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ORIGINAL PAPER

The effect of amendments on *Lolium perenne* roots arbuscular mycorrhizal fungi colonization when cultivated in contaminated soil

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Abstract

Arbuscular Mycorrhizal Fungi that colonize the roots of plants growing on lands contaminated by heavy metals may influence the phytostabilization process reducing the translocation of metals to the aboveground parts of the plant. This study aimed to evaluate the effects of soil amendments (lime and lignite) on the concentration of the bioavailable form of heavy metals ($CaCl_2$ extraction) in soil and on the colonization of Arbuscular Mycorrhizal Fungi in the roots of *Lolium perenne* when cultivated in contaminated soil. During the experiment, the bioavailability of Pb, Cd, and Zn in soil was significantly reduced after application of the amendments, causing an increase of *L. perenne* shoot dry biomass. It was observed that the higher dose of lime (0.5%) resulting in amplified values of relative mycorrhizal intensity. However, independently of the dose, the treatments increased the occurrence of arbuscules in *L. perenne* roots, with the highest value observed after the application of 0.25% lime with 5% lignite. The results for the first time present the effect of lime and lignite application on the *L. perenne* roots colonization by Arbuscular Mycorrhizal Fungi indicating the increase of occurrence of arbuscules. These findings suggest that in order to explain the different responses of Arbuscular Mycorrhizal Fungi to the applied treatment further investigations are needed to identify the spore morphology. The results of the experiment were implemented to stabilize heavy metals during remediation of a spoil heap in Ruda Śląska, Poland.

Keywords Arbuscular mycorrhiza · Heavy metals · Lignite · Lime · Phytostabilization

Introduction

An incessant growth of industry and other anthropogenic activities have been a cause of some adverse changes in the environment which may indirectly affect human health. A by-product of these activities are heavy metals which lead to soil contamination (Jiang et al. 2018). The most commonly occurring metal(loid)s in contaminated soil are Pb, Cd, Zn, As, Cr, Cu, Hg, and Ni (Laghlimi et al. 2015). Those might negatively impact the environment by affecting not only the quality of soil, but the microbial activity of crops and the physiological status of plants as well, leading to a decrease in food quality and safety (Liu et al. 2018; Shahid et al. 2015; Yang et al. 2015a, b). The adverse and toxic effects of heavy metals on plants, such as changes in their metabolism and growth limitation, are well known (Emamverdian et al. 2015; Wani et al. 2018). Therefore, it is necessary to deal with contaminants in the soil and seek for solutions that lead to a proper management of degraded areas while reducing environmental hazards associated with them.

Phytoremediation of contaminated soils reduces the risk for human health and supports the environmental restoration of post-industrial and degraded areas. It allows for an effective stabilization and/or elimination of heavy metals at low costs restoring the biological balance in areas excluded from food production (Fiorentino et al. 2018). There are several approaches where plants are used for heavy metal contaminated land restoration including phytoextraction, phytostabilization, phytodegradation, and phytovolatilization (Hanus-Fajerska and Koźmińska 2016). Among them, phytostabilization seems to demonstrate the highest application potential in terms of effectiveness to deal with severely heavy metal contaminated soils. The method consists in

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stabilizing the metals in the rhizosphere and consequently limiting their mobility in soil and translocation to the above-ground biomass (Barbosa and Fernando 2018; Parmar and Singh 2015). Aided phytostabilization consists in using soil amendments along with selected plant species. Such combination can substantially amplify the effect of reducing the concentration of heavy metal bioavailable form in soil and at the same time decrease the metals uptake by plants by increasing the soil pH, organic matter content, etc. (Jin et al. 2016; Liu et al. 2018; Martínez-Martínez et al. 2019; Radziemska 2017, Radziemska et al. 2017). Aided phytostabilization is recommended as a suitable and cost-effective technique for heavily metal-polluted soils (Bidar et al. 2016; Gonneau et al. 2017), especially to prevent wind and water erosion on post-mining tailings (Barbosa and Fernando 2018; Luo et al. 2019). This remediation strategy can be used on a large scale, both in rural and industrial areas (Bidar et al. 2016). Aluminosilicates, organic materials, lignite, liming agents, and different phosphorus compounds have been successfully tested in the past as soil amendments (Briceño et al. 2018; Cui et al. 2016; Fresno et al. 2018; Goulding 2016; Shackira and Puthur 2019). The literature data and a number of experiments show that the most desirable features of plant species for phytostabilization are high tolerance to heavy metals, low level of metal accumulation in shoots, and ability to develop extensive root system (Burges et al. 2016; Jutsz and Gnida 2015; Radziemska 2018). Many authors indicated *Lolium perenne* as a promising species for aided phytostabilization (Kacprzak et al. 2014; Radziemska 2017), including metal contaminated soils from post-industrial areas and mine tailings deposits (España et al. 2019; Hechelski et al. 2019).

