Nano-Phytoremediation of Heavy Metal Contaminated Wastewater Ecosystems and Wetlands by Constructed-Wetlands planted with Water-logging-tolerant Mycorrhizal Fungi and Vetiver Grass

A G Khan

Grad Life Member Western Sydney University

Alumni Sydney University

Researchgate..net/profile/abdul_khan206.

Linkedin.com/in/abdul-khan-0603115a/

E'Mail: lasara1937@gmail.com

Wetlands & Their Signficane

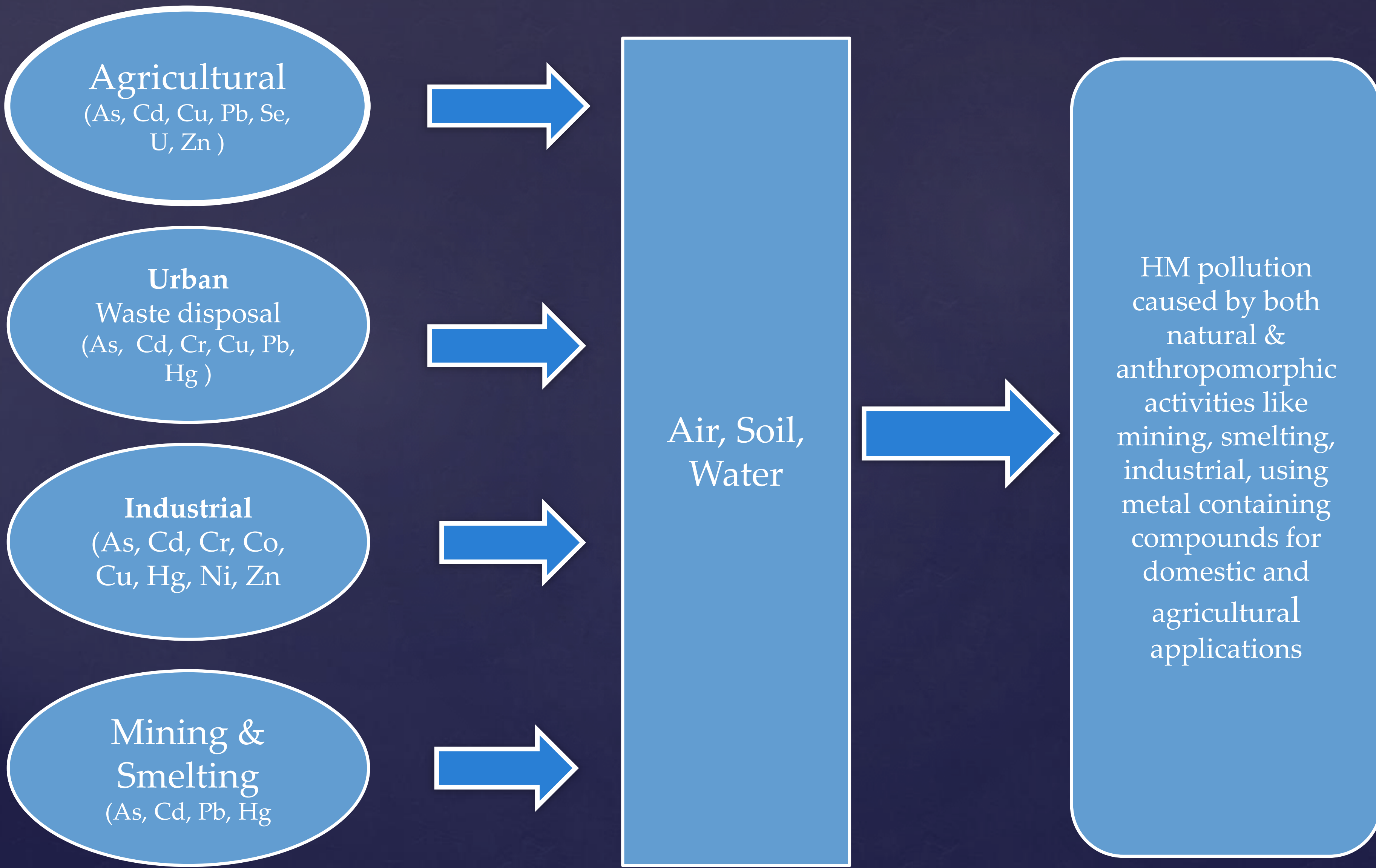
- Wetlands & Aquatic Ecosystems:
  - important part of ecological systems
  - National Resources

Becoming polluted

  - By toxic heavy metals from man-initiated industrial, mining & smelting of metalliferous ores , & agricultural activities
  - Need to well manage
  - Loss of wetlands & aquatic ecosystems may end with lost Flora & Fauna
  - Influence BIODIVERSITY

Need to be well managed and protected

Potential sources of heavy metals (HM) in the environment



HMs in soil, air, and water environments effects Human Health

VARIOUS PHYTOREMEDIAL MECHANISMS

BIOLOGICAL MECHANISMS INVOLVED IN PHYTOREMEDIATION

PHYTODEGRADATION
(Breakdown or transformation of HMs by enzymes(Nano particles) within plant tissues

PHYTOFILTERATION
(Sequestration of HMs)

BIODEGRADATION
Rhizosphere BD of HMs by Microflora

PHYTOSTABILIZATION
(Stabilise HMs in soil by roots

PHYTOEXTRACTION
ACCUMULATION OF HMs in shoot

Phytoremediation Techniques for Decontaminating HM-Pollute Aquatic Ecosystems and Wetlands

- 1. Phytostabilization (stabilize HMs in the rhizospheres of aquatic macrophytes
- Phytofiltration (Sequestration of HMs in the roots of aquatic macrophytes)
- Phytoextraction (accumulating HMs into shoots)

Aquatic Macrophytes Suitable for Phytoremediation of HM-polluted Aquatic Ecosystems & Wetlands –

