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REGULAR ARTICLE

Arbuscular mycorrhiza of introduced and native grasses colonizing zinc wastes: implications for restoration practices

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Abstract The analysis of mycorrhizal status of grasses introduced under conventional phytoremediation programmes (including covering of the waste area with a layer of uncontaminated soil and watering) and of grasses that spontaneously established in later successional stages, was carried out on the slopes of the 1- to 30-year-old tailings of ZG Trzebionka Mining Company located near Chrzanów (southern Poland). Most grasses were mycorrhizal and the parameters of mycorrhization were higher on the older parts of the waste. Grasses selected for restoration practices were well developed only if the waste was covered with a layer of soil and continuous irrigation took place, both being expensive practices. The field experiment showed that the introduction of standard inoculum containing mycorrhizal fungi indeed improved the development of grasses during early stages, but still was not effective enough due to high erosion of the substratum. A slight improvement was observed when AgroHydroGel was used to lock moisture in the soil, but the plants did not survive for longer time periods. On the contrary, much better results were obtained when vegetatively multiplied grasses selected from specimens originating from natural succession were used to stabilize the vegetation on the bare industrial waste.

Keywords Arbuscular mycorrhiza · Grasses · Heavy metals · Industrial waste · Restoration · Natural succession

Introduction

The exploitation of zinc/lead ores in the Małopolska region of Southern Poland has a long tradition (Szuwarzyński 2000). Long-term mining activities produced large areas of tailings, causing pollution and land degradation, if stable vegetation cover had not developed. Flotation is currently the most widespread method of metal ore enrichment (Yarar 1998), involving both mechanical and chemical processing of metalliferous minerals. Post-flotation wastes are extremely harsh substratum, poorly suitable for plant growth. The sediment is composed of particles ranging mostly between 0.1 and 0.02 mm in diameter, making it extremely difficult for any biological reclamation. Compaction and low porosity result in unfavourable air–water conditions restricting water infiltration during rainfall and decreasing water recharge by capillary rise from deeper layers during dry periods. These factors favour wind erosion in dry periods and water erosion during

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rainfalls (Strzyszcz 1980, 2003). In addition, the slopes of the heaps slide down and are difficult to stabilize (Strzyszcz 2003).

Phytostabilization of such areas involves mostly the use of grasses (Pierzynski et al. 2002a, b) that are selected towards their tolerance to toxic metals, drought, and low nutrition levels. In addition, ideal candidates should be able to establish quickly on the waste and persist for long time periods. The ability to form extensive root systems and not to accumulate potentially toxic elements in the shoots, minimizing the risk of metal translocation into the food chain (Prasad 2006), are also important selection criteria. Grasses that were so far used in phytoremediation practices in the study area belonged to *Arrhenatherum elatius*, *Bromus inermis*, *Dactylis glomerata*, *Lolium perenne*, *Festuca ovina*, *Festuca rubra*, and *Poa pratensis* (Trafas 1996). Although their establishment is relatively fast in areas covered with a layer of humus, they usually disappear from the site within a few subsequent vegetation seasons and are slowly substituted by grasses that originate from natural succession (Turnau et al. 2006a). Symbiotic endophytes were often suggested