Heavy metal uptake may be supported by the activity of soil microorganisms, including Arbucular Mycorrhizal Fungi (AMF) (Gu et al. 2017). AMF are one of the most widespread representatives of microbiota (Della Mónica et al. 2015; Öpik et al. 2013). AMF occur in many habitats, including contaminated sites, where plant growth is limited (Bąba et al. 2016; Gunathilakae et al. 2018; Nowak et al. 2019; Rajtor and Piotrowska-Seget 2016). In recent years, the effect of AMF on phytoremediation process has been considered in many studies (Cabral et al. 2015; Das et al. 2017; Schneider et al. 2016). They showed that the role of AMF in the phytoremediation process is equally important as the role of plants (Yang et al. 2016). Also, the impact of AMF on heavy metal accumulation in plants has been described in detail (Liu et al. 2015; Zhan et al. 2016).

It is assumed that the presence of AMF may have a significant influence on the efficiency of heavy metal accumulation by host plants which subsequently could improve the phytostabilization process (Rusinowski et al. 2019). AMF may aid plant growth in heavy metal contaminated soils by facilitating the supplementation of essential nutrients in host plants (Cely et al. 2016). It could also improve the tolerance of host plants to heavy metals pollution by, e.g., chelating metals inside the hyphae or its precipitation in polyphosphate granules in the soil (Latef et al. 2016).

In various studies lime was used to increase the pH of soil to reduce the bioavailability of heavy metals (Heyburn et al. 2017). Also there is data available from the investigations of lignite sorption capability in metals immobilization (Briceño et al. 2018). Using information about the proven properties of both lime and lignite as soil additives (Gucwa-Przepióra et al. 2007), it was proposed to test a combination of them to improve the properties of soil heavily contaminated with Pb, Cd, and Zn. The purpose of this study was to investigate and assess the application effects of two different combined doses of lignite and lime on soil parameters, including pH and heavy metals' bioavailability. Moreover, the literature showed the impact of lime on AMF colonization (Heyburn et al. 2017; Seguel et al. 2016), but there is no data available on the effects of a combined application of lignite and lime on *Lolium perenne* roots AMF colonization process. Therefore study attempted also to assess the impact of soil amendments on the mycorrhizal colonization parameters and Pb, Cd, and Zn concentration in perennial ryegrass (*Lolium perenne* cv. STADION). This study was performed in the year 2017, at the facility of the Institute for Ecology of Industrial Areas, Katowice, Poland.

Material and methods

Experiment design

The soil for the pot experiment was collected from a spoil heap of a closed-down zinc smelter located in Ruda Śląska (Upper Silesia, Poland). The soil was highly contaminated with heavy metals, particularly Pb, Cd, and Zn. Soil samples were collected in 2017 from spots where the highest concentration of the bioavailable forms of heavy metals was found, from a depth of 0–25 cm. The selection of spots was based on data from a previous chemical analysis of soil from the site and the GIS data. The collected soil was air-dried, passed through a 4 mm sieve and transferred into plastic pots. Five pots (0.0015 m^3 volume each) for each treatment were prepared and labeled as follows:

- Non-amended soil (C),
- Soil amended with 5% (w/w) lignite and 0.5% (w/w) lime (LL_I),
- Soil amended with 5% (w/w) lignite and 0.25% (w/w) lime (LL_II).

To control the stabilization process, soil samples were collected three times: at the beginning of the experiment and subsequently after two and six weeks. Soil samples were air-dried and sieved through < 2 mm sieve to remove large particles for pH and electric conductivity determination and then through < 0.25 mm. The concentrations of the bioavailable forms ($CaCl_2$ extractable fraction) and pseudo-total concentrations of heavy metals (Pb, Cd, and Zn) were measured. Subsequently, *Lolium perenne* cv. STADION was sown and cultivated in the growing chamber under controlled conditions: temperature 24 °C, 300 µmol (photons) m^{-2} s^{-1}, and humidity 50%. The aboveground parts of the plants were harvested after six and thirteen weeks after the sowing. Additionally, at the end of the experiment, root samples were collected. The root samples were divided into two parts, one for each experimental treatment and one for replication, to estimate the mycorrhizal colonization parameters. The remaining roots and the aboveground parts of the plants, were washed with tap water to remove soil particles, and then again with deionized water. Subsequently, plant material was dried for 3 days at 70 °C and, after constant weight was achieved, the samples were milled to a homogeneous powder (< 1 mm) for further analysis (Pb, Cd, and Zn concentration). The possible limitations of the experiment are related to the scale as it was conducted in pots, where limited amount of soil is provided and might only in some extend reflect the process in open-environment. Another limitations is related to conduct experiment in controlled conditions, which do not reflect the fluctuations of environment variables during field implementation. However, to investigate organisms behavior limiting effect of variables is required to draw initial conclusions before full-scale implementations.

