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Arbuscular mycorrhiza of *Deschampsia cespitosa* (Poaceae) at different soil depths in highly metal-contaminated site in southern Poland

Ewa Gucwa-Przepióra[1]*, Janusz Błaszkowski[2], Renata Kurtyka[3], Łukasz Małkowski[1], Eugeniusz Małkowski[3]

[1] Department of Plant Systematics, University of Silesia, Jagiellońska 28, 40-032 Katowice, Poland

[2] Department of Plant Pathology, West Pomeranian University of Technology, Słowackiego 17, 71-434 Szczecin, Poland

[3] Department of Plant Physiology, University of Silesia, Jagiellońska 28, 40-032 Katowice, Poland

Abstract

This study presents root colonization of *Deschampsia cespitosa* growing in the immediate vicinity of a former Pb/Zn smelter by arbuscular mycorhizal fungi (AMF) and dark septated endophytes (DSE) at different soil depths. AMF spores and species distribution in soil profile were also assessed. Arbuscular mycorrhiza (AM) and DSE were found in *D. cespitosa* roots at all investigated soil levels. However, mycorrhizal colonization in topsoil was extremely low with sporadically occurring arbuscules. AM parameters: frequency of mycorrhization of root fragments (*F*%), intensity of root cortex colonization (*M*%), intensity of colonization within individual mycorrhizal roots (*m*%), and arbuscule abundance in the root system (*A*%) were markedly higher at 20–40, 40–60 cm soil levels and differed in a statistically significant manner from AM parameters from 0–10 and 10–20 cm layers. Mycorrhizal colonization was negatively correlated with bioavailable Cd, Pb and Zn concentrations. The number of AMF spores in topsoil was very low and increased with soil depth (20–40 and 40–60 cm). At the study area spores of three morphologically distinctive AMF species were found: *Archaeospora trappei*, *Funneliformis mosseae* and *Scutellospora dipurpurescens*. The fourth species *Glomus tenue* colonized roots of *D. cespitosa* and was observed in the root cortex at 20–40 and 40–60 soil depth, however, its spores were not found at the site.

Keywords: arbuscular mycorrhiza (AM), soil depth, heavy metals, Glomeromycota, DSE, grasses, *Glomus tenue*

Introduction

Arbuscular mycorrhizal fungi (AMF) are a natural constituent of soil in most ecosystems. It has been estimated that over 80% of all vascular plants form arbuscular mycorrhizas [1]. AMF have a positive effect on plant mineral nutrition and increase tolerance of plants to drought, heavy metals and other environmental stress factors [2–5]. These fungi are a crucial contributor to the formation and maintenance of soil structure as well as constitute a significant component of soil organic matter [6].

Heavy metal contamination of soil is a worldwide problem that affects large number of sites [7–10]. Particularly high concentrations of Cd, Pb, Zn were recorded in soils contaminated by nonferrous mining and smelting activities [11–13]. At such sites in Silesia, an industrial region of southern Poland, total concentration of Cd, Pb and Zn in soil exceeds 500, 14000, 12000 mg kg^{-1} respectively [14–16].

A very high concentration of these metals in soil results in poor development of natural vegetation [17] and has a negative influence on the development of AMF [18–20]. Nevertheless, mycorrhizal plant species, the most common of which are grasses, are capable of growing on highly contaminated soils, e.g. *Agropyron repens* [21,22], *Festuca ovina* sensu lato [23] or *Deschampsia cespitosa* [16,17,22,24]. These mycorrhizal plants and the associated with their roots AM fungi play an important part in areas affected by industry, such as former mining areas or waste sites, since they are able to accelerate their revegetation [25–29].

Most mycorrhizal studies are generally restricted to the main rooting zone (0–20 cm soil depth) [30–32]. There are a few studies where AMF have been examined at different soil levels. It was found that the percentage of roots colonized by AM fungi [33–35], the amount of their extraradical hyphae [36] as well as their spore abundance and species richness decreased [37] with increasing soil depth. However, there is a dearth of data on vertical distribution of AM at the sites contaminated with heavy metals.