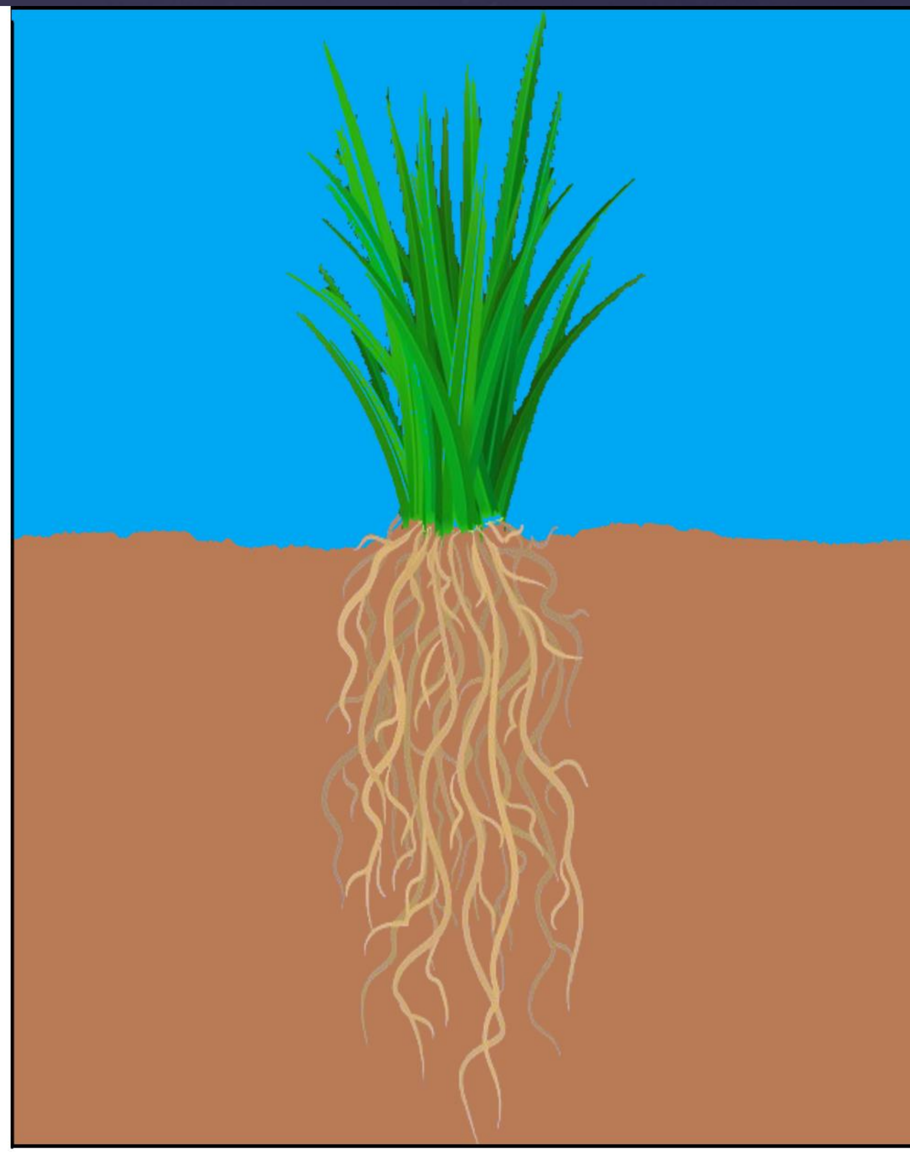
- Be able to grow as a hydrophyte
- Have quick, rapid, and fast growth rate;
- Non-invasive
- Be vegetatively propagated and sterile;
- Yield high biomass;
- Tolerant to multiple heavy metals (HM) toxicity;
- Great accumulator of different HM;
- Large, deep and penetrating root system

ALL THE AABOVE CHARACTERISTICS ARE POSSED BY Vetiver Grass

Vitiveria (Chrysopogon) zizinioides (Figure 1)

Shoot:
Densley tufted, awlness, stiff, erect leaves glabrous, Non-invasic, Sterile

Roots:
Large and deep penetrating root system, can grow upto about 4 metres.



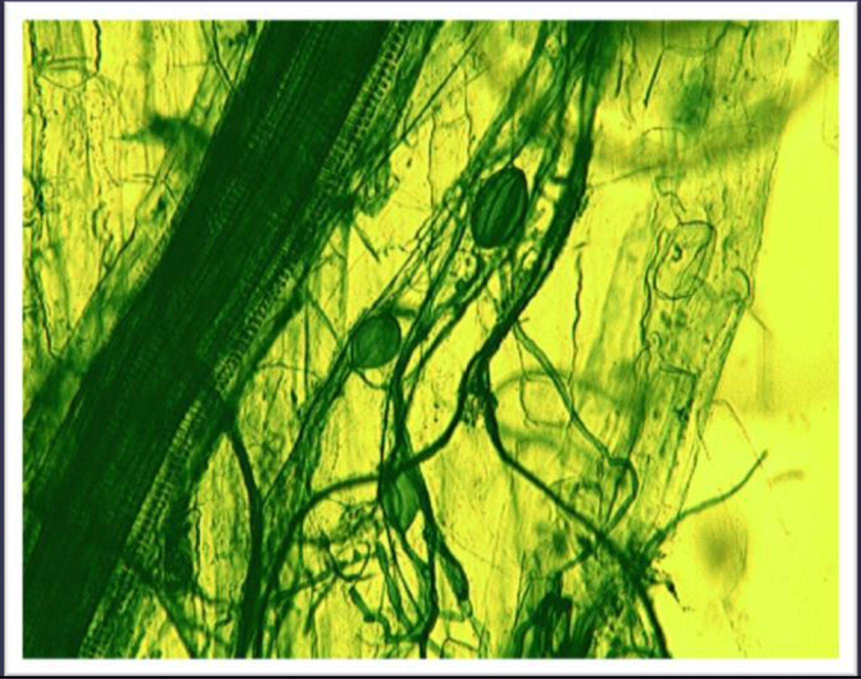
Root:
- Large and Deep
- No Rhizome or Stolon.
- Propogated by Slips.

