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# Comparison of root colonization by arbuscular mycorrhizal fungi in energy crop species cultivated on arable land contaminated with heavy metals

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## Introduction

Arbuscular mycorrhiza is a widespread terrestrial symbiosis, involving 80 – 85% of vascular plants in almost all ecosystems (Bonfante, Genre, 2010; Bothe et al., 2010; Zhu et al., 2012). AM fungi increase plant tolerance to heavy metals by developing mechanisms allowing immobilization metals within or near the root and preventing their translocation to the shoot. Therefore, AMF could be involved in phytostabilization of heavy metals contaminated soils (Rahmaty, Khara, 2011). Except soil contamination with heavy metals, fertilization seems to be an important factor affecting the development of arbuscular mycorrhiza. To improve yields and quality of crops, different fertilizers are used, including chemical fertilization and microbial inoculation (Dong et al., 2012; Pogrzeba et al., 2017).

The aim of the presented study was to explore the effect of heavy metals on arbuscular mycorrhizal fungi (AMF) root colonization of selected energy crop species: *Miscanthus* x *giganteus*, *Panicum virgatum* and *Spartina pectinata* cultivated on heavy metals contaminated arable land under different fertilisation (NPK vs. microbial inoculation).

## Material and methods

The experiment was conducted on contaminated arable land in Bytom (Upper Silesia), Poland (50°20'43.0"N 18°57'19.6"E) on the experimental site of the Institute for Ecology of Industrial Areas. Soil was characterized by heavy metals contamination, resulting from short distance from closed-down Pb/Zn smelter. Total soil Pb, Cd and Zn concentration exceed the maximum threshold values proscribed by Polish government (Dz.U. 2016 poz. 1395), excluding this area from food production. In October 2016, root samples of *M.* x *giganteus*, *P. virgatum* and *S. pectinata* were collected for estimation of mycorrhizal development. Roots were prepared according to a modified method of Phillips and Hayman (1970):

Digestion in 7% KOH in 80°C → Acidification in 5% lactic acid → Staining with 0.05% aniline blue → Washing in 0.3% acetic acid and storing in lactoglicerol

Subsequently, the evaluation of AMF colonization was conduct according to Trouevolt et al. (1986) method.

## Results

Studies show the presence of arbuscular mycorrhiza structures, i.e. intracellular hyphae, arbuscules and vesicles in all examined plants (Fig. 2-4). Furthermore, it has been shown, that influence of soil fertilization on AMF root colonization is species-dependent. Both NPK fertilization and microbial inoculation resulted in reduction of mycorrhizal colonization in *Miscanthus* x *giganteus*, whereas in *Panicum virgatum* degree of AMF root colonisation was increased. Soil fertilisation had no effect on degree of *Spartina pectinata* root mycorrhization, since they were almost fully colonized independly on fertilization (Fig. 1). Additionally, due to the presence of dark septate endophytic fungi only in *S. pectinata* (Fig. 4j), we hypothesized, that they may contribute in enhancing intensity of root colonization by arbuscular mycorrhizal fungi.

