Effects of Sewage Sludge Application and Arbuscular Mycorrhizal Fungi Interactions on the Heavy Metal Phytoremediation in Chrome Mine Tailings.

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Introduction

- The soil, having vital importance for the natural environment is the foundation of the food system.

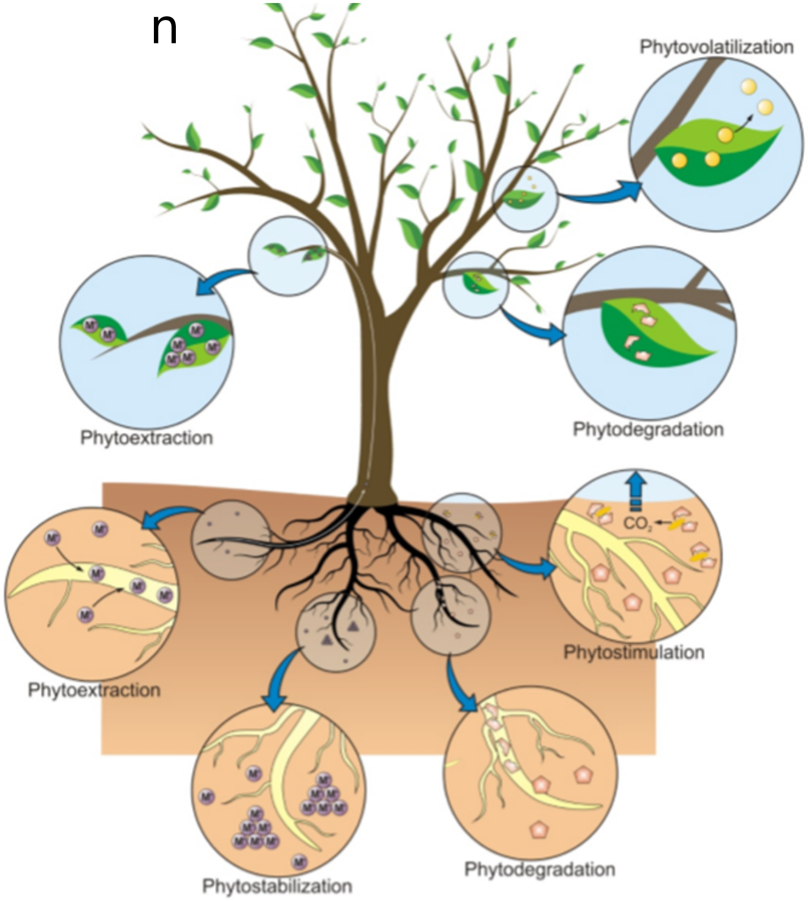
- Soil pollution becomes substantial problem for human beings, living and non-living entity as the soil is a non-renewable resource.

- Soil remediation is needed for removal of pollutants to retrieve the soil supply efficiently.

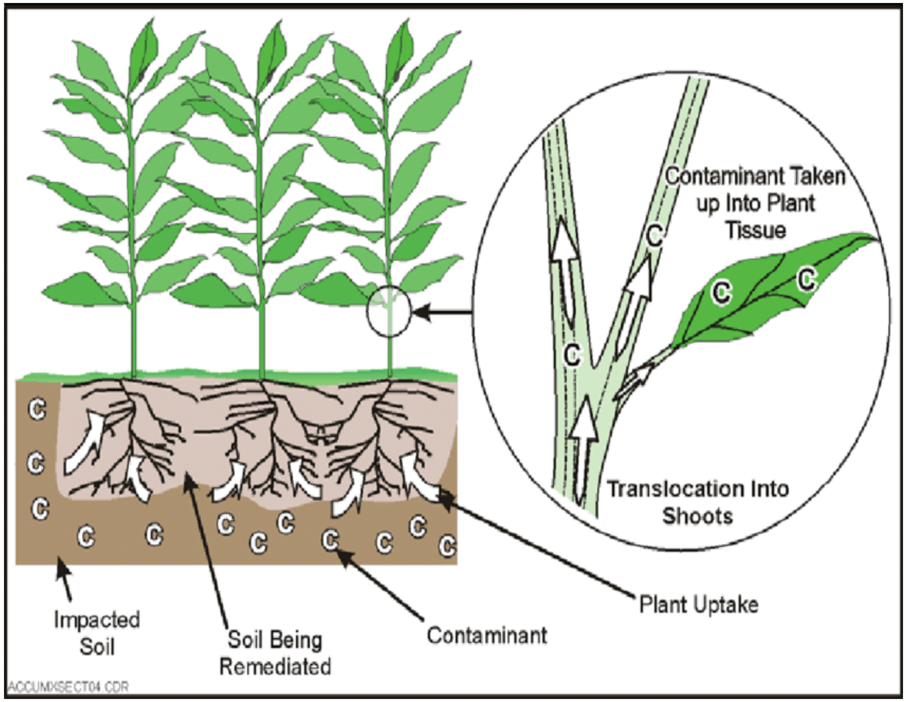
Phytoremediation

- Phytoremediation is a low-cost, feasible, green technology using metal-accumulating plants to remove toxic metals, pesticides, and other hazardous materials from soil and water.

Phytoremediation



What is Phytoextraction?



- The soil contaminant is absorbed by the plant root.
- The roots translocate the contaminant into the different portions of the plant below and above ground.
- The phytoextraction is mostly used for metal uptake from the contaminated soil.

