1, 3	0	0	NA
3.	Amaranthus spinosus L.	3	0	0	NA
4.	Gomphrena globosa L.	3	0	0	NA
	CYPERACEAE				
5.	Carex filicinia Nees.	1, 2, 3	0	0	NA
II.	MYCORRHIZAL PLANTS				
	PORTULACACEAE				
6.	Portulaca oleracea L.	1, 2, 3	47	140	LFSC, LMSS, SSNS, SRBF
	ASTERACEAE				
7.	Trianthema decandra L.	2, 3	38	94	LFSC, LMCC, SCVS
8.	Eclipta prostrata L.	1, 2, 3	92	425	ABRT, GGGT, LFSC, LFLV, LGSP, LMSS, SRBF, SSNS
9.	Vernonia cinerea L.	1, 2	45	125	GGGT, LGSP, LINR
10.	Physalis minima L.	1	85	385	AELG, GGGT, LFSC, LGSP, LINR, LMSS, SRBF, SSNS
	EUPHORBIACEAE				
11.	Euphorbia rosea Retz.	1, 2, 3	55	220	GGGT, LCRD, LFLV, LFSC, LINR, LPVN, SSNS
12.	Phyllanthus maderaspatensis L.	2	62	320	GGGT
13.	Acalypha indica L.	2	20	45	LFSC
	COMMELINACEAE				
14.	Commelina bengalensis L.	1, 2, 3	58	180	LCRD, LFSC, LMCC, LMSS, SRBF, SSNS
	POACEAE				•
15.	Chloris bournei Rang & Tad.	1, 2, 3	82	420	ABRT, ASCB, LFSC, LGSP, SCVS, SSNS
16.	Cyanodon dactylon Pers.	1, 2, 3	78	380	ASCB, LCRD, LFSC, LFLV, LMSS, SSNS
17.	Paspalidium punctatum Acamu.	1, 2, 3	92	520	LFSC, LGSP, LINR, LMSS, SRBF, SSNS
18.	Paspalum vaginatum S.W.	1, 2, 3	98	640	ABRT, LFSC, LGSP, LMSS, SRBF, SSNS

[&]quot;Sites are the same as in Table 1.

^bAbbreviations: NA, not available; ABRT, Acaulospora bireticulata; AELG, Acaulospora elegans; ASCB, Acaulospora scrobiculata; GGGT, Gigaspora gigantea; LCRD, Glomus claroideum; LFSC, Glomus fasciculatum; LFLV, Glomus fulvum; LGSP, Glomus geosporum; LINR, Glomus intraradices; LMCC, Glomus macrocarpum; LMSS, Glomus mosseae; LPVN, Glomus pulvinatum; SCVS, Sclerocystis clavispora; SRBF, Sclerocystis rubiformis; SSNS, Sclerocystis simuosa.

They were screened for VAM colonization in the roots and for the presence of VAM spores in rhizosphere soils (Table 3). Among the 18 plant species, 13 were positive in VAM colonization in roots and mycorrhizal plants, but the percentage of VAM colonization varied. The 5 species were nonmycorrhizal plants and were Gisekia pharnaceoides Digera muricata, Amaranthus spinosus, Gomphrena globosa, and Carex filicinia, showing no VAM spores and sporocarps in their root-zone soils. This result indicates that mycorrhizal condition is limited and agrees with the widespread occurrence of mycorrhizae reported in natural ecosystem [7]. It is of interest to observe that Amarantaceae, Aizoaceae, and Cyperaceae members of five plant species were nonmycorrhizal in the present study. Such families have already been reported as nonmycorrhizal in polluted sites [36], and mycorrhizal in nonpolluted sites [37, 40]. Mycorrhizal and nonmycorrhizal plant species occurring together in different sites have previously been reported [22]. The presence of mycorrhizal infection in plants of industrially polluted natural habitats has also been reported previously [12].

The absence of VAM association on the nonmycorrhizal plant species may be due to volatile substances of plant extracts, secreted by root, stem, and leaf cells; the extract contains biologically active substances, such as to hormones, vitamins, antibiotics, and antiseptics, and prevents microbial growth inside plant cells, especially root cells. Alternately, the above mentioned absence of VAM association may also be due to the presence of heavy metals in the site [16]. The present investigation showed the occurrence of both host specific and nonhost specific association of VAM fungi.

