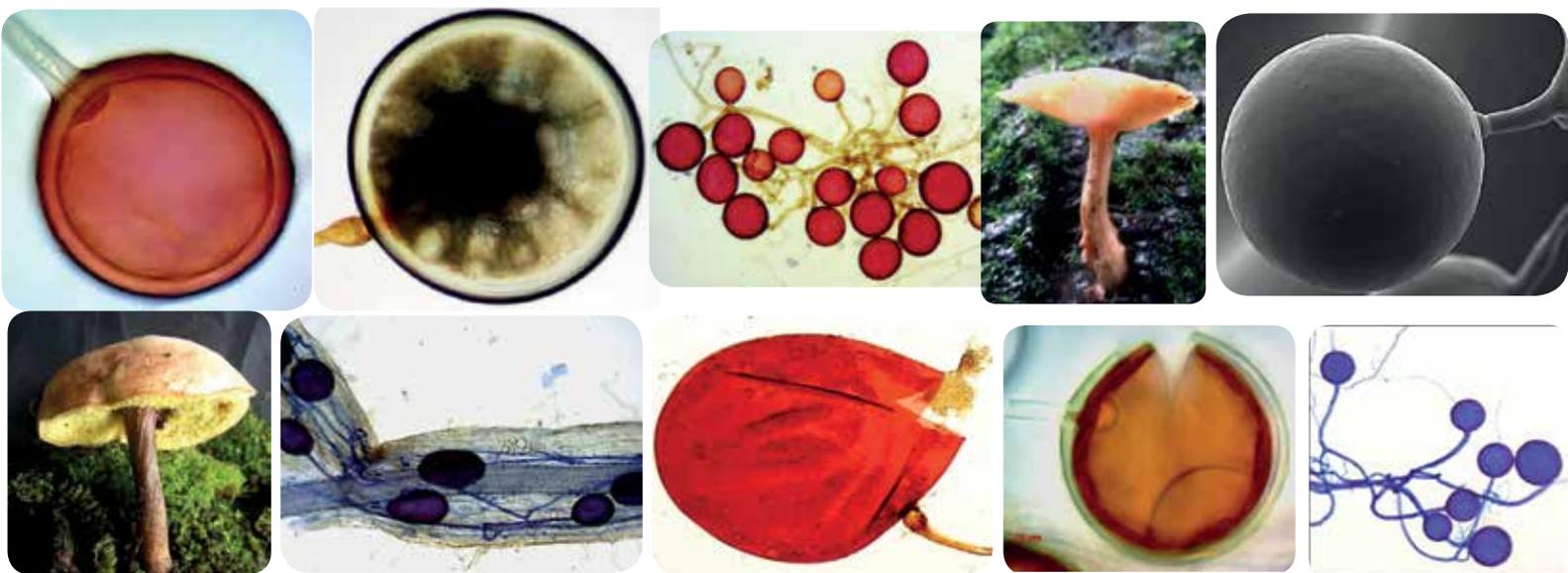




MYCORRHIZA NEWS

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RESEARCH FINDING PAPER

Effects of Vesicular-arbuscular Mycorrhiza Colonization on Biomass Production of Economically Important Crop Plants Grown Directly over Industrial Sludge

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Introduction

In India, more than 53% of the land area is degraded. This is mainly attributed to rapid industrialization and land disturbance due to effluent discharge. Mycorrhizal fungi are known to improve the revegetation of such disturbed areas (Draft and Nicolson 1974; Draft and Hasckaylo 1976; Khan 1981).

Bioremediation is a technology that has gained great popularity and is currently attracting attention as a remedial measure where microorganisms are used to detoxify wastes. Some industrial set-ups engaged in manufacturing distilleries and chemicals produce huge quantities of alcohol and thereby generate spent wash as well as sludge (two major pollutants) in mammoth proportions. There are several reports on increased plant growth even under various environmental stresses, when infected with vesicular-arbuscular mycorrhizal (VAM) fungi (Fidler 1985; Jeffries 1987; Azcon, Atrach, and Barea 1988). The present study— Effects of Vesicular-arbuscular Mycorrhiza Colonization on Biomass Production of Economically Important Crop Plants Grown Directly over the Sludge—was conducted to explore the impact of VAM colonization on economically important crop plants grown in industrial sludge.

Materials and Methods

The sludge used in the present study was collected directly from the industrial area of Satharia, Jaunpur,

powdered and mixed thoroughly. Viable seeds of *Vigna mungo* (Urad bean), *Vigna radiata* (Mung bean), *Vigna catijung* (Cow pea), *Cajanus cajan* (Pigeon pea), *Sorghum vulgare* (Indian millet), and *Pennisetum typhoides* (Pearl millet) were selected and sown in earthen pots containing only the sludge. Seedlings grown in sludge-free soil were treated as control. After 50 days, the plants were uprooted and their aerial parts and roots were collected (Draft and Nicolson 1974). Substrates were collected from the control (sludge-free soil) and treated (sludge) pots, and the VAM spores were isolated by wet sieving and decanting method and then identified. Elemental analysis of the 50 days old crop plants was carried out using a flame photometer (Systronics, India). Analysis of total biomass was carried out in 90-day old *Vigna mungo*, *Vigna radiata*, *Vigna catijung*, and 120-day old *Sorghum vulgare*, *Pennisetum typhoides* and their harvest indices were calculated.