Enhancement of Phytoremediation

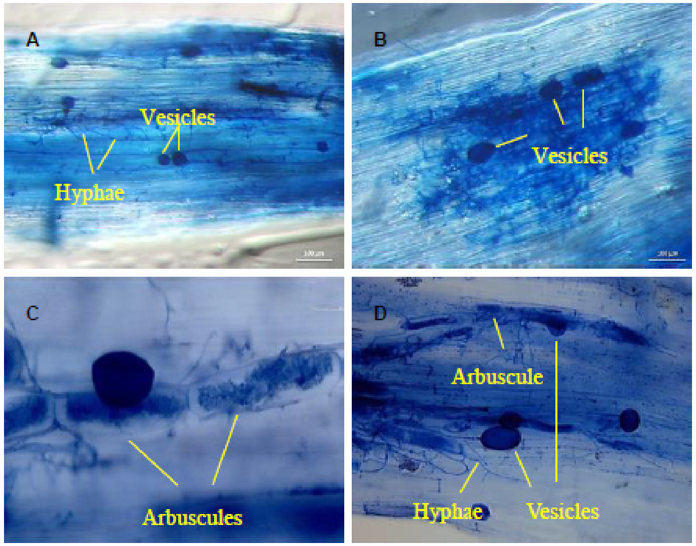
1. Mycorrhizal association enhances phytoremediation

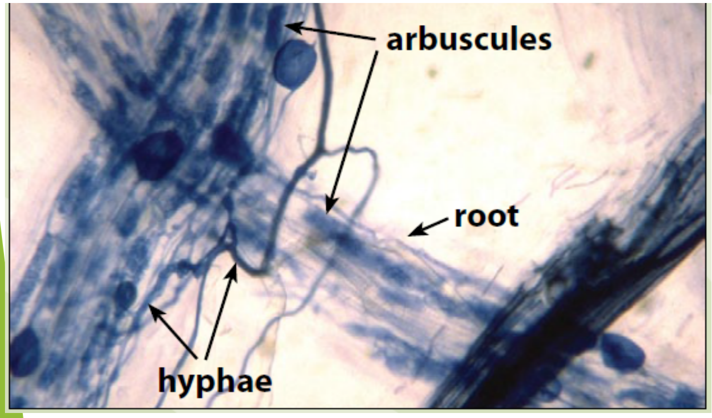
Mycorrhiza is a fungus which grows in association with the roots of a plant in a symbiotic or mildly pathogenic relationship.

