

Arbuscular mycorrhizal fungi (AMF) root colonization dynamics of *Molinia caerulea* (L.) Moench. in grasslands and post-industrial sites

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Abstract

The aims of this studies were: (i) to examine the influence of heavy metal content (Zn, Cd, Pb, Fe, Cu) and other physico-chemical soil parameters on the level of root colonization of ~~Molinia caerulea~~ *Molinia caerulea* and (ii) to relate root colonisation parameters and soil variables to ~~Molinia caerulea~~ (~~Molinia caerulea~~) *Molinia caerulea* abundance in two contrasting habitats (grasslands and heavy metal contaminated sites).

The sites differ significantly in terms of bio-available heavy metal contents, particularly Zn (34 times more than grasslands), soil texture, CaCO₃, organic matter (LOI%), Mg and nitrate content. Principal Component Analysis showed the strong negative correlations between frequency of mycorrhization (F), arbuscular abundance (A%) and intensity of root cortex colonisation (M%) and concentration of bio-available Zn and Cd. Moreover, no positive correlation between root colonization of ~~Molinia~~ *Molinia* and its abundance was found. The frequency of mycorrhization of root fragments (F%) was only slightly different between these two habitats, whereas the intensity of root cortex colonisation (M%) and relative arbuscular abundance (A%) were significantly lower (3 and 4 times respectively) on the post-industrial sites.

The bioavailable Zn content in the substratum of post-industrial sites was strongly negatively correlated with species richness, Shannon diversity index and Evenness. In contrast, these relationships were not statistically significant in grasslands. Based on obtained results we could draw a model of possible relationships between root colonization of ~~Molinia~~ *Molinia*, HM content and ~~Molinia~~ *Molinia* abundance on grasslands and post-industrial sites. Bioavailable Zn content in the soil is a one of main factors influencing the ~~Molinia~~ *Molinia* community diversity. In the grasslands, lower amounts of bioavailable Zn, resulted in higher species richness (R) and species diversity (H) which in turn lead to higher root colonization. On the other hand, on the post-industrial sites, the elevated bioavailable Zn content strongly decreases the plant species richness (R) and species diversity (H) and this caused the decline in root colonization parameters. The low species richness on Zn-polluted sites allowed ~~Molinia~~ *Molinia* to reach higher abundance since the competition with other species is reduced.

Keywords: Arbuscular mycorrhizal fungi; Heavy metals; [Contaminated sites](#); [Grasses](#); Physico-chemical soil properties; Reclamation

1 Introduction

Wastes connected with zinc and lead industry such as: washing and flotation sludge, metallurgical slag, sediments from ship ports, are extremely harsh substrates for plant growth and soil biota due to: high concentrations of heavy metals (HM) (Cd, Pb, Zn), low porosity, low amounts of macronutrients and unfavorable air-water conditions (Doni et al., 2015; [Yi and Sung, 2015](#); Shetty et al., 1994; Stalmachová and Sierka, 2014; [Wójcik et al., 2014](#)).

The traditional and most common approach to these sites is the technical reclamation, which comprises levelling off sites, covering wastes with fertile topsoil, sowing grass-herb mixtures and planting trees, frequently ignoring the ecological principles and the role of biological diversity, mainly the relationships between abiotic and biotic environments ([Rahmonov and Oleś, 2010](#); [Tropek et al., 2012](#); [Woźniak et al., 2003](#)). Therefore, such management is often unsuccessful and resulted in plant cover that needs to be maintained only at high expense ([Jochimsen, 2001](#)). Moreover, created in this way plant cover is characterised by lower species and functional diversity ([Tropek et al., 2012](#)) and delivered a reduced range of ecosystem services ([Lacková et al., 2012](#)). In contrast, spontaneous or directed succession is inexpensive, and spontaneously revegetated sites usually exhibit a higher natural value as a result of significant biodiversity (Prach and Pyšek, 2001; [Tropek et al., 2012](#)). Therefore there is still a need for examined factors that affect the successful colonization of grasses in contrasting habitats (i.e. semi-natural and post-industrial sites).

Successful survival and growth of plants on a site is dependent on the abiotic, as well as the biotic, properties of the soil (Gucwa-Przepióra et al., 2016; [Rahmonov and Szymczyk, 2010](#); [Woźniak, 2010](#)). Among the biotic properties plant-microbe interactions (including arbuscular mycorrhizal fungi, AMF) in the rhizosphere are primary determinants of plant health and soil fertility ([Jeffries et al., 2003](#); [Markowicz et al., 2015](#); [Markowicz et al., 2015](#); [Woźniak et al., 2015](#)), as well as the rate of plant succession (Entry et al., 2002; Kumar et al., 2003; [Li-Ping et al., 2009](#)); [Li-Ping et al., 2009](#)).

Many factors can have an influence on mycorrhizal colonization of plant roots both in polluted and unpolluted sites ([Berthelin et al., 1995](#); [Carrenho et al., 2007](#); [Kapoor et al., 2002](#); [Magurno et al., 2014](#); [Mejstrik, 1972](#); [Weissenhorn et al., 1995](#); [Zarei et al., 2008](#)). Among them heavy metal content in the soil (HM) seems to be one of the most important one ([Leyval et al., 1995](#); [Zarei et al., 2008](#)).

An extensive body of literature is focused on beneficial plant-mycorrhizal relations in postindustrial waste sites rich in potentially toxic metals, low in macronutrients and with poor water holding capacity ([Gucwa-Przepióra and Błaszowski, 2007](#); [Gucwa-Przepióra and Turnau, 2001](#); [Moora and Zobel, 2010](#); [Orłowska et al., 2002](#)). It has been stated that mycorrhizal fungi improve plant nutrition ([Smyth and Box, 1997](#); [Entry et al., 2002](#); [Li-Ping et al., 2009](#); [Kuimei et al., 2012](#); [Entry et al., 2002](#); [Li-Ping et al., 2009](#); [Kuimei et al., 2012](#) (please remove 'Kuimei et al., 2012')) [Qian et al., 2012](#)) through increasing absorption of P, N, K ([Li-Ping et al., 2009](#); [Kuimei et al., 2012](#); [Kuimei et al., 2012](#) [Qian et al., 2012](#)), increase pathogenic resistance, phyto-hormone production and take part in formation of soil aggregates ([Göhre and Paszkowski, 2006](#); [Hildebrandt et al., 2007](#); [Kuimei et al., 2012](#); [Smith and Read, 2008](#)). AMF alleviate also the water deficit for plants due to increased water uptake from soil with the help of hyphae ([Smyth and Box, 1997](#); [Entry et al., 2002](#); [Entry et al., 2002](#)). AMF contribute to immobilisation of HM in the soil beyond plant rhizosphere by root exudates (e.g. glomalin) that bind metals in the soil, passive adsorption to fungal chitin cell walls (important sink of HM since large surface area), chelation of metals inside the fungal vesicles. It was found ~~that~~ that sequestration of some metals (e.g. Pb) in the roots was correlated with an increase in the number of fungal vesicles in highly colonised species ([Göhre and Paszkowski, 2006](#); [Hildebrandt et al., 2007](#)).

Plants play also important role in the restoration of contaminated soils. They naturally reduce the mobility and bio-availability of heavy metals via sorption, sequestration of HM in the vacuoles, precipitation (secretion of chelatin agents in the soil such as histidine, organic acids, and complex reactions) ([Göhre and Paszkowski, 2006](#); [Violante et al., 2010](#)). Despite the fact, that plants have different physiological adaptations that enable them to adjust to adverse conditions during spontaneous succession and cope with a variety of stresses, the vegetation development on sites contaminated by heavy metals is very slow ([Leyval et al., 1997](#); [Medina and Azcón, 2010](#)). Among plants that successfully colonize these sites are grasses due to their tolerance to toxic metals, drought and low nutrition levels and the possibility to develop abundant root systems inhabited by diverse microbial communities ([Bothe et al., 2010](#); [Mardukhi et al., 2011](#); [Ryszka and Turnau, 2007](#); [Turnau et al., 2010, 2008](#)). Both annual and perennial grasses can be colonized by arbuscular mycorrhizal fungi AMF ([Ammani et al., 1994](#); [Newsham and Watkinson, 1998](#)), that can help plants to alleviate heavy metal stress and maintain water supply ([Hildebrandt et al., 2007](#); [Krishnamoorthy et al., 2015](#); [Mohammadi et al., 2011](#); [Pawłowska et al., 1996](#); [Pawłowska and Charvat, 2004](#); [Turnau et al., 2012](#)). However, there are also studies which show that some grasses respond differently to AMF colonization. Some of these such as *Dactylis glomerata*, ~~*Festuca rubra*, *F. tenuifolia*~~, [Festuca rubra](#), [F. tenuifolia](#), [Deschampsia caespitosa](#), can be even highly dependent on mycorrhizal symbiosis ([Gucwa-Przepióra et al., 2013](#); [Ryszka and Turnau, 2007](#); [Turnau et al., 2008, 2012](#)). Under natural conditions these species ([Grime et al., 1987](#); [van der Heijden, 2002](#); [Newsham et al., 1995](#)) are not strongly dependent on mycorrhiza, although in extreme situations this might be different ([Turnau et al., 2008, 2012](#)).

A good example of a species that can survive successfully on post-industrial wastes is a wet meadow species *Molinia caerulea* ([Ryszka and Turnau, 2007](#); [Turnau et al., 2008](#)).

The aims of this study were: (i) to examine the influence of soil heavy metal content and chosen physico-chemical soil parameters on the level of root colonization of *Molinia caerulea* and (ii) to relate those mycorrhizal colonisation and soil parameters to *Molinia caerulea* abundance.

We expected that successful colonization by *Molinia* of post-industrial sites strongly depends on AMF in comparison to grasslands.