Results, Discussion, and Conclusion

The present study critically analyses the effect of sludge on VAM infection, plant biomass, and crop productivity. Physicochemical properties of the sludge indicated that its water-holding capacity was 122% while that of the garden soil was about 19%. Among the chemical parameters, pH was found to be very high in the sludge (9.82) while the electrical conductivity (EC) value was found to be 8.63 mS/cm, thereby indicating extreme salinity. The sludge

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was found to contain various organics like sugars, phenols, sucrose, starch, nitrogen, and proteins as well as elements such as N, P, K, and Ca. The percentage of germination and seedling vigour index were not affected even when the sludge (alone) was used for raising the plants. Chemical properties like pH have been reported to have a definite influence on VAM diversity, spore population, and spore germination (El-Kherbawy, Angle, Heggo, *et al.* 1989). As given in Table 1, the percentage of VAM infection was more in the 50-day old plants grown directly in the sludge (21%–80%) when compared to the control. Maximum infection was noted in *Vigna catijung* (90%) whereas in control it was only 50%. The pH and EC values of the sludge did not affect the VAM spore occurrence, germination and root colonization.

It has been well established that mycorrhizal colonization can enhance the efficiency of nutrient uptake (in addition to P) (Eivazi and Weir 1989; Harrison and Dixon 1994). Therefore, elements such as N, P, K, and Ca in the roots of 50-day old plants

were analysed to find out if the mycorrhizal symbiosis could improve the efficiency of nutrient uptake in plants. The studied elements (mg/g Dry Weight (DW)) were found to be higher in the plants grown directly in the sludge. The phosphorus uptake was more, followed by nitrogen, calcium, and potassium. Table 2 clearly indicates that mycorrhizal symbiosis is one of the mechanisms by which nutrient uptake in plants could be improved. The improved N, P, K, and Ca uptake by VAM plants could be due to the increased amounts of unidentified organic acids (Fabig, Veilhauer, Moawad, *et al.* 1989), chelating compounds (Jayachandran, Kshwab, and Hetrick 1989) or CO₂ (a result of the symbiosis) (Knight, Allen, Jurinak, *et al.* 1989).

After studying the elemental composition of the roots, interest was to find out if the increased uptake and mycorrhizal effectiveness could improve plant growth and productivity and hence, their biomass and harvest indices were calculated (Table 3).

Table 1 VAM colonization in 50 days old plants (grown directly in sludge)

Crop plants	Infection (in %)	
	Control	Sludge
<i>Vigna radiata</i>	66	80 (121)
<i>Vigna mungo</i>	53	73 (138)
<i>Vigna catijung</i>	50	90 (180)
<i>Cajanus cajan</i>	53	65 (123)
<i>Sorghum vulgare</i>	66	86 (130)
<i>Pennisetum typhoides</i>	60	86 (143)

Table 2 Elemental analyses of 50 days old plant roots, grown in sludge

Element (in mg/g DW)							
Nitrogen (N)		Phosphorus (P)		Potassium (K)		Calcium (Ca)	
Control	Sludge	Control	Sludge	Control	Sludge	Control	Sludge
122	180 (148)	33	68 (206)	11	16 (145)	3	5 (167)
109	200 (183)	34	75 (220)	11	14 (127)	4	7 (175)
119	228 (192)	48	74 (154)	9	13 (144)	3	5 (167)
95	165 (174)	43	85 (198)	10	15 (150)	3	5 (167)
89	116 (130)	14	28 (200)	8	12 (150)	3	7 (233)
86	129 (150)	22	35 (159)	8	10 (125)	2	4 (200)

Table 3 Total biomass and harvest indices of 90 days old *Vigna radiata*, *Vigna mungo*, *Vigna catijung*, and 120 days old *Sorghum vulgare* and *Pennisetum typhoides* grown sludge

Crop plants	Biomass (in g)		Harvest index (in %)	
	Control	Sludge	Control	Sludge
<i>Vigna radiata</i>	11.20	14.54 (130)	40	54 (145)
<i>Vigna mungo</i>	11.28	15.04 (133)	42	58 (138)
<i>Vigna catijung</i>	26.07	44.89 (173)	59	84 (142)
<i>Cajanus cajan</i>	35.27	59.27 (168)	48	64 (133)
<i>Sorghum vulgare</i>	18.05	25.96 (144)	41	54 (132)
<i>Pennisetum typhoides</i>	16.29	22.42 (138)	42	53 (126)

Total biomass increased significantly in sludge plants (30%–73%) and the harvest index was also found to be higher (26%–45%) over the control. There is a lot of literature evidence to indicate VAM inoculation significantly increases the dry weight of different plants (Tanner and Clayton 1985; Sharma and Srivastava 1991).

Through the study ‘Effects of Vesicular-arbuscular Mycorrhiza Colonization on Biomass Production of Economically Important Crop Plants Grown Directly over the Sludge’, it can be concluded that application of industrial sludge is not harmful to plants and it was also found that the increased level of pH and EC did not affect VAM spore populations, spore germination, root colonization or mycorrhizal effectiveness. The study further supports the occurrence of mycorrhiza, VAM in particular, in disturbed environments.

