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# The arbuscular mycorrhizal fungus *Glomus mosseae* induces growth and metal accumulation changes in *Cannabis sativa* L.

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**Abstract**

The effect of arbuscular mycorrhiza on heavy metal uptake and translocation was investigated in *Cannabis sativa*. Hemp was grown in the presence and absence of 100 $\mu g\,g^{-1}$ Cd and Ni and 300 $\mu g\,g^{-1}$ Cr(VI), and inoculated or not with the arbuscular mycorrhizal fungus *Glomus mosseae*. In our experimental condition, hemp growth was reduced in inoculated plants and the reduction was related to the degree of mycorrhization. The percentage of mycorrhizal colonisation was 42% and 9% in plants grown in non-contaminated and contaminated soil, suggesting a significant negative effect of high metal concentrations on plant infection by *G. mosseae*.

Soil pH, metal bioavailability and plant metal uptake were not influenced by mycorrhization. The organ metal concentrations were not statistically different between inoculated and non-inoculated plants, apart from Ni which concentration was significantly higher in stem and leaf of inoculated plants grown in contaminated soil. The distribution of absorbed metals inside plant was related to the soil heavy metal concentrations: in plant grown in non-contaminated soil the greater part of absorbed Cr and Ni was found in shoots and no significant difference was determined between inoculated and non-inoculated plants. On the contrary, plants grown in artificially contaminated soil accumulated most metal in root organ. In this soil, mycorrhization significantly enhanced the translocation of all the three metals from root to shoot. The possibility to increase metal accumulation in shoot is very interesting for phytoextraction purpose, since most high producing biomass plants, such as non-mycorrhized hemp, retain most heavy metals in roots, limiting their application.


*Keywords:* Industrial hemp; Heavy metals; Arbuscular mycorrhizal symbiosis; *Glomus mosseae*; Phytoremediation

## 1. Introduction

Phytoextraction is the use of higher plants to remove inorganic contaminants, primarily metals, from

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polluted soil (Lasat, 2002). In this approach, plants capable of accumulating and tolerating high levels of metals, termed hyperaccumulators, are grown in contaminated soil. At maturity, metal-enriched aboveground biomass is harvested and a fraction of soil metal contamination removed. Unfortunately, to date most of the identified metal hyperaccumulators are small and slow growing (Khan et al., 2000). These properties have an adverse impact on the potential for metal phytoextraction and restrict the employment of this technology. In the effort to contribute to overcome this difficulty, during the last three years we have investigated the metal tolerance and accumulation abilities of industrial hemp, *Cannabis sativa*, a fast growing and high biomass producing plant. We demonstrated that, although hemp cannot be defined as a hyperaccumulator for cadmium, nickel and chromium, but as a metal tolerant organism that has evolved mechanisms allowing it to cope with high metal concentration in soil, the amount of Cd and Ni accumulated in its shoot was not negligible (Linger et al., 2002; Citterio et al., 2003). In addition, hemp is an industrial crop with multiple non-food uses and is an excellent rotation and companion crop. The possibilities of easily growing hemp in different climates and using its biomass in non-food industries can make heavy metal contaminated soils productive, and, although slowly, restore them at the same time. It signifies economic advantage along with a better quality of soil. Therefore, any effort, to enhance *C. sativa* capacity to absorb and translocate metal from root to shoot, is of interest.