Analysis of soil

The physicochemical properties of soil, such as pH and electrical conductivity (EC), were measured according to standardized methods. Soil pH was analyzed in H_2O and 1 M KCl (ratio 1:2.5 m/v) with a combination of glass and calomel electrode (OSH 10–10, METRON, Poland) and a pH-meter (CPC-551, Elmetron, Poland). The EC was measured using an ESP 2 ZM electrode (EUROSENSOR, Poland) according to the Polish norm PN-ISO 11265:1997, using the same device as for pH. To analyze the concentrations of the bioavailable forms of metals (Cd, Pb, and Zn) soil extraction with 0.01 M $CaCl_2$ was used according to the method described by Pogrzeba et al. (2017). Afterward, the concentrations of the bioavailable forms of Cd, Pb, and Zn were determined in filtrates by flame atomic absorption spectrometry (iCE 3500 FAAS, Thermo Scientific). Pseudo-total metal concentrations were analyzed by extraction using *aqua regia* digestion according to ISO 11466:1995 and measured using flame atomic absorption spectrometry (iCE 3500 FAAS, Thermo Scientific).

Heavy metals concentration in plants

Concentrations of Pb, Cd, and Zn were determined using flame atomic absorption spectroscopy (iCE 3500 FAAS, Thermo Scientific) after hot plate digestion of dried milled biomass samples at 80 °C in nitric and perchloric acid (4:1 v/v) (Schierup and Larsen, 1981).

Translocation and bioconcentration factors

The translocation factor (TF), which was used to estimate the translocation of metals from roots to shoots, was calculated according to Malik et al. (2010):

$$\mathrm{TF} = \frac{\text{metal concentration in aboveground parts of the plant}\left(\mathrm{mg\,kg^{-1}}\right)}{\text{metal concentration in belowground parts of the plant}\left(\mathrm{mg\ kg^{-1}}\right)}.$$

Bioconcentration factor (BCF) was estimated according to Zhuang et al. (2007) formula:

$$\mathrm{BCF} = \frac{\text{metal concentration in the plant tissue}\left(\mathrm{mg\ kg^{-1}}\right)}{\text{metal concentration in the soil}\left(\mathrm{mg\ kg^{-1}}\right)}.$$

Arbuscular mycorrhiza colonization

To estimate the AMF colonization parameters, the roots were prepared according to Philips and Hayman (1970) method. The roots were cut into 2 cm segments, and then rinsed in 7% KOH for 24 h. Afterward, the roots were acidified in 5% lactic acid for 24 h and stained with 0.05% aniline blue in lactic acid for 24 h. The percentage of mycorrhizal root colonization was assessed with a microscope Zeiss Axio Imager D2 (Zen 2 software, Zeiss, Germany) according to Trouvelot et al. (1986) method. Then the colonization parameters were calculated using MYCOCALC software (http://www.dijon.inra.fr/mychintec/Mycocal-prg/download.html). The parameters measured were: the mycorrhizal frequency (F%), relative mycorrhizal intensity (M%), and relative abundance of arbuscules (A%).

Statistical analysis

All analyses were performed using Statistica 13.1 (Dell, US). Initially, the dataset was checked for normal distribution. The results were then used for the selection of tests to define significant difference of data between three or more independent groups (F-test for normal distribution, Kruskal–Wallis test for distribution other than normal). To remain concise and clear in data presentation, as long as the test selection algorithm lead to post hoc tests (ANOVA), the results of post hoc tests were presented on Figures and Tables. Fisher LSD post hoc test was used for denote significant differences between groups (experimental treatments, $P \leq 0.05$). All presented means were expressed with ± SE.

Results and discussion

Soil characteristics

The physical and chemical parameters of the soil are presented in Table 1. The soil pH in the control variant during the experiment was slightly acidic, while the application of lime and lignite caused an increase of pH to neutral. Also Lahori et al. (2017) confirmed the effect of lime on pH increase, but they used hydrated lime ($Ca(OH)_2$) alone or combined with other additives. They also noticed a drop in soil pH without additives, which could be related to the influence of the rhizosphere on increasing soil pH acidity. Similar results were obtained by Singh and Kalamdhad (2016). For all investigated treatments, the electrical conductivity (EC) was low (121–176 µS cm^{-1}). During the experiment, a reduction of the EC value was observed for control, while an increase and no changes of the value were observed for LL_I and LL_II treatments, respectively. The first visible changes in the EC value between the control and LL_I treatments occurred after 6 weeks from the start of the experiment. Pseudo-total Pb concentration was 22 693 mg kg^{-1}, Cd 100 mg kg^{-1} and Zn was 41 461 mg kg^{-1} in initial soil samples. The permissible heavy metal concentration in the soil used for the experiment was surpassing limits for industrial areas defined in Polish regulations (Official Journal of Laws of the Republic of Poland of 2016, item 1395). The total lead concentration exceeded the limits set in the regulation 38-fold, whereas the total cadmium and zinc concentration did so sevenfold and 41-fold, respectively.