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Arbuscular Mycorrhizal Fungi- Mediated Phytoremediation of Heavy Metal-Contaminated Sites

Akshaya S.¹

Owing to our increasing needs and demands, the past few decades have seen a dramatic acceleration in activities which have been contributing to biosphere pollution (Varma, Prasad, and Tuteja 2017). One of the needs of the hour being abatement of pollution, several measures with the aim of achieving this in a cost-effective, durable, and environment-friendly way have been adopted by communities across the globe. Bioremediation (one such measure) is a biotechnological method that uses biological processes or activities of organisms to transform contaminants into inert substances (Pandey, Larroche, and Soccol 2018; Kaur, Mavi, and Raghav 2019). This method is currently being used for the treatment of various wastes and the remediation of contaminated air, water, and soil, and has been found to be more efficient when compared to conventional physico-chemical methods (for example, soil flushing, soil leaching, electro-chemical methods, soil washing) which cause significant changes to the physical, chemical, and biological characteristics of the treated resource (Varma, Prasad, and Tuteja 2017; Jin, Luan, Ning, *et al.* 2018; Pandey, Larroche, and Soccol 2018). When plants are used for the ecological rehabilitation/reclamation of the contaminated site, the technique is termed as phytoremediation, an alternative *in-situ* phytorestorative (bioremediation) technology which exploits plants and their rhizosphere to remove the contaminants or lower (through accumulation) their bioavailability in soil and water (Muthukumar and Bagyaraj 2010; Atanagana, Khasa, Chang, *et al.* 2014; Dal Corso, Fasani, Manara, *et al.* 2019). Plants require various chemical elements for their life processes and have developed a number of adaptations for acquiring these elements under a wide range of environmental conditions (Dal Corso, Fasani, Manara, *et al.* 2019). Among these elements are heavy metals (HMs) which naturally occur in the Earth's crust. While HMs such as iron (Fe), zinc (Zn), copper (Cu), nickel (Ni), cobalt (Co), molybdenum (Mo) (micronutrients essential for plant biochemistry) become toxic to the plants only when in excess, others such as cadmium (Cd), mercury (Hg), silver (Ag), lead (Pb), chromium (Cr) cause toxicity even at low concentrations (Gaur and Adholeya 2004; Dal Corso, Fasani, Manara, *et al.* 2019). Geologic (processes) and anthropogenic activities (for example, use of agrochemicals and long-term

application of urban sewage sludge, industrial waste disposal, waste incineration, vehicle exhausts) increase HM concentration to levels that are harmful to many living forms. Their transport in the form of leachates and gases and in particulate phases has led to their rapid accumulation in soil, water, and living systems (Gaur and Adholeya 2004; Ferrol, Tamayo, and Vargas 2016; Mishra, Singh, and Arora 2017). Uptake, translocation, sequestration, and detoxification are the key processes in plants for HM homeostasis (Shi, Zhang, Chen, *et al.* 2019; Dal Corso, Fasani, Manara, *et al.* 2019). Although plants possess several defence strategies to avoid or tolerate HM intoxication, that is, for HM homeostasis, these mechanisms do fail and jeopardize the plants when their threshold is crossed (Ferrol, Tamayo, and Vargas 2016; Mishra, Singh, and Arora 2017).

In-loco strategies/mechanisms employed by plants and their rhizosphere for soil reclamation (HM decontamination) include phytoextraction—accumulation of pollutants in harvestable biomass; phytovolatilization—conversion of pollutants to volatile form and their subsequent release to the atmosphere; phyto(rhizo) stabilization—limiting the mobility and bioavailability of pollutants in soil by plant roots (Joner and Leyval, 2003; Ali, Khan, and Sajad 2013; Dal Corso, Fasani, Manara, *et al.* 2019). In spite of many exemplary properties possessed by plants, often, physico-chemical properties of the waste or the contaminated site prevent plant establishment because of the absence of/limited nutrients and microbial activities. In cases such as these, the employment of microbial symbionts such as bacteria (for example, *Rhizobium*) and fungi (for example, AMF, mushrooms) is an alternative strategy for safe and efficient management of the contaminated site (Varma, Prasad, and Tuteja 2017). Mycorrhizae, one of the world's ancient symbioses that exist between plants and fungi, are important from the perspective of plant growth, nutrition, diversity, nutrient cycling and ecosystem functioning. Arbuscular mycorrhizal fungi (AMF) are important soil microorganisms, obligate biotrophs that establish mutualistic symbiotic associations with many plant species and are known to benefit plants under various environmental stress conditions (Varma, Prasad, and Tuteja 2017; Shah, Venkatramanan, and Prasad 2019). They improve the antioxidative status of the host plant and can trigger differential expression of genes involved in HM accumulation in both partners (Shi, Zhang, Chen, *et al.* 2019). Mycorrhizal fungi have been found to be inherently more capable in dealing with metals, when