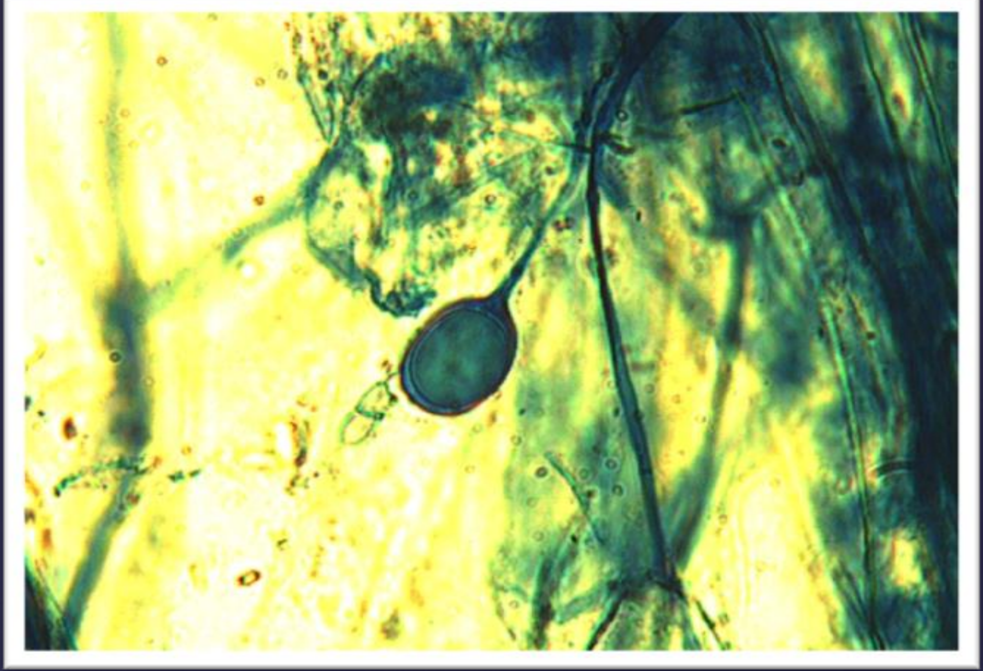
ARBUSCULAR MYCORRHIZA

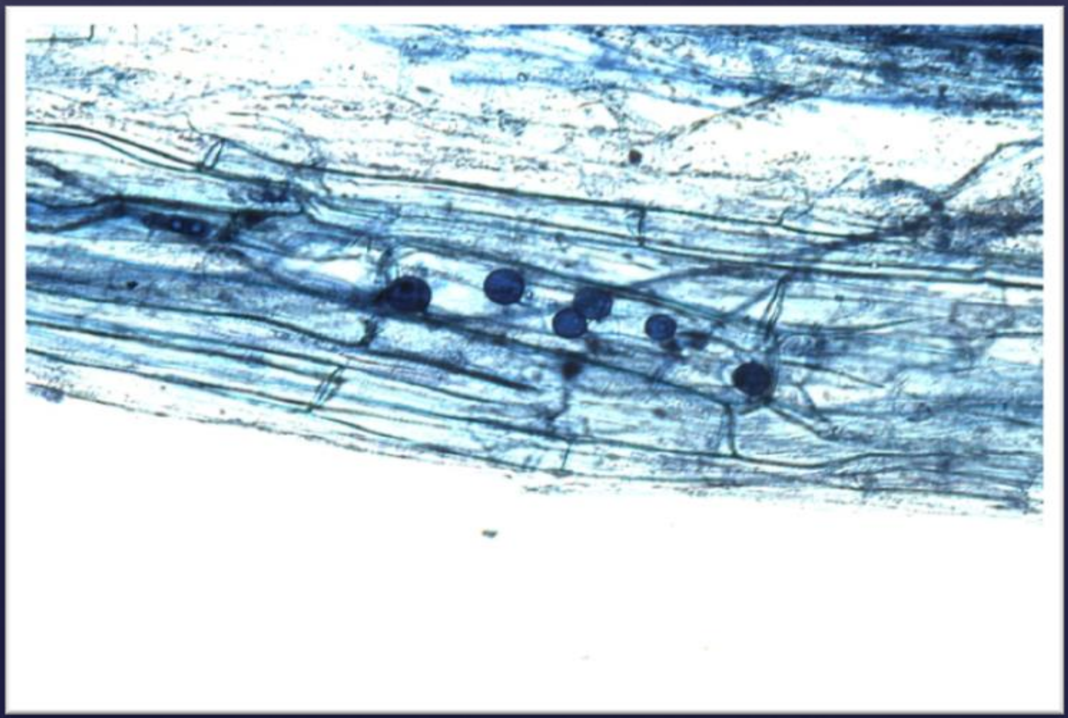
- The most common Mycorrhizae
- Are highly evolved non-pathogenic symbiotic association between roots of most vascular plants and certain specialized soil fungi (Basidiomycetes, Ascomycetes and Zygomycetes); they colonize the cortical tissues of roots during periods of active plant growth both in natural environment and in cultivation.
- Display characteristic root infections with coils (arbuscules), and in some cases vesicles, and spores of various morphological features in their rhizospheres
- Play important role in survival of plants growing on HM polluted soils, on saline, or xerophytic, or aquatic conditions.

Benefits of Mycorrhiza

- Mycorrhiza promotes the growth of host plants and increases productivity
- Improves water relations
- Protects roots from pathogenic fungi
- Saves from heavy metal toxicity
- Protects from adverse temperatures, pH, high salinity, toxic stress etc
- Reduces soil erosion
- Increases soil biological activity Influences the development of plant community







Illustrations:




Spores of *Glomus* spp.

Sclerocystis

Entrophospora

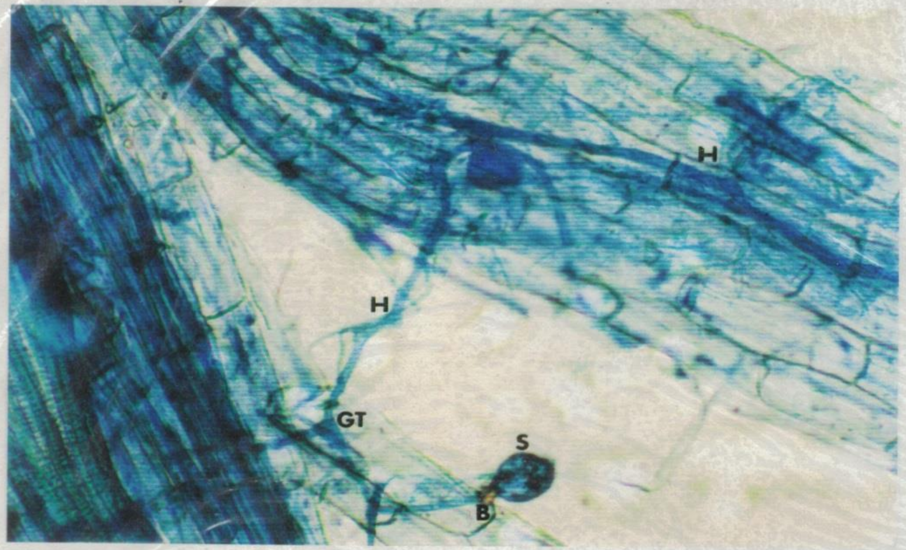


Fig 3(a): Microphotograph showing extension of a germ tube from an extra-matrical pregerminated spore (S) of *Gigaspora spp.* on the surface of the *Acacia dealbata* root with internal and external hyphae (H). Note the bulbous base (B) of the germinating spore (S) and two germ tubes (GT) arising from it (x100).