2 Materials and methods

2.1 The study area

The study area is situated at an altitude of 250–350 m a. s. l. in the southern part of Poland (Fig. 1) belonging to the Silesian Uplands (Southern Poland). The mean annual temperature is ca. 8.8 °C, average annual precipitation is 700–800 mm, prevailing winds are from a westerly direction (SW, W, NW), the duration of snow cover is about 50–70 days (Kondracki, 2011). Waste heaps, mine pits and sedimentation ponds are characteristic features of the Silesian landscape (Pełka-Gościński, 2014). Sometimes wastelands serve as secondary habitats for many plants even for rare and protected species (Chmura et al., 2011). These wastelands, being in many cases extreme habitats for plant colonisation (due to a high content of heavy metals, low nutrient levels, unfavourable water and air conditions), are currently subject to spontaneous succession as a result of industrial restructuring (Woźniak et al., 2011).

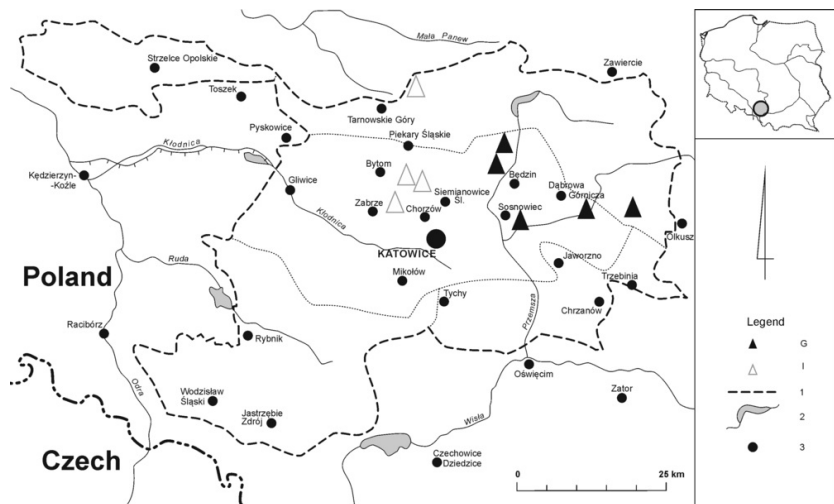


Fig. 1 Location of *Molinia caerulea* sampling plots: G—grassland sites, I—post-industrial sites; 1—grassland sites, I—post-industrial sites; 1—the borders of the Silesian Uplands (according to Kondracki 2001); 2—lakes and rivers, 3—lakes and rivers; 3—towns and cities

This region is known for its deposits of important mineral resources: hard coal, zinc, lead and iron ores, sand, gravel, and dolomite. Exploitation of mineral resources began in the early Middle Ages and was intensified in the second half of the 18th century due to changes in the forms of economic activity, technical and scientific progress and urbanization. The lead and zinc bearing ores and their connected excavations for the lead and zinc industry were concentrated between Bytom and Tarnowskie Góry in the west and Olkusz, Chrzanów and Zawiercie in the east (Pełka-Gościński, 2014). As a result of industrial extraction a variety of waste was formed primarily connected with lead and zinc ore mining and secondly with the processing of lead and zinc ores by smelting. Secondary wastes of Zn-Pb smelters (occupying an area of 152.6 ha) were stored near steel works and mining and metallurgical factories in Bytom, Piekary Śląskie, Miasteczko Śląskie, Radzionków, Ruda Śląska, Świętochłowice (Chropaczów, Lipiny), Ruda Śląska (Nowy Bytom), Katowice (Wełnowiec, Szopienice). The area occupied by wastes from the flotation process of non-ferrous metal ores is currently about 300 ha, and is deposited on stony and loamy ground, and contains 2.9% Zn and 0.6% Pb. The large-scale exploitation and processing of minerals that has taken place over many years (in Bytom from the twelfth century) has caused the widespread deterioration of water, air and soils (e.g. due to heavy metal contamination with Pb, Zn, Cd, As) which in some places has exceeded various national and international thresholds (Ullrich et al., 1999).

2.2 Study species

Molinia caerulea (L.) Moench (purple moor-grass) is an expansive polycarpic perennial grass widespread in Europe, south-western and northern Asia, North America and the British Isles (Taylor et al., 2001). In Poland it occurs

mainly in the lowlands but is fairly frequent in the Polish mountains (Mirek and Piękoś-Mirkowa, 2007). This grass occurs in a variety of habitats such as moorland, mire, river banks, grazed and ungrazed grasslands (the *Molinion* alliance) and wastelands (coal mine heaps, metal contaminated sites, sites of sand-pit excavation) and wet coniferous forests (the *Molinio-Pinetum*) (Grime et al., 1988; Kompała-Bąba et al., 2005; Kompała-Bąba and Bąba, 2013; Kompała and Woźniak, 2001; Ryszka and Turnau 2007; Salim et al., 1995; Szarek-Lukaszewska and Grodzińska, 2011; Rostański, 2006). It forms tussocks (0–20 cm in diameter) or extensive swards. It has condensed rhizomes, but in heavily crowded areas of tussocks, it can extend vertically and lift new daughter tillers between 2 and 10 cm above the surrounding tiller level. *Molinia caerulea* has a well-developed root system, forming a dense tangle at the top, which can penetrate deep into the soil to more than 80 cm (Ryszka and Turnau, 2007; Taylor et al., 2001; Turnau et al., 2008). Their roots differ in terms of length and diameter (Jefferies 1915, 1916). It can be accompanied by various rhizosphere micro-organisms such as AMF, diazotrophs and rhizobacteria (Eason et al., 1991; Jefferies, 1916; Hamelin et al., 2002; Harley and Harley, 1987; Reinhold-Hurek and Hurek, 1998). *M. caerulea* with the arbuscular type of endomycorrhiza is efficient at stabilizing the post-flotation wastes substratum (Ryszka and Turnau, 2007). It reproduces mainly vegetatively by stolons but it can produce seeds but germination and survival of seedlings on mine tailings are extremely low (Turnau et al., 2012).

This expansive grass prefers moist to wet, poor to moderately poor sites, from highly acidic (pH \leq 4) to alkaline soil reaction (pH > 7), sandy to heavy clay and loamy, mineral-humic to soil rich in organic matter (peaty gleys or deep peats) (Zarzycki et al., 2002). Generally it tolerates a wide range of irradiance (open, sub-montane grasslands, mires and some lighter patches of *Betula pubescens* woodland) (Taylor et al., 2001). It is considered to be a facultative halophyte but not an indicator of resistance to increased heavy metal content in soil (Zarzycki et al., 2002). It prefers flat ground or gentle to moderate slopes. Purple moor-grass produces an abundance of persistent litter, so sites contain little bare soil (Grime et al., 1988; Ryszka and Turnau, 2007).

2.3 Data collection

In July 2011, 9 localities inhabited by *Molinia caerulea* in the Silesian Uplands (southern Poland) were chosen for detailed investigations. Five of these were located in meadows (mainly purple-moor grass meadows) (Table 1, Fig. 1, marked G) and the other four were on post-industrial areas associated with the excavation and processing of lead and zinc ores (washing and flotation settlings, waste produced from zinc and lead smelters) (Table 1, Fig. 1, marked I). The site geographical coordinates were noted in the field. On post-industrial sites *Molinia caerulea* occurred mainly in floristically poor patches (containing 4 to 30 species, with an average species richness of 13.75, average values of Shannon diversity indices of 2.502 and evenness 0.756) that consisted of *Silene vulgaris*, *Cardaminopsis arenosa*, *Festuca ovina*, *Leontodon hispidus*, *Cardaminopsis arenosa*, *Festuca ovina*, *Leontodon hispidus*, *Thymus pulegioides* (Table 1, marked I). *Molinia caerulea* covered more than 35% of a given patch (average 68.7%). In contrast in non-industrial sites *Molinia caerulea* was present in species-rich patches (containing 11 to 48 species, with an average species richness of 29.8, an average values of Shannon diversity indices of 1.452 and evenness 0.565) mainly with wet meadow species of the *Molinietalia* order (*Equisetum palustre*, *Lysimachia vulgaris*, *Juncus inflexus*, *Lysimachia vulgaris*, *Juncus inflexus*, *Angelica sylvestris*, *Cirsium rivulare*), some species of fens of the *Scheuchzerio-Caricetea* class (*Carex nigra*, *C. panicea*). It covered between 17.5% to 87.5% of a given patch (average 44.5%). The vegetation characteristics of each locality are shown in Table 1.

Table 1 Vegetation characteristics of the study locations.

alt-text: Table 1

Location/Geographical coordinates	Habitat type	Vegetation type	Vegetation characteristics*
Psary N 50°17'56" E 18°59'53"	grassland (G)	overgrown wet meadow <i>Molinietum caeruleae</i>)	cover: herbs (100%), No. = 38 species, H = 2.75, E = 0.76: <i>Molinia caerulea</i> (62.5%), <i>Geranium palustre</i> , <i>Lysimachia vulgaris</i> , <i>Solidago canadensis</i> , <i>Alnus glutinosa</i> , <i>Impatiens parviflora</i>
Sławków N 50°18'14" E 19°20'35"	Grassland (G)	purple moor-grass meadow (<i>Molinietum caeruleae</i>)	cover: herbs (100%), mosses (50%), No. = 48 species, H = 3.52, E = 0.91: <i>Molinia caerulea</i> (17.5%), <i>Centaurea oxylepis</i> , <i>Sanguisorba officinalis</i> , <i>Arrhenatherum elatius</i> , <i>Deschampsia caespitosa</i>
Ostrowy Górnicze N 50°17'08" E 19°15'52"	grassland (G)	grassland <i>Juncus effusus</i> - <i>Carex brizoides</i> community	cover: herbs (90%), No = 11 species, H = 1.88, E = 0.78: <i>Molinia caerulea</i> (37.5%), <i>Juncus effusus</i> , <i>Urtica dioica</i> , <i>Carex brizoides</i>
Dąbrowa Górnicza Strzemieszyce	grassland (G)	purple moor-grass meadow	cover: herbs (90%), mosses (50%), No = 28 species, H = 2.51, E = 0.75: <i>Molinia caerulea</i> (17.5%),