Metal uptake by plants can be influenced by soil microorganisms that intimately associate with the plant roots to form the rhizosphere community (Shilev et al., 2001). It is well known that mycorrhizal fungi are a major component of the rhizosphere and form mutualistic associations (mycorrhizae) with most plant species (Azcòn-Aguilar and Barea, 1992). Of these, the arbuscular mycorrhizae (AM) are by far the most widespread (Smith and Read, 1997). Benefits to the plant include improved nutrition (see Clark and Zeto, 2000, for review), through extensive extraradical hyphal networks, which explore the soil, absorb nutrients and translocate them to the roots (Giovannetti et al., 2002), and root system modifications, generally resulting in a more extensive length and increased branching, and therefore in a more efficient nutrient absorption (Berta et al., 2002). In addition, AM fungi have been shown to enhance tolerance of biotic and abiotic stresses, including heavy metals (Leyval et al., 2002): as they are a direct link between soil and roots, they can be very important for heavy metal availability and toxicity to plants (Leyval et al., 1997). The AM symbiotic status changes the chemical composition of root exudates (Laheurte et al., 1990; Barea et al., 2002) and influences the soil pH (Li et al., 1991), thus quantitatively and qualitatively affecting the microbial populations in the rhizosphere and/or in the rhizoplane (Azcòn-Aguilar and Barea, 1992; Linderman, 1992; Barea, 1997). All these factors, alone or in combination, can influence metal mobility or availability; nevertheless, the role of AM fungi in the uptake and in the transfer of heavy metals to the plant is still poorly understood and literature results are conflicting. Some reports indicate that AM fungi enhance plant accumulation and tolerance of heavy metals, as occurs in *Helianthus annuus* or *Glycine max* when associated with *Glomus intraradices* and *Glomus mosseae*, respectively (Davies et al., 2001; Jamal et al., 2002). Other show that AM fungi enhance heavy metal uptake and translocation, but at high metal concentration and low pH they are disadvantageous for the plant, whose growth is depressed (Galli et al., 1994). By contrast reduced heavy metal concentrations were found in other mycorrhizal associations. Joner and Layval (1997) showed that in the subterranean clover colonized by *G. mosseae*, the transfer from the fungus to the plant is restricted, due to fungal immobilization. Similar results were obtained for uranium uptake by mycorrhizal carrot roots under root-organ culture conditions (Rufyikiri et al., 2003). The bulk of evidence seems to suggest a species-specific effect of AM associations on root metal uptake.

In order to optimize the heavy metal mobilization/absorption and/or the root to shoot translocation in *C. sativa*, the present study was designed to evaluate the influence of root colonization by the AM fungus *G. mosseae* on hemp ability to tolerate and accumulate Cd, Ni and Cr.

## 2. Materials and methods

### 2.1. Experimental design

Three pots for each treatment, with a diameter of 0.45 m and a depth of 0.65 m were used to germinate and grow *C. sativa* L. cv Carmagnola. 84 kg of 3% organic matter soil, pH 7.5–8.0 obtained by mixing sterilised quartz sand (0.5 mm coarse grade) with autoclaved sowing potting compost (Duemme, Reggio Emilia, Italy), were used to prepare each control pot (*C*) and each heavy metal contaminated pot (HM). The compost characteristics were: organic C = 20% (weigh percentage on dry product), organic N = 1%, organic matter = 35%, pH: 7. Soil for HM pots was contaminated with 300 $\mu g\,g^{-1}$ potassium dichromate, 100 $\mu g\,g^{-1}$ nickel chloride and 100 $\mu g\,g^{-1}$ cadmium sulphate. The soil contamination was performed before sowing by adding the right amount of heavy metals dissolved in distilled water to 12 kg of soil and mixing throughout. Seven 12 kg-layers of this contaminated soil were used to fill up each HM pot.

Additional 504 kg of 3% organic matter soil, pH 7.5–8.0 obtained as above reported but substituting 148 kg of quartz sand with inoculum of *G. mosseae* BEG 12 (Biorize, Dijon, France), were used to prepare the remaining two type of pots: one type for hemp growth in the presence of the AM fungus *G. mosseae* (*G.m.*) and another type for hemp growth in the presence of the AM fungus *G. mosseae* and heavy metals (*G.m.* + HM). The contamination of the soil for the latter type of pot was performed with the same heavy metal concentrations and with the same method used for the HM pot type. Before sowing, the total and bioavailable metal concentrations in soils were measured in 3 soil samples collected from each pot (Fig. 1), applying the USEPA 3051a metal extraction methodology reported below. The fraction of phosphorous available for plant was quantified by the Olsen method (Olsen et al., 1954). It was $0.02 \pm 0.001$ mg g$^{-1}$.