Recently Gucwa-Przepióra et al. [16] studied the effect of phytostabilization practices on the AM development in the roots of *Deschampsia cespitosa* (L.) PB in soils contaminated by nonferrous mining and smelting activities. In addition, they investigated the colonization at different soil depths. They

* Corresponding author. Email: ewa.gucwa-przepiora@us.edu.pl

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demonstrated that mycorrhizal colonization was very limited in the upper soil layers, where bioavailable concentrations of Cd, Pb and Zn were high, and increased with soil depth. In contrast, in non-metal contaminated soils a decrease in mycorrhizal colonization with soil depth was observed [33–35].

In this paper we focused on evaluating the effect of soil depth on AMF colonization parameters in *Deschampsia cespitosa* roots growing spontaneously in the immediate vicinity of the former Zn/Pb smelter, where interactions among heavy metals (Zn, Pb and Cd) and soil constituents were long-lasting. The abundance of AMF spores and AMF species in soil were also investigated. In addition the colonization of roots by dark septated endophytes (DSE) was assessed.

Material and methods

Site description

The experimental site is located at a former mine and smelter area, situated between the towns Bytom and Piekary Śląskie, in the Upper Silesia industrial region of southern Poland (N 50°21′59.64″, E 18°58′17,90″). The mine and smelter operated for approximately 70 years. In 1989 the production stopped, all the facilities were closed down and dismantled. The study area, where *D. cespitosa* grew spontaneously in large numbers is located in the immediate vicinity of the former smelter.

Collection of soil and root samples

On 3 August 2005 root samples of *D. cespitosa* were taken from the investigated area in order to check its mycorrhizal status and to examine the vertical distribution of AM development. An approx. 70 cm deep trench was dug along three large *D. cespitosa* specimens. Four blocks of soil approx. 1 m long and 15 cm wide containing roots of *D. cespitosa* were taken, each at different soil level: 0–10, 10–20, 20–40 and 40–60 cm. From a single block of soil four samples of plant roots for mycorrhizal studies were collected. From the same block of soil also three samples were taken to conduct chemical analyses of soil and to determine arbuscular fungi spores and species. At the laboratory the soil samples were air-dried and sieved through 2 mm screen for chemical analyses and for identification of spores.

Mycorrhizal studies

EVALUATION OF ROOT COLONIZATION PARAMETERS. For the estimation of mycorrhizal development *D. cespitosa* roots were prepared according to the modified method of Phillips and Hayman [38]. After careful washing with tap water, the roots were softened in 7% KOH for 24 h, washed in water, acidified in 5% lactic acid in water for 1–24 h, and stained with 0.01% aniline blue in 5% lactic acid for 24 h at room temperature. The stained roots were stored in lactoglycerol until they were used for slide preparation. Five parameters of mycorrhizal colonization were evaluated microscopically using thirty 1-cm root fragments per sample and calculated as percentages: frequency of mycorrhization of root fragments (*F*%), intensity of root cortex colonization (*M*%), intensity of colonization within individual mycorrhizal roots (*m*%), arbuscule abundance in the root system (*A*%) and arbuscule richness in root fragments where the arbuscules were present (*a*%; http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html) [39].

ISOLATION AND IDENTIFICATION OF AMF SPORES. AMF spores were extracted from 50 g of sieved (<2 mm) air-dried soil from rooting zone by wet sieving and decanting [40]. Morphological properties of spores and their subcellular structures were determined on spores mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG) [41] and a mixture of PVLG and Melzer's reagent (1:1, v/v). Spores were crushed to varying degrees by applying pressure to the cover slip and then stored at 65°C for 24 h to clear their contents from oil droplets. These were examined under an Olympus BX 50 compound microscope equipped with Normarski differential interference contrast optics [42].