The values of the bioavailable forms of the investigated heavy metals significantly decreased after application of the amendments when compared to the control, irrespective of the dose. A decrease by 70% and 90% of bioavailable Pb was observed in LL_I treatment for sampling date T1 and T2, respectively, and by 80% in LL_II treatment. Similar results were found for Cd bioavailable forms: Both treatments decreased the Cd bioavailable concentration by about 87% when compared to the control. For Zn bioavailable form, the treatments resulted in a decrease by about 90% in comparison to the values in the control. It demonstrates that soil pH might play a significant role as a factor inhibiting the bioavailability of these metals in the treated soil. Changes of soil pH may influence the availability and mobility of heavy metals via hydrolysis, complexation by organic or inorganic ligands, redox reactions (Singh and Kalamdhad 2013). Moreover, obtained results are in agreement with Cao et al. (2018), who showed a decrease in heavy metals bioavailability after lignite and lime application to a heavy metal contaminated soil. Lahori et al. (2017) showed decrease of DTPA-extractable fraction of Pb, Cd, Cu, and Zn, after lime application. Also, Amoah-Antwi et al. (2020) showed reduction in bioavailability of heavy metals such as Pb, Cd, and Zn, after application to soil 5% and 10% brown coal waste.

Heavy metal concentration and plant biomass production

The concentration of heavy metals in *L. perenne* roots and shoots and biomass production is presented in Table 2. The heavy metal concentration differed firmly between plant

Table 1 Physicochemical parameters and heavy metals concentration of soil

	Value								
	Control			LL_I			LL_II		
	T0	T1	T2	T0	T1	T2	T0	T1	T2
Physicochemical soil parameters									
pH (H_2O)	6.56 ± 0.03b	6.42 ± 0.03b*	6.53 ± 0.01c	6.86 ± 0.01a*	7.05 ± 0.02a	7.21 ± 0.01a	6.86 ± 0.02a*	7.05 ± 0.02a	7.14 ± 0.01b
pH (KCl)	6.02 ± 0.03b	5.97 ± 0.03c*	6.02 ± 0.01b	6.41 ± 0.01a*	6.47 ± 0.02b*	6.61 ± 0.01a	6.38 ± 0.02a*	6.55 ± 0.02a	6.68 ± 0.01a
EC (µS cm^{-1})	176 ± 0.01a	155 ± 0.01a*	145 ± 0.02b*	107 ± 0.03b*	155 ± 0.01a	177 ± 0.04a	157 ± 0.06a	121 ± 0.03a*	159 ± 0.0 ab
Bioavailable heavy metals concentration ($CaCl_2$ extraction)									
Pb (mg kg^{-1})	1.70 ± 0.05 a	1.75 ± 0.09 a	1.58 ± 0.06a	0.46 ± 0.0b	0.43 ± 0.1b	0.35 ± 0.05b	0.27 ± 0c	0.29 ± 0.0b	0.35 ± 0.03b
Cd (mg kg^{-1})	5.40 ± 0.22a	5.44 ± 0.07a	5.05 ± 0.17a	0.86 ± 0.03b	0.74 ± 0.08b	0.57 ± 0.03 b*	0.58 ± 0.03b*	0.50 ± 0.05c*	0.81 ± 0.01b
Zn (mg kg^{-1})	122 ± 1.40a	127 ± 1.90a	108 ± 0.73a*	9.54 ± 0.68b	7.69 ± 0.89b*	6.37 ± 0.24c*	6.63 ± 0.2b*	5.85 ± 0.55b*	10 ± 0.26b

Values are mean, ± SE, $n = 5$. Asterisks (*) denote significant differences between parameters while considering the time of collection within one variant, and lower case letters (a, b, c) denote significant differences between different variants separately for the time of collection, according to Fisher LSD test ($P \leq 0.05$). T0—initial soil sampling; T1—second soil sampling (two weeks after amendment application); T2—third soil sampling (six weeks after amendment application). C – control, LL_I—5% lignite and 0.5% lime, LL_II—5% lignite and 0.25% lime

Table 2 Concentration of heavy metals in shoots and roots of *Lolium perenne* and shoot dry weight

	Variant		
	C	LL_I	LL_II
HM concentration in shoots			
Pb (mg kg^{-1})	21.659 ± 2.6a	19.720 ± 2.6a	19.483 ± 3.0a
Cd (mg kg^{-1})	9.631 ± 0.6a	6.102 ± 0.5b	5.608 ± 0.4b
Zn (mg kg^{-1})	436.8 ± 17.5a	330.51 ± 17b	321.995 ± 11.1b
HM concentration in roots			
Pb (mg kg^{-1})	2760.7 ± 50.1a	1680.79 ± 31.5c	1986.721 ± 58.3b
Cd (mg kg^{-1})	258.67 ± 32.3a	221.397 ± 11.8a	260.752 ± 30.1a
Zn (mg kg^{-1})	13,945.2 ± 465.0a	11,629.236 ± 671.4b	7667.332 ± 716.1c
Shoot dry weight			
	1.850 ± 0.36b	2.113 ± 0.33a	2.430 ± 0.32a

Values are mean, ± SE, $n = 10$. Lower case letters (a, b, c) denote significant differences between different variants, separately for the parameters, according to the Fisher LSD test ($P \leq 0.05$). C—control, LL_I—5% lignite and 0.5% lime, LL_II—5% lignite and 0.25% lime

shoots and roots. Heavy metals were predominantly accumulated in the roots.