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compared to vascular plants, owing to their diversity in metal-contaminated soils (Coninx, Martinova, and Rineau 2017). *Funneliformis mosseae*, *Rhizopagus irregularis*, *Glomus intraradices*, *G. versiforme*, and *Scutellospora reticulata* are some examples of AMF that are capable of thriving in HM-contaminated soils (Alori and Fawole 2012; Lingua, Bona, Todeschini, *et al.* 2012; Ferrol, Tamayo, and Vargas 2016). They expand the interface between plants and soil through an extensive network of extraradical hyphae, thereby contributing to the plants' uptake of macronutrients as well as micronutrients (or HMs) beyond the depletion zone that develops around roots and thus provide a new pathway. Inside the roots, they develop arbuscules to facilitate nutrient exchange between the symbionts. AMF also improve the soil structure and act as filters, blocking xenobiotics within their mycelium. They have gained prominence all over the world in treating various kinds of HM-contaminated soils (Ferrol, Tamayo, and Vargas 2016; Varma, Prasad, and Tuteja 2017; Shah, Venkatramanan, and Prasad 2019). The use of mycorrhizal plants in phytoremediation of contaminated sites has been described as mycorrhizoremediation (Abdul 2006; Giasson, Jaouich, Cayer, *et al.* 2006; Channabasava, Lakshman, and Muthukumar 2015) and as mycorrhiza-assisted remediation (MAR) as well (Chibuikwe 2013). It has also been reported that combinations of AMF and plant growth-promoting rhizobacteria (PGPR) (for example, *Rhizobium*) and, AMF interaction with other soil fungi (for example, *Fusarium concolor*, *Trichoderma koningii*), earthworms, and so on facilitate the growth of plants and phytoremediation in metal-contaminated soils (Malekzadeh, Alikhani, Savaghebi-Firoozabadi, *et al.* 2011; Chibuikwe 2013).

The AMF provide an attractive system to advance plant-based environmental clean-up (Varma, Prasad, and Tuteja 2017). AMF-mediated phytoremediation takes place in two processes—phytostabilization (also known as phytoimmobilization) and phytoextraction (also known as phytoaccumulation, phytoabsorption or phytosequestration) (Ali, Khan, and Sajad 2013; Shah, Venkatramanan, and Prasad 2019). Their key elements are chelation and sequestration, which result in inactivation and removal of toxic HMs from sensitive sites, and metal transport systems, which control HM acquisition and efflux. Over the years, genome sequencing and transcriptomic analyses of various AMF have resulted in the identification of a number of genes encoding putative transport proteins (for example, CTR family of Cu transporters—RiCTR1 and RiCTR3, iron permease—RiFTR1) that mediate the uptake of HMs from polluted soils. AMF handle metal pollutants through phytostabilization—a mechanism in which HMs are immobilized/

sequestered within the soil. AMF-mediated HM immobilization in the soil takes place as illustrated in Figure 1(B). The mechanisms include extracellular chelation by excreted ligands (such as glomalin, a glycoprotein produced by hyphae), biosorption of metals to the fungal cell wall, enhanced efflux via transporters located at the plasma membrane, reduced uptake via transporters located at the plasma membrane, intracellular chelation, subcellular compartmentation and vacuolar compartmentation. Since free HMs in the cytosol could represent a serious threat, the two symbionts have developed mechanisms to sequester and deliver them in bound forms (for example, chaperones, apo-metalloproteins, metallothioneins, organic acids). In the vacuoles, HMs are stabilized with polyP (polyphosphate) granules and some of these vacuoles are translocated to the fungal spores. Most of the metals taken up by the fungi are utilized for their own metabolism while the surplus metal is translocated to the aerial parts (Ferrol, Tamayo, and Vargas 2016; Coninx, Martinova, and Rineau 2017; Shah, Venkatramanan, and Prasad 2019). AMF also immobilize metal ions by adsorbing them through their cell wall components such as chitin and extracellular slime and function as biological barriers for HM entry into host plants (Ali, Khan, and Sajad 2013; Shi, Zhang, Chen, *et al.* 2019).

The AMF can colonize the roots extensively in metal-contaminated soils and it has been seen that mycorrhizal plants produce considerably more biomass and grow faster when compared to the non-mycorrhizal ones because, because of the enhanced phosphate, water and micronutrient uptake. Apart from this, the AMF hyphae also take up HMs and transport them to the plants. This second process by which HMs are extracted by the above ground plant tissues—with the aid of AMF—is known as phytoextraction (Figure 1(A)) (Abbaslou and Bakhtiari 2017; Shah, Venkatramanan, and Prasad 2019). It has been observed that effective phytoextraction of HM-contaminated soils could be achieved by employing hyperaccumulators (for example, *Brassica juncea*, *Helianthus annuus*, *Arundo donax*, *Eichhornia crassipes*), that is plants which show the ability to accumulate extraordinarily high amounts of metals and organic pollutants in aboveground tissues without suffering phytotoxic effects. Hyperaccumulators are also easy to cultivate and harvest, have a high-growth rate, produce more above-ground biomass, are widely distributed and have highly branched root system, show faster root-to-shoot translocation, are resistant to diseases and infestation by pests, and can repel herbivores to avoid food chain contamination (Rascio and Navari-Izzo 2011; Ali, Khan, and Sajad 2013; Atanagana, Khasa, Chang, *et al.* 2014; Shah, Venkatramanan,

and Prasad 2019; Dal Corso, Fasani, Manara, *et al.* 2019). For achieving the desired reduction in soil HM concentration, it is necessary to select and cultivate hyperaccumulators for several cycles (including harvest and removal of the HM-enriched biomass). Indigenous plant species are preferred because of their adaptations to the native environmental conditions and reduced need for management (Shah, Venkatramanan, and Prasad 2019). Proper integration of hyperaccumulating plants and AM fungi could be a key solution to the issue of HM pollution

(Miransari 2011). The mycorrhizal pathway of phytoextraction of HM involves acquisition by high-affinity metal transporters located in the extraradical mycelium, followed by metal translocation along the hyphae to intracellular structures, and then transfer to the root at the symbiotic interface. Once the HMs reach the intraradical mycelium, release from the fungus in the apoplast of the symbiotic interface is facilitated by ion-specific carriers, pumps or channels. Metals released by the fungus at the interface are then taken up by the specific plant.