Soil analyses

Physical and chemical properties of the soil samples collected from four levels were analyzed. Soil texture was determined by hydrometer method developed by Prószyński [43]. Soil pH (H_2O) and electrical conductivity (*EC*) were measured in water suspension (soil to solution ratio 1:2.5) after 24 h of equilibration. Cation exchange capacity [cmol(+)/kg] was determined according to Houba et al. [44]. The fraction of bioavailable metals was obtained by extraction of 3 g of air-dried soil (ground to <0.25 mm) with 30 ml 0.01 M $CaCl_2$ for 5 h; total metal content was determined after extraction of air-dried soil ground to <0.25 mm with concentrated $HClO_4$ and HF. Concentration of metals was analyzed by using flame atomic absorption spectrophotometry (Varian Spectra AA300).

Statistical analysis

Normality tests Kolmogorov–Smirnov with the Lilliefors correction for the mycorrizal colonization and soil parameters data were run. The applied normality tests showed that the distribution of most of the variables was normal. Data, which did not meet test requirements, were transformed. Then data were subjected to one-way ANOVA. Significant differences among mean values were calculated using the LSD test. Pearson's correlation coefficients were calculated between particular mycorrhizal parameter (*F*%, *M*%, *m*%, *a*%, *A*%) and the concentration of metals (Cd, Zn, Pb, Cu). The probability level of 0.05 or less value was considered to be statistically significant. The statistical analysis was performed using the computer software Statistica version 7.1 (StatSoft Inc., Tulsa, OK, USA).

Results

Soil properties

The substrate sandy silt loam was characterized by high content of organic matter and relatively low cation exchange capacity (Tab. 1). Soil pH was close to 7.0 in the upper layer and decreased with increasing soil depth with the lowest value of 5.18 in the 40–60 cm layer (Tab. 2). Soil *EC* was low and did not exceed 130.5 μS cm^{-1}. Highest *EC* levels were found in the top 10 cm of soil and they decreased with soil depth. The soil pH showed a similar trend (Tab. 2). The total concentrations of Cd, Pb and Zn were very high in the topsoil (0–10 cm) and decreased with the increasing soil depth. The same trend was observed in concentrations of bioavailable Cd, Pb and Zn (Tab. 2). The concentrations of bioavailable Cd were very high (almost 90 mg kg^{-1}) in the topsoil (0–10 cm) and decreased with the increasing soil depth, being 14-fold lower in the deepest layer (40–60 cm). Statistically significant differences were found in bioavailable Cd concentrations among all investigated soil layers (Tab. 2). The concentrations of bioavailable Zn were

Tab. 1 General properties of the soil.

Property	Value
Organic matter content (%)	8.52 ±0.12
Cation exchange capacity [cmol(+)/kg]	6.67 ±0.24
Sand [1–0.05 mm; (%)]	37.3
Silt [0.05–0.002 mm; (%)]	56.3
Clay [< 0.002 mm; (%)]	6.4

Values represent mean of three replicate samples ±*SE*. Soil was collected from the top 30 cm layer.

also markedly higher in the upper soil layers and differed in a statistically significant manner from the content of the two deeper soil layers. The concentration of bioavailable forms of Pb decreased with soil depth, however, it was very low even in the 0–10 cm layer (7.0 mg kg^{-1}).

AM root colonization

Arbuscular mycorrhiza was found in *D. cespitosa* roots in all soil levels investigated. However, in topsoil the mycorrhizal colonization was extremely low with sporadically occurring arbuscules (Fig. 1). Vesicles were absent at the 0–10 cm soil level, while in the 10–20 cm layer they were noticed only in one sample. There was no AM coils detected in the roots from two upper soil levels. The relative arbuscule richness (*A*%) did not exceeded 3% and the intensity of root cortex colonization (*M*%) was also very low (below 5%; Fig. 1). On the contrary, *D. cespitosa* roots from the depth below 20 cm comprised properly developed AM structures, like arbuscules, vesicles and coils. Roots were heavily mycorrhizal, with more than 35% of root cortex colonized by AMF. Almost all AM parameters (*F*%, *M*%, *m*%, and *A*%) increased considerably and were markedly higher at the 20–40, 40–60 cm soil levels when compared to the upper levels (0–10, 10–20 cm). These differences were statistically significant (Fig. 1).