N 50°18'12" E 19°18'56"		(<i>Molinietum caeruleae</i>)	<i>Epipactis palustris</i> , <i>Lysimachia vulgaris</i> , <i>Carex flacca</i>
Gródków N 50°22'28" E 15°06'00"	grassland (G)	Community <i>Molinia caerulea</i>	Cover: herbs (100%), No = 24 species, H = 1.85, E = 0.58: <i>Molinia caerulea</i> (87.5%), <i>Briza media</i> , <i>Geranium palustre</i> , <i>Juncus inflexus</i>
Miasteczko Śląskie N 50°30'41" E 18 56'04"	post-industrial site (wastes of zinc smelters: slag from roll down furnaces) (I)	Community <i>Molinia caerulea</i>	cover: herbs (80%), No. = 4 species, H = 0.56, E = 0.40: <i>Molinia caerulea</i> (87.5%), <i>Deschampsia flexuosa</i> , <i>Sorbus aucuparia</i> , <i>Cardaminopsis halleri</i>
Dąbrówka Wielka (Dołki) N 50°21'18" E 19°00'36"	post-industrial site (gangue) (I)	Community <i>Molinia caerulea</i>	cover: herbs (70%), No. = 30 species, H = 2.75, E = 0.81: <i>Molinia caerulea</i> (37.5%), <i>Cardaminopsis arenosa</i> , <i>Daucus carota</i> , <i>Festuca ovina</i> , <i>Lotus corniculatus</i>
Świętochłowice (Chropaczów) N 50°19'06" E 18°54'20"	post-industrial site wastes produced in zinc smelter (metallurgical slag, dolomite, muffles, brick chamotte) (I)	Community <i>Molinia caerulea</i>	cover: herbs (90%), No. = 9 species, H = 0.99, E = 0.45: <i>Molinia caerulea</i> (87.5%), <i>Silene vulgaris</i> , <i>Festuca ovina</i> , <i>Epipactis palustris</i> , <i>Betula pendula</i> , <i>Rumex acetosa</i>
Bytom (Żabie Doły) N 50°20'19" E 18°59'53"	p Post-industrial site flotation sludge mixed with finely ground dolomite with higher amounts of Zn, Pb (I)	Community <i>Molinia caerulea</i>	cover: herbs (70%), No. = 12 species, H = 1.51, E = 0.61: <i>Molinia caerulea</i> (62.5%), <i>Silene vulgaris</i> , <i>Cardaminopsis arenosa</i> , <i>Deschampsia caespitosa</i> , <i>Thymus pulegioides</i>

Explanation: *vegetation characteristics: layer (percentage cover) no. **—** mean number of species in a sample plot, H **—** mean value of Shannon-Wiener diversity index; E **—** mean value of the **e** Evenness, G **—** grasslands (semi-natural habitats), I **—** post-industrial sites.

On July 2011 one sample plot (5 × 5 m) was randomly established in each of the locations. Within each plot, five (2 × 2 m) subplots were randomly placed and the abundance of *Molinia caerulea* was estimated according to the abundance classes: 0-5%, 6-25%, 26-50%, 51-75, 76%-5%, 6-25%, 26-50%, 51-75, 76%-100%. Root samples of 5 individuals of *Molinia caerulea* per plot were collected to check their mycorrhizal status and mixed soil samples (individuals of *Molinia caerulea* were shaken to obtain soil or waste material from the root zone) were taken in order to conduct physico-chemical analyses. In total 45 samples were collected (9 sample plots **×** 5 subplots).

2.4 Soil analyses

The soil samples were air-dried in the laboratory and sieved through a 2 mm or 0.25 mm mesh in order to make further analyses. The content of bio-available metals (Zn, Cd, Pb, Fe, Cu) was determined using flame atomic absorption spectrophotometry (Varian Spectra AA300). In order to extract bio-available forms of metals, 3 g of air-dried soil (grain <0.25 mm) were shaken with 30 ml 0.01 M CaCl₂ for 5 h. Soil texture was determined by the hydrometer method developed by Prószyński (Bednarek et al., 2005). Soil pH (1 M KCl) and electrical conductivity (EC) were measured in water suspension (soil to solution ratio 1:2.5 (w:v)) after 24 h of equilibration. The organic matter content in the soil was determined as weight loss on ignition (LOI%) at 550 °C in a muffle furnace. Total N was calculated by the Kjeldahl method using a Kjeldahl Digestion Unit for nitrogen analysis (DKL 42/26) and a UDK 129 Kjeldahl distillation unit (this allowed the separation of nitrogen from the digested mixture by steam distilling). The calcium carbonate content in the soil was determined with the application of the Scheibler volumetric method (Bednarek et al., 2005). The maximum water holding capacity (WHC%) was determined by the gravimetric method. Other soil parameters (P₂O₅, K₂O, Mg, Ca, Na, Cl, salinity, N

NO₃-N, **NO₂-N**

NH₄) were analysed according to standard methods of soil analyses (Ostrowska, 1991).

2.5 Evaluation of root colonization parameters

In order to estimate the mycorrhizal development the *Molinia caerulea* roots were carefully rinsed with tap water for some minutes, then softened in 7% KOH for 24 h, washed again with tap water and acidified in 5% lactic

acid in water for 12–24 hours. They were then stained using 0.05% aniline blue in lacto-glycerol for 24 h at room temperature. The stained roots were left in 5% lactic acid in order to eliminate undesired dye particles and kept for slide preparation. For the quantification of AMF colonization, 30 one cm sections were selected randomly and left on slides under a microscope (magnification 80x) and percentage root colonization (PRC) was calculated according to the Phillips and Hayman (1970) procedure. Parameters of mycorrhizal colonization were evaluated according to the Trouvelot method (Trouvelot et al., 1986) which included F% mycorrhizal frequency in root fragments, M% intensity of mycorrhizal colonisation, A% arbuscule abundance in the root system.

2.6 Statistical analyses

The sampled individuals within-sites could be potentially more similar than those coming from distant localities. Therefore, the differences in the bio-available heavy metal content (Zn, Pb, Cd, Cu, Fe) and other physico-chemical parameters in the soils between the grasslands (G) and post-industrial sites (I) and *Molinia caerulea* root colonisation parameters (A%, M%, F%) were analysed statistically with the Generalized Mixed Models (Restricted maximum likelihood method) with habitat (grasslands, post-industrial sites) as fixed variable and sites treated as a random variable (Zuur et al., 2009). Prior to the analyses, the assumptions: normality of variables were tested with Anderson-Darling test. In the case where strong departures were found, the variables were properly, log or arcsine transformed or the non-parametric alternative (Mann-Whitney U test) was used (Sokal and Rohlf, 1995).

In order to visualise the differences among grasslands (G) and post-industrial sites (I) with reference to selected soil properties (bio-available heavy metal content), *Molinia caerulea* abundance and *Molinia* root colonisation parameters, a PCA analysis was performed. It was performed on correlation matrix, data were standardized prior to the analysis. Moreover, the Pearson correlation coefficient between the bio-available heavy metal content and percentage contribution of each variable to the variance explained by first two PCA axes were performed. These analyses were performed using STATISTICA 10 (StatSoft, Inc., 2011) and R 3.0.2 (R Core Team, 2013).

3 Results

3.1 Heavy metals and other physico-chemical parameters of the soils occurring in examined habitats

The first two PCA axes together explained 78% of the variation in the dataset (Fig. 2). The first PCA axis, which explained 63% of the variation, was highly correlated with available Zn content ($r = 0.88$, $p < 0.001$, Pearson correlation) and Cd content ($r = 0.58$, $p < 0.001$) (Table 2). This axis separated the post-industrial sites (I) connected with excavation and processing of lead and zinc ores: Miasteczko Śląskie (MS), Świętochłowice (Chropaczów) (CH), Dąbrowka Wielka – Dołki (D) and Bytom – Dołki (D) and Bytom – Żabie Doły (ZD) from grasslands (G): Psary (P), Grodków (G), Dąbrowa Górnicza Strzemieszyce (DGS), Sławków (SI) and Ostrowy Górnicze (OG) occurred in intermediate positions. The second PCA axis shows a strong correlation with available Fe content and separated the Ostrowy Górnicze (OG) site from the others. The similar results were obtained, when the contributions of each variable to the variance explained by the PCA axes were taken into account (Table 3).

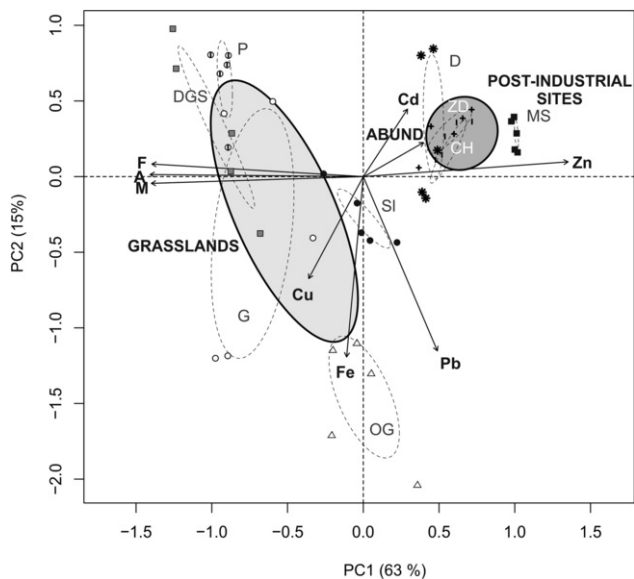


Fig. 2 Principal Component Analysis (PCA) of selected soil properties (bio-available metal content in the soil), *Molinia caerulea* abundance and mycorrhizal root colonization parameters of *Molinia* in grasslands and post-industrial sites. The samples from the

same locations are marked with the same symbols while the habitats, are marked with different letters: grasslands (G): locations P—Psary Φ , G—Grodków Φ , DGS—Psary Φ , G—Grodków Φ , DGS—Dąbrowa Górnicza Strzemieszycze ■, OG—Ostrowy Górnicze Δ , SL—Sławków ●; post-industrial sites (I): D—Dąbrowka Wielka (Dołki) *, S—Miasteczko Śląskie ■, ZD—Bytom (Żabie Doły) +, CH—Świętochłowice (Chropaczów) I. F%—mycorrhizal frequency in root fragments, M%—intensity of mycorrhizal colonisation, A%—arbuscule abundance in the root system.