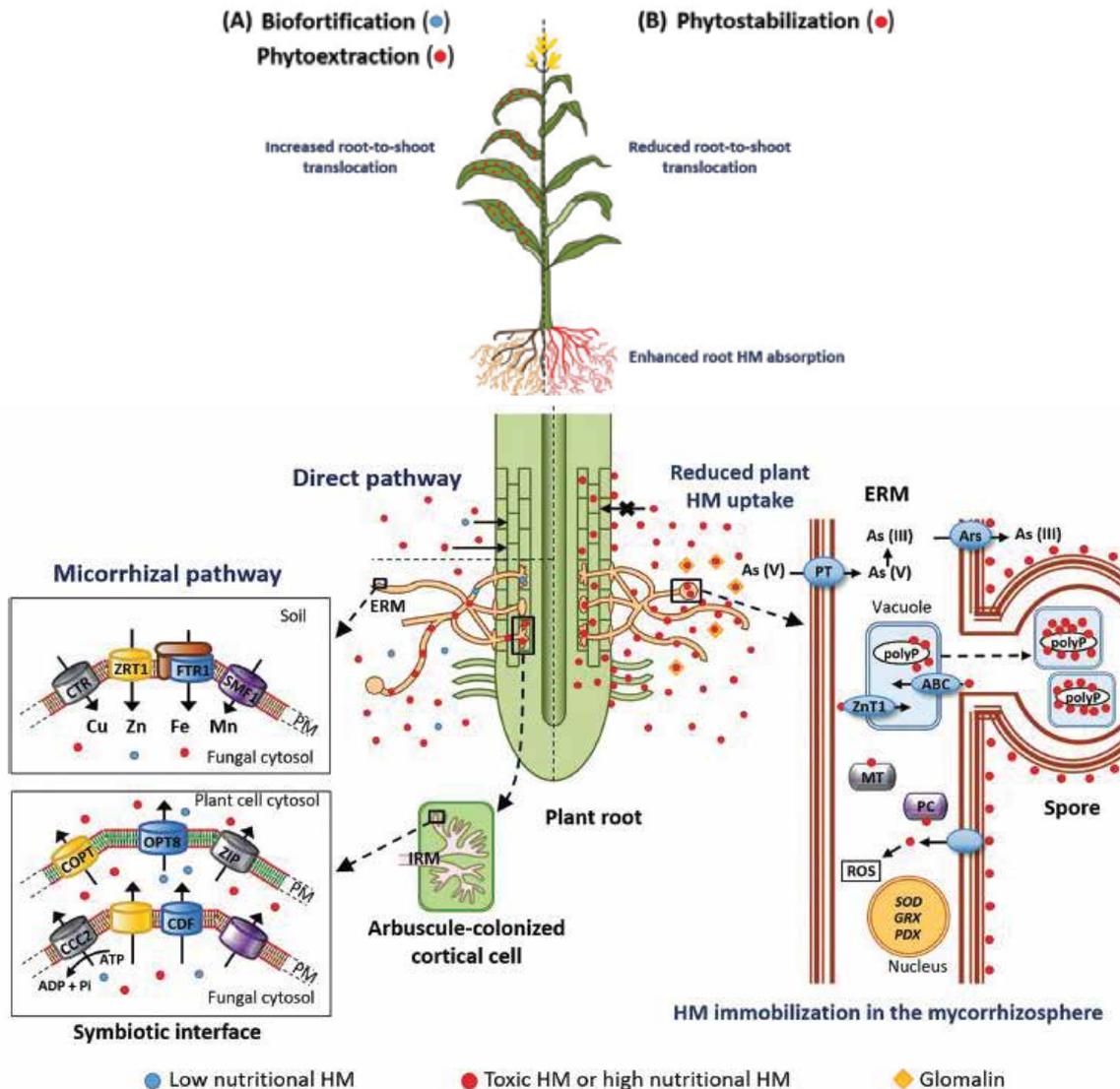


Figure 1 AMF-mediated HM uptake

A: Phytoextraction CTR (fungal Cu transporter), CCC2 (Cu-ATPase), CDF (cation diffusion facilitator), COPT (plant Cu transporter), FTR1 (Fe permease), OPT (oligopeptide transporter), SMF1 (Mn transporter). ZRT1 (Zn transporter), ZIP (Zn-Fe permease);

B: Phytostabilization ABC (ABC transporter), Ars (arsenite pump), GRX (glutaredoxin), MT (metallothionein), PC (phytochelatin), PDX (pyridoxine synthase), PT (P transporter), ROS (reactive oxygen species), SOD (superoxide dismutase), ZnT1 (Zn transporter)

Source Ferrol, Tamayo, and Vargas (2016)

Transporters expressed in the periarbuscular membrane (Ferrol, Tamayo, and Vargas 2016). Sequestration sites in plant cells mainly include cell walls, vacuoles, and the Golgi apparatus. HM ions are known to bind to the functional groups of cell wall components (cellulose, hemicellulose, lignin, pectin). Some plants have been found to even enhance their lignin biosynthesis in response to HM exposure (Shi, Zhang, Chen, *et al.* 2019). It has also been shown that the use of chelating agents such as ethylenediaminetetraacetic acid (EDTA) and ethylenediamine disuccinate (EDDS) as soil amends, in combination with AMF, enhances the bioavailability of HMs, hence, improving the phytoextraction process (Wang, Pan, Shah, *et al.* 2018; Shah, Venkatramanan, and Prasad 2019). Mycorrhizal fungi also enhance HM accumulation in host plants by enhancing their defence systems and by inducing transcriptional overexpression of host genes involved in HM transport, detoxification, and sequestration (Shi, Zhang, Chen, *et al.* 2019).