Along with the decrease in the content of the bioavailable fraction of heavy metals, the translocation of metals to shoots of *L. perenne* also decreased (Xiao et al. 2019). In the treatments with lime and lignite, the Pb concentration in shoots was lower by about 9% (LL_I) and 10% (LL_II) than the concentration in the control, however values were not significantly differ. The used amendments significantly decreased the Cd concentration in shoots—by about 37% (LL_I) and 42% (LL_II). Also compared to the control, the Zn concentration in the amended treatments was lower by about 24% (LL_I) and 26% (LL_II). Lower Cd-uptake by *L. perenne* after lignite application was also confirmed by Simmler et al. (2013).

Concerning the roots, lime and lignite addition resulted in a similar effect causing a significant decrease of Pb and Zn concentrations in the amended treatments compared with the control, except for Cd. The concentration of Pb in the roots of the control treatment was higher by 40% and 30% than in the case of LL_I and LL_II, respectively, and for Zn by 18% and 48%. It might be attributed to the absorption of the metal on the root surface which, consequently, limits the transport to the aboveground parts of the plant (Pogrzeba et al. 2019). Radziemska et al. (2018) reported a reduction of Cu concentration in the aboveground parts of *L. perenne* after the application of lime and diatomite to the soil. Moreover, a lower heavy metal concentration in the roots of the amended treatments may be associated with the mycorrhizal root colonization, especially that the ability of AMF to stabilize heavy metals by hyphae on the surface of the roots was previously reported (Wu et al. 2016).

Zhang et al. (2018) showed that contamination of soil with Cd may adversely affect the growth and biomass of *L. perenne*. This is confirmed by our research, where after the application of amendments, along with decrease fraction of bioavailability of heavy metals, increase in dry mass of shoots was observed. Compared to the control, the dry weight of the shoots was higher by 12% (LL_I) and 24% (LL_II). Also, this phenomena were observed in previous studies after application of lime (Hartley and Lepp 2008) and $FeSO_4$ with lime to the soil (Moreno-Jimenez et al. 2017).

Translocation and bioaccumulation of heavy metals

A characteristic feature of *L. perenne* is an ineffective heavy metals translocation process from roots to shoots. The potential of ryegrass as a candidate species for phytostabilization was expressed in the low translocation factor (TF), which is typically used to estimate the phytoremediation potential of plants. According to Lee et al. (2014), the plant species suitable for phytostabilization should be characterized by TF < 1. This findings are confirmed with numerous other studies, which have reported *L. perenne* to be a suitable species for the phytostabilization process (Norini et al. 2019; Radziemska et al. 2018). Zn was translocated more effectively than the other two metals in both of the amended treatments (Table 3). Research of Gu et al. (2017) shows that AM fungi can effectively support the plant in the uptake and translocation of Zn into shoots, which was confirmed by the results of BCF and TF in case of *L. perenne* inoculated with mycorrhiza. The addition of lime and lignite significantly increased Pb and decreased Cd translocation. The addition of 0.25% lime enhanced Zn translocation, while no significant differences between LL_I treatment and control were observed. Bioaccumulation of Cd and Zn was lower after amendments application. *L. perenne* root colonizing by mycorrhiza can additionally inhibit metal translocation from roots to shoots (Gu et al. 2017; Zhang et al. 2018),

Table 3 The efficiency of *L. perenne* in translocating metals from roots to shoots

	Control	LL_I	LL_II
TF_{Pb}	0.00 ± 0.0 b	0.01 ± 0.0 a	0.01 ± 0.0 a
TF_{Cd}	0.03 ± 0.0 a	0.02 ± 0.0 b	0.02 ± 0.0 b
TF_{Zn}	0.03 ± 0.0 b	0.03 ± 0.0 b	0.04 ± 0.01 a
BCF_{Pb}	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.0 a
BCF_{Cd}	0.10 ± 0.01 a	0.06 ± 0.01 b	0.06 ± 0.0 b
BCF_{Zn}	0.01 ± 0.00 a	0.01 ± 0.00 b	0.01 ± 0.0 b

Values are means ± SE ($n = 5$). Lower case letters (a, b, c) denote a significant difference between variants in a row according to the Fisher LSD test at $P \leq 0.05$. TF Translocation factor, BCF Bioaccumulation factor. C—control, LL_I—5% lignite and 0.5%lime, LL_II—5% lignite and 0.25%lime

because heavy metals can be retained in mycorrhizal structures for example by bind to hyphae (Malcová et al. 2003; Joner et al. 2000). Moreover, Begum et al. (2019) showed that a symbiosis between AMF and host plants could enable selective transport mechanisms of both essential elements and heavy metals. For example, the presence of mycorrhiza may increase Pb uptake in polluted areas (Yang et al. 2016). A limited translocation of Pb and Cd to the aboveground parts of the plant might occur either due to the complexation of these metals in the root cells or lack of specific transport channels (Xin et al. 2018), especially for metals which are not essential for physiological processes, like cadmium and lead. Cd^{2+} and Pb^{2+} are ions which may become extremely toxic for the plant (Chen et al. 2017): They disturb redox homeostasis (Khanna et al. 2020) and lead to the inhibition of plant growth and yield reduction (Chen et al. 2017). Specific transport channels not only play a crucial role in metals transport, but also prevent the accumulation of heavy metals in toxic levels. One of the prevention mechanisms is a limited translocation of heavy metals to shoots (Hossain et al. 2012).