The prepared pots were placed in field conditions (inside Milan Botanical garden) to grow hemp in natural environment. The hemp seeds were germinated at the end of April 2002 to reach a plant density of about 100 m$^{-2}$ (15 plants per pot). During cultivation, minimal and maximal temperature ranged from 5 to 15 and 25 to 34 °C, respectively. Drinking water and natural precipitation were used as water source for plants; the excess of water flowing from the bottom of the pots was poured back on the respective soil. The total amount of precipitation in Milan during the four months of cultivation was about 950 mm as calculated by the information from the Lombardia Regional Agency for Agriculture Development (ERSAL).

The plants were analysed about four months after germination at ripeness stage. Plants were harvested by upsetting the pots. The soil was carefully removed and the roots were kept intact as far as possible, to assess the plant reaction to heavy metal and/or to *G. mosseae* presence. Plant growth (dry biomass), the mycorrhizal colonization indices, the heavy metal content in roots, stems, leaves and seeds were determined for each treatment.

### 2.2. Evaluation of mycorrhizal colonization

*G. mosseae* root colonization was evaluated in mycorrhizal roots of each treatment, after trypan blue staining (Trouvelot et al., 1986). The parameters of mycorrhizal infection were the extent of root cortex colonization (M%) and the arbuscule abundance in the whole root system (A%).

### 2.3. Plant growth measurements

Plant growth was assessed by determining plant organ dry weight (DW) just after plant harvest, 152 d from seed germination. Leaves, stem and roots of each plant were separated, cut in small parts and placed in

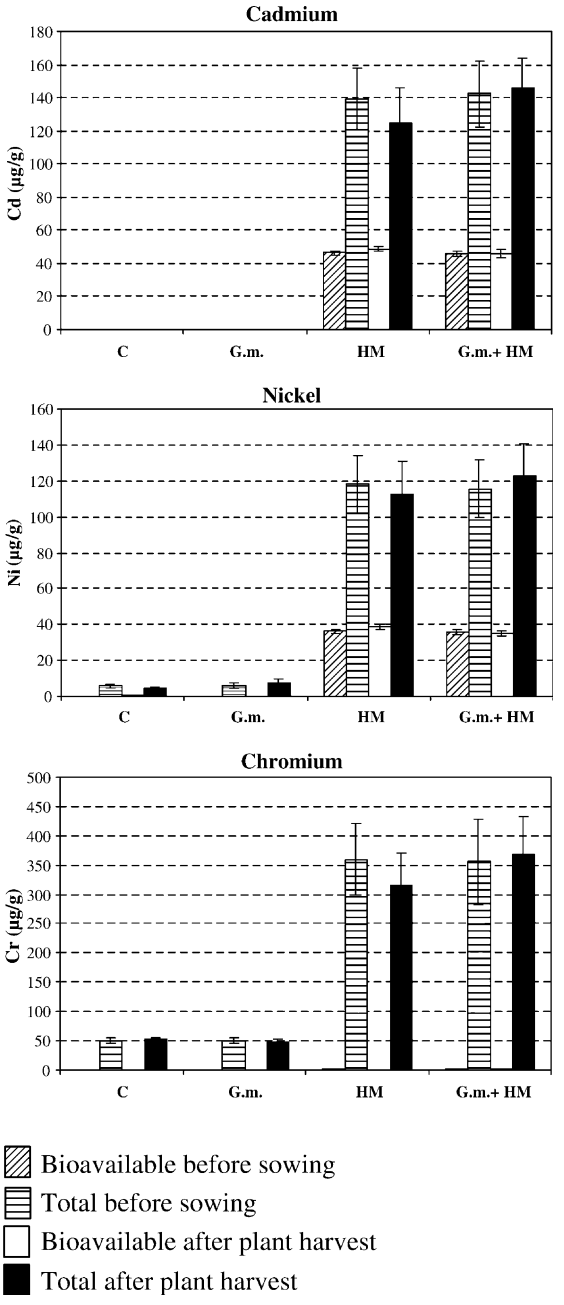


Fig. 1. Bioavailable and total content (µg g$^{-1}$) of Cd, Ni and Cr in soil before sowing and after plant harvest. *C* = control soil; *G.m.* = inoculated plants grown in non-contaminated soil; HM = non-inoculated plants grown in artificially contaminated soil; *G.m.* + HM = inoculated plants grown in contaminated soil. Data are the mean ± SE of nine soil samples (three from each pot).

a dry cabinet at 40 °C until a constant weight was reached. Then they were weighed and used for the other determinations.