Selection of the appropriate mycorrhizal fungi can enhance the accumulation of HMs in host plants and increase the efficiency of phytoremediation, however, some mycorrhizal fungi do lead to less HM accumulation (Shi, Zhang, Chen, *et al.* 2019). Hence, the outcome of the symbiosis established with metallophytes depends on the fungal isolate as well as the plant species and some other factors (biotic factors like mutation, interaction and abiotic factors like nutrients, temperature, pH) (Ferrol, Tamayo, and Vargas 2016; Abatenh, Gizaw, Tsegaye, *et al.* 2017; Shi, Zhang, Chen, *et al.* 2019). To be able to exert a beneficial effect on the process, the mycorrhizal fungi must first be able to establish the symbiosis and therefore, the ability of the fungi to survive in the metal-contaminated area is an important prerequisite for its use (Coninx, Martinova, and Rineau 2017). An important aspect to be considered during phytoremediation is the disposal of the contaminated biomass. Appropriate storage or disposal of the hazardous biomass is absolutely essential to ensure that it does not pose any risk to the environment. Main constituents of the biomass (such as lignin, hemicellulose, cellulose, mineral matter) vary with the species involved. Post-phytoremediation harvest management techniques include composting and compaction, combustion and gasification, phytomining and pyrolysis. Among these, phytomining (defined as the natural uptake and pre-concentration of bioavailable metal species from the environment into the plant biomass) has been found to be a less expensive and environment-friendly method for recovery of dispersed metals from soils (Mohanty 2016). While advantages of MAR are simplicity, cost effectiveness, and safety (in terms of topsoil

protection, secondary pollution, waste generation), the major disadvantage is that it is a relatively slow process (Chibuike 2013; Jin, Luan, Ning, *et al.* 2018). Although, the importance of AMF in improving plant nutrition and in alleviating HM toxicity has been well established, many questions regarding various mechanisms of HM homeostasis in AM still remain unanswered. Understanding them would be extremely useful in enhancing the efficiency of MAR. Apart from this compatibility between various AM fungal isolates and plant varieties and their possible use for the remediation of HMs still needs exploration. More efforts are required towards accomplishment of tasks like planting, maintenance of the remediation site, and fruitful conversion of the harvested biomass, for completely harnessing the benefits of this method of soil remediation (Chibuike 2013; Zadehbagheri, Azarpanah, and Javanmardi 2014; Dal Corso, Fasani, Manara, *et al.* 2019).

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NEWS HIGHLIGHTS



Controlling mycorrhizal colonization

According to a study published in *Theoretical and Applied Genetics*, certain genes control the chemical signals and pathways that call the specific mycorrhizal fungi towards the roots. They allow the plant to recognize the specific fungus as a 'good guy', and help build arbuscules. Such information could be crucial in developing cultivars with a higher affinity for the fungus, reduce the overuse of fertilizers and pesticides, and so on.¹

From the thawing permafrost

A study, that could have implications for researchers and computer models that predict nitrogen pathways at both regional and global levels, has been conducted by a team of scientists from the Center for Ecosystem at NAU. Ericoid mycorrhizal and ectomycorrhizal were found to be involved in their respective plant partner's nitrogen uptake (from the permafrost).²

Fossil fungi

A new research that backs up previous findings of the symbiotic relationship between fungi and the first plants has been brought to light. The discovery of the new fungus would aid in filling the gaps in fossil records and completing the timeline for the emergence of terrestrial multicellular life.³

An alarming loss

Disappearance of *Thecostele alata* from its ecosystem in Bangladesh (Chittagong region) along with 31 other native orchids has left researchers worried about their future. Experts say that if orchid habitat is destroyed, we could potentially also see the extinction of the unique resident fungi. And the truth is that not much is known about these fungi as some of them have never been described at the species level.⁴

Mitti ke doctor

The Soil Health Cards (SHC) programme, implemented in 2015, assesses soil fertility in terms of availability of key nutrients and physical parameters. The receptivity of farmers to the programme has led to the emergence of soil health specialists who encourage the farmers to switch to balanced fertilizer (for example, bio-fertilizers and pesticide application for sustainable agriculture without compromising productivity).⁵

Recognition and accommodation of beneficial organisms

A study published in *Nature Plants* describes the discovery of a common genetic basis for all the symbioses observed in nature. It was discovered that three genes are shared exclusively by the plants that form intracellular symbiosis and lost in plants unable to form this type of beneficial relationship.⁶

¹ Details available at <https://phys.org>

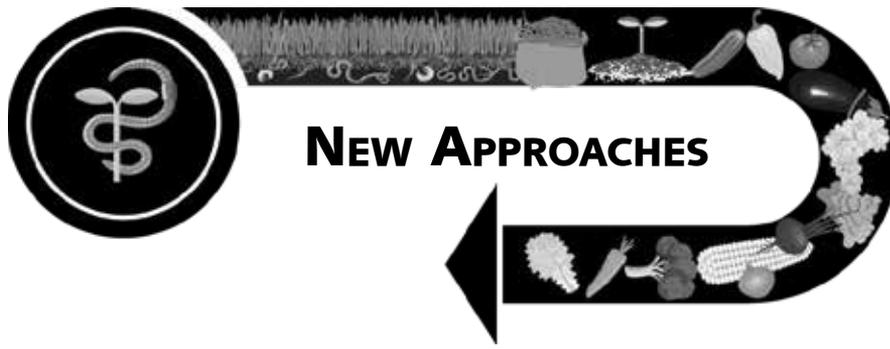
² Details available at www.sciencedaily.com