Coarse AMF (mycelium above 2 µm diameter) dominated in *D. cespitosa* roots. The fine endophyte, usually considered *Glomus tenue* (Greenall) I. R. Hall, characterized by mycelium of ca. 1 µm diameter, was observed in the *Deschampsia* root cortex only in the two deeper layers (20–40 and 40–60 cm) where it formed well-shaped arbuscules (Fig. 2).

Dark septated endophytes were found in all soil levels investigated. The hyphae of DSE could be readily distinguished from AM hyphae by their dark brown colour, thicker lateral wall, and frequent septa. However they were not abundantly developed. The regularly septated hyphae were sporadically accompanied by sclerotia. The mycelium did not stain with aniline blue and remained brownish. DSE were observed in the cortex together with AMF but mainly in the root fragments where arbuscules were absent.

Relationships between AM colonization and soil properties

The interrelations between the mycorrhizal parameters (*F*%, *M*%, *m*%, *a*%, *A*%) and bioavailable metal concentrations, total

Tab. 2 Chemical and physical soil properties in different soil layers.

Soil depth (cm)	*EC* (µS cm^{-1})	pH (H_2O)	Bioavailable metal concentrations (mg kg^{-1})			Total metal concentrations (mg kg^{-1})		
			Cd	Zn	Pb	Cd	Zn	Pb
0–10	121.1 ±4.21 a	6.47 ±0.026 a	89.89 ±1.745 a	598.7 ±32.3 a	7.03 ±0.37 a	598.75 ±125.7 a	7770.8 ±2276 a	9785.4 ±2422 a
10–20	66.7 ±3.46 b	6.23 ±0.005 a	55.61 ±2.735 b	463.0 ±24.95 c	1.39 ±0.12 ac	155.85 ±39.20 a	2049.8 ±469 b	1719.58 ±428 b
20–40	45.5 ±4.21 c	5.66 ±0.150 b	13.00 ±0.46 c	167.3 ±11.75 b	BDL b	22.38 ±12.93 b	359.5 ±91.9 b	128.21 ±17.8 b
40–60	42.3 ±1.09 c	5.18 ±0.049 c	6.42 ± 0.455 d	117.0 ± 4.03 b	0.46 ±0 07 bc	9.53 ±4.09 b	201.2 ±61.8 b	85.10 ±14.56 b

Values are means ±*SE* (n = 3, except for bioavailable metal concentration where n = 4). Means followed by the same letter in a column are not significantly different from each other using LSD test ($P \leq 0.05$). BDL – below detection limit.

Tab. 3 Pearson's correlation coefficients between mycorrhizal parameters and soil properties.

Mycorrhizal parameters	Correlation coefficient							
	Bioavailable metal concentration (mg kg^{-1})			Total metal concentration (mg kg^{-1})			pH (H_2O)	*EC* (µS cm^{-1})
	Cd	Zn	Pb	Cd	Zn	Pb		
F%	−0.79*	−0.81*	−0.80*	−0.80*	−0.76*	−0.53	−0.74*	−0.83*
M%	−0.83*	−0.88*	−0.70*	−0.68*	−0.64*	−0.63*	−0.80*	−0.75*
m%	−0.27	−0.45	−0.31	−0.72*	−0.67*	−0.66*	−0.77*	−0.76*
a%	−0.07	−0.20	−0.16	−0.67*	−0.61*	−0.60*	−0.89*	−0.75*
A%	−0.75*	−0.82*	−0.62*	−0.36	−0.30	−0.01	−0.46	−0.36

a% – arbuscule richness in root fragments where the arbuscules were present; *A*% – arbuscule abundance in the root system; *F*% – frequency of mycorrhization; *m*% – intensity of colonisation within individual mycorrhizal roots; *M*% – intensity of root cortex colonization. * Correlation coefficient was statistically significant at $P \leq 0.05$.