### 2.4. Analytical methods for heavy metal quantification

Total cadmium, chromium and nickel were quantified in the plant and soil samples applying the USEPA 3051a protocol. The harvested plants were carefully washed with tap water and then with distilled water before analysis. All the samples (soil and plant) were dried at 100 °C overnight. For each sample 10 ml of $HNO_3$ and 2 ml of $HClO_3$ were added to 0.2 g of dry soil or 0.5 g of dry plant matter. The samples were digested by using the ETHOS HPR 100/10 microwave lab station (FKV, Bergamo, Italy) reaching the 180 °C temperature by different steps. After their complete mineralization, they were diluted to 10 ml with MilliQ water (Millipore, Bedford, MA, USA) and centrifuged at 3000*g* for 15 min at 25 °C. The supernatants were then diluted again with MilliQ water to reach 0.2% $HNO_3$. Standards (from ENEA Research Centre, Roma, Italy) and blanks were run with all sample series for quality control. In particular "Antartic marine sediment certified reference material for trace element" was used as standard. Chemical analyses were carried out by graphite furnace atomic absorption spectroscopy (GFAAS; SIMA 6000, Perkin-Elmer). The limits of detection were 2, 50 and 10 $\mu g\,kg^{-1}$ for Cd, Ni and Cr, respectively. At least 9 soil samples before and after plant growth, 15 plants for each treatment and 3 samples for each plant organ were analysed.

For bioavailable metal quantification in soil samples, the protocol of Lindsay and Norwell (1969) suitable for metal extraction from non-acid soils was applied. 10 g of soil were extracted with 20 ml of 5 mM DTPA (Sigma), 0.1 M trietanolamine (Merck) and 0.01 M $CaCl_2$ (Merck), for 2 h at 20 °C under stirring. Samples were then filtered and heavy metal concentrations were determined by GFAAS. Nine soil samples for each treatment were analysed.

### 2.5. Statistical analysis

Data were statistically analysed by Statgraphics plus program for Windows (version 5.0, Manugistic, MD, USA): t-student test, for two-sample comparison, or ANOVA and Duncan test, for multiple sample comparison, were applied when normality and homogeneity of variance were satisfied. Data, which did not conform to the assumptions, were alternatively transformed into logarithms or were analysed by Mann Whitney or Kruskal-Wallis non-parametric procedures (for two or multiple sample comparison, respectively).

## 3. Results

### 3.1. Soil heavy metal content

Total and bioavailable concentrations of soil Cd, Ni and Cr, determined by AAS before sowing and after plant harvest, are reported in Fig. 1. Before sowing, in non-contaminated soils, Cr and Ni concentrations were about 50 and 5 $\mu g\,g^{-1}$, whereas Cd was below the instrument detection limit. The contaminated soils contained a metal concentration corresponding to the sum of the metals naturally present in the 3% organic matter and the metals artificially added. The available Cd, Ni and Cr for plant was assessed measuring the amount of DTPA-extractable metals. By this methodology, as expected on the basis of literature data (Han et al., 2002), a considerable quantity of bioavailable Cd and Ni and a negligible amount of Cr (less than 1%) were estimated in HM and *G.m.* + HM soils. No statistically significant difference in both total and bioavailable metal contents was found between inoculated and non-inoculated soils before seed germination.

After plant harvest, the measurement of total metals in the four different soils was not statistically different from that obtained before sowing (Fig. 1) as expected on the basis of the results previously achieved (Citterio et al., 2003). The only difference, although not statistically significant, registered after hemp harvest, was the lower percentage of bioavailable Cd and Ni in inoculated soil (31% and 29%) compared with that in non-inoculated soil (39% and 34%).