- Myco comes from fungi

- Rhiza comes from roots

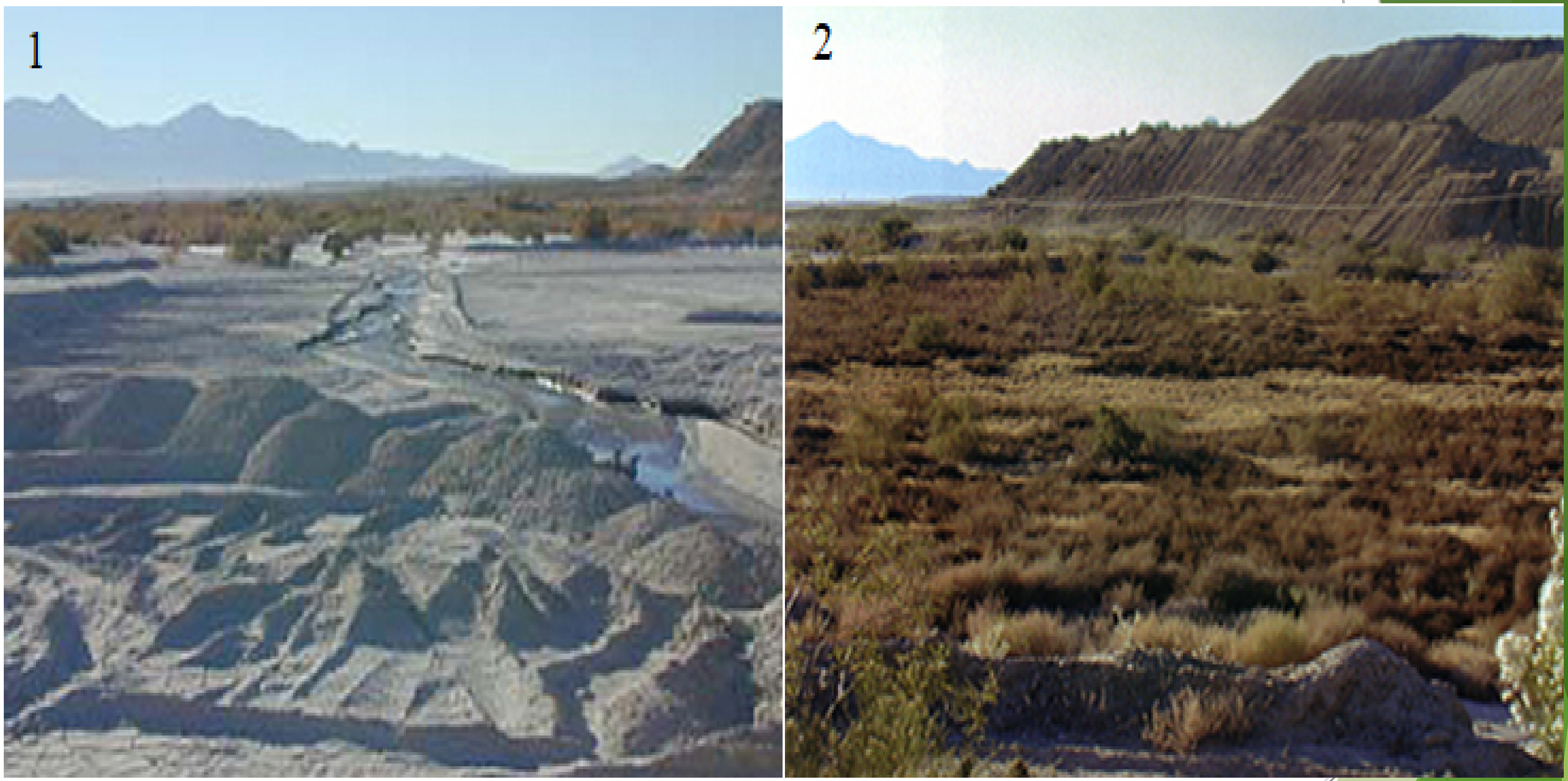
AMF - Arbuscular Mycorrhizal Fungi





- **Arbuscular mycorrhizal fungi** are soil borne microorganisms that form a **symbiotic association** named arbuscular mycorrhiza with plants.
- AMF promote stabilization of trace elements in rhizosphere of plants through the **hyphae, arbuscules and vesicles** favouring phytostabilization and phytoextraction.
- hyphal networks enable the host plant nutrient (predominantly phosphate), water and heavy metal uptakes

2. Sewage sludge application enhances phytoremediation



1) Mine tailings unamended
2) Mine tailings three years after biosolids amendment

The aim of the study

to investigate the effects of sewage sludge application and arbuscular mycorrhizal fungi interactions on the heavy metal phytoremediation in chrome mine tailings

The objectives of this study

- **to investigate the effect of different AMF species interactions**

AMF

- **to investigate sewage sludge addition on heavy metal phytoremediation in mine tailings**

Sludge

- **to investigate sunflower plants on heavy metal phytoremediation in mine tailings**

Sunflower

Material Methods

Sludge

- The sewage sludge was taken from an advanced wastewater treatment plant in Istanbul, Turkey.
- Applied to the mine tailings in two different concentrations of **20** and **30 g dry sludge/kg mine**

Mine Tailing Soils

The mine tailings were supplied from a Chrome Mine in Kütahya, Turkey

Mycorrhiza Species

- *Glomus mosseae*
- *Glomus intraradices*

indigenous to central Anatolia

Hyperaccumulating Plant

Sunflower

The Pot Experiments

- The pot experiments were carried out in a greenhouse for 3 months between August and October 2018.

- Eight different pot sets were prepared in three replicates (total of 24 pots).

- The control pots contained only mine tailings.

- pH of mine tailings was in a range of 7,9-8,5

- The bu 1, 7 g/cm^3

Pot Sets

- The pots contained mine tailings amended with sludge (20 and 30 g dry sludge/kg mine tailings) and/or inoculated with AMF species of ***G. mosseae*** or ***G. intraradices***

Contents of the Pots

No	Pots Sets	Mine Tailings (kg)	Sludge (dry) (g)	Sludge dose (g/kg tailings)	AMF species
1	M (contr)	2 kg	-	-	-
2	MMos	2 kg	-	-	*G. mosseae*
3	MS20	2 kg	40 g	20	-
4	MS20Mos	2 kg	40 g	20	*G. mosseae*
5	MS20Int	2 kg	40 g	20	*G. intraradices*
6	MS30	2 k	60	30	

Initial metal contents of chrome mine tailings and sewage sludge used in the study

- The sewage sludge and soil samples heavy metal concentrations characterised by using ICP-OES equipment after digesting the samples according to EPA 3051.

- The soil and metal concentration is showed in the table

Metals	Cr	Mn	Fe	Ni	Cu	Zn	Al	Cd	Pb	Si	Co	Mo
Mine Tailings	1218	672	26424	1585	8.84	17.1	29273	0	3.73	446	44.4	1.4
Sludge	709	461	86321	411	676	1815	72886	4	34	363	7	3

Plant sampling and analyses

Plant metal characterization

- Plant shoots were separated from the roots and placed in the papers bag to be dried at 70 ^{0}C for 2 days
- weighed and powdered to have homogenous samples.
- digested according to EPA 3052
- Metals were determined by using ICP-OES equipment