For 18 host plant species investigated at the three sites, 11 plant species occurred with the association of more than two VAM species and could be attributed to the absence of preferred host-fungus relationship [25]. Previous report also proved such nonhost specific associations [34]: Among the identified VAM species, Acaulospora bireticulata, Glomus fasciculatum, G. geosporum, G. mosseae, and Sclerocystis sinuosa had broad host ranges and G. fasciculatum was found in 11 plant species, and dominant and commonest symbiont. Particularly, VAM species occurred with the association of unique host plants, as exemplified by Phyllanthus maderaspatensis and Acalypha indica with Gigaspora gigantea and G. fasciculatum, respectively. Such host specific VAM associations have also been reported earlier [8].

The casual relationship for such host specific associations are poorly understood, as reported previously [1]. The occurrence of the 10 host plants in all the three sites, differences in their percent root colonization, the composition of associated VAM species, and their VAM colonization are very unique, and the maximum colonization was recorded among those common plants with mycorrhizal incidence [7, 8].

Table 4. Distribution of VAM fungi in the soils taken from three sites.

VAM fungal species ^a	Sites					
- VAIVI Tungai species	Site 1	Site 2	Site 3			
ABRT	+	+	+			
AELG	+	-	_			
ASCB	+	_	-			
GGGT	+	+	+			
LCRD	-	-	+			
LFSC	+	+	+			
LFLV	~	+	-			
LGSP	+	+	+			
LINR	+	+	-			
LMCC	+	-	+			
LMSS	+	+	+			
LPVN	+	-	-			
SCVS	-	-	+			
SRBF	+	+	+			
SSNS	+	+	+			

Abbreviations as in Table 3.

A total of 15 different VAM fungal species were observed in the root-zone soils of the host plants (Tables 3, 4). Among the 15 species, 8 species belonged to genus *Glomus*, 3 to *Acaulospora*, 3 to *Sclerocystis*, and one to *Gigaspora*. The occurrence of several species of VAM fungi in polluted habitats in the present study constitutes a new finding, while Gildon and Tinker [11] have isolated only one species of effluent tolerant VAM fungi, *G. mosseae*.

The number of VAM fungal spores present in root-zone soils of the three sites varied, ranging from 45–640/100 g of soil (Table 3). This is in agreement with the earlier findings [4, 7] for polluted soils.

There was a significance correlation between the number of spores and root colonization, as exemplified by *A. indica* and *Paspalum vaginatum*. A similar correlation between spore numbers and percentage root colonization was also reported previously [41]. Several suggestions to explain the correlations and variations in root colonization have been offered: One attractive hypothesis by Iqbal and Quershi [16] suggested that the failure in some plants was due to volatile substances of the root cells and the presence of heavy metals in root-zone soils.

Among the 13 mycorrhizal plants, *Eclipta prostrata* and *Physalis minima* were maximally associated with 8 VAM species, and 8 mycorrhizal plants occurred at all three sites (Table 3). All the plants showed variations in their percent root colonization and species composition of their associated VAM fungi. The highest percentage of VAM colonization was observed in *P. vaginatum* (98%), *Paspalidium punctatum* (92%), and *E. prostrata* (92%) (Table 3). The lowest percentage of VAM colonization was recorded in *A. indica* (20%). In the present study, the acidic nature of the three

polluted sites may have favored more VAM spore number and more occurrence of mycorrhizal incidence with the host plants, inducing influence on the growth rate of plants, as reported previously [7, 21].

Distribution of VAM Fungal Species

At the site-1, 6, 3, 1, and 2 species of Glomus, Acaulospora, Gigaspora, and Sclerocystis, respectively, were recorded. At site-2, 5, 2, 1, and 1 species of Glomus, Sclerocystis, Gigaspora and Acaulospora, respectively, were recorded. At the site-3, 5, 3, and 1 species of Glomus, Sclerocystis, and Acaulospora, respectively, were recorded. Among the 15 VAM species isolated, Gi. gigantea, G. fasciculatum, G. geosporum, G. mosseae, A. bireticulata, S. rubiformis, and S. sinuosa were encountered in all the three sites (Table 4). A. elegans, A. scrobiculata, and G. pulvinatum (at site-1), G. fulvum (at site-2), and G. claroideum and S. clavispora (at site-3) were found only in one particular site (Table 4). Although VAM species are not known to be strictly site specific, [29] it was interesting to find specific associations of VAM species of root-zone soils of certain host plants. In the present study, G. fulvum was observed only in the site-2, whereas A. elegans, A. scrobiculata, and G. pulvinatum were at site-1, and G. claroideum and S. clavispora only in site-3. The pattern of VAM fungi distribution at the three sites varied, because of edaphic and climatic factors. In addition, volatile substances enumerated from root and heavy metals reduced the rate of germination and colonization of the VAM fungi [23].