### 3.2. Mycorrhizal colonization

Fig. 2 shows the degree of mycorrhizal colonization (M%) and the relative abundance of arbuscules (A%) in inoculated and non-inoculated plants grown in artificially contaminated and non-contaminated soil. No mycorrhization was observed in roots of non-inoculated hemp plants, whereas all the plants roots inoculated with *G. mosseae* were mycorrhized. Percentages of mycorrhizal colonization and of arbuscules were 42 and 37.8 in plants grown in *G.m.* soil and 9 and 6.1 in plants grown in *G.m.* + HM soil. The differences between the mean percentages were statistically significant (Fig. 2). The behaviour of fungal structures was apparently normal.

### 3.3. Plant growth

On average, mycorrhizal plants grown in metal contaminated (*G.m.* + HM) and non-contaminated soils (*G.m.*) produced lower root and shoot yields than the corresponding non-mycorrhizal ones (HM and *C*).

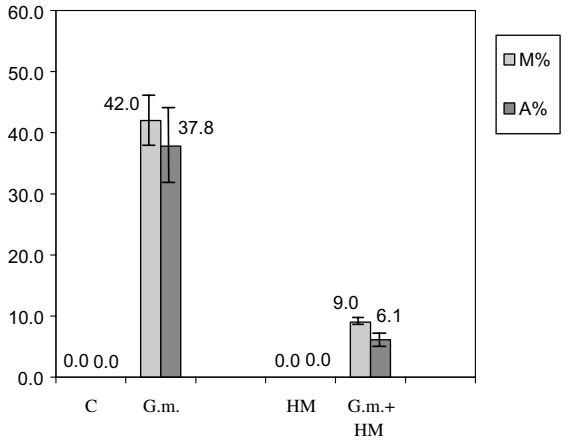


Fig. 2. Mycorrhizal colonization (M%) and the relative abundance of arbuscules (A%) in inoculated and non-inoculated plants grown in metal contaminated and non-contaminated soil. Mean percentages ± SE relative to 15 plants for each treatment (five from each pot) are reported. *C* = plants grown in control soil; *G.m.* = inoculated plants grown in non-contaminated soil; HM = non-inoculated plants grown in artificially contaminated soil; *G.m.* + HM = inoculated plants grown in contaminated soil. *G.m.* and *G.m.* + HM are statistically different ($P \leqslant 0.05$).

Inoculated plants grown in non-contaminated soil showed a mean dry biomass of 1.8 ± 0.2 g, a value statistically different from that of non-inoculated plants grown in the same type of soil (4.0 ± 0.7 g). Similarly, in contaminated soil the growth of inoculated plants (4.2 ± 0.4 g) was less than that of non-inoculated plants (4.8 ± 0.4 g), although the difference was not statistically significant. The biomass reduction of the mychorrizal plants was related to the lesser development of roots, stems and leaves (Table 1) and was directly correlated to the percentage of *G. mosseae* infection.

### 3.4. Metal accumulation in C. sativa tissues

The metal concentrations determined by AAS in hemp organs at ripeness are reported in Table 2. Low levels of all the metals were found in root and shoot of plants grown in absence of added metals, since the bioavailable metal concentrations in soil were very low (Fig. 1). On the contrary, in plants grown on artificially contaminated soil the concentrations of Cd, Ni and Cr were higher, although they were lower than those obtained in the previous experiments in which we used a different hemp variety. As expected, the higher concentrations of Cd, Ni and Cr were found in roots.

Mycorrhization did not increase the organ metal concentrations, apart from Ni whose concentration was significantly higher in stem and leaf of inoculated plants grown in contaminated soil (*G.m.* + HM).