³ Details available at www.sciencealert.com

⁴ Details available at <https://blogs.scientificamerican.com>

⁵ Details available at <https://indianexpress.com>

⁶ Details available at www.sciencedaily.com



New Approaches and Techniques

Digitizing Microscopic Observations of Arbuscular Mycorrhizal Fungal Colonization

Since the mid-twentieth century, a number of methods have been proposed to assess the presence of mycorrhizal fungal structures and the level of mycorrhizal colonization in plants' root cortexes. When tracking the temporal development of colonization, researchers are forced to choose and adapt a particular method, which often reduces or makes harder the possibility of comparing it with similar studies. The harmonization of methodologies for the assessment of radicular endophytic colonization is a current necessity as the functionality of mycorrhizal symbionts for plants can be described only by indicators obtained based on microscopic analysis. The MycoPatt model is an objective system that has been proposed for assessing mycorrhization. The model takes into account the linear segmentation of root samples where each root fragment that is considered for microscopic evaluation corresponds to a number of 15 microscopic fields at 400 \times . Each structure is encoded in a data collection table in a 10 \times 10 matrix and, the evaluation and coding can be done directly at the microscope (by applying the eyepiece micrometer grid) or by applying 10 \times 10 grids to the images captured. The penetration of hyphae into intracellular spaces as well as occurrence of arbuscules, vesicles, intraradical spores, and auxiliary cells can be observed. The model could also be adapted for thicker roots and used without any changes, at different microscopic magnification. Collected data is transferred to an Excel data table which automatically converts each microscopic field into a coloured

mycorrhizal map (for each 1 cm fragment) and this is assembled into a complex model that follows the pattern of mycorrhizal development throughout the root system (Figure 1). It is also possible to correlate the obtained data with root architecture and plant dependence to mycorrhiza. The background formulae are adaptable and the coding can be changed with minimal effort, depending on the user's needs. The coloured map developed for arbuscular mycorrhizal (AM) aids in microscopic evaluation. This could also be adapted for dark septate or fine root endophytes. The main advantage of MycoPatt is that it tracks the establishment and development of symbiosis with AM fungi along the root length, highlighting the entry areas in the cortex and branches. MycoPatt evaluates the expansion of AM in detail where each indicator is calculated separately (horizontally and vertically) for each microscopic field and in this way, the objectively made final data report presents the actual level of structural extension in the roots. Another result is the achievement of a clear separation between mycorrhized and non-mycorrhized areas. Up to date, it is the only method that allows the evaluation of all AM structures at once and, their exact position in plant roots. The efficiency of the model is attributed to increased resolution of observation, speed of analysis, and fidelity of produced data. Examples of fields where the model could be used include bioproduct production, agronomy, plant breeding, phytosociology research, and so on.