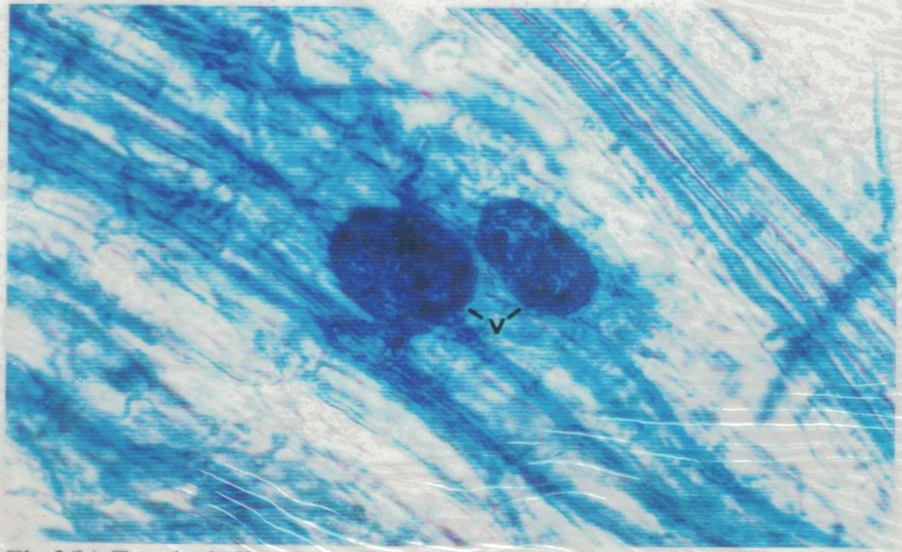
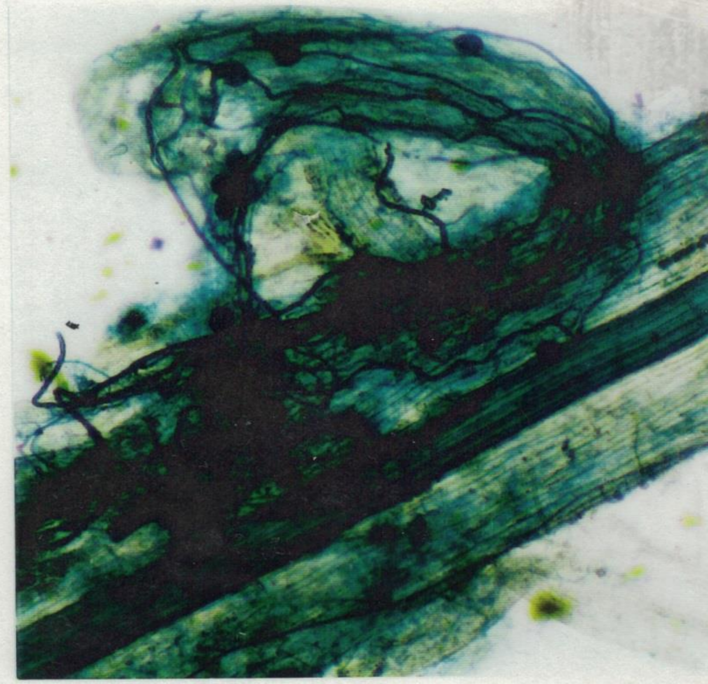


Fig 3(b): Terminal elliptical vesicles (V) in the inner cortical layer of *Acacia dealbata* root.(microphotograph, 100x; fungal structure stained with trypan blue)



2.8b.

HOW PLANTS SURVIVE IN HM-CONTAMINATED WATERS -1

Plants either:

- Accumulate HM into their root or shoot tissues (HYPERACCUMULATORS) and survive OR
  tolerate saline, hydrophytic, xerophytic, and heavy metal contaminated soils
- Universal and ubiquitous AMF forming symbiotic associations with roots of these plants known to benefit plant nutrition, growth, and survival on such soils.
- Toxic metals in rhizosphere dislodge biological molecules hindering their functions & toxic to plant cells, change enzymes, proteins or membrane transport systems
- Indigenous AMF and associated rhizospheric microbes (PGPM) have mechanisms to degrade HMs

HOW PLANTS SURVIVE IN HM CONTAMINATED WATERS- 2

Mechanism involves:

- Sequestration of HMs by root cell wall component or by intra-cellular metal-binding proteins or peptides (Nano-particles) (Metallothionins –MTs), or Phytochelatins (PCs) synthesised by plant from GSH (Glutathione) in the cytoplasm or Symplast of the plant root cells widely found in plants.
- These nano-molecules have a Potential role in detoxification of HMs by forming HM-binding complexes (nano-molecules) in the cytoplasm of root cells
- Positive relationship between levels of PCs and HM tolerance.
- Recent studies have identified specific genes for PC-Synthase for specific HMs indicating their role in HMs (Cd, As) tolerance
- Role of PC in HM accumulating plants still unclear to establish relationship – need more research re HM binding and movement in plant

ROLE OF AMF IN PHYTOEXTRACTION & PHYTOSTABILIZATION OF HMs -1

In addition to providing nutrients to plants, AMF can:

* **Improve soil structure by forming soil aggregates which protect organic matter.**

* **Increase decomposition rates in the myco-rhizosphere**

* **Act as bioprotectant against phytopathogens by producing antibiotics & cell wall lysing enzymes**

* **Produce root exudates (organic acids) increasing solubilization & mobilization of HMs**