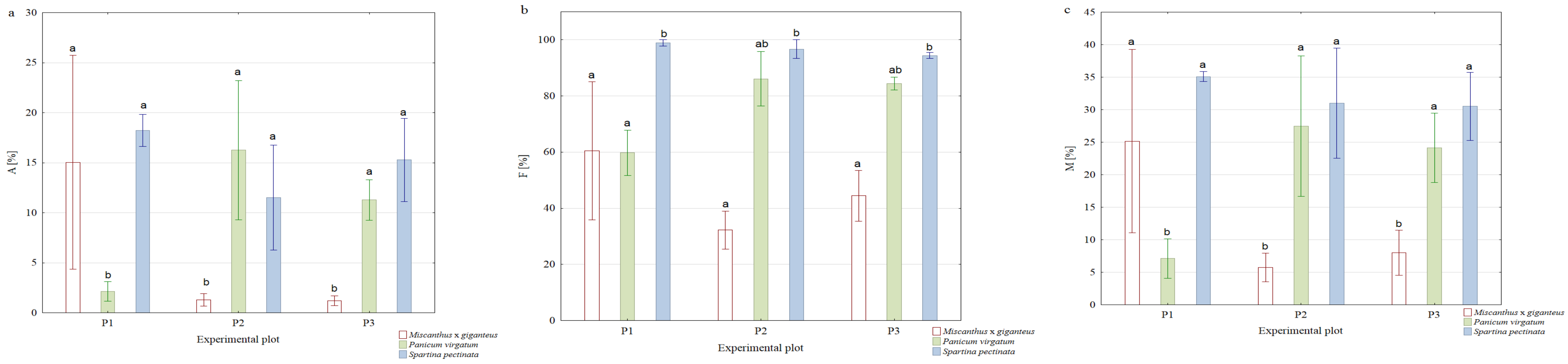


**Fig. 1.** Mycorrhizal colonization in *Miscanthus* x *giganteus, Panicum virgatum, Spartina pectinata* roots. a: the relative arbuscular richness (A%), b: mycorrhizal frequency (F%), c : relative mycorrhizal roots (M%). Values are means ± SE (n=3). Lower case letters (a, b) denote significant differences between plants in each experimental plot at $P \leq 0.05$ according to Fisher LSD test

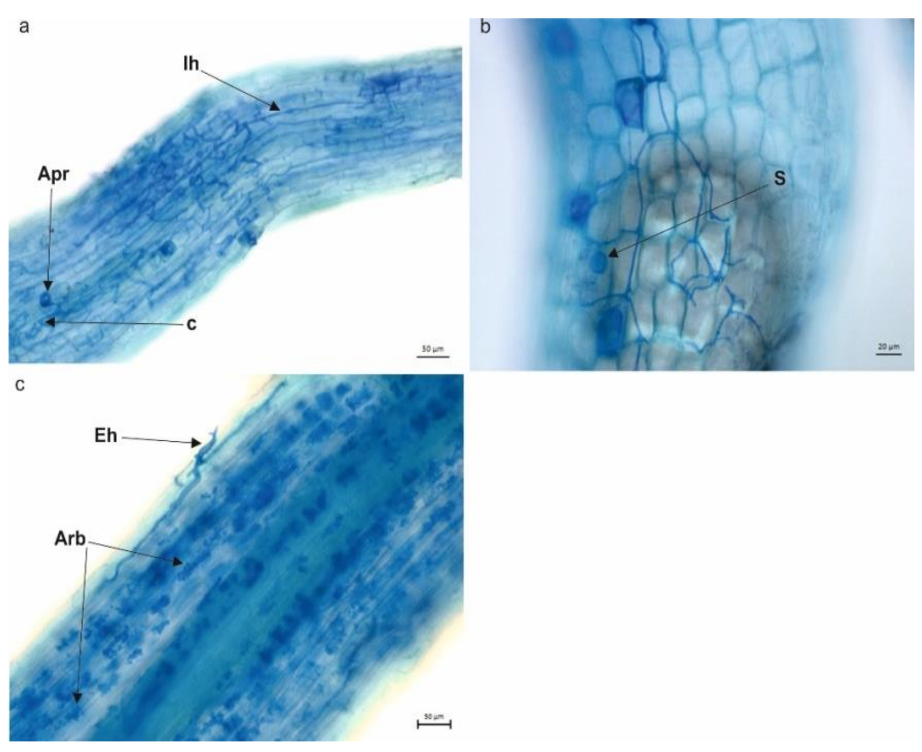


**Fig. 2.** Mycorrhizal structures in *Miscanthus* x *giganteus* roots. Signs: Apr (appressorium); c (coil); Ih (intracellular hyphae); S (spore); Eh (extracellular hyphae); Arb (arbuscules)