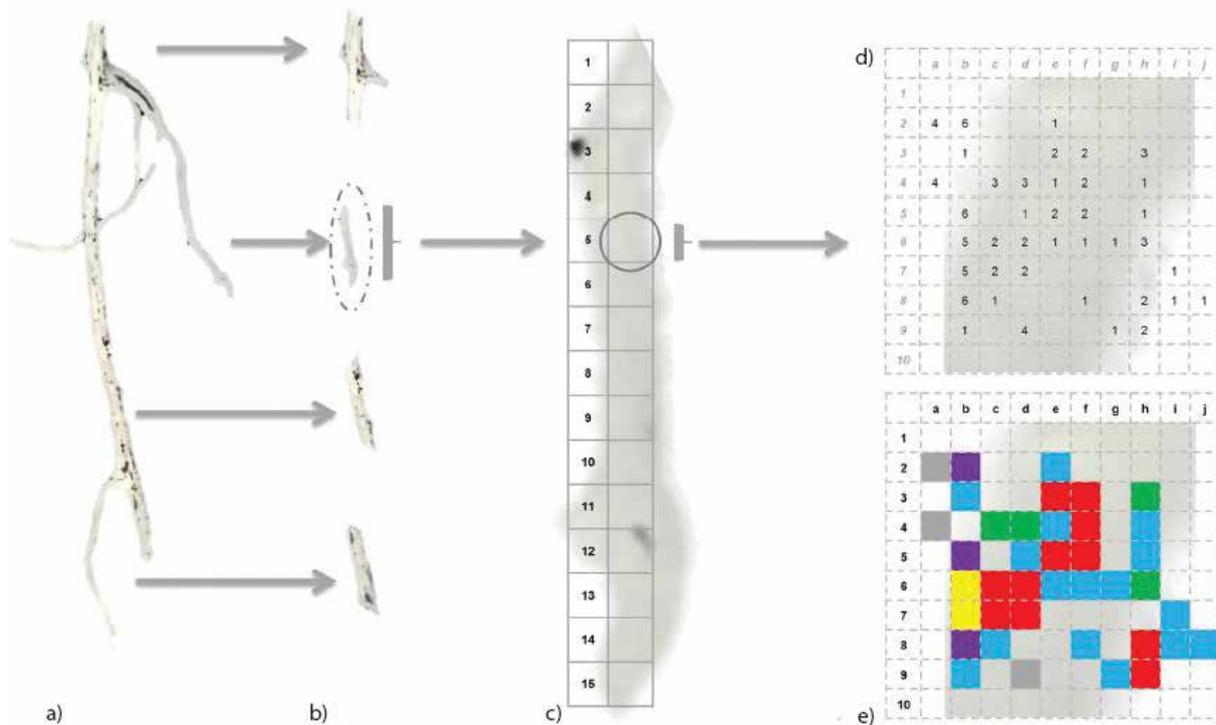


Figure 1 Schematic framework of MycoPatt model: (a) Root system; (b) Selection of 1 cm segments; (c) Segment analysis flow: 15 microscopic fields/segment; (d) MycoPatt grid overlay on each microscopic field with the assignment of values for mycorrhizal structures: (1) hyphae, (2) arbuscules, (3) vesicles, (4) spores, (5) auxiliary cells, (6) entry points; (e) Conversion of values into colours for development of mycorrhizal map: (1) blue, (2) red, (3) green, (4) gray, (5) yellow, (6) purple

Harmonization of mycorrhiza studies based on MycoPatt methodology could lead to the creation of a unitary system of data collection and therefore increase the flexibility of research and applications. Numeric expression of colonization patterns along with the creation of new sets of indicators (adapted for a realistic description of symbiotic mechanisms) could open a new stage in mycorrhizology. Evolution of this methodology could lead to digitization of AM systems

which might also allow the creation of databases that supplement the existing ones (Stoian, Vidican, Crişan, *et al.* 2019).

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RECENT REFERENCES

The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals:

- *Agroforestry Systems*
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- *Botanical Studies*
- *Environmental Microbiology*
- *Frontiers in Microbiology*
- *Frontiers in Plant Science*
- *Fungal Biology*
- *Fungal Ecology*
- *Genes*
- *Journal of Soils and Sediments*
- *Mycorrhiza*
- *Mycoscience*
- *New Phytologist*
- *Oecologia*
- *Plant and Soil*
- *Plants*
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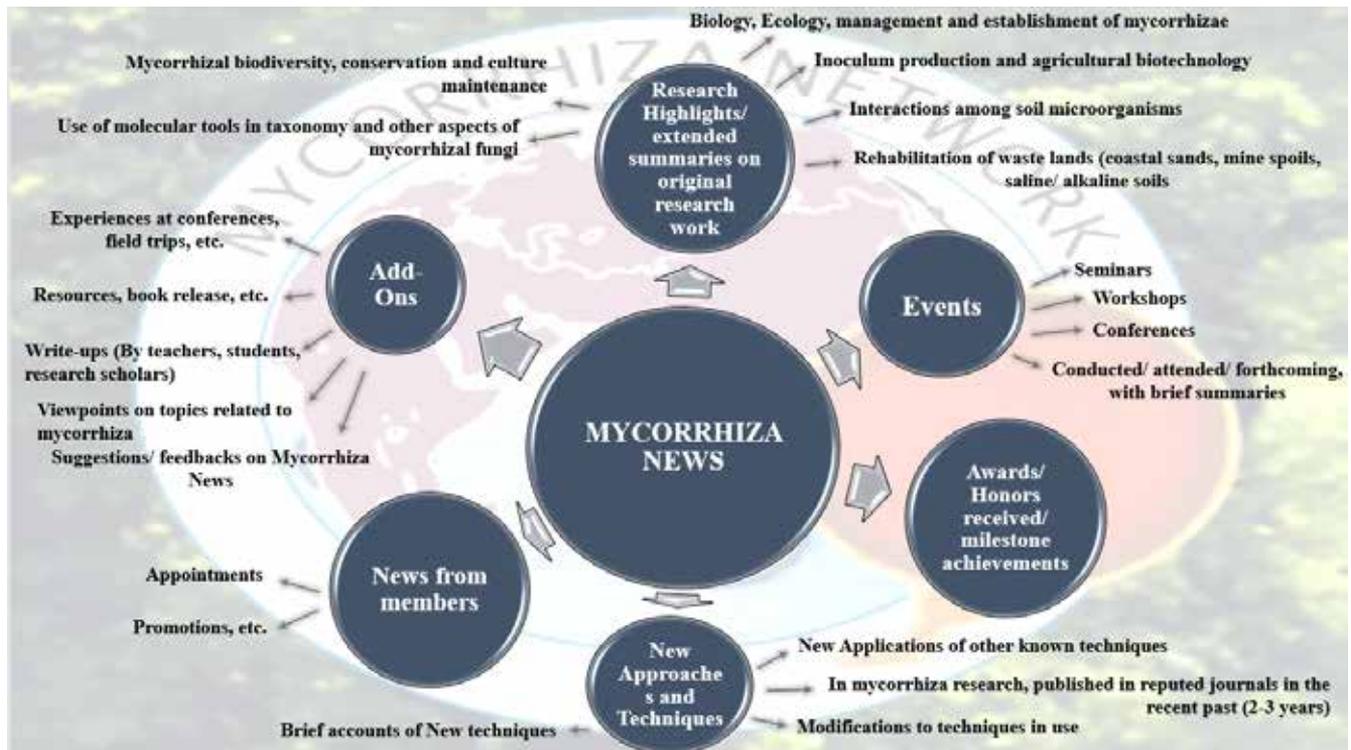
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