* **Convert toxic forms of HMs to less toxic to other living systems**

* **Play role in Absorption, Sequestration, and Transportation of HMs**

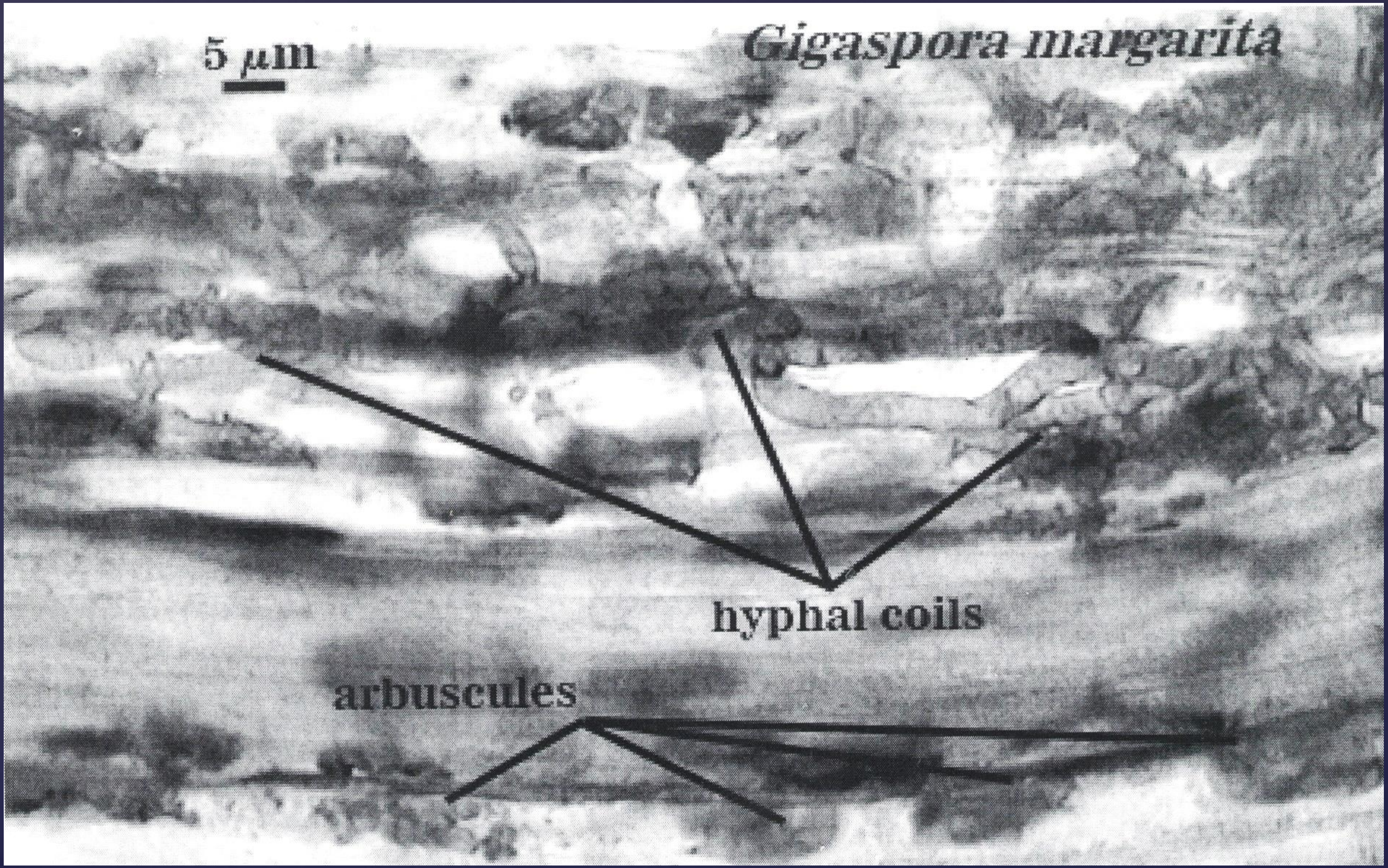
IN DOING SO, AMF PROVIDE HYPERACCUMULATING PLANTS SPECIAL DEFENCE AND hm TOLERANCE