In this study, although the volatile substances of root cells partly inhibited the development of VAM fungal association, industrial polluted soil was the main factor for causing the absence of VAM association in plant roots, because the association of VAM fungi in the polluted site was less, compared with the association of VAM fungi in nonpolluted sites. Also, the nonmycorrhizal families showed VAM association in nonpolluted sites, but showed no association of VAM fungi in polluted sites [37].

The present study described the occurrence of VAM fungi in polluted soil sites; whereas we previously showed the occurrence of VAM fungi in nonpolluted soil sites [37], one earlier report showed the significance of VAM fungi on plant growth in the experimental plant Prosopis juliflora and comparative analysis on the effect of VAM fungi on plant growth in nonpolluted and polluted soils [36]. The distribution of plants in nonpolluted soil showed more VAM association than in the presently described polluted soil. The previous study [37] showed a total of 14 plants, belonging to 6 families: Among the 14 plants, 9 plants belonged to three nonmycorrhizal families such as Aizoaceae, Amarantaceae, and Euphorbiaceae. Furthermore, although 9 plants belonged to nonmycorrhizal families, all 14 plants of the nonpolluted soil sites were mycorrhizal and showed 100% mycorrhizal association [37]. The present study on polluted sites showed only 72.2% (13 plants) mycorrhizal association among the 18 plants analyzed. The remaining 28.8% (5 plants) belonged to nonmycorrhizal families such as Aizoaceae, Amarantaceae, and Cyperaceae. The association of VAM fungi was not developed, although such families showed VAM association in nonpolluted sites in the previous study [37]. It might be mainly due to the polluted nature of the soil and also partly due to the volatile substances of the root cells.

When compared with the nonpolluted soil of the earlier study [36], the polluted soil in the present study showed only minor differences in macronutrients (N, P, and K) as well as in micronutrients (Mn and Fe) levels. The N, P, and K levels of the polluted soils in this study were 35.8 to 45.8, 15.3 to 30.2, and 124 to 148 mg/kg, respectively. Similar to these values, those of the nonpolluted soil from the earlier report [36] were 40.64, 35.68, and 137 mg/kg, respectively, and the Mn and Fe levels of the polluted soils were 4.6 to 5.6 and 2.1 to 2.3 ppm, respectively. Similarly, those of the nonpolluted soil from the earlier report [36] were 4.8 and 2.2 ppm, respectively. However, there were differences in the pHs and the levels of heavy metals such as Cu and Zn between the polluted and nonpolluted soils. The polluted soil of the present study showed an acidic pH of 5.8 to 6.9, whereas the nonpolluted soil of the earlier study showed a pH of 7.2, a nonacidic pH [36].

A major difference between the polluted soil and the nonpolluted soil was heavy metal levels: The levels of Cu and Zn in the polluted soils in this study were 147 to 231 and 621 to 711 ppm, respectively, whereas those of the nonpolluted soil in the earlier study [36] were only 3 and 5 ppm, respectively, comparatively small amounts.

The influence of VAM fungi on plant growth in heavy metal polluted soil is most likely due to restriction of heavy metals in the internal hyphae of VAM fungi and also by absorption and translocation of P and other nutrients (like N and K) by their extraradical hyphae, a hyphal extension of VAM fungi, present outside the plant's root system [36]. The earlier study [36] reported that the VAM fungi G. fasciculatum which was inoculated to Prosopis juliflora plants treated with tannery effluents, showed less uptake of heavy metals (Cr and Zn) from the soil to the leaf and root than the control plants. It revealed that due to the presence of VAM fungal association, the uptake of heavy metals from soil by roots to aerial parts of plants was highly restricted, and the function of VAM fungi to bind heavy metals in their internal hyphae was to protect the plants from heavy metal toxicity [9, 36], thereby supporting survival of the plants in polluted soil. Also, our previous study revealed that VAM fungi G. fasciculatum inoculated to experimental plant Phyllanthus niruri showed higher uptakes of K and Ca than the control plant, without VAM inoculation [37], and that VAM fungi G. fasciculatum,

inoculated to plant *Datura metal*, showed higher P uptake than the control [38].

In conclusion, VAM fungi are associated with the majority of plants in the industrial polluted sites, thereby supporting the survival of plants in acidic soils that are polluted with heavy metals of the industrial effluents.

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