Mycorrhizal colonization studies

Phytoremediation processes involving plants capable of symbiosis with indigenous AM fungus may bring promising effects (Miransari 2010). However, AMF colonization in contaminated soils depend on availability of nutrients and climate (Smith and Read 2010). Due to the potential of *L. perenne* in phytostabilization, determination of AMFs' root colonization in soil contaminated with heavy metals and the effect of used soil additives on the AM fungi was investigated. Microscopic observations showed that root samples of *L. perenne*, both treated and control, were colonized by AMF. In all examined roots the structures characteristic for AM fungi such as arbuscules (Arb), intracellular hyphae (Ih), and vesicles (v) were found (Figs. 1, 2, 3). To describe the characteristics of the mycorrhizal structure development the following parameters were used: mycorrhizal frequency (F%), relative mycorrhizal intensity (M%), and relative abundance of arbuscules (A%). Also, dark septate endophytes (DSE) and microsclerotia were found in the observed root fragments irrespectively of the treatments, which was confirmed by Zhang et al. (2013) in plant roots on Pb- and

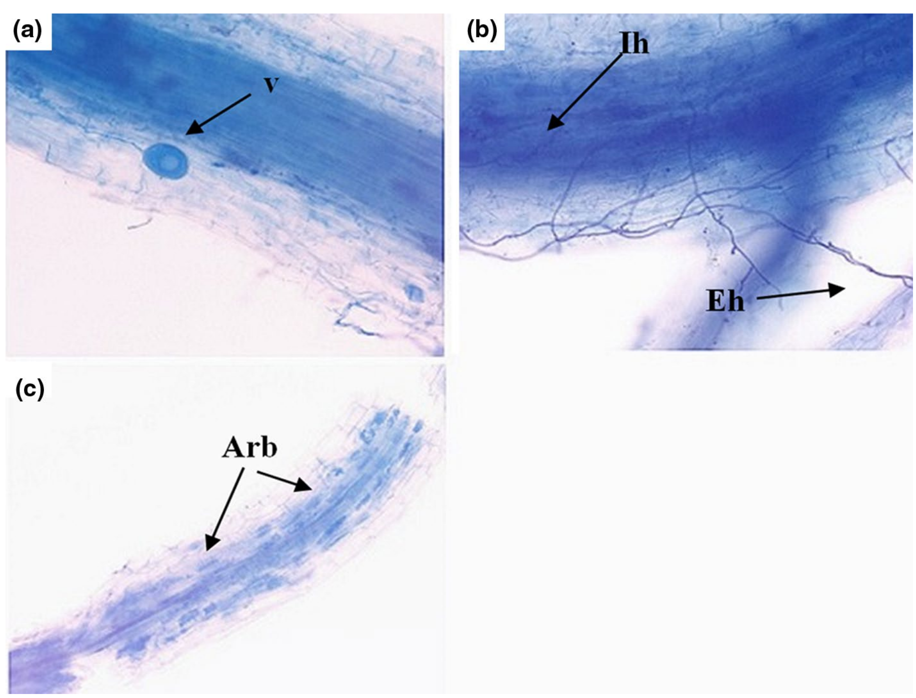

Fig. 1 Mycorrhizal structures in *L. perenne* roots in the control variant. **a** v (vesicle); **b** Ih (intracellular hyphae), Eh (extracellular hyphae); **c** Arb (arbuscules)