Taking into account the dry weight and the metal concentrations of the single plant organs, the mean content (μg) of metals in the shoot and in the whole plant was calculated for each type of soil (Table 3). On average, considering the whole plants, the amount (μg) of metals was lower, although not statistically significant, in inoculated plants. By contrast, considering only the shoot, the metal quantities were generally higher in inoculated plants, but also in this case, the differences were not statistically significant. Using these values the distribution of heavy metal in shoot and root was determined and reported in Fig. 3. It can be observed that in plants grown in non-contaminated soil the greater part of absorbed Cr and Ni was found in the shoot and there was no significant difference between inoculated and non-inoculated plants. On the contrary, plants grown in artificially contaminated soil accumulated most of metal in the root organ. In this soil mycorrhization enhanced the translocation of all the three heavy metals from root to shoot: the amount of metal in the shoot compared with the total absorbed metal was in fact statistically different in inoculated and non-inoculated

Table 1
Dry biomass of hemp plants inoculated or not with *Glomus mosseae* and grown in the absence or presence of the Cd, Ni and Cr concentrations reported in Fig. 1

| | Dry weight (mean + SE) | | | |
|---|---|---|---|---|
| | Root | Stem | Leaf | Seed |
| *C* | 0.6 ± 0.18 A | 2.5 ± 0.38 A | 0.5 ± 0.10 A | 0.6 ± 0.11 A |
| *G.m.* | 0.2 ± 0.01 B | 1.1 ± 0.21 B | 0.3 ± 0.02 B | 0.4 ± 0.01 A |
| HM | 0.8 ± 0.12 A | 3.1 ± 0.27 A | 0.5 ± 0.03 A | 0.6 ± 0.08 A |
| *G.m.* + HM | 0.6 ± 0.09 A | 2.8 ± 0.29 A | 0.5 ± 0.02 A | 0.5 ± 0.10 A |

*C* = plants grown in control soil; *G.m.* = inoculated plants grown in non-contaminated soil; HM = non-inoculated plants grown in artificially contaminated soil; *G.m.* + HM = inoculated plants grown in contaminated soil.
Root, stem and leaf data are the mean of 15 plants (five from each pot), whereas seed values are the mean of female plants.
Means with the same letter are not significantly different ($P \leqslant 0.05$).

Table 2
Cd, Ni and Cr concentrations in plant organs

| | Root | Stem | Leaf | Seed |
|---|---|---|---|---|
| *Cadmium concentration* ($\mu g\,g^{-1}$) | | | | |
| *C* | n.d. | n.d. | n.d. | n.d. |
| *G.m.* | n.d. | n.d. | n.d. | n.d. |
| HM | 217.2 ± 26.9 A | 12.0 ± 1.4 A | 12.1 ± 0.9 A | 7.7 ± 1.1 A |
| *G.m.* + HM | 163.9 ± 20.0 A | 16.6 ± 1.8 A | 11.2 ± 0.7 A | 7.8 ± 0.6 A |
| *Nickel concentration* ($\mu g\,g^{-1}$) | | | | |
| *C* | 4.3 ± 0.9 A | 0.8 ± 0.4 A | 4.8 ± 0.4 A | n.d. |
| *G.m.* | 7.6 ± 1.0 A | 0.2 ± 0.1 A | 4.9 ± 0.6 A | 2.3 ± 1.3 A |
| HM | 112.2 ± 14.4 B | 19.4 ± 2.0 B | 12.8 ± 0.4 B | 17.0 ± 2.8 B |
| *G.m.* + HM | 122.5 ± 13.7 B | 29.1 ± 2.5 C | 17.6 ± 1.5 C | 17.1 ± 1.7 B |
| *Chromium concentration* ($\mu g\,g^{-1}$) | | | | |
| *C* | 1.0 ± 0.2 A | 0.8 ± 0.4 A | 1.8 ± 0.2 A | n.d. |
| *G.m.* | 1.8 ± 0.2 A | 0.5 ± 0.2 A | 2.9 ± 0.4 A | 0.2 ± 0.2 A |
| HM | 47.5 ± 6.0 B | 1.0 ± 0.2 A | 4.3 ± 0.3 B | 0.2 ± 0.2 A |
| *G.m.* + HM | 45.7 ± 7.2 B | 1.3 ± 0.2 A | 4.1 ± 0.3 B | 0.1 ± 0.1 A |

Mean values of 15 plants (five from each pot) for each treatment ± standard error are reported. Seed means are referred to only female plants. Means with the same letter are not significantly different ($P \leqslant 0.05$).
n.d.: below detection limit.