The ellipses cover 95% confidence limits for standard deviations of points that belong to a particular site (dotted lines) or habitat type—grasslands or post-industrial areas (solid line).

alt-text: Fig. 2

Table 2 The correlations between available heavy metals, mycorrhizal colonisation parameters, *Molinia coerulea* abundance (%) and PCA axes.

alt-text: Table 2

Variable/Axis	PC1	PC2	PC3
Pb [mg/kg]	0.18	-0.68	-0.11
Cd [mg/kg]	0.58	0.28	0.11
Zn [mg/kg]	0.88	0.01	-0.16
Cu [mg/kg]	0.30	-0.70	0.21
Fe [mg/kg]	0.15	-0.78	-0.24
F (%)	-0.98	0.04	-0.09
M (%)	-0.91	0.17	0.01
A (%)	-0.93	0.09	0.02
abundance (%)	0.27	0.23	-0.17

Table 3 Percentage contribution of chosen variables (available heavy metals, mycorrhizal colonisation parameters, *Molinia coerulea* abundance (%) to the variance explained by PCA axes.

alt-text: Table 3

Variable/Axis	PC1	PC2	PC3
Pb [mg/kg]	0.57	24.87	0.73
Cd [mg/kg]	12.31	10.26	0.72
Zn [mg/kg]	27.21	0.00	1.59
Cu [mg/kg]	11.51	15.77	2.80
Fe [mg/kg]	1.44	17.52	3.70
F (%)	16.29	0.06	0.51
M (%)	14.13	0.92	0.01
A (%)	14.89	0.23	0.01
Abundance (%)	5.96	6.78	1.71

Strong differences were found between the grasslands and post-industrial sites in reference to bio-available heavy metal content in the soils. The average content of available Zn was 34 times, Cd 14 times, Pb 5 times, and Cu 2 times higher on the post-industrial sites than those recorded on grasslands. On the other hand, the content of available Fe was significantly lower on the post-industrial sites (Fig. 3, Table 2). These results were confirmed by GLM

models (Table 4).

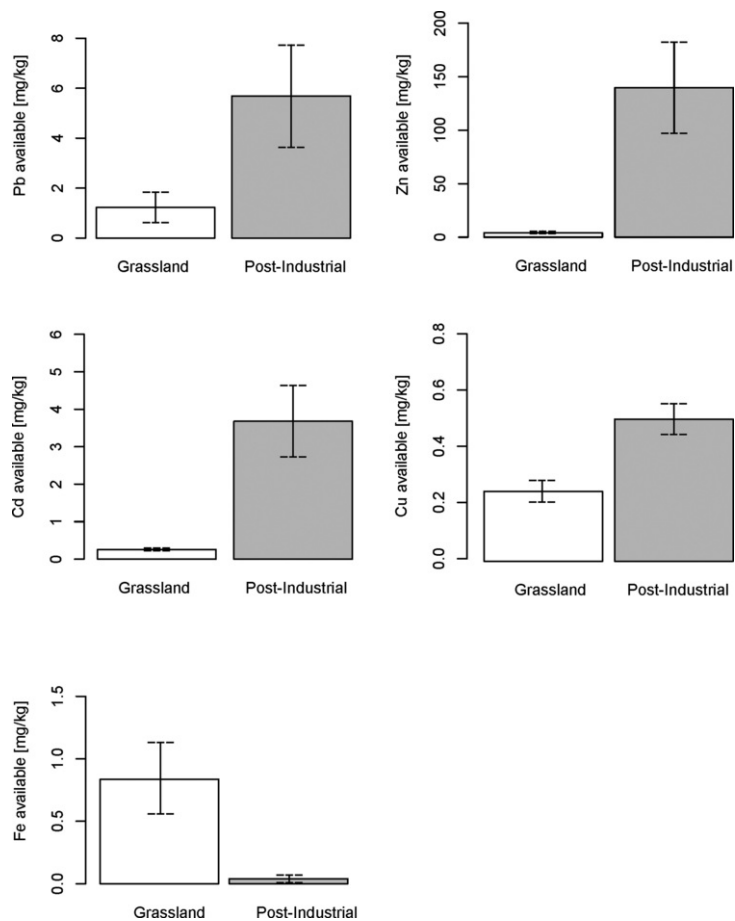


Fig. 3 Differences in mycorrhizal frequency of the root fragments (F%), intensity of mycorrhizal colonization (M%), arbuscule abundance of the root system (A%) in *Molinia caerulea* roots taken from grasslands (n = 25) and post-industrial sites (n = 20). Bar indicates mean values of the parameters and whiskers are standard errors. **Mean values followed by different letters are significantly different from each other using Student's t-test (p < 0.05).**

alt-text: Fig. 3

Table 4 The results of Generalized Mixed Models (Restricted maximum likelihood method, REML) analyses. The differences in *Molinia caerulea* root colonisation parameters (F%, A%, M%) and bio-available heavy metal contents in the soils between grasslands and post-industrial sites. The habitat (grasslands, post-industrial sites) was treated as fixed variable while location (see Table 1) as random variable.

alt-text: Table 4

		Fixed effects				Random effects (Std. Dev.)		AIC	BIC	LogLik
		Estimate	Std. Error	t value	p-value	(Intercept)	Residual			
F(%)	(Intercept)	88.83	2.13	43.77	0	9.17	0.0011	325.92	332.96	-158.96
	grasslands	9.29	2.72	3.41	0.01					

M(%)	(Intercept)	2.65	0.92	2.87	0.01	4.13	0.0001	258.11	265.15	-125.05
	grasslands	5.45	1.24	4.40	0.00					
A(%)	(Intercept)	0.90	0.56	1.60	0.01	2.51	0.0079	215.55	222.59	-103.77
	grasslands	3.15	0.75	4.15	0.00					
Pb	(Intercept)	5.68	3.15	1.86	0.01	5.87	3.69	266.97	274.01	-129.48
	grasslands	-4.46	0.88	-5.59	0.00					
Cd	(Intercept)	3.67	0.51	7.14	0.00	3.22	0.54	118.93	125.98	-55.46
	grasslands	-3.42	0.73	-4.62	0.00					
Zn	(Intercept)	139.68	67.49	2.17	0.05	134.19	32.49	466.81	473.86	-229.41
	grasslands	-135.63	21.54	-6.42	0.00					
Cu	(Intercept)	0.51	0.07	6.64	0	0.17	0.13	4.22	2.82	-6.10
	grasslands	-0.26	0.10	-2.51	0.04					
Fe	(Intercept)	0.04	0.03	2.17	0.01	1.027	0.50	99.15	106.19	-45.57
	grasslands	0.79	0.71	1.13	0.00					

The two types of habitats did not differ significantly in reference to soil reaction (pH in KCl near 6.30), soil electrical conductivity (EC), total N (Kjeldahl), K₂O, P₂O₅, Ca, Na, Cl or salinity content. A higher sandy fraction (%) content was recorded in grassland soils (G) compared with post-industrial sites (I) which had higher fine fraction content of clay% and silt%. Significant differences were also found in the water holding capacity (WHC%), calcium carbonate (CaCO₃), organic matter content (LOI%), Mg, N

~~NO₃~~, and ~~N~~ ~~NO₃~~ and N

NH₄. These values were higher in the soil from post-industrial sites (I) (Table 5).

Table 5 Differences in the physico-chemical soil parameters of grasslands (n = 25) and post-industrial sites (n = 20) (\oplus U Mann-Whitney test). The significant differences were marked in bold.

alt-text: Table 5

Physico-chemical soil parameters	U	Z	P
pH-KCl	217.5	-0.73	0.46
EC [ms/cm]	180.5	-1.58	0.12
WHC [%]	24.00	-5.15	0.001
Sand [%]	0.00	5.70	0.001
Silt [%]	25.00	-5.13	0.001
Clay [%]	0.00	-5.70	0.001

LOI [%]	41.00	-4.76	0.001
CaCO ₃ [%]	128.00	-2.78	0.01
N Kjeldahl [%]	243.00	0.15	0.88
P ₂ O ₅ [mg/100 g]	250.00	0.11	0.99
K ₂ O [mg/100 g]	150.00	-2.27	0.02
Mg [mg/100 g]	100.00	-3.41	0.001
Ca [mg/dm ³]	175.00	-1.70	0.08
Na [me/100 g]	225.00	0.56	0.57
Cl [mg/dm ³]	250.00	-0.01	0.99
salinity [g/dm ³]	212.5	-0.84	0.39
N — N_3O [mg/kg d.m.]	100.00	-3.41	0.001
N_4H [mg/kg d.m.]	100.00	-3.41	0.001

3.2 Mycorrhizal colonization parameters and abundance of *Molinia caerulea* on grasslands and post-industrial sites

Arbuscular mycorrhizal fungi were found in roots of *Molinia caerulea* individuals growing both on grasslands and on post-industrial sites. However, significant differences in the colonization of *Molinia* roots by arbuscular mycorrhizal fungi (AMF) were detected among grasslands (G) and post-industrial sites (I) (Fig. 4, Table 4). The difference in the frequency of mycorrhization of root fragments (F%) of *Molinia caerulea* was only slight (1%) but significantly lower in the post-industrial sites (I). In contrast, the intensity of root cortex colonisation (M%) was three times lower on the post-industrial sites (I) and relative arbuscular richness (A%) four times lower. The PCA showed a strong negative correlation between the A%, F% and M% mycorrhizal colonisation parameters and heavy metal content in soils (Fig. 2, Tables 2 and 4).