Mycorrhization Rates

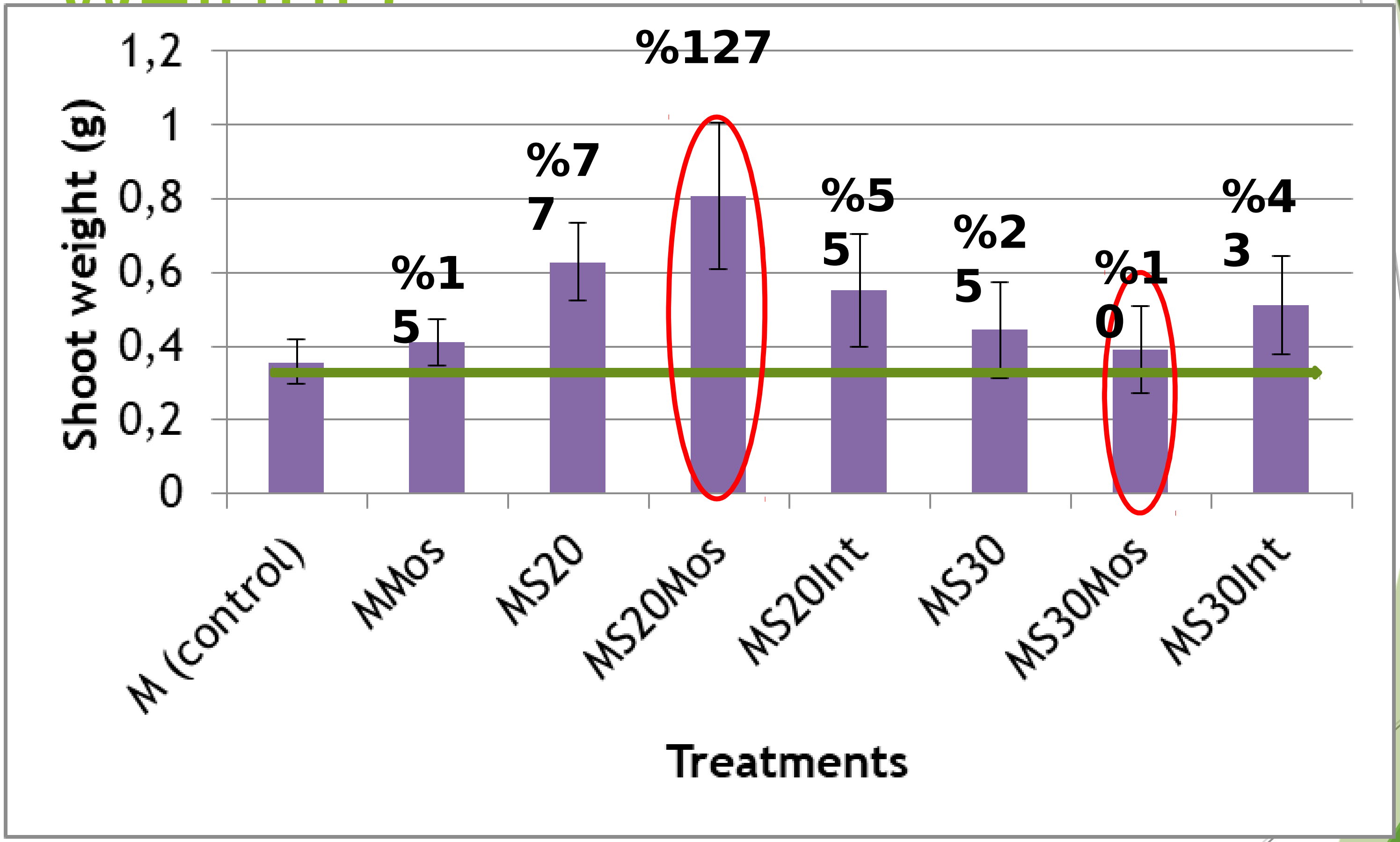
- Plant roots were put in 50 ml falcon tubes containing 70% ethyl alcohol and stored at +4 ^{0}C
- washed with KOH at 60 ^{0}C for 5 hours then washed with HCl and distilled water three times for 1 minute.
- Roots were packed in tulle and waited in lactic acid, glycerol distilled water and %0,05 trypan blue for 7 days in the darkness.
- The roots were aligned on the lam and the rates were determined by microscopic observation.

Glomalin Related Protein Determination

determined according to Bradford assay.