Table 3
Metal mean μg in shoot and entire plant

| | Cadmium | | Nickel | | Chromium | |
|---|---|---|---|---|---|---|
| | Shoot | Plant | Shoot | Plant | Shoot | Plant |
| *C* | n.d. | n.d. | 3.9 ± 0.8 A | 5.4 ± 1.0 A | 2.7 ± 0.8 A | 3.2 ± 0.9 A |
| *G.m.* | n.d. | n.d. | 2.2 ± 0.3 A | 3.2 ± 0.4 A | 1.6 ± 0.4 A | 2.0 ± 0.4 A |
| HM | 43.2 ± 3.8 A | 197.1 ± 25.2 A | 73.3 ± 9.4 B | 154.8 ± 19.0 B | 4.7 ± 0.6 B | 37.9 ± 5.6 B |
| *G.m.* ± HM | 49.7 ± 4.6 A | 137.1 ± 16.9 A | 88.4 ± 8.4 B | 150.6 ± 14.9 B | 5.0 ± 0.5 B | 27.4 ± 4.0 B |

Data are the mean ± SE of 15 plants (five from each pot).
Means with the same letter are not significantly different ($P \leqslant 0.05$).

plants, revealed by applying ANOVA test after percentage transformation.

## 4. Discussion

To study the effect of mycorrhizal fungi on plant ability to tolerate and accumulate heavy metals is not straightforward. The results depend not only on the specific species of interacting plant and fungus but also on the experimental conditions. Temperature, light, pot size, soil composition etc. can influence the results (Galli et al., 1994; Joner and Layval, 2001). We chose to perform our experiment in seminatural conditions using big pots containing well characterised soil and adding as homogeneously as possible known concentrations of heavy metals. *G. mosseae* was selected on the basis of literature data reporting its presence in heavy metal contaminated soils (Chaudhry et al., 1999; Khan et al., 2000; Hayes et al., 2003), and its ability to enhance heavy metal uptake in other plant species (Jamal et al., 2002; Vivas et al., 2003).

In our experimental conditions, *G. mosseae* negatively affected hemp growth. Growth decrement was proportional to the degree of mycorrhization, significantly lower in plants developed in artificially contaminated soil. These last observations are consistent with papers reporting that elevated levels of metals reduced AM root colonization (Chao and Wang, 1990, 1991; Leyval et al., 1997). The reduction of hemp size at ripeness should be discussed in terms of net costs:benefits of the symbiosis (Johnson et al., 1997) and of mycorrhizal plant dependency (Azcòn and Barea, 1997).

Though the relationships between plants and AM fungi are generally reported as mutualistic (Smith and Read, 1997), neutral or negative plant responses have sometimes been observed (Johnson et al., 1997). The reason is to be sought in those genetic and environmental factors which determine a net cost of mycorrhizal association exceeding net benefit (Baath and Hayman,