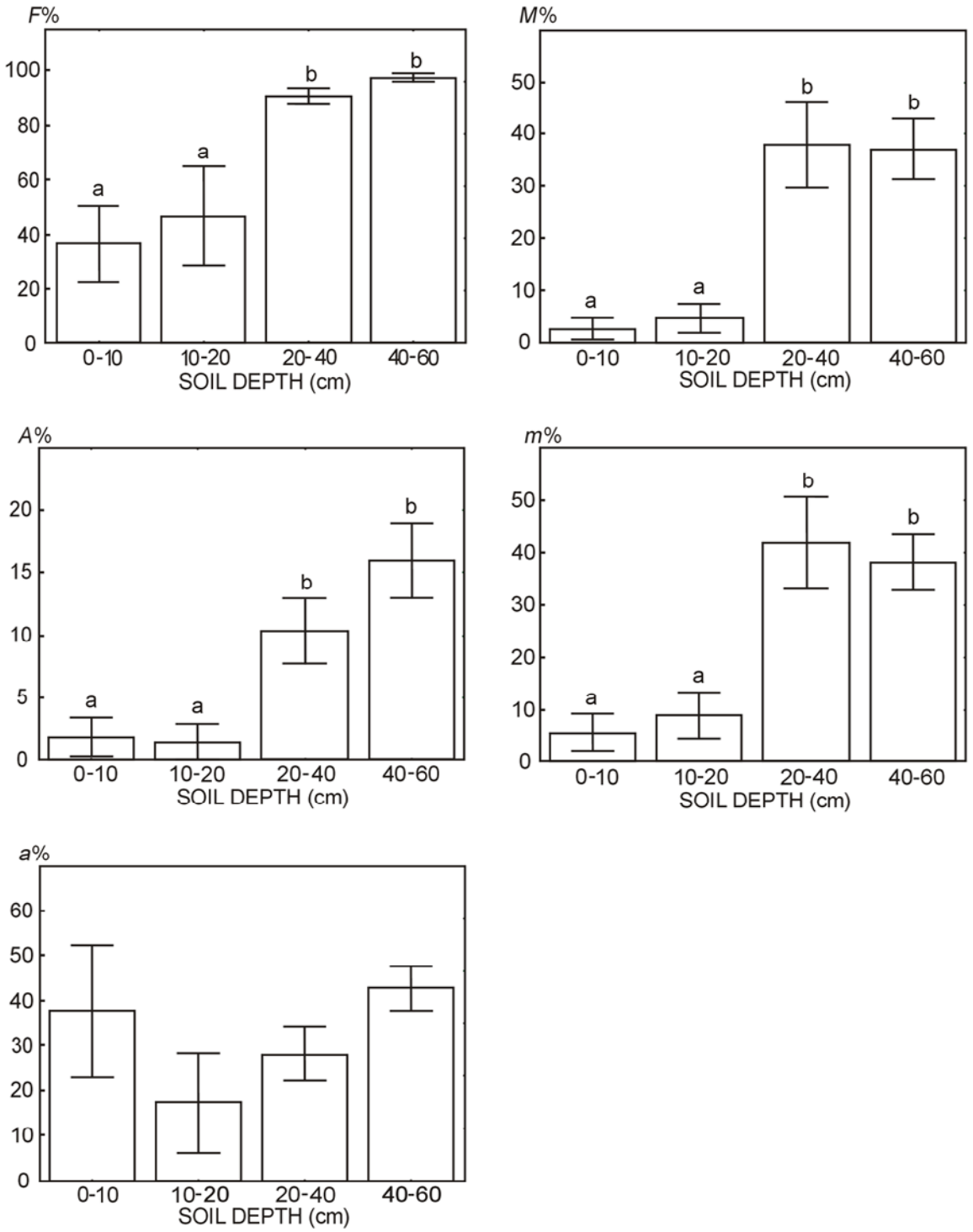

Fig. 1 Differences in frequency of mycorrhization (*F*%), intensity of root cortex colonization (*M*%) intensity of colonisation within individual mycorrhizal roots (*m*%), arbuscule abundance in the root system (*A*%) and arbuscule richness in root fragments where the arbuscules were present (*a*%) in *Deschampsia cespitosa* roots at different soil depth. Values are means ($n = 4$). Means followed by different letters are significantly different from each other using LSD test ($P \leq 0.05$). Lack of letters denotes a lack of statistical differences among the means.