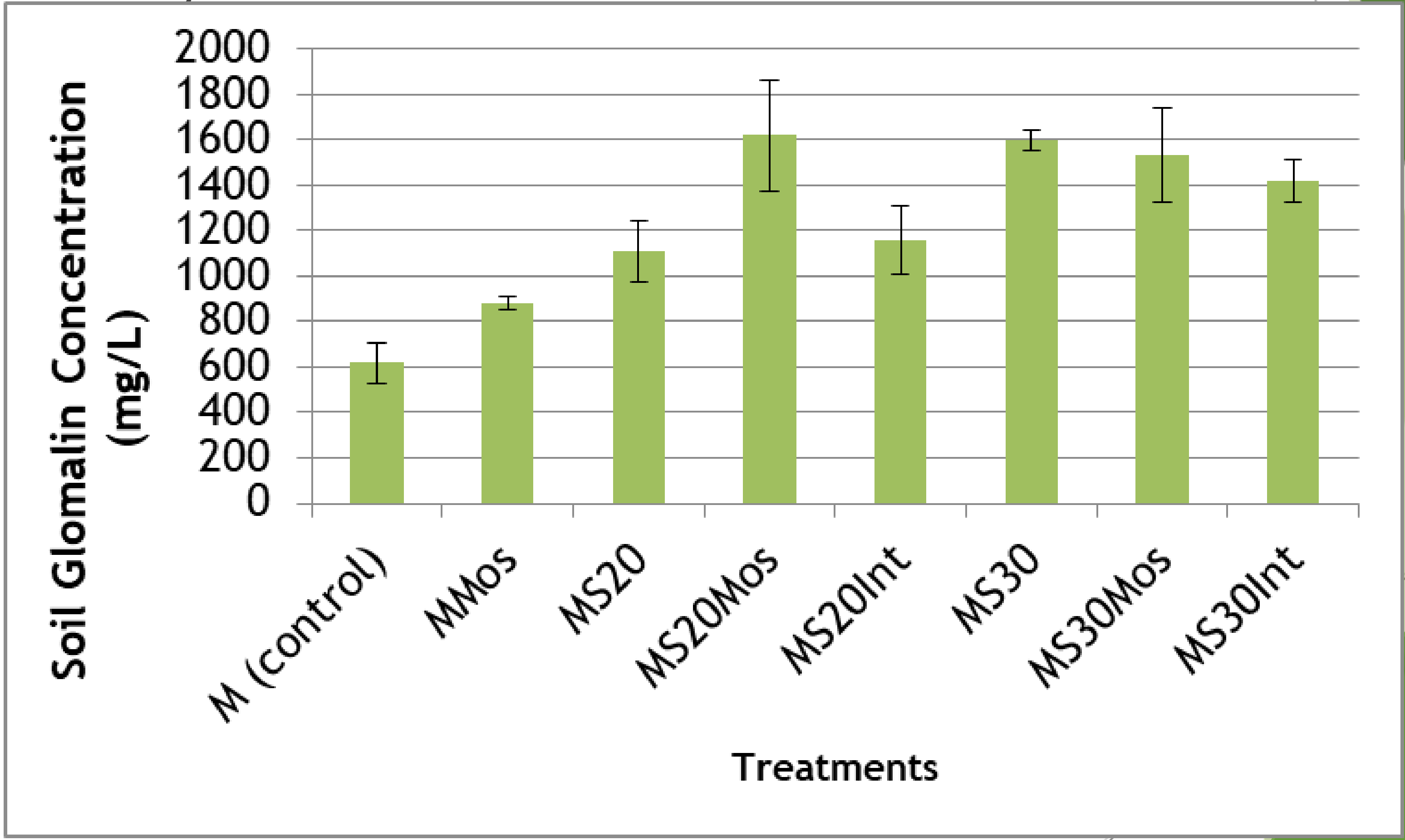
RESULTS AND DISCUSSION

Plant Growth (Sunflower Shoots Dry Weight)