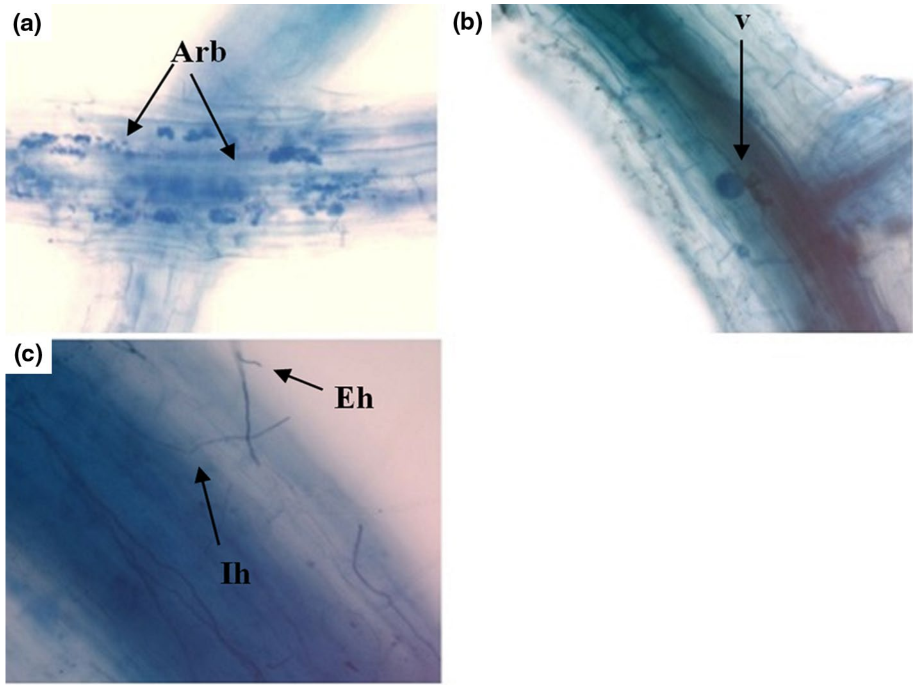

Fig. 2 Mycorrhizal structures in *L. perenne* roots in the soil amended with 5% of lignite and 0.5% of lime. **a** Arb (arbuscules); **b** v (vesicle); **c** Ih (intracellular hyphae), Eh (extracellular hyphae)

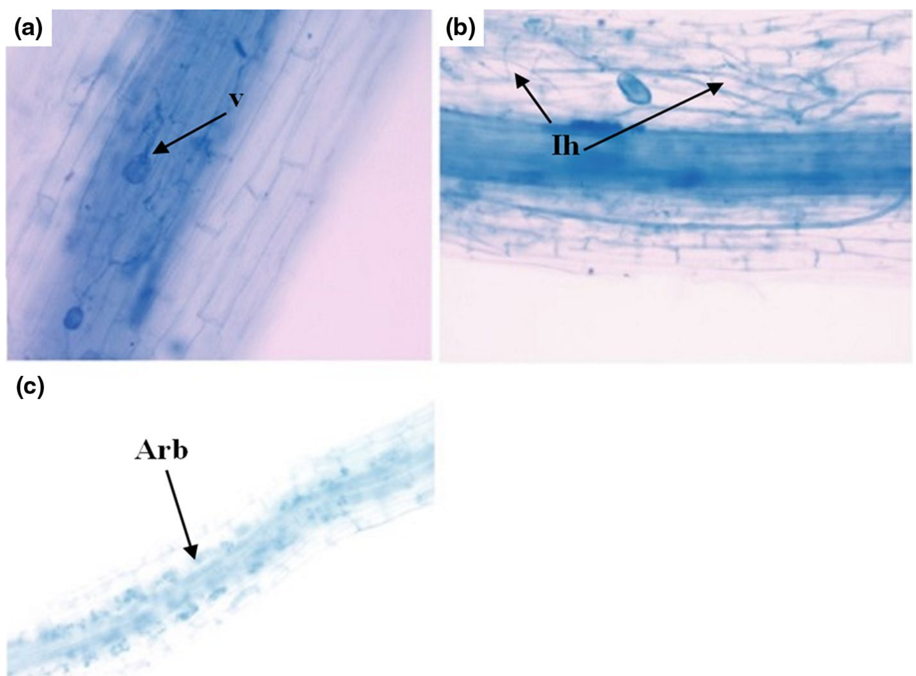

Fig. 3 Mycorrhizal structures in *L. perenne* roots in the soil amended with 5% of lignite and 0.25% of lime. **a** v (vesicle); **b** Ih (intracellular hyphae); **c** Arb (arbuscules);

Zn-slag heaps. Despite reports showing the negative impact of high levels of heavy metals in soil on AMF root colonization (Chang et al. 2018; Rusinowski et al. 2019), high value of frequency of mycorrhizal colonization approx. 95% was observed for *L. perenne* roots in the presented study (Fig. 4). This phenomena is consistent with previous reports, showed a strong symbiotic relationship between AMF and *L. perenne*, confirmed by studies conducted on various soil types in 40 geographically dispersed sites (Hazard et al. 2013), grasslands, arable lands (Hazard et al. 2014), and contaminated soil (Zhang et al. 2018). What differentiate this study from the similar research, is the use of autochtonous AMF communities originated from collected soil, whereas most of the research are focused on investigation

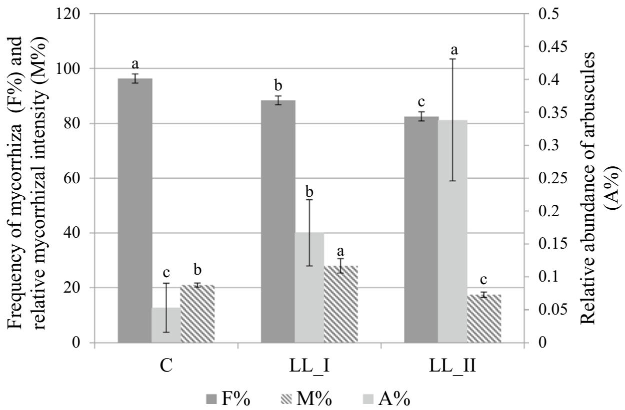

Fig. 4 *L. perenne* root Arbuscular Mycorrhiza Colonization. Lower case letters (**a**, **b**, **c**) denote significant differences between different variants separately for each parameter. F%—mycorrhizal frequency; M%—relative mycorrhizal intensity; A%—the relative arbuscular richness

of AMF provided via inoculation (Berthelot et al. 2018; Orłowska et al. 2010).