metal concentrations, the soil pH (H_2O) or *EC* were assessed (Tab. 3). All mycorrhizal parameters were negatively correlated with bioavailable Cd, Pb and Zn concentrations. However in the case of *m*% and *a*% the correlations were low and were not statistically significant. Negative correlation coefficients were also found between particular mycorrhizal parameter (*F*%, *M*%, *m*%, *a*%, *A*%) and the total concentration of metals (Cd, Zn, Pb) but for parameter *A*% they were statistically insignificant. The same trend was observed between all mycorrhizal parameters and soil pH or *EC* (Tab. 3).

AMF spore abundance and species diversity

Spore numbers were very low and their vertical distribution differed considerably among soil layers in metal contaminated soil. The abundance of spores was highest in deeper soil layers, where 15 and 11 spores per 150 g of soil were found at 20–40 and 40–60 cm soil depth respectively, whereas AMF spores were absent in the 0–10 cm soil layer (Tab. 4).

Spores of only three morphologically distinctive AMF species were found at the site (*Archaeospora trappei*, *Funneliformis mosseae*, *Scutellospora dipurpurescens*), of which *S. dipurpurescens*

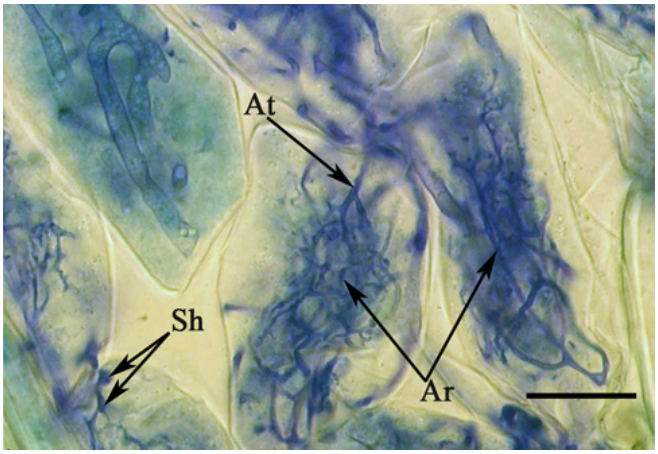

Fig. 2 Fine endophyte *Glomus tenue* mycelium in the cortex of *Deschampsia cespitosa* roots from 40–60 cm soil layer. Ar – arbuscules; At – arbuscule trunk; Sh – swollen hyphae. Scale bar: 20 μm.

occurred most frequently (Tab. 4). However, *S. dipurpurescens* was recorded only in the two deeper and moderately polluted soil layers. In addition, in roots of *D. caespitosa* mycorrhizal structures of *Glomus tenue*, the so-called fine endophyte, were identified. Most likely the fungus rarely produces extraradical spores or they are difficult to extract due to their small size [43]. *Glomus tenue* was observed in root cortex at 20–40 and 40–60 cm soil depths. At present the systematic position of the fine endophyte is not clear [45].

Discussion

The results presented in the current study show that AM colonization level of *Deschampsia cespitosa* roots was low in topsoil (0–20 cm) and increased with soil depth (20–60 cm; Fig. 1). Among the examined soil properties *EC* is not likely to have an effect on the above mentioned differences in mycorrhizal colonization as its decline is observed when *EC* exceeds 500 μS cm^{-1} [46] whereas, the highest *EC* was found in the 0–10 cm soil level and it was 121 μS cm^{-1} (Tab. 2).