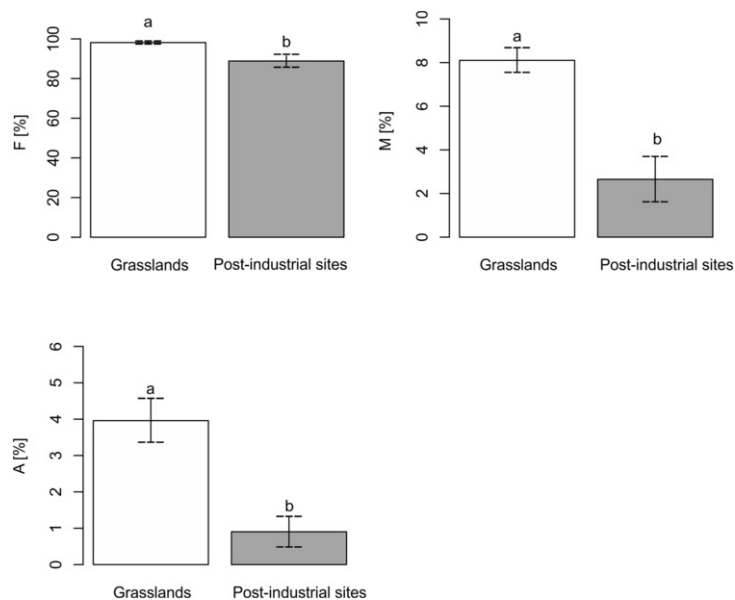


Fig. 4 Differences in the content of bio-available metal concentrations (mg/kg) in the soils of grasslands (n = 25) and post-industrial sites (n = 20). Bars indicate mean values and whiskers standard errors.

Abundance of *Molinia caerulea* on both habitat types could serve as easy-to-measure 'coefficient' of species performance. The abundance of *Molinia caerulea* was significantly different on the grasslands in comparison to the post-industrial sites (Mann-Whitney U test, $U = -3.41$, $Z = -3.57$, $p = 0.0003$). Significant negative correlation was found between abundance of *Molinia caerulea* and grasslands. *Molinia caerulea* in well-preserved, species rich patches of grasslands has the lowest abundance (below 18%), whereas in patches, which developed on post-industrial sites it covered more than 35% of it. Among the AMF parameters only the mycorrhizal frequency (F%) on grasslands was significantly negatively related with *Molinia caerulea* abundance ($r = -0.43$, $p < 0.001$). There were no significant relationships between the M%, A% parameters and *M. caerulea* abundance on both habitat types.

4 Discussion

4.1 Mycorrhizal colonization of *Molinia caerulea* roots on sites with different HM content

On the basis of our results we didn't confirmed existence of positive correlation between root colonization of *Molinia* and its abundance. However, the significant differences in the colonisation of *Molinia caerulea* roots by arbuscular mycorrhizal fungi between grasslands and post-industrial sites were found. Although the frequency of mycorrhization of root fragments (F%) was only slightly different between these two habitats, the intensity of root cortex colonisation (M%) and relative arbuscular abundance (A%) were significantly lower (three and four times respectively) on the post-industrial sites.

The frequency of root colonisation of *Molinia caerulea* (F%) was significantly higher compared to other grasses studied on post-industrial sites reported by [Gucwa-Przepióra et al. \(2013\)](#) and [Regvar et al. \(2006\)](#). Conversely, the values of other parameters (M%, A%) were comparable with results obtained for other grasses such as *Deschampsia caespitosa* ([Gucwa-Przepióra et al., 2013](#); A% did not exceed 3% and M% was below 5%), *Calamagrostis varia* and *Sesleria caerulea* ([Regvar et al., 2006](#)).

The lower values of M%, A% parameters on post-industrial sites can be connected with high HMs content in the soil because they may delay, reduce or eliminate production of AMF propagules ([Berthelin et al., 1995](#); [Carrenho et al., 2007](#); [Kapoor et al., 2002](#); [Mejstrik, 1972](#); [Weissenhorn et al., 1995](#); [Zarei et al., 2008](#)). High concentration of bio-available forms of heavy metals such as Cd and Zn, has even more toxic effect on plants and mycorrhizal fungi than their total content in the soil ([Gucwa-Przepióra et al., 2013](#); [Pawłowska and Charvat, 2004](#); [Regvar et al., 2006](#); [Wang et al., 2007](#); [Zarei et al., 2008](#)). We confirmed these results since we found a negative correlation between mycorrhizal colonization parameters (F%, M%, A%) of *Molinia caerulea* on post-industrial sites and concentration of bio-available Zn and to the smaller extent Cd. Similar results were obtained also by [Leyval et al. \(1995\)](#) or [Ietswaart et al. \(1992\)](#) in case of mycorrhizal root colonization of *Agrostis capillaris* specimens coming from sandy soil polluted by a zinc smelter, which in laboratory experiment were treated with Zn. Comparable to [Gucwa-Przepióra et al. \(2013\)](#), we did not find any significant relationships between bio-available Pb and mycorrhiza parameters (A%, M%, F%) of *Molinia caerulea*. The probable explanation of this fact was the low content of Pb in the studied soil that had no toxic influence on species and arbuscular mycorrhizal fungi. [Zarei et al. \(2008\)](#) found that the levels of mycorrhization declined considerably for F%, A% and M% parameters and they were even more affected by available higher Pb than Zn concentrations in contaminated soils. [Zak and Parkinson \(1982\)](#) suggested that a lack of mycorrhizal colonization observed in some grasses (*Agropyron trachycaulum*) can be found in the case of poor or absent mycorrhizal inoculum in mine spoils.

The bioavailable Zn content in the substratum of post-industrial sites was strongly negatively correlated with species richness (R; $r = -0.68$, $p < 0.0001$), Shannon diversity index (H; $r = -0.65$, $p < 0.0001$) and Evenness (E; $r = -0.62$, $p < 0.0001$). In contrast, these relationships were not statistically significant in grasslands. Based on our results we could draw a model of possible relationships between *Molinia* AMF root colonization, HM content and *Molinia* abundance on grasslands and post-industrial sites. Bioavailable Zn content in the soil is a one of main factors influencing the *Molinia* community diversity. In the grasslands, lower bioavailable Zn content, resulted in higher species richness (R) and species diversity (H) which in turn resulted in higher root colonization. On the other hand, on the post-industrial sites, the elevated bioavailable Zn content strongly decreased the species richness (R) and species diversity (H) and this caused the decline of root colonization parameters. The low species richness on Zn-polluted sites caused that *Molinia* reached higher abundance since the competition with other species is reduced.

According to [Regvar et al. \(2006\)](#) a reduced mycorrhizal colonization level may enhance colonization success of some plants at highly metal polluted sites. [Grime et al. \(1987\)](#), [van der Heijden \(2002\)](#), [Newsham et al. \(1995\)](#) stated that *Molinia caerulea*, similarly to *Festuca ovina*, *Bromus erectus* under natural conditions is not strongly dependent on mycorrhiza. [Turnau et al. \(2006\)](#) confirmed that *Molinia caerulea* belongs to facultative mycorrhizal grasses. Other studies conducted by [Pawłowska et al. \(1996\)](#) and [Regvar et al. \(2006\)](#) revealed that plants with the high mycorrhizal colonization frequency were the most common and more abundant at the less polluted sites such as *Molinia caerulea* meadows (*Molinietum caeruleae*). However, the frequency and intensity of mycorrhizal colonization of species occurring in the floristic composition of *Molinietum caeruleae* meadow varied from very common to incidental (single) infection of only a few roots [Mejstrik \(1972\)](#).

4.2 Other physico-chemical soil parameters and mycorrhizal colonization of *Molinia caerulea* roots in grassland and post-industrial sites

Apart from the HMs concentration, other factors can also have an influence on mycorrhizal colonization of species' roots such as: type of waste, soil reaction and electrical conductivity, water holding capacity and nutrient pool (Oliveira et al., 2001; Regvar et al., 2006; Ryszka and Turnau, 2007; Turnau et al., 2006, 2010; Zubek et al., 2013). In our research we found that electrical conductivity (EC) and soil reaction did not have an impact (data not shown) on almost all mycorrhizal colonization parameters of *Molinia caerulea* roots both on grasslands and post-industrial sites. The mean values of EC were higher on post-industrial sites (0.51 mS/cm) compared with those in the grasslands (0.34 mS/cm) and were not significantly greater than those indicated by Pattinson et al. (2000) as serious limitation of the mycorrhizal colonization. Soil reaction and EC had also no effect on colonization level of roots of other species growing on post-industrial sites (Gucwa-Przepióra et al., 2013; Oliveira et al., 2001; Zarei et al., 2008). In our studies a negative correlation was found between all mycorrhizal colonization parameters (A%, M%, F%) and water holding capacity (WHC%; data not shown) which is considered to be a key factor in the survival of plants on polluted soils (Ryszka and Turnau, 2007; Turnau et al., 2010).

Contrary to Zarei et al. (2008), we found that M% and A% parameters of *Molinia caerulea* root colonisation were positively correlated with sand% and negatively with clay% and silt% in the soil (higher values of clay and silt particles were found mainly in the soils of post-industrial sites). Since sandy soils are more porous and less fertile they can increase a plants' dependence on mycorrhizal associations and stimulate the optimum AMF development (Carrenho et al., 2007). High content of sand in the soil is responsible for the decrease of cation exchange capacity and potential for cation adsorption which trigger HMs concentration in the soil. The finest fractions are typically the most enriched in Zn, Pb, Cd and possibly cause the development of mycorrhiza in conditions of strong metal stress (Cabała et al., 2009).

In our study, no significant correlations were found between other soil variables (K₂O, N%), and *Molinia* root colonization parameters on the post-industrial sites and grasslands, which is similar to results obtained by Zarei et al. (2008). The CaCO₃ content was negatively correlated with A%, M% colonization parameters, whereas available P was not correlated with any of mycorrhizal parameters of *Molinia caerulea* roots. Lingfei et al. (2005) found that mycorrhizal colonization parameters were negatively correlated with total N, available P and soil organic matter, but positively correlated with soil pH. In contrast root colonization parameters correlated positively with CCE (calcium carbonate equivalent) and negatively with available phosphorus (Zarei et al., 2008).