The effectiveness of mycorrhizal fungi depends on the soil pH (Carballar-Hernández et al. 2017; Li et al. 2017; Hazard et al. 2013). However, previously conducted studies do not give a clear answer how soil pH changes affect the colonization of AMF. According to Lin et al. (2017), the development of indigenous mycorrhizal fungi can be inhibited at the highest pH levels, whereas Parihar et al. (2019) showed that high pH favors high values of mycorrhizal frequency. The effect of the application of lime and lignite on the mycorrhizal colonization in the *L. perenne* root system is shown in Fig. 4. There were significant differences in mycorrhizal colonization parameters (F%, M%, and A%) among experimental variants. Lower values of mycorrhizal frequency (F%) were noted in samples with higher pH value and lower concentration of bioavailable forms of heavy metals, when compared to the control treatment. The frequency of mycorrhiza in the amended treatments was considerably lower than in the control: by 8% (LL_I) and 14% (LL_II), respectively. This phenomenon may be related to the increase in organic matter added in form of lignite, as confirmed by the results of studies by Yang et al. (2015a, b), where F% was negatively correlated with the content of organic matter in the soil. Amendments slightly changed mycorrhizal intensity dependent on dose of lime: higher lime dose increased of M% by about 30%, whereas lower lime dose decreased M% by 17%, compared to the control. Interestingly, both of used amendments amplified significantly the development of arbuscules, and at the same time it led to a reduction of other mycorrhizal structures such as hyphae and vesicles. Previous studies presented various impacts of soil treatments such as liming (Gomes et al. 2015; Heyburn et al. 2017; Nava et al. 2016) or application of lignite in combination with calcium phosphate (Gucwa-Przepióra et al. 2007) on mycorrhizal colonization. The studies of Gucwa-Przepióra et al. (2007), showed an increase in the development of AMF in *Deschampsia cespitosa* roots caused by the application of mixed calcium phosphate and lignite, but indicated that EC and pH did not affect native AM colonization level. Differences between presented study and Gucwa-Przepióra et al. (2007) are associated with the use of phosphorus compounds which modify soil properties in terms of primary macronutrients availability (particularly P), what was not true for this study. Nava et al. (2016) showed a positive influence of liming on mycorrhizal colonization effectiveness in *Acca sellowiana*. The discrepancy in the results may be due to the fact that, the effectiveness AMF colonization may depend on the mycorrhizal species and their tolerance to practices in crop management (Junior et al. 2019), as well as on host plants and environmental conditions (Oehl et al. 2003). However, there is no information how changes in soil properties caused by lime and lignite can affect on native AMF colonization in the phytostabilization process involving *L. perenne*.

Conclusion

The major findings of presented research are as follows:

- The application of lime and lignite decrease the investigated heavy metals (Pb, Cd and Zn) bioavailable fraction ($CaCl_2$ extractable) in the contaminated soil.
- Used amendments increased the shoot dry biomass, as well as decreased heavy metals concentration in the roots and shoots of *L. perenne*.
- Structures characteristic for AMF was observed in *L. perenne* roots irrespective of the amendments dose
- The amended treatments increased the arbuscules occurrence (A%) in *L. perenne* roots, while the dose of 0.5% lime and 5% lignite resulted in a better relative mycorrhizal intensity colonization value (M%) than 0.25% lime and 5% lignite dose.

The novelty of conducted experiment is related to effect of applied amendments on the native AMF *L. perenne* root colonization. Despite this fact, further analyses would be required, based on spore morphology, to explain the different responses of both AMF and microorganisms communities to the different treatments. Moreover, effect of this approach should be further validated in the field scale application.

In general, phytostabilization of heavily contaminated soil could serve as solution, where the other methods of remediation can not be applied, from technical and economical point of view. Additional advantage of such approach is related to the improvement in soil biodiversity, particularly plant – AMF symbiosis. The results of the experiment were implemented to stabilize heavy metals during remediation of a spoil heap in Ruda Śląska, Poland, aimed at an environmental restoration of the site in order to enable its use as public space for recreational purposes. Phytostabilization, in this case, was an important part of the investment which mitigated the risk of further contaminants spread.

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Authors contribution The authors contributions are as follows: ASB performed the experimental work, analyzed the data and wrote the manuscript (60%), JK planned and designed the research and wrote the manuscript (10%), SR performed the statistical analysis and wrote the manuscript (10%), ASS and IRK contributed to the manuscript including language corrections (5%), MP planned and designed the research and contributed to the manuscript (10%).

Declarations

Conflict of interest The authors declare no conflicts of interest.

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