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# Mycorrhizal functioning as part of the survival mechanisms of barley (*Hordeum vulgare* L) at long-term heavy metal stress

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**ABSTRACT** Pot experiment was design to study the inside and outside root colonization of arbuscular mycorrhizal fungi (AMF) on the barley (*Hordeum vulgare* L) at various types and loadings of several heavy metals or toxic elements. Soils of the pots were originating from a long-term field experiment, where 13 metal salts, such as the Al, As, Ba, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, Sr, Zn was applied in 4 gradients (0, 30, 90, 270 $mg.kg^{-1}$ dry soil), 12 years prior the study. Beside the relative and absolute frequency (F%, M%), the arbusculum richness (a%, A%) and the sporulation intensity ($g^{-1}$ dry soil) of the AM fungi the biomass production and the element accumulation of the barley was also measured. The inside mycorrhizal colonization of the roots proved to be much less sensitive to the long-term heavy metal stress. Except the increased mycorrhizal sporulation at Ni (36 $g^{-1}$ soil), several toxic elements, such as the Al, As, Ba, Cr, Cu, Pb, Se, Sr and Zn could reduce the spore-numbers of the AM fungi significantly. This lower density, however was not affecting to the biomass production of the barley. At some metals with lower AMF sporulation an increased root (and fungal) biomass were found at the As, Ba, Cr, Cu and Cd elements. Increased arbusculum richness (A%) could be developed, on the other hand at the Hg and Pb metals. Such various mechanisms of the mycorrhizal functioning can offer a general aid for the host-plants to cope with the environmental stress, which could result a more balanced shoot (and yield) biomass production. **Acta Biol Szeged 49(1-2):65-67 (2005)**

**KEY WORDS**

heavy metal stress
mycorrhiza colonization
sporulation
long-term effects

Accumulating heavy metals and/or toxic elements are posing serious problems in the soil-plant-animal-human food chain (Kádár 1995). The long-term effect of those elements may threaten the microbial survival and the soil-functioning (Takács et al. 2000; Mikanová et al. 2001) in the different environments. The same influence of the drought and salinity was found on the Hungarian steppe (Hortobágy), with a reduced species diversity of the arbuscular mycorrhizal (AM) fungi (Landwehr et al. 2002). There was also an interrelation found, between the mycorrhizal status and the impact of salinity at some dominant halophytes (Füzy et al. 2003). Microbial abundance and the stress adaptation level of several microbial groups, such as the heterotrophs, oligotrophs and micromycetes were well correlating in those environmentally stressed ecosystems (Biró et al. 2002). Trustable field-experiments are necessary, therefore to follow the adaptation, selection processes of the soil- and rhizobiota. There are different effects of the rhizosphere microorganisms recorded, as a function of their adaptation level. The tolerant fungi in general can protect the host-plants more efficiently in comparison with the non-tolerant ones (Kaldorf et al. 1999; Takács and Vörös 2003; Vivas et al. 2003). There could be different mechanisms of this protection both from the macro- and the micro-symbiont sides. Relatively less study is known, however about the long-term effect of the heavy metals at a well-separated way from the cocktails of the other influencing elements (Biró et al. 1999). Among these stressed conditions there could be a various pattern found between the biotic and the abiotic environmental parameters (Tsimilli-Michael and Strasser 2002). The stress buffer effect of the arbuscular mycorrhyzal fungi and their colonization behavior was studied in heavy-metal spicked soil on a long-term level, but among controlled conditions.

## Materials and Methods

### Experimental background

Long-term field experiment was set up in 1991 on a calcareous chernozem soil by a single mass application of 13 microelement salts (Al, as, Ba, cd, Cr, Cu, Hg, Ni, Pb, Se, Sr and Zn) in 4 concentration levels (0, 30, 90 and 270 $mg.kg^{-1}$ soil). More detailes about the soil characteristics at Biró et al. 1998; Kádár 1995. The soils of this experimental background were sampled after a 12 years of metal-adaptation processes. Pot experiment was conducted by using the heavy metal contaminated soils (500 g in each) and the barley (*Hordeum vulgare* L) as test plants in a controlled light chamber.