The soil reaction, which decreased with depth in a statistically significant manner (Tab. 2), also could not be the factor that would cause substantial differences in mycorrhizal colonization. Since experiments have shown that in soil with pH between 5.5 and 6.5 spores of most of AM fungi species are able to germinate [47]. There is also lack of data on inhibition of mycelium growth or root colonization in this pH range [2].

Data presented in Tab. 3 also shows statistically significant negative correlations between total Cd, Zn and Pb concentrations and most of the mycorrhizal parameters (*F*%, *M*%, *m*% and *a*%). However, research data indicates that the link between total metal concentrations in soil and their toxic effect on plants and mycorrhizal fungi is considerably less distinct than the concentrations of bioavailable metal forms [48–51]. Thus the main reason for the low value of *F*%, *A*%, *M*% and *m*% found at the depth of 0–20 cm might be the high concentration of bioavailable Cd and Zn, which was observed in this soil layer (Tab. 2). The negative correlation between bioavailable Cd, and Zn concentrations in soil and mycorrhizal parameters (*F*%, *M*%, *A*%; Tab. 3) support the notion that metal content is the main factor behind the low level of AM colonization in topsoil. Also a negative correlation between the concentration of bioavailable forms of Pb and *F*%, *M*% and *A*% (Tab. 3) has been found. However, taking into account very low concentrations of bioavailable forms of Pb (max. 7 mg kg^{-1}) when compared to Cd (90 mg kg^{-1}) and Zn (599 mg kg^{-1}) Pb could not have had a significant effect on mycorrhizal colonization of the roots of *D. cespitosa*.

A toxic effect of Cd, Zn and Pb on mycorrhizal fungi as well as their resistance to these metals was also reported by other authors [26,52–55]. In the present field experiment with spontaneous vegetation mycorrhizal colonization was high in soil layers with the concentration of bioavailable Cd lower than 20 mg kg^{-1} (Fig. 1, Tab. 2). This observation agrees well with the results obtained in laboratory experiments. Joner and Leyval [56] observed that the AM fungi hyphal growth was reduced at extractable Cd soil concentration higher than 20 mg Cd kg^{-1} of soil, while Repetto et al. [57] and Rivera-Becerril et al. [58] observed a decrease in mycorrhizal parameters (*F*%, *M*%, *A*%) even at the concentration of 2–3 mg of bioavailable Cd kg^{-1} of substrate. Chen et al. [59], in turn, recorded a toxic effect of Zn on *Z. mays* mycorrhizal root colonization.

In the present study root samples were generally co-colonized by AMF and DSE. However DSE were rare and occurred mainly in root fragments where AM colonization was poorly developed. DSE are ubiquitous root-associated fungi common in stressful environments [60]. Some DSE have been reported to be either weak or serious pathogens, whereas others appeared to improve host growth. This range of host responses is attributable to the great diversity of fungal taxa and strains of these endophytes [60–62]. It is possible that DSE may improve *D. cespitosa* growth at the investigated area, particularly in case where the AM colonization in roots was poorly developed, but their role has to be experimentally studied.

It was documented that in agroecosystems, non-contaminated by heavy metals, such as grasslands, vineyards and maize fields the largest number of AM fungi spores and species

Tab. 4 AMF species (Glomeromycota) associated with *Deschampsia cespitosa* rooting zone at different soil depth.

Soil depth (cm)	AMF species	Number of spores per 150 g dry soil
0–10		0
10–20	*Archaeospora trappei* (R. N. Ames & Linderman) J. B. Morton & D. Redecker	1
20–40	*Scutellospora dipurpurescens* J. B. Morton & Koske	15
40–60	*Scutellospora dipurpurescens* J. B. Morton & Koske	9
40–60	*Funneliformis mosseae* (T. H. Nicolson & Gerd.) C. Walker & A. Schüßler	2

For each soil level 3 soil samples were taken, 50 g of dry soil per sample, but they were pooled together for each soil depth because of very low number of spores.

richness occurred in the topsoil layers (0–20 cm) and they decreased with the increasing soil depth [37]. By contrast, in *D. cespitosa* rooting zone AMF spore numbers and species richness increased with the increasing soil depth and this increase was positively correlated with the diminishing concentration of Cd and Zn in the deeper layers of soil (Tab. 2, Tab. 4).