4.3 *Molinia caerulea* as a successful colonizer of the post-industrial sites

Our results clearly show that *Molinia caerulea* develops dense and stable vegetation patches on heavy metal contaminated ~~waste~~ sites in spite of parameters of mycorrhizal colonization (F%, A%, M%) of its roots are significantly lower compared with those measured for *Molinia* individuals coming from grasslands. Contrary to our expectations, it seems for *Molinia caerulea* to become successfully established on heavy metal contaminated wastes, any additional support such as inoculation with mycorrhiza is needed. Turnau et al. (2012) found in experiments with introducing of plantlets obtained by splitting of *Molinia caerulea* tussocks on bare zinc waste substrates that it can survive without additional fertilisation, watering or introduction of mycorrhizal inoculum. This is a plant suitable to cover heavy metal contaminated sites due to its native origin, common occurrence, low soil requirements (particularly P and N), and ability to accumulate organic matter what improves microbial activity (Turnau et al., 2006, 2008, 2012). Its abundant, fibrous root system play an important role in binding fine particles of wastes and in this way prevents a given site from soil erosion, particularly water erosion of heavy metals (Ryszka and Turnau, 2007; Turnau et al., 2008). In this way it can be used in phytostabilisation of polluted soils. Moreover, this species is resistant to taking up large amount of heavy metals what is explained by increased content of Si in its tissue (Kabata-Pendias, 2001). One of mechanisms, which this species uses in keeping lower concentration of HMs in its shoot, are small amounts of polisaccharides that can bind heavy metals within intracellular spaces (Broadley et al., 2003). This grass also has a high aesthetic value. It changes colour from green to yellow through the year which makes heaps covered by *Molinia caerulea* an attractive element in post-industrial landscapes.

Understanding the interplay between environmental conditions, dynamics of plants and AM fungal communities is a crucial step in ecosystem management, rehabilitation and restoration (García de León et al., 2016). The latest studies on vegetation development of post-industrial sites also confirmed that soil and environmental biota enhance the ecosystem function (Stefanowicz et al., 2015) and conservation value of those sites (Harabiš et al., 2013). Some species that spontaneously colonized spoil material since they affect nutrient availability and support microbial performance can be recommended for the use in reclamation works instead of cultivars (Stefanowicz et al., 2015). Moreover, our results confirmed that the knowledge about biology of species spontaneously colonizing the post-industrial sites is of prime importance. It is well-known phenomenon the lack of success of commercial cultivars used in reclamation even if financial input was large and some technical works were undertaken (covering with topsoil, watering, seeding with seeds). In spite of high biomass, such cultivars frequently depend on well-established mycorrhiza in a given site, high fertilizer input and inorganic and organic amendments that affect the rates at which plant are infected by AMF over time. Their populations decrease with time when the introduced soil is removed by water erosion (Zak and Parkinson, 1982; Turnau et al., 2010, 2006). These cultivars are mostly non-mycorrhizal species and their populations decrease with time Turnau et al. (2006). They are followed in the succession by facultative mycorrhizal species such as *Molinia caerulea*. In presence of environmental stress, *Molinia caerulea* can act as a nurse plant allowing for the appearance of seedlings of other plant and animal (e.g. ants) species during the course of succession (Turnau and Ryszka, 2007; Ryszka and Turnau, 2007; Turnau et al., 2012). Ants by building underground corridors and nests can transport seeds of other plants increasing species diversity of a given sites as well as spores of AMF. This grass can be accompanied by some microorganisms, among them nitrogen fixing bacteria which by supplying it with nitrogen enable it to growth better (Hamelin et al., 2002; Reinhold-Hurek and Hurek, 1998). Nutrients can be stored during winter in swollen intercalary basal nodes and release more than ones during spring what helps *Molinia* to regrowth after drought period (Jefferies, 1915; Turnau et al., 2012).

5 Conclusions

Our results revealed the strong, negative influence of HM contents, especially Zn and other factors such as soil parameters: soil texture, WHC(%), CaCO₃ (%) and species diversity on root colonization of *Molinia caerulea*. We confirmed that *Molinia caerulea* is a facultative mycorrhizal species, since we did not find any relationship between its abundance and root colonization parameters. An efficient and lower cost restoration of HMs contaminated sites would be the introduction of *Molinia caerulea* individuals obtained from post-industrial sites. *Molinia caerulea* can work as a “bio-enhancer” in the re-vegetation of such post-industrial sites and in this way can support natural colonization processes that are considered by engineers as slow. Forming dense and stable vegetation cover, this species can be used in phytoremediation of metal contaminated sites.

Uncited reference

Magurno et al. (2014) we cited this paper in introduction.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2016.07.013>.

References

- Ammani K., Venkateswarlu K. and Rao A.S., Vesicular arbuscular mycorrhizae in grasses: their occurrence, identity and development, *Phytomorphol.* **44** (3-4), 1994, 159-168.
- Bednarek R., Dziadowiec H., Pokojaska U. and Prusinkiewicz Z., *Badania ekologiczno-gleboznawcze*, 2005, PWN; Warszawa, (in Polish).
- Berthelin J., Munier-Lamy C. and Leyval C., Effect of microorganisms on mobility of heavy metals in soils, In: Huang P.M., Berthelin J., Bollag J.-M., McGill W.B. and Page A.L., (Eds.), *Environmental impact of soil component interactions, Vol. 2, Metals, other inorganics, and microbial a* *Impact of Soil Component Interactions, Vol. 2, Metals, Other Inorganics, and Microbial Activities*, 1995, CRC Press; 3-17.
- Bothe H., Regvar M. and Turnau K., Arbuscular mycorrhiza, heavy metal and salt tolerance, In: Sherameti I. and Varma A., (Eds.), *Soil heavy mHeavy Metals. Soil Biology Series*, 2010, Springer; Heidelberg. Berlin. New York, 87-111.
- Broadley M.R., Bowen H.C., Cotterill H.L., Hammond J.P., Meacham M.C., Mead A. and White P.J., Variation in the shoot calcium content of angiosperms, *Journal of Experimental. Exp. B* **54**, 2003, 1431-1446.
- Cabała J., Krupa P. and Misz-Kennan M., Heavy metals in mycorrhizal rhizospheres contaminated by Zn-Pb mining and smelting around Olkusz in southern Poland, *Water Air Soil Pollut.* **199**, 2009, 139-149.
- Carrenho R., Trufem S.F.B., Bononi V.L.R. and Silva E.S., The effect on different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize, *Acta Bot. Bras.* **21** (3), 2007, 723-730.
- Chmura D., Molenda T., Błońska A. and Woźniak G., Sites of leachate inflows on coalmine heaps as refuges of rare mountainous species, *Pol. J. Environ. Stud.* **20** (3), 2011, 551-557.
- Doni S., Macci C., Peruzzi E., Iannelli R. and Masciandaro G., Heavy metal distribution in a sediment phytoremediation system at pilot scale, *Ecol. Eng.* **81**, 2015, 146-157.
- Eason W.R., Newman E.I. and Chuba P.N., Specificity of interplant cycling of phosphorus: The role of mycorrhizas, *Plant Soil* **137**, 1991, 267-274. Entry J.A., Rygielwicz P.T., Watrud L.S. and Donnelly P.K., Influence of adverse soil conditions on the formation and function of Arbuscular mycorrhizas. *Advances in Environmental Research* **7**, 2002, 123-138.
- Göhre V. and Paszkowski U., Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation, *Planta* **223**, 2006, 1115-1122.
- García de León D., Moora M., Öpik M., Neuenkamp L., Gerz M., Jairus T., Vasar M., Bueno C.G., Davison J. and Zobel M., Symbiont dynamics during ecosystem succession: co-occurring plant and arbuscular mycorrhizal fungal communities, *FEMS Microbiol. Ecol.* **92**, 2016, fiw097, <http://dx.doi.org/10.1093/femsec/fiw097>.
- Grime J.P., Mackey J.M.L., Hillier S.H. and Read D.J., Floristic diversity in a model system using experimental microcosms, *Nature* **328**, 1987, 420-422.
- Grime J.P., Hodgson J.G. and Hunt R., Comparative Plant Ecology, A Functional Approach to Common British Species, 1988, Unwin Hyman; London, UK.