The effect of the indigenous arbuscular mycorrhizal fungi (AMF) was studied on the biomass production (shoot and

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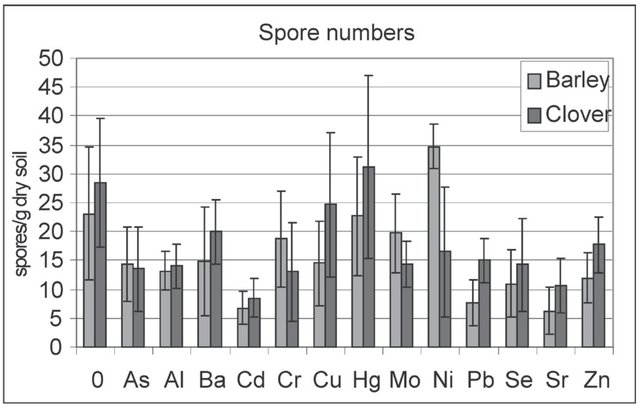


**Figure 1.** The effect of the long-term heavy metal loadings on the spore numbers of arbuscular mycorrhizal (AM) fungi in 1 g of rhizosphere soil of barley (*Hordeum vulgare* L) and clover (*Trifolium repens* L). The cumulative effect of the 30, 90 and 270 mg.kg$^{-1}$ metal doses are shown in comparison to the zero metal treatment. Results on the clover are not presented in the study.

root DW) and the element allocation after a three month of growth.

### Chemical analysis

Plant available metal content was assessed by the method of Lakanen and Erviö 1971. Plant metal contents were assessed after wet digestion with $HNO_3+H_2O_2$ with ICP-AES.

### Mycorrhizal parameters

The mycorrhizal colonization was estimated in the cleared and aniline-stained root samples. The five-class system of Trouvelot et al (1985) was used, which allow the calculation of the mycorrhizal frequency (F%) and intensity (M%), furthermore the relative and absolute arbusculum richness(a%, A%) in the sample or in the whole root systems. Results are shown for calculating the statistically relevant least significant differences ($LSD_{5\%}$) among the various metals and loadings.

## Results and Discussion

Respond of the barley-mycorrhiza system was found to be rather variable, as a function of the type and doses of the heavy metals on the particular soil. After the 12 years of heavy metal stress, there were non-significant metal accumulation found in the shoot biomass of the barley at the As, Al, Hg, Cu, Ni and Pb, while a clear dose-effect could be realized at the Ba, Cd, Cr, Mo, Sr, Se and Zn. This element accumulation however was not accompanied with the shoot-dry-weight reduction. No significant differences were found as a cumulative effect of the studied microelements. An especially enhanced root biomass production was realized on the other hand at the Cd, as a protecting mechanism of the host-plant against the toxic element accumulation. The dry weight of the roots has increased from 0,6 g (control) to 1,2; 1,0 and 1,5 as a function of the increasing heavy metal dosis (30, 90, 270 mg.kg$^{-1}$ dry soil, respectively).

The functioning of the arbuscular mycorrhizal fungi was highly depending from the studied microelements. The mycorrhizal colonization was less affected inside the root-system, which means, that except the reduced effect of Cd, no significant changes was found in the mycorrhizal intensity (M%). The arbusculum richness (A%), however was improving at the Hg and Pb elements, significantly. This increased activity enhanced the phosphorous accumulation in the shoot from 2451- to 3078 mg.kg$^{-1}$ in case of the Pb. There was a strong dose-dependency found at the arbusculum richness in general, which means that the mycorrhizal functioning (A%) was significantly enhanced from 40 (control) to 53 at the 90 mg.kg$^{-1}$ metal dosis. The sporulation of the mycorrhizal fungi was found as the most sensitive parameter at the long-term heavy metal stress. This fact was not supporting the other findings on the short-term effects of metals at the same experimental background (Biró et al. 1998). Through the adaptation, selection processes the finally reduced sporulation, however supports the hypothesis, that the AM fungi outside the root system can be more efficiently targeted by the metal toxicity. According to the Figure 1 a significantly reduced sporulation was recorded at the Al, As, Ba, Cr, Cu, Pb, Se, Sr and Zn microelements, while the Ni loadings could increase the spore numbers from 22 (control) to 36 peace.g$^{-1}$ dry soil. As it was previously reported by (Takács et al. 2000) for the Cd, Zn and Ni microelements those reduced spore numbers was found to be positively correlating with the spore types and the species richness at the long-term heavy metal contamination. According to Meharg (2003) such diversity of the mycorrhizal fungi on metal polluted sites can be in considerable contrast to the above ground flora, which is always greatly diminished on highly metal contaminated sites. This suggests that mycorrhizal fungi are inherently more flexible in coping with metal pollutants (Vivas et al. 2003) compared to the vascular plants. Such mycorrhizal behavior and the high versatility of the mycorrhizal parameters inside and outside the root systems may be key to the function on polluted sites.


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