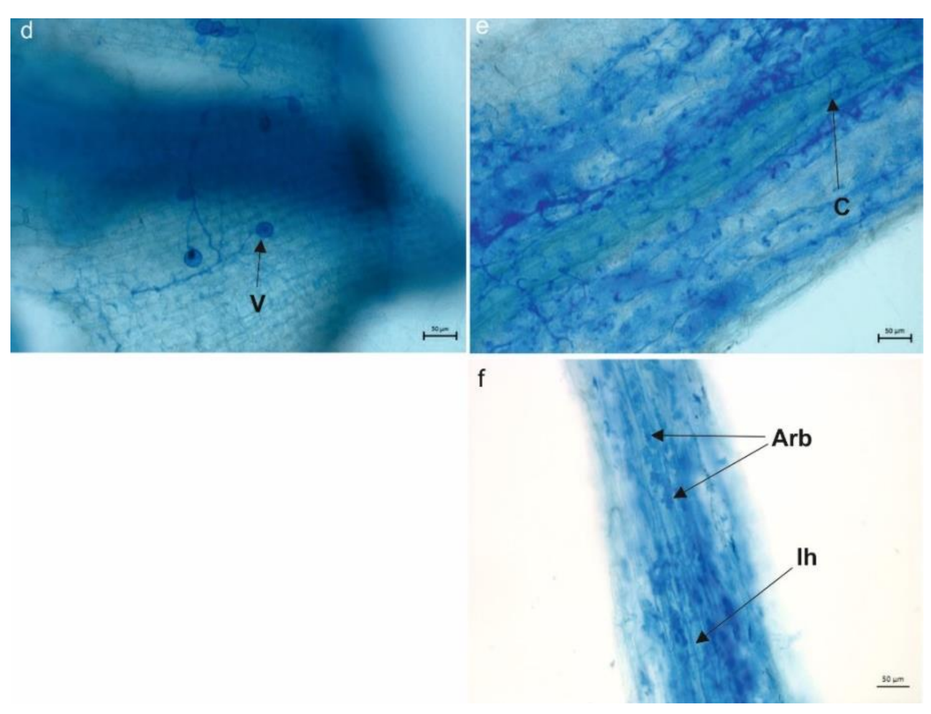


**Fig. 3.** Mycorrhizal structures in *Panicum virgatum* roots. Signs: v (vesicle); c (coil); Ih (intracellular hyphae); Arb (arbuscules)

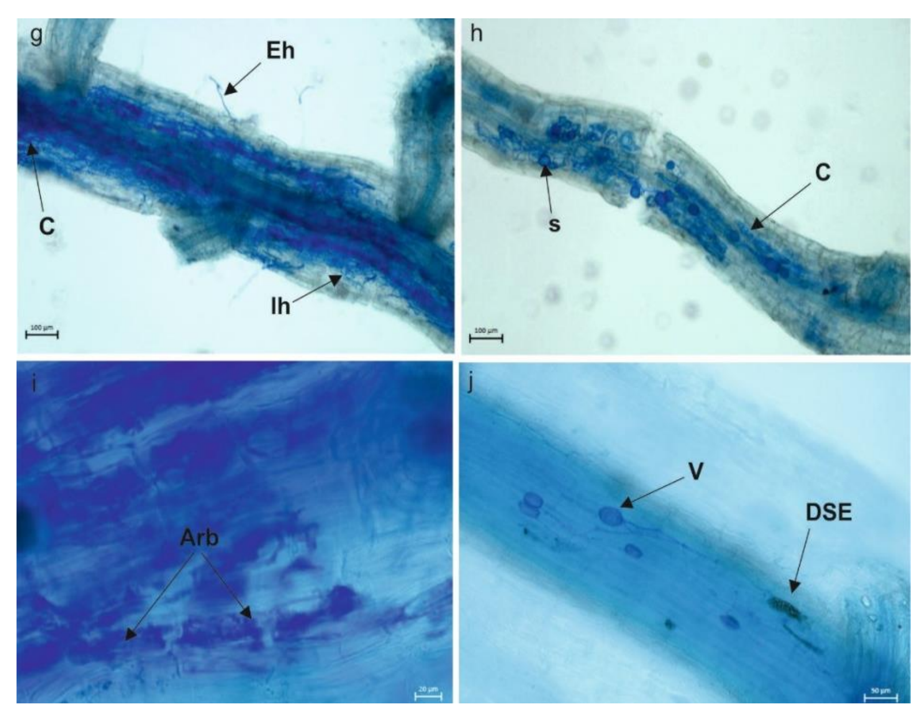


**Fig. 4.** Mycorrhizal structures in *Spartina pectinata* roots. Signs: Eh (extracellular hyphae); c (coil); Ih (intracellular hyphae); s (spore); Arb (arbuscules); v (vesicle); DSE (dematiaceous septate hyphae and microsclerotia of dark septate fungal endophytes)

## Conclusions

Soil contamination with heavy metals did not result in restriction of mycorrhizal colonization, which could be a consequence of AMF adaptation to extreme conditions. Nevertheless, application of both fertilizers reduced root colonization by arbuscular mycorrhizal fungi (AMF) in *Miscanthus* x *giganteus*, which could be resulted from depletion of AMF diversity in soil and also increased competition between microorganisms derived from inoculum and native AMF. In turn, positive influence of fertilization on degree of AMF root colonization in *Panicum virgatum* could eventuate from differences in specific fungal-plant interactions. Among tested species, the highest level of mycorrhizal colonization was found for *Spartina pectinata* roots in each experimental variant. It indicates the highest susceptibility of this species to development of mycorrhizal structures regardless of heavy metal pollution and fertilization.

## References

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