The maximum spore number observed in *D. cespitosa* rooting zone was low (15 spores per 150 g of soil) when compared with the spore abundance reported from metal contaminated sites by other authors. Zarei et al. [55,63,64] found from 60 to 131 spores per 150 g of dry soil at a site adjacent to a Zn and Pb open pit mine. Pawłowska et al. [65] determined 30–38 spores per 150 g of substrate from calamine spoil mound, del Val et al. [19] 45–345 spores per 150 g of soil contaminated by addition of sewage sludge and Wu et al. [66] 132–492 spores per 150 g of soil contaminated by As/Pb/Zn mines. Only Ortega-Larrocea et al. [67] reported low spore abundance (0–19 spores per 150 g) at a metal contaminated site with very high concentrations of Cd, which was similar to the metal content measured by us in *D. cespitosa* rooting zone (Tab. 2). These data suggests that Cd and Zn concentrations are a major factor contributing to the low abundance of spores in soil.

Scutellospora dipurpurescens was the most abundant AMF species. Its spores were found in deeper soil layers (20–40, 40–60 cm) where the concentration of bioavailable Cd and Zn was still high when compared to the concentration of bioavailable metals reported by other authors [19,48,55,64]. Two other species *Funneliformis mosseae* and *Archaeospora trappei* were represented by 2 and 1 spore respectively (Tab. 4). Of the three morphologically distinctive AMF species found in the rooting zone of *D. cespitosa* only *Funneliformis mosseae* was detected many times in metal contaminated soils [19,55,64,66–70].

S. dipurpurescens was capable of growing and sporulating in soil highly contaminated with metals, which might suggest that this species is particularly resistant to Cd, Pb and/or Zn. However, Oehl et al. [37] observed that AMF species from genus *Scutellospora* in grasslands preferentially occurred in deeper soil layers in contrast to other AMF species. On the basis of this data it is possible to conclude that the presence of *S. dipurpurescens* at the investigated area is not connected with an exceptional resistance of this species to the metals, but with its ability to grow and sporulate in deeper soil layers, where the concentration of bioavailable Cd and Zn was considerably lower than in topsoil (Tab. 2, Tab. 4). This preference of *S. dipurpurescens* to lower soil depth could be the main reason for a dearth of data on the presence of this species in metal contaminated soils. To the best of the authors' knowledge there are only few papers, which documented the presence of *S. dipurpurescens* in metal contaminated soils from rhizosphere of *Agrostis capillaris* [71–73] and *Molinia caerulea* [74].

No spore of the fine endophyte *Glomus tenue* was found at the investigated site. *Glomus tenue* rarely or never produces extraradical spores [42] (Tab. 4). However, *Glomus tenue* colonized the root cortex of *D. cespitosa* in two deeper soil layers (20–40 and 40–60 cm; Fig. 2). It was reported that *Glomus tenue* is a common AMF in grassland soils especially those which are very low in phosphorus and it was frequently found in association with plant species colonizing primary habitats [75]. In our study the fine endophyte occurred along with a coarse AMF in *Deschampsia* roots (Fig. 1, Fig. 2), much as in the roots of the numerous cultivated and uncultivated plant species examined by Błaszkowski [76]. There are a few papers on *G. tenue* colonizing roots of plants growing in soils contaminated by heavy metals [71,72,77]. Most studies dealing with the AMF at such sites did not consider the fungus [19,55,64,66–70]. Therefore further studies on the occurrence and role of *G. tenue* occurring alone and with other AM fungi in the roots of plants colonizing contaminated sites are needed.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: study conception: EGP, EM; field research: EGP, JB, ŁM; writing the manuscript: EGP, JB, RK, ŁM

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