ROLE OF AMF IN PHYTOEXTRACTION & PHYTOSTABILIZATION OF HMs -2

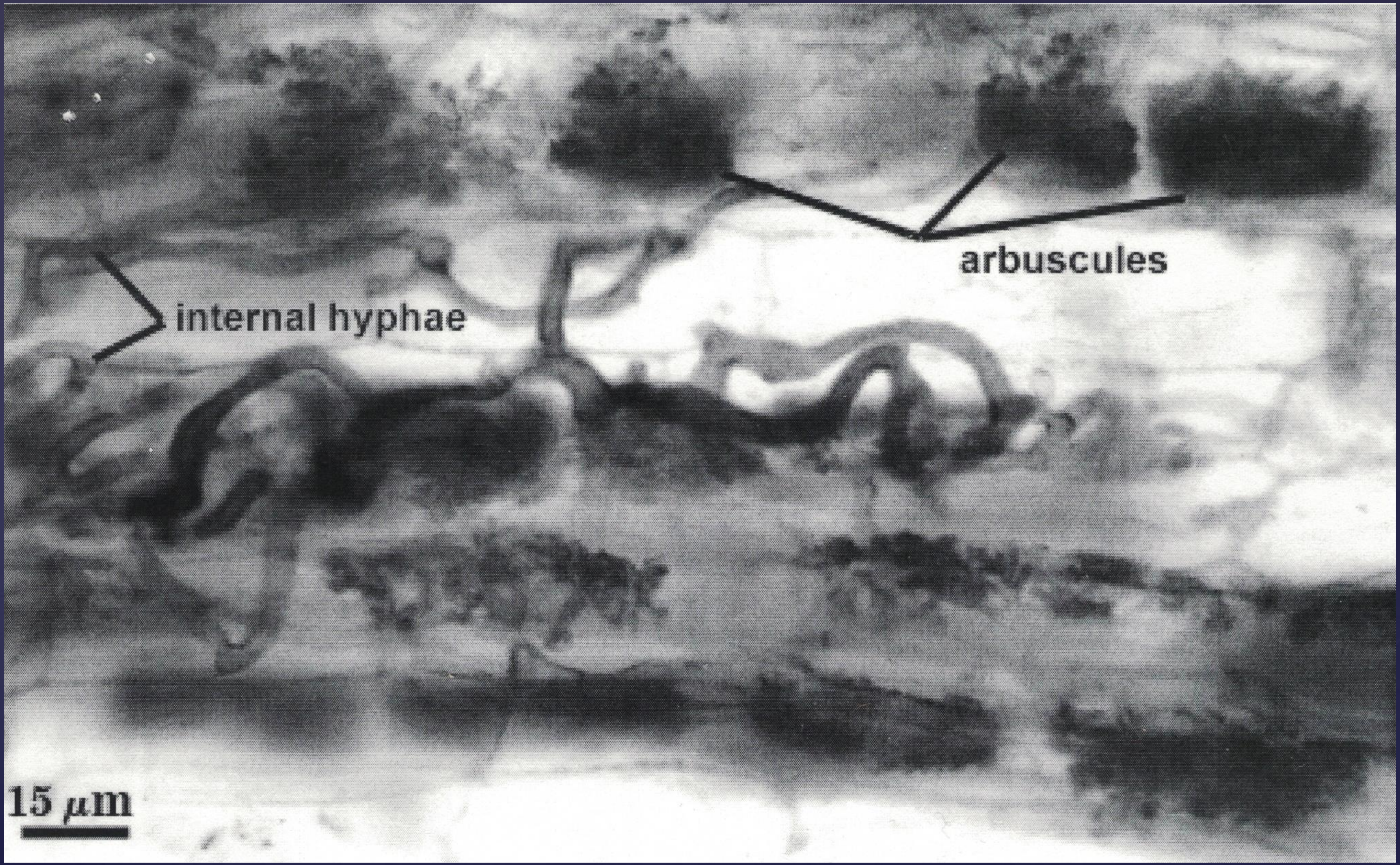
- High metal-affinity Transporters located in the External Fungal Hyphae make HM immobilize
- Heavy Metal tolerance by AMF due to:
  - Chelation of HMs by protein Glomalin produced by AMF
  - Followed by HMs translocation along the hyphae to root intracellular structures
  - Then, compartmentalization of HMs in the root cortical cell vacuoles
  - Then transfer to the root at the symbiotic interface (Arbuscules)
  - HMs stabilized in the root cortical cell vacuole & translocated to the AMF spores (vesicles)

NEXT FEW SLIDES SHOW INTERNAL AMF STRUCTURES IN ROOT CORTICES OF AQUATIC MACROPHYTES

ILLUSTRATION



ILLUSTRATION



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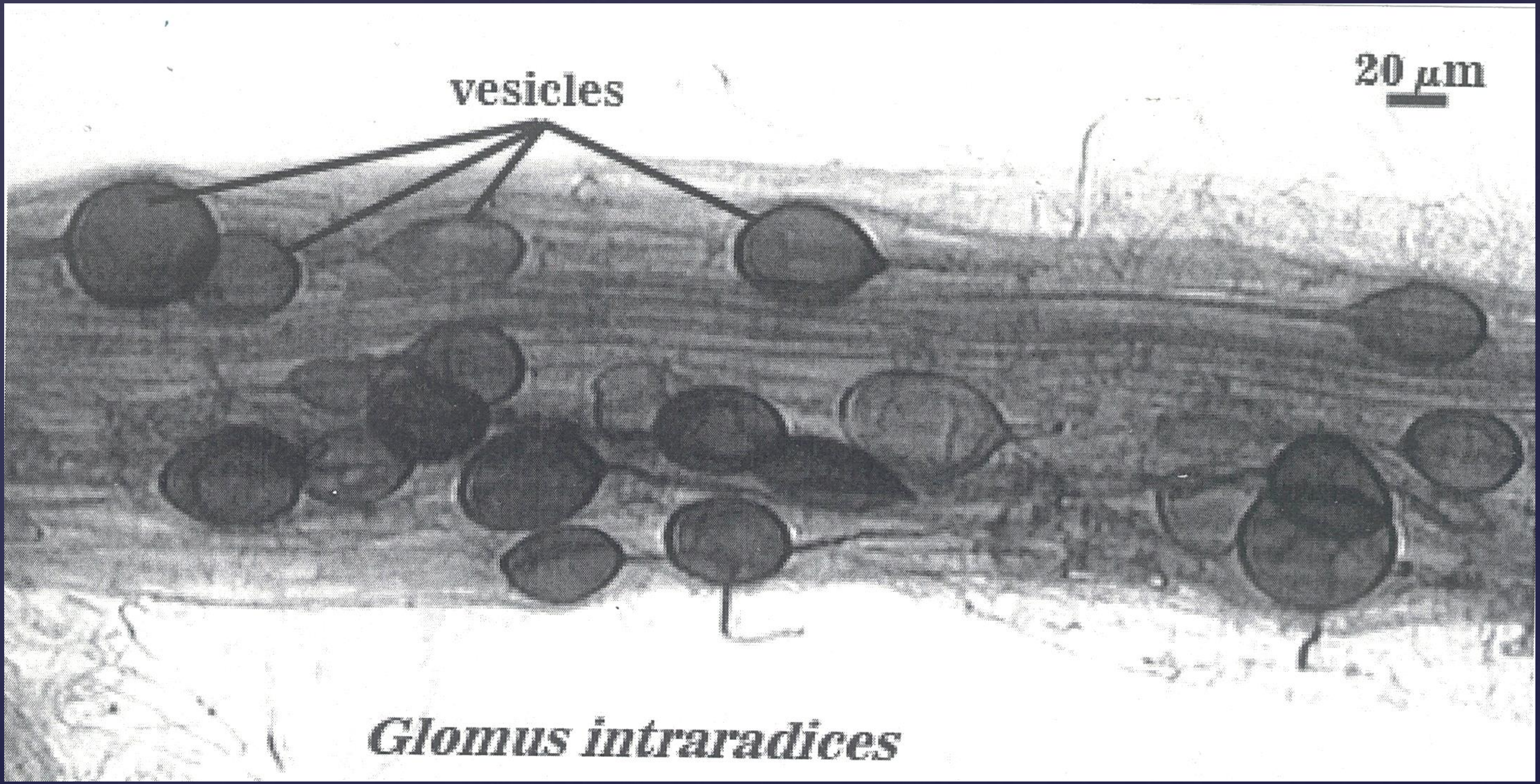