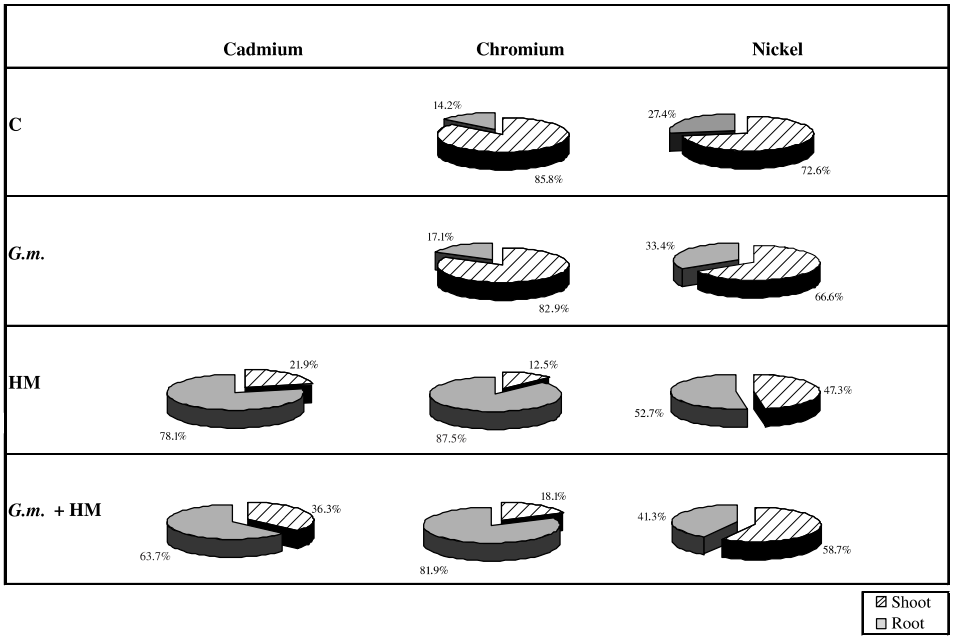


Fig. 3. Heavy metal distribution (percentage) in plant shoot and root.

1984; Fitter, 1991). In our system, the factors could be related to the characteristics of hemp, which is a high biomass plant with a wide root system, efficiently scavenging for nutrients: no additional effect could therefore be expected from AM, as the cost of the symbiosis for the plant, in terms of organic carbon, exceeded the benefits conferred by the fungus. Hemp is probably a poorly mycorrhizal dependent plant; in the literature, there are, to our knowledge, no data in this regard, except contradictory citations concerning a plant of the same family (*Humulus lupulus* L., Harley and Harley, 1987).

Hemp characteristics combined with our experimental conditions including the fungal inoculum, could be responsible for the small effect (positive or negative) of AM on heavy metal uptake. On average, only in the case of nickel, was the metal concentration statistically higher in stem and leaves of inoculated plants. For the other two metals (Table 2), no significant difference between inoculated and non-inoculated plants was observed. This lack of a substantial mycorrhizal effect on metal uptake should also be related to the lack of the mycorrhizal influence on soil pH and metal bioavailability. Only a slight non-significant decrease in bioavailable Cd and Ni percentages was measured in inoculated pots. The metal distribution in plant organs was, on the contrary, different: mycorrhizal plants grown in contaminated soil translocated a higher percentage of all three metals from root to shoot (Fig. 3). This disagrees with that part of the literature data that suggests that AM attenuate the toxic effect of metals, retaining them in the fungal structure with the subsequent restriction of metal transfer to the plant (Joner and Layval, 1997; Vivas et al., 2003). On the other hand, it was also reported that this AM protective effect depends on the plant species and even on the plant variety (Rivera-Becerril et al., 2002) and that AM fungi do not necessarily prevent metal uptake by the host plant; on the contrary, they may be active in the uptake process, since mycorrhizal plant species growing on mine tailings have been shown to be metal accumulators (Hayes et al., 2003). We may speculate that the mycorrhizal association *G. mosseae–C. sativa* (var. Carmagnola) simulates the hyperaccumulating plant species, enhancing the root to shoot metal translocation to sequester the exceeding toxic metals in the shoot cell vacuoles by means of molecules such as metallothioneins and phytochelatins (Assunçao et al., 2003). In this regard, in a different experiment with the same mycorrhizal association, we noticed a qualitative and quantitative difference in plant organ phytochelatins correlated to fungal presence (data not shown). Moreover, heavy metal stress has recently been shown to increase the trancription of a phytochelatin synthetase gene in mycorrhizal pea roots, in which the expression of three metallothioneins genes was also enhanced (Rivera-Becceril, unpblished data).

In general, it is currently accepted that the presence of heavy metals in the soil induces changes in the pattern of plant gene and protein expression (Robinson et al., 1994; Prasad, 1995; Hajduch et al., 2001). Recent findings by Repetto et al. (2003) showed that also mycorrhizal

symbiosis modulates the expression of several plant proteins. The significance of these changes and their relation to plant tolerance toward xenobiotics are not yet clear.

Ongoing research is presently aimed at investigating the role of *G. mosseae* in metal translocation from hemp root to shoot, since one of the limits of phytoextraction technology is related to the retaining of metals in roots of plants producing high biomass, that for this reason cannot be considered hyperaccumulators. At the same time, the effect of hemp mycorrhization on metal uptake in natural field, without any restriction in soil exploring by hyphae should be evaluated.

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