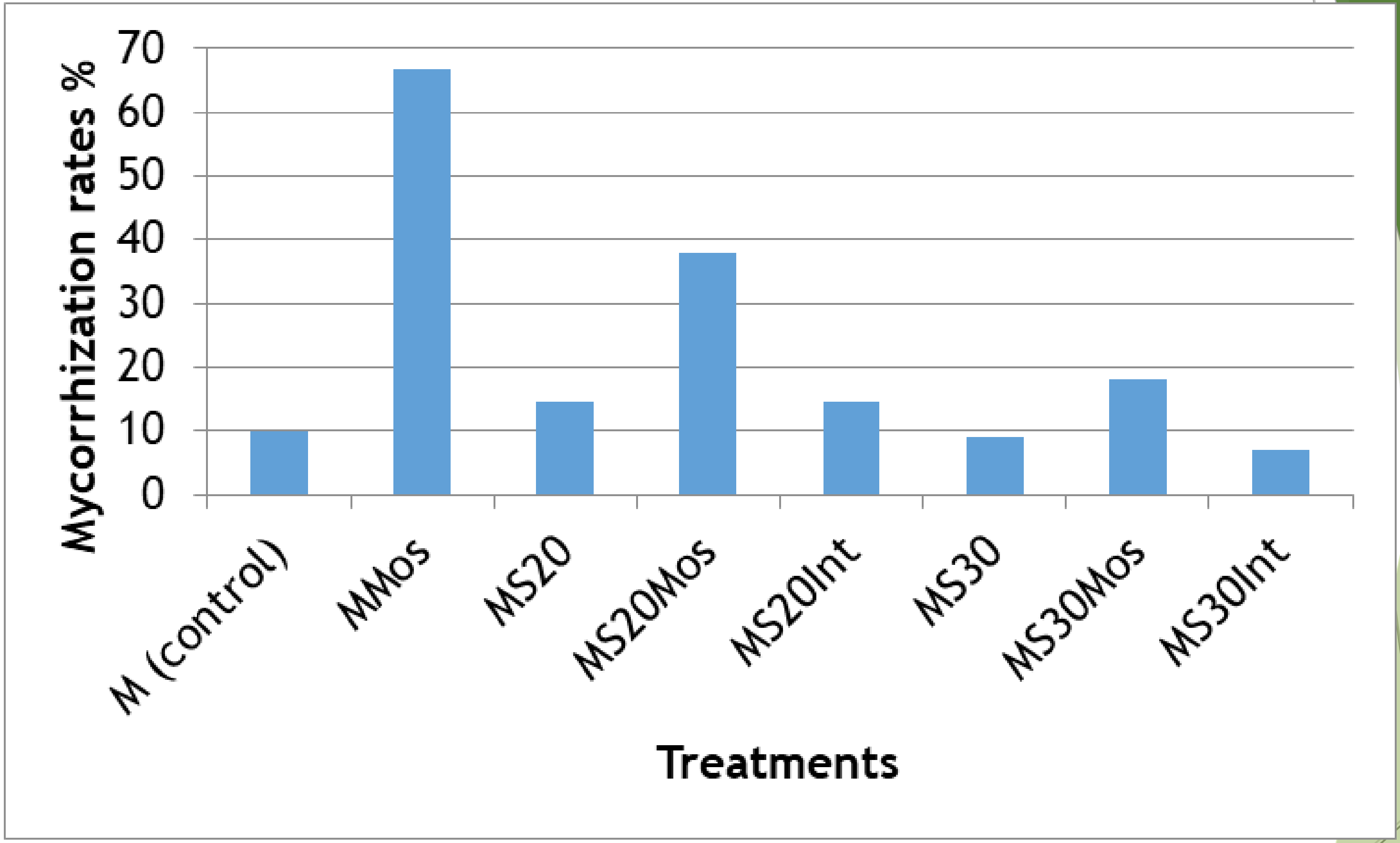


Glomalin Related Protein

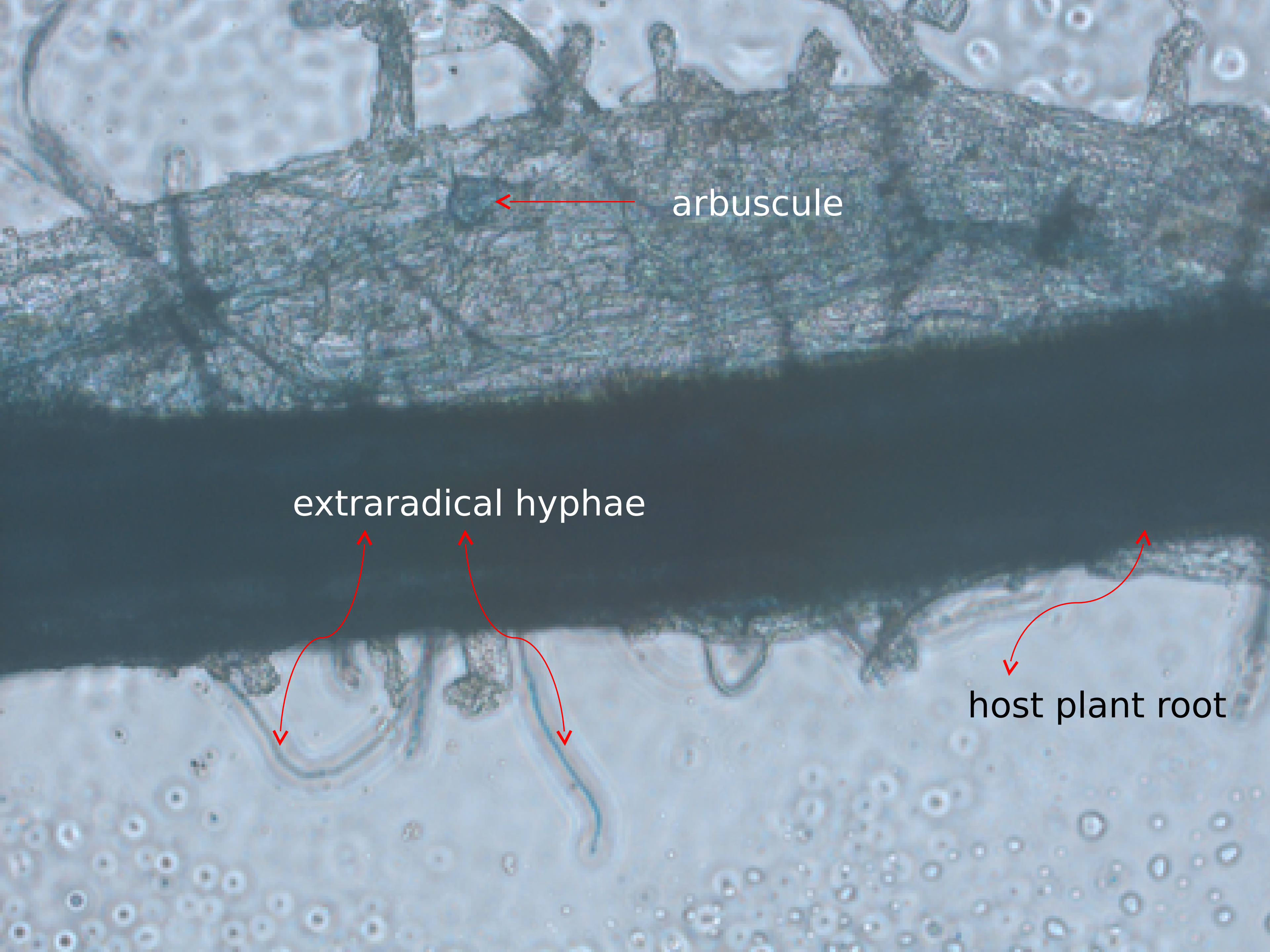
- Glomalin related protein increased with the mycorrhizal colonization and addition of sewage sludge.