ROLE OF AM IN HM ACQUISATION AND DISTRIBUTION

AMF PLAY IMPORTANT ROLE IN HM ACQUISATION BUT IT DEPENDS ON:

1. AMF --- introduced Non HM tolerant versus Indigenous tolerant
2. PLANT specie – HM Accumulating or HM Excluding
3. HM involved – metal speciation

During last 10 - 15 years, knowledge about mechanism of HM acquisition by AMF

- Several HM tolerance and acquisition factors (CHELATION, SEQUESTRATION & TRANSPORTATION are being identified
- Many unanswered questions still remain to be explored further

POSITIVE EFFECTS

Increase plant Biomass

Enhance plant Growth

Increase plant nanomaterial … Enzymes

PHYTOREMEDIATION OF HMs IN SOILS

Reduce HM Translocation (PHYTOSTABIIZATION)

Increase HMs Extraction

(PHYTOEXTRACTION)

NEGATIVE EFFECTS

Decrease biomass

Reduce root elongation

Leaf Necrosis

NANOMATERIALS

PLANTS &

MYCORRHIZAE….

COMBINED

IN SITU NANO-PHYTOREMEDIATION STRATEGY TO MYCORRHIZOREDATE

HM-CONTAMINATED SOILS

Combination of Plants, Nanomaterials, & AM in environmental soil Management USING BIOENERGY PLANTS

AMF Effects

External Hyphae

Uptake mobile nutrients – improve plant growth

Alleviate HM toxicity to plant roots

METHODS OF AMF INOCULUM PRODUCTIONS

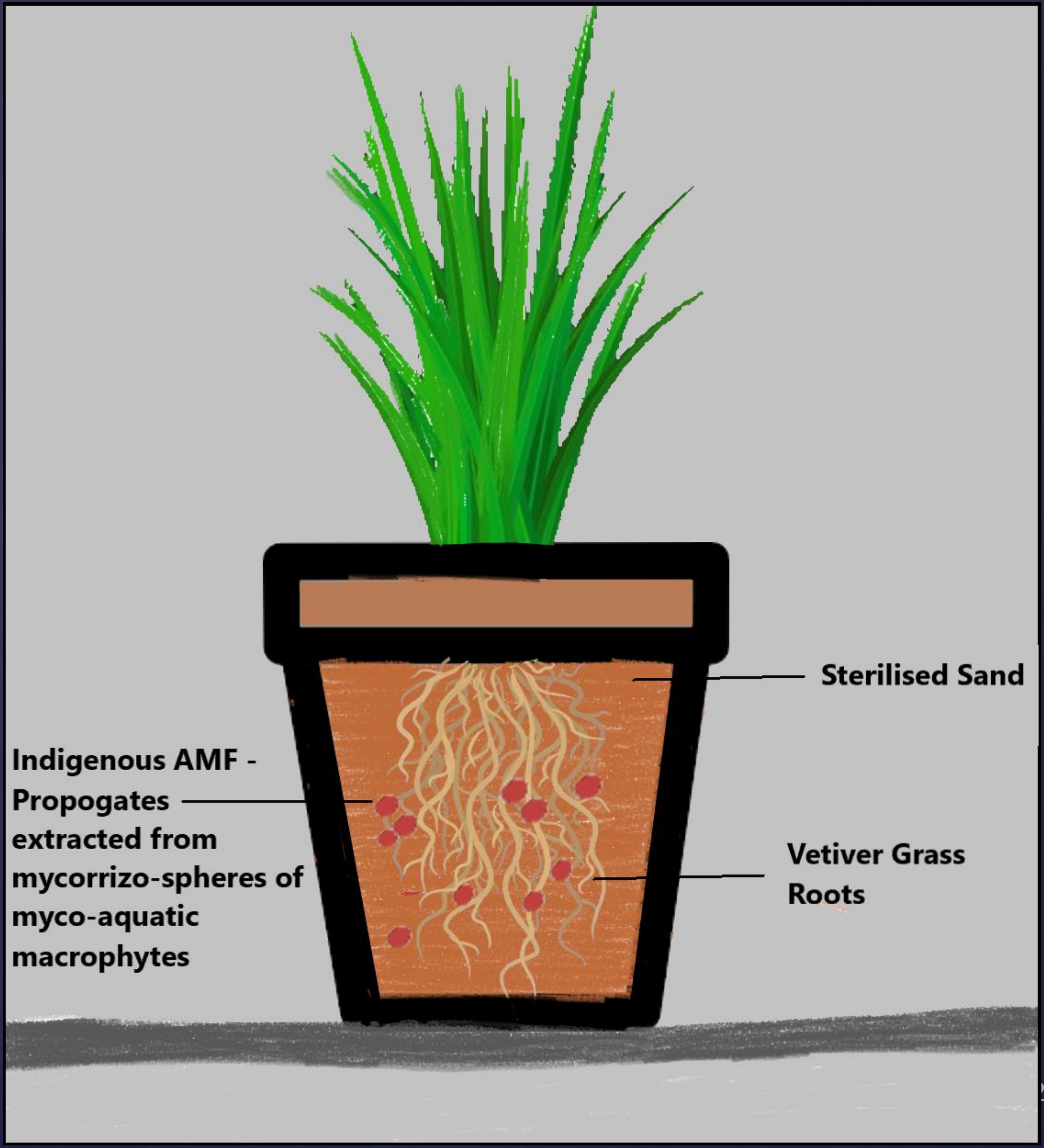
1. Common method – using POT CULTURE technology (Fig 1)

2. Other methods – Monoxenic Cultures utilizing split-plate cultures & RiT-DNA transformed carrot roots --- good for physiological & genetical studies

   {Mohammad A & Khan AG. 2002. Monoxenic in vitro production and colonization potential of AM fungus *Glomus intraradices* . Indian J Experimental Biology 40, 1087-1091.