- Gucwa-Przepióra E. and Błaszczkowski J., Arbuscular mycorrhiza of plant spontaneously colonizing the soda heap in Jaworzno (southern Poland), *Acta Soc. Bot. Pol.* **76**, 2007, 69-74.
- Gucwa-Przepióra E. and Turnau K., Arbuscular mycorrhiza and plant succession on zinc smelter spoil heap in Katowice-Wełnowiec, *Acta Soc. Bot. Pol.* **70** (2), 2001, 153-158, <http://dx.doi.org/10.5586/asbp.2001.020> [Gucwa-Przepióra E., Nadgórska-Socha A., Fojcik B. and Chmura D., Enzymatic activities and arbuscular mycorrhizal colonization of *Plantago lanceolata* and *Plantago major* in a soil root zone under heavy metal stress. *Environ. Sci. Pollut. Res.* **23**, 2016, 4742-4755.](#)
- Gucwa-Przepióra E., Błaszczkowski J., Kurtyka R., Małkowski Ł. and Małkowski E., Arbuscular mycorrhiza of *Deschampsia caespitosa* (Poaceae) at different soil depths in highly metal-contaminated site in southern Poland, *Acta Soc. Bot. Pol.* **82** (4), 2013, 251-258.
- Hamelin J., Fromin N., Tarnawski S., Teyssier-Cuvelle S. and Aragno M., nifH gene diversity in bacterial community associated with the rhizosphere of *Molinia caerulea*, an oligonitrophilic perennial grass, *Environ. Microbiol.* **4**, 2002, 477-481.
- Harabiš F., Tichanek F. and Tropek R., Dragonflies of freshwater pools in lignite spoil heaps—restoration management, habitat structure and conservation value, *Ecol. Eng.* **55**, 2013, 51-61.
- Harley J.L. and Harley E.L., A check-list of mycorrhiza in the British flora, *New Phytol. Suppl.* **105**, 1987, 1-102.
- ~~157, 243-265.~~ [Hildebrandt et al., 2007](#) [U. Hildebrandt, M. Regvar and H. Bothe](#), Arbuscular mycorrhiza and heavy metal tolerance, *Phytochemistry* **68** (1), 2007, 139-146.
- Ietswaart J.H., Griffioen W.A.J. and Ernst W.H.O., Seasonality of VAM infection in three populations of *Agrostis capillaris* (Gramineae) on soil with or without heavy metal enrichment, *Plant Soil* **139** (1), 1992, 67-73 <http://dx.doi.org/10.1007/BF00012843>.
- Jefferies T.A., Ecology of the purple heath grass (*Molinia caerulea*), *J. Ecol.* **3**, 1915, 93-109.
- Jefferies T.A., The vegetative anatomy of *Molinia caerulea*, the purple heath grass, *New Phytol.* **15**, 1916, 49-71.
- Jeffries P., Gianinazzi S., Perotto S., Turnai K. and Barea J.-M., The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility, *Biol. Fertil.* **37**, 2003, 1-16.
- Jochimsen M., Vegetation development and species assemblages in a long-term reclamation project mine spoil, *Ecol. Eng.* **17** (2-3), 2001, 187-198.
- Kabata-Pendias A., Trace ~~elements in soils and plants~~ [3rd. eds Elements in Soils and Plants, 3rd ed.](#), 2001, CRC Press; Boca Raton, London, New York, Washington.
- Kapoor R., Gird B. and Mukerji K.G., Soil factors in relation to distribution and occurrence of vesicular arbuscular mycorrhiza, In: Mukerji K.G., Manoharachary C. and Chamola B.P., (Eds.), *Techniques in Mycorrhizal Studies*, 2002, Kluwer Academic Press; Netherlands, 51-85.
- Kompała A. and Woźniak G., Grasses in anthropogenic plant communities, In: Frey L., (Ed), *Studies on Grasses in Poland*, 2001, W. Szafer Institute of Botany, Polish Academy of Sciences; Kraków, 329-351.
- Kompała-Bąba A. and Bąba W., The spontaneous succession in a sand-pit—the role of history traits and species habitat preferences, *Pol. J. Ecol.* **61** (1), 2013, 13-22.
- Kompała-Bąba A., Błońska A., Bąba W. and Czyba M., Grasses in the plant communities which develop on the waste sites of zinc-lead industry (Upper Silesia, S Poland), In: Frey L., (Ed), *Biology of Grasses*, Szafer, 2005 W. Institute of Botany, Polish Academy of Sciences; Kraków, 269-283.
- Kondracki J., Geografia regionalna Polski, 2011, Wydawnictwo Naukowe PWN; Warszawa, (in Polish).
- Krishnamoorthy R., Kim C.G., Subramanian P., Kim K.Y., Selvakumar G. and Sa T.M., Arbuscular mycorrhizal fungi community structure, abundance and species richness changes in soil by different levels of heavy metal and metalloids concentration, *PLoS ONE* **10** (6), 2015, 1-15 [Kumar, A., Raghuvanshi, R., and Upadhyay R. S., Vesicular-arbuscular mycorrhizal association in naturally revegetated coal mine spoil. *Trop. Ecol.* **44**\(2\), 2003, 253-256.](#)
- Lacková E., Kupec P., Polanská J. and Stalmachová B., Social forest functions of reclaimed spoil heaps in the Ostrava-Karvina district, [Journal of Forest Science Institute of Agricultural and Food Information, For. Sci. Inst. Agric. Food Inf](#) 2012, 203-212, (ISSN 1212-4834).
- Leyval C., Singh B.R. and Joner E., Occurrence and infectivity of arbuscular-mycorrhizal fungi in some Norwegian soils influenced by heavy metals, soil properties, *Water Air Soil Pollut.* **84**, 1995, 203-216.

Leyval C., Turnau K. and Haselwandter K., Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects, *Mycorrhiza* **7**, 1997, 139-153.

Li-Ping W., Kui-mei Q., Shi-long H. and Bo F., Fertilizing reclamation of arbuscular mycorrhizal fungi on coal mine complex substrate, *Procedia Earth and Planetary Science Planet. Sci.* **1**, 2009, 1101-1106.

Lingfei et al., 2005. [L. Lingfei, Lingfei L., Anna Y.Y. Anna and Z. Zhiwei, Zhiwei Z.](#), Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China, *FEMS Microbiol. Ecol.* **54**, 2005, 367-373.

Magurno F., Sasvári Z. and Posta K., From monoculture to the Norfolk system: assessment of arbuscular mycorrhizal fungi communities associated with different crop rotation systems, *Symbiosis* **64**, 2014, 115-125.

Mardukhi B., Rejali F., Daei G., Ardakani M.R., Malakouti M.J. and Miransari M., Arbuscular mycorrhizas enhance nutrient uptake in different wheat genotypes at high salinity levels under field and greenhouse conditions, *Comptes Rendus - Biologies* **334** (7), 2011, 564-571, <http://dx.doi.org/10.1016/j.crvi.2011.05.001> [Markowicz A., Woźniak G., Borymski S., Piotrowska-Seget Z. and Chmura D., Links in the functional diversity between soil microorganisms and plant communities during natural succession in coal mine spoil heaps. *Ecol. Res.* **30**, 2015, 1005-1014.](#)

Medina A. and Azcón R., Effectiveness of the application of arbuscular mycorrhizal fungi and organic amendments to improve soil quality and plant performance under stress conditions, *J. Soil Sci. Plant Nutr.* **10** (3), 2010, 354-372.

Mejstrik V.K., Vesicular arbuscular mycorrhizas of the species of a *Molinetum caeruleae* L. association: the ecology, *New Phytol.* **71**, 1972, 883-890.

Mirek Z. and Piękoś-Mirkowa H., Trawy gór, In: Frey L., (Ed), *Księga polskich traw. Instytut Botaniki im. W. Szafera*, 2007, Polska Akademia Nauk; Kraków, 203-228, (in Polish).

Mohammadi K., Khalesro S., Sohrabi Y. and Heidari G., A review: beneficial effects of the mycorrhizal fungi for plant growth, *J. Appl. Environ. Biol. Sci.* **1** (9), 2011, 310-319.

Newsham K.K. and Watkinson R., Arbuscular mycorrhizas and the population biology of grasses, In: Cheplick G.P., (Ed), *Population Biology of Grasses*, 1998, Cambridge University Press; Cambridge, UK, 286-310.

Newsham K.K., Fitter A.H. and Watkinson A.R., Multi-functionality and biodiversity in arbuscular mycorrhizas, *Trends Ecol. Evol.* **10**, 1995, 407-411.

Oliveira R.S., Dodd J.C. and Castro P.M.L., The mycorrhizal status of *Phragmites australis* in several polluted soils and sediments of an industrialised region of Northern Portugal, *Mycorrhiza* **10**, 2001, 241-247.

Orłowska E., Zubek S., Jurkiewicz A., Szarek-Lukaszewska G. and Turnau K., Influence of restoration on arbuscular mycorrhiza of *Biscutella laevigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds, *Mycorrhiza* **12** (3), 2002, 153-159, <http://dx.doi.org/10.1007/s00572-001-0155-4>.

Ostrowska A., Metody analizy i oceny właściwości gleb i roślin, 1991, Instytut Ochrony Środowiska; Warszawa, (in Polish).

Pattinson G.S., Sutton B.G. and McGee P.A., Leachate from a waste disposal centre reduces the initiation of arbuscular mycorrhiza, and spread of hyphae in soil, *Plant Soil* **227** (1), 2000, 35-45.

Pawłowska T.E. and Charvat I., Heavy-metal stress and developmental patterns of arbuscular mycorrhizal fungi, *Appl. Environ. Microbiol.* **70** (11), 2004, 6643-6649.

Pawłowska T.E., Błaszczowski J. and Ruhling A., The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland, *Mycorrhiza* **6**, 1996, 499-505.

Pełka-Gościński J., Environmental aspects of relief transformation (Silesian Upland, [Southern Poland](#)) *Environ. Socio-economic southern Poland*, *Environ. Socio Economic Stud.* **2** (4), 2014, 13-20.

Phillips J.M. and Hayman D.S., Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection, *Transactions of the British Mycological Society, Bri. Mycol. Soc.* **55**, 1970, 158-161 [Prach K., Bartha S., Joyce C.B., Pysek P., Van Diggelen R. and Wiegleb G., The role of spontaneous vegetation succession in ecosystem restoration: A perspective. *Appl. Veg. Sci.* **4**, 2001, 111-114.](#) [Qian K., Wang L. and Yin N., Effects of AMF on soil enzyme activity and carbon sequestration capacity in reclaimed mine soil. *Int. J. Min. Sci. Technol.* **22**, 2012, 553-557.](#)

R Core Team, R: A Language and Environment for Statistical Computing, 2013, R Foundation for Statistical Computing; Vienna, Austria <http://www.R-project.org/>.

Rahmonov O. and Oleś W., Vegetation succession over an area of a medieval ecological disaster: The case of the Błędów, Desert [Poland, Poland](#), *Erdkunde* **64** (3), 2010, 241-255.

Rahmonov O. and Szymczyk A., Relations between vegetation and soil in initial succession phases in post-sand excavations, *Ekologia (Bratislava)* **29** (4), 2010, 412-429.