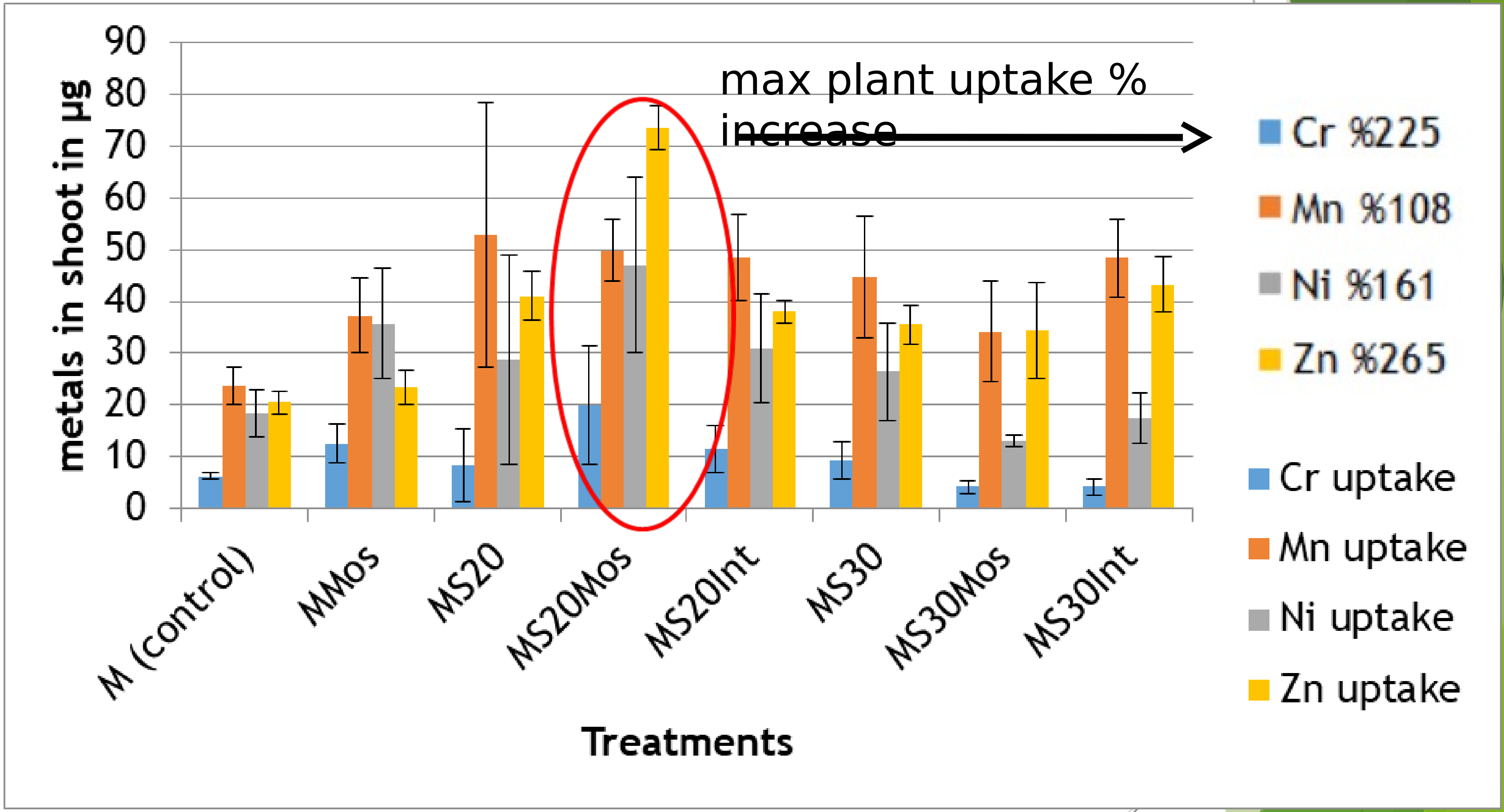
Mycorrhization Rates



- Mycorrhizal relationship decreases trough sewage sludge addition.
- Sludge application increases the vesicles.


arbuscule
extraradical hyphae
host plant root

Plant Shoots Heavy Metals Uptakes



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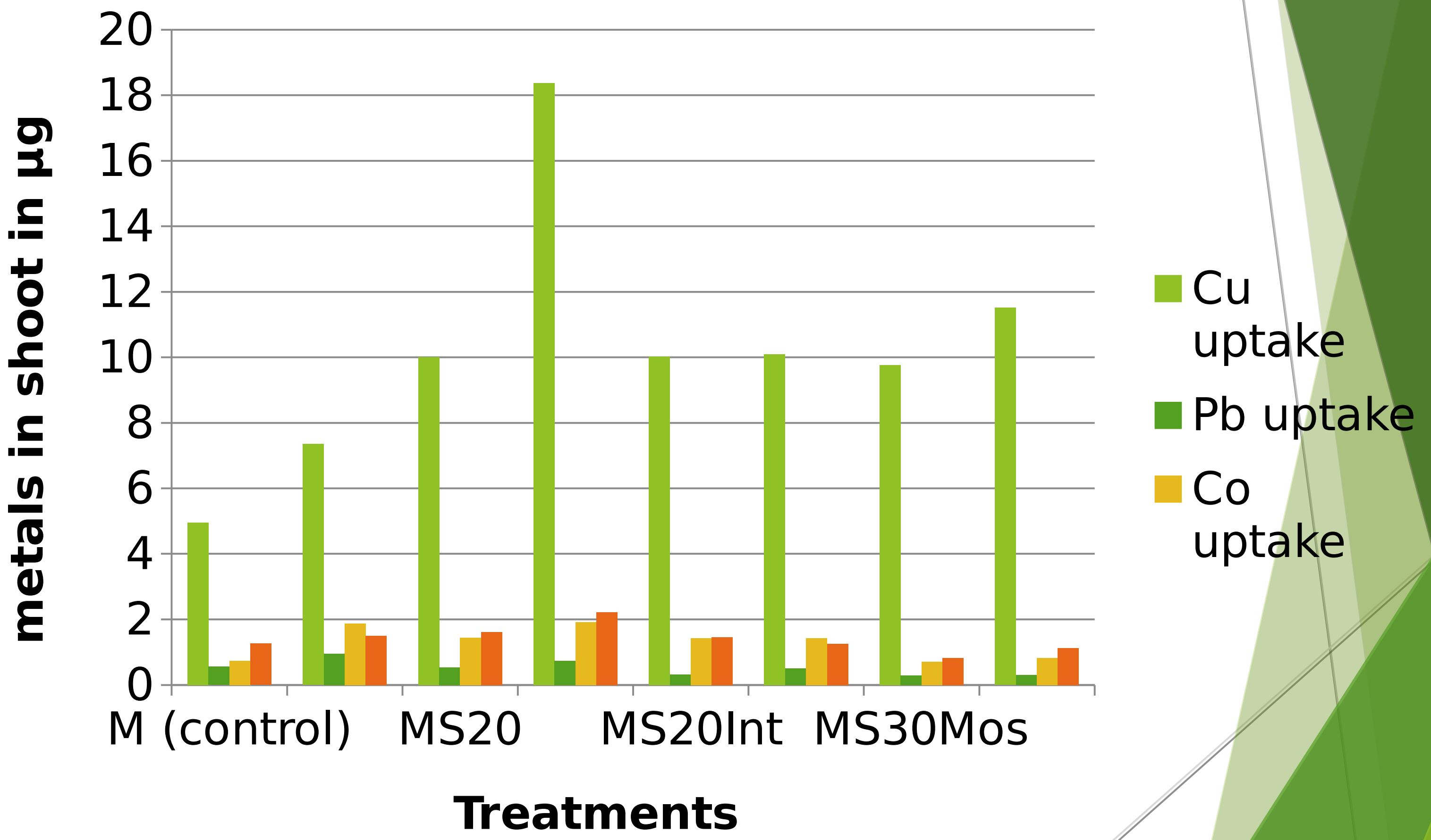