3. Aeroponically/hydrologically produced sheared-root inoculum technique can produce commercial quantities of AMF inocula

   {Khan A.G. 2007. Producing mycorrhizal inoculum for phytoremediation. *Methods in Biotechnology 23, 89-98.*


Sterilised Sand
Indigenous AMF - Propogates extracted from mycorrizo-spheres of myco-aquatic macrophytes
Vetiver Grass Roots

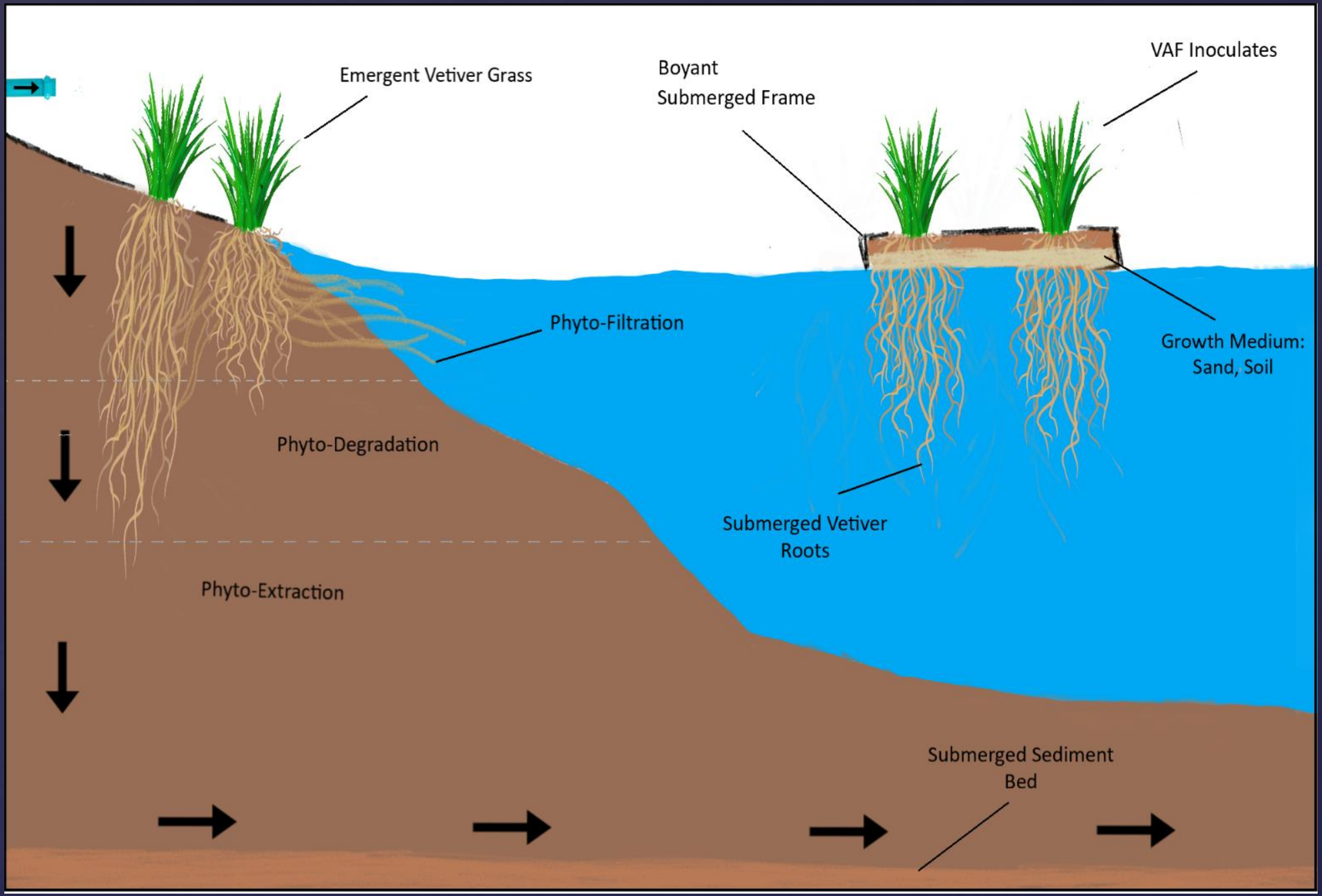
CONSTRUCTION OF FLOATING WETLAND

Step 1:Extraction of heavy metal-adapted AMF spores from the rhizosphere of HM-adapted aquatic macrophytes growing at the land-water interface of the contaminated water ecosystem, by using Wet-Sieving and Decanting Technique

Step 2: Establishing POT-CULTURES by growing surface sterilized Vetiver grass cutting into a pot containing 1kg soil inoculated with AMF spores extracted as Step 1.

Step 3: Selecting heavily infected/AMF –preoccupied mycorrhizal Vetiver grass plants from the pot-cultures above and transferring them into floating polystyrene raft as Constructed Wetland for effective and efficient uptake of HM-contaminants from the polluted aquatic ecosystem.

FIG 2


Emergent Vetiver Grass
Boyant
Submerged Frame
VAF Inoculates
Phyto-Filtration
Growth Medium:
Sand, Soil
Phyto-Degradation
Submerged Vetiver
Roots
Phyto-Extraction
Submerged Sediment
Bed

IMPROVING MODERATELY HM CONTAMINATED WATERS USING NANO MYCORRHIZO PHYTOREMEDIATION STRATAEGY

- SYMBIOTIC OR FREE LIVING N-FIXING MICROBES (IN ROOT NODULES OR IN RHIZOSPHERE) SECRETING HM-AFFINITY TRANSPORTER NANO-MOLECULES
- External Fungal Hyphae of AMF FUNGI SECRETING HM-AFFINITY TRANSPORTER NANOMATERIALS IMMOBILIZING OR TRANSLOCATING HMs INTO ROOT CELL
- IDEAL PLANT (eg *Vetiver Zizinoides*) SECRETING PHYTOCHELATING NANO-MOLECULES TRANSLOCATING HMs IN ROOTS OR SHOOT

Concluding Remarks

Please remember that Mycorrhizae are not a miracle.

It is only a helping hand. Although I showed lots of benefits.

Inoculum developed with certain fungi work very well under certain conditions with some crops , but not with others.

Inoculum must be developed with local isolates and existing conditions.

A Nano-Phytoremediation strategy to MYCORHIZOREMEDIATE HM contaminated WATERS depends on the:

- AM fungal isolate
- The Plant spp. , and
- Nutritional (including HMs) status of contaminated soils.

More data is needed before adopting this strategy for nano-phytoremediation

Thank You for Your Kind Attention

Contact Email details:

Professor (Rtd.) Dr AG Khan
lasara1937@gmail.com