- Regvar M., Vogel-Mikuš K., Kugonič N., Turk B. and Batič F., Vegetation and mycorrhizal successions at a metal polluted site: ~~Ind~~ication for the direction of phytostabilisation?, *Environ. Pollut.* **144**, 2006, 976–984.
- Reinhold-Hurek B. and Hurek T., Life in grasses: diazotrophic endophytes, *Trends Microbiol.* **6**, 1998, 139–144.
- Rostański A., Spontaniczne kształtowanie się pokrywy roślinnej na zwałowiskach po górnictwie węgla kamiennego na Górnym Śląsku, 2006, Wydawnictwo Uniwersytetu Śląskiego; Katowice, (in Polish).
- Ryszka P. and Turnau K., Arbuscular mycorrhiza of introduced and native grasses colonizing zinc wastes: implications for restoration practices, *Plant Soil* **298**, 2007, 219–229.
- Salim K.A., Gordon D.B., Shaw S. and Smith C.A., Variation in *Molinia caerulea* (L.) Moench, the purple moor grass, in relation to edaphic environments, *Ann. Bot.* **75**, 1995, 481–489.
- Shetty K.G., Hetrick B.A.D., Figge D.A.H. and Schwab A.P., Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil, *Environ. Pollut.* **86**, 1994, 181–188.
- Smith S.E. and Read D., Mycorrhizal ~~symbiosis~~^{3rd edn}~~Symbiosis~~, ^{3rd ed.}, 2008, Academic; New York.
- Smyth C.R. and Box P.O., Establishment and growth of mycorrhizal and Rhizobium inoculated high-elevation native legumes on an unamended coal mine spoil dump in South-eastern British Columbia, *Proceedings of the 21st Annual British Columbia Mine Reclamation Symposium in Cranbrook* 1997, 32–45.
- Sokal R. and Rohlf F.R., Biometry. The Principles and Practice of Statistics in Biological Research, 1995, W.H Freeman and Company; New York.
- Stalmachová, B., Sierka, E., 2014. Managed succession in reclamation of post-mining landscape. Technická univerzita v Košiciach, 1. Vyd., Košice, p. 85.
- StatSoft, Inc, STATISTICA (~~data analysis software system~~), ^{vData Analysis Software System}, Version 10, 2011 www.statsoft.com.
- 85. Stefanowicz et al., 2015.** ~~A.M.~~ Stefanowicz ^{A.M.}, ~~P.~~ Kapusta ^{P.}, A. Błońska, ~~A.~~ Kompała-Bąba ^{A.} and ~~G.~~ Woźniak, ^{Woźniak G.}, Effects of *Calamagrostis epigejos*, *Chamaenerion palustre* and *Tussilago farfara* on nutrient availability and microbial activity in the surface layer of spoil heaps after hard coal mining, *Ecol. Eng.* **83**, 2015, 328–337.
- Szarek-Lukaszewska G. and Grodzińska K., Grasslands of a Zn–Pb post-mining area (Olkusz ore-bearing region, S Poland), *Pol. Bot. J.* **56** (2), 2011, 245–260.
- Taylor K., Rowland A.P. and Jones H.E., *Molinia caerulea* (L.) ~~Moench~~^{Ecolmoench}, *J. Ecol.* **89**, 2001, 126–144.
- Tropek R., Kadlec T., Hejda M., Kocarek P., Skuhrovec J., Malenovsky I., Vodka S., Spitzer L., Banar P. and Konvicka P., Technical reclamations are wasting the conservation potential of post-mining sites. A case study of black coal spoil dumps, *Ecol. Eng.* **43**, 2012, 13–18.
- Trouvelot A., Kough J.L. and Gianinazzi-Pearson V., Mesure du taux de mycorhization VA d'un systeme racinaire. Recherche de methodes d'estimation ayant une signification fonctionnelle, In: Gianinazzi-Pearson V. and Gianinazzi S., (Eds.), *Physiological and ~~genetical aspects of m~~Genetical Aspects of Mycorrhizae*, 1986, INRA; Paris, 217–221.
- Turnau K., Orłowska E., Ryszka P., ^{Zubek} Sz., Anielska T., Gawroński S. and Jurkiewicz A., Role of mycorrhizal fungi in phytoremediation and toxicity monitoring of heavy metal rich industrial wastes in southern Poland, In: Twardowska I., Allen H.E., Häggblom M.M. and Stefaniak S., (Eds.), *Soil and Water Pollution Monitoring, Protection and Remediation*, 2006, Springer, 3–23.
- Turnau K., Anielska T., Ryszka P., Gawroński S., Ostachowicz B. and Jurkiewicz A., Establishment of arbuscular mycorrhizal plants originating from xerothermic grasslands on heavy metal rich industrial wastes ~~new~~ solution for waste revegetation, *Plant Soil* **305**, 2008, 267–280.
- 3–23 Turnau et al., 2010.** ~~K.~~ Turnau ^{K.}, ~~P.~~ Ryszka ^{P.} and ~~G.~~ Wojtczak ^{G.}, Metal tolerant mycorrhizal plants: a review from the perspective on industrial waste in temperate region, In: H. Koltai and Y. Kapulnik, (Eds.), *Arbuscular Mycorrhizas: Physiology and Function*, 2010, Springer Science ⁺ ⁺ Business Media B.V, 257–276.
- Turnau K., Gawroński S., Ryszka P. and Zook D., Mycorrhizal-based phytostabilization of Zn–Pb tailings: lessons from the Trzebionka ^{Mining-Wmining} works (Southern Poland), In: Kothe E. and Varma A., (Eds.), *Bio-Geo Interactions in Metal Contaminated Soils*, *Soil Biol.* **31**, 2012, 327–348.
- Ullrich S.M., Ramsey M.H. and Helios-Rybicka E., Total and exchangeable concentration of heavy metals in soils near Bytom, an area of Pb/Zn mining and smelting in Upper Silesia, Poland, *Appl. Geochem.* **14**, 1999, 187–196.
- van der Heijden, M.G.A., 2002. Arbuscular mycorrhizal fungi as a determinant of plant diversity: in search of underlying mechanisms and general principles. ~~Fn-]In~~ van der Heijden, M.G.A., Sander, I.R.,

(Eds.), Mycorrhizal Ecology. Ecological Studies, [157, 243-265](#).

Violante A., Cozzolino V., Perelomov L., Caporale A.G. and Pigna M., Mobility and bioavailability of heavy metals and metalloids in soil environments, *J. Soil. Sci. Plant Nutr.* **10** (3), 2010, 268-292.

Wójcik M., Sugier P. and Siebielec G., Metal accumulation strategies in plants spontaneously inhabiting Zn-Pb waste deposits, *Sci. Tot. Environ.* **487**, 2014, 313-322.

Wang Y., Shi J., Lin Q., Chen X. and Chen Y., Heavy metal availability and impact on activity of soil microorganisms along a Cu/Zn contamination gradient, *J. Environ. Sci. Environ. Sci.* **19**, 2007, 848-853.

Weissenhorn I., Leyval C. and Berthelin J., Bioavailability of heavy metals and abundance of arbuscular mycorrhiza in a soil polluted by atmospheric deposition from a smelter, *Biol. Fertil. Soils* **19**, 1995, 22-28.

Woźniak et al., 2003. ~~G. Woźniak G., A. Pasierbiński and A. Rostański, Pasierbiński A., Rostański A.~~ The diversity of spontaneous woodland vegetation on coals mine heaps of Upper-Silesian Industrial Region, *Arch. Environ. Prot.* **29** (2), 2003, 93-105.

Woźniak G., Chmura D., Błońska A., Tokarska-Guzik B. and Sierka E., Applicability of the concept of functional groups for analysis of spatiotemporal vegetation changes on manmade habitats, *Pol. J. Environ. Stud.* **20** (3), 2011, 623-631.

Woźniak G., Markowicz A., Borymski S., Piotrowska-Seget Z., Chmura D. and Besenyei L., The relationship between successional vascular plant assemblages and associated microbial communities on coal mine spoil heaps, *Community. Ecol.* **16** (1), 2015, 23-32.

Woźniak G., Diversity of ~~vegetation on coal mine h~~[Vegetation on Coal-Mine H](#)eaps of the Upper Silesia (Poland), 2010, W. Szafer Institute of Botany, Polish academy of Sciences; Kraków, 320 pp.

Zak J.C. and Parkinson D., Initial vesicular-arbuscular mycorrhizal development of slender wheatgrass on two amended mine spoils, *Can. J. Bot.* **60**, 1982, 2241-2248, <http://dx.doi.org/10.1139/b82-275>.

Zarei M., Saleh-Rastin N., Gholamreza S.J., Gholamreza S. and Francois B., Arbuscular mycorrhizal abundance in contaminated soils around a zinc and lead deposits, *Eur. J. Soil Biol.* **44**, 2008, 381-391.

Zarzycki K., Trzcinińska-Tacik G., Różański W., Szelaż Z., Wotek J. and Korzeniak U., Ecological ~~indicator values of vascular p~~[Indicator Values of Vascular P](#)lants of Poland, 2002, W. Szafer Institute of Botany, Polish Academy of Sciences; Kraków, 183 p.

Zubek S., Błaszczkowski J., Seidler-Łożykowska K., Bąba W. and Mleczko P., Arbuscular mycorrhizal fungi abundance, species richness and composition under the monocultures of five medicinal plants, *Acta Sci. Pol. Hortorum Cultus* **12** (5), 2013, 127-141.

Zuur A.F., Ieno E.N., Walker N.J., Saveliev A.A. and Smith G.M., Mixed Effect Models and Extensions in Ecology with R, 2009, Springer; New York.

Appendix A. Supplementary data

The following is Supplementary data to this article:

[Multimedia Component 1](#)

Highlights

- Effects of soil heavy metal content on *Molinia caerulea* mycorrhizal root colonization were tested.
 - Higher bioavailable heavy metal content, particularly Zn reduced root colonization of *Molinia caerulea*.
 - *Molinia caerulea* abundance does not strongly depend on the presence of mycorrhiza.
 - *Molinia caerulea* could be a promising species in reclamation work.
-

Queries and Answers

Query: The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly

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Woźniak

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