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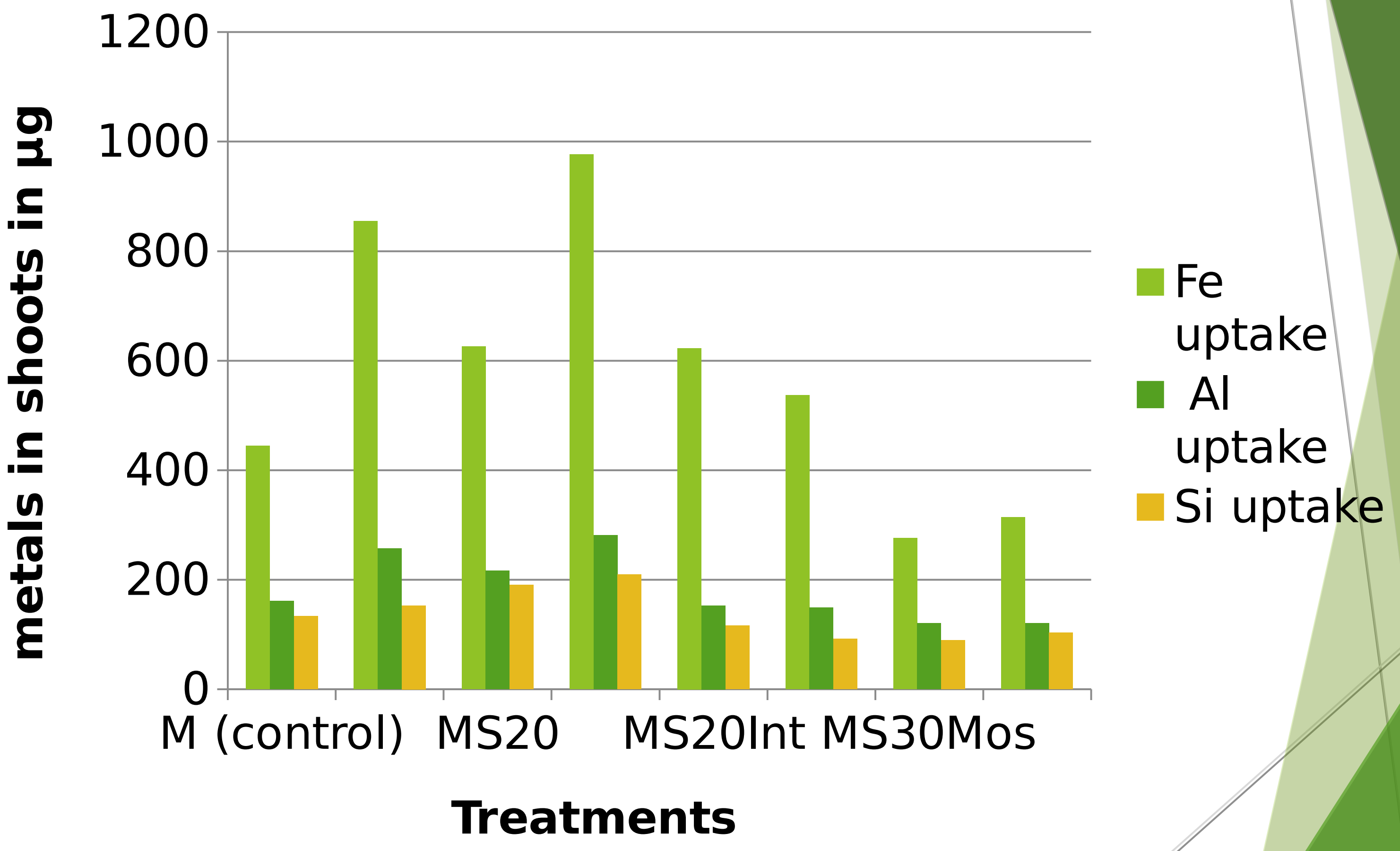

Conclusion:

- The sewage sludge application improved the growth of plants by supplying nutrients and increasing water holding capacity of the mine tailings.

- AMF association improved the efficiency of phytoremediation by enhancing the metal uptake of the plants.

- *G. mosseae* was found to be more effective than *G. intraradices* for sunflower mycorrhizal colonization.

- The combined AMF and 20g/kg sludge amendments resulted with the highest plant heavy metal uptakes and phytoremediation efficiency.

Conclusion:

- 30 g/kg of sludge application led to a negative effect on the mycorrhizal symbiotic relationship between plant and AMF due to increased soil nutrient concentration. The *Glomus mosseae* association supressed the plant growth due to the carbon competition between the host plant and the mycorrhizal fungi.

- The correlation between glomalin accumulation and certain metals uptake in plants shoot indicates the fact of metals sequestration by mycorrhizal fungi in the presence of glomalin.

- Soil bulk density may have crucial importance for phytoremediation efficiency with AMF association.

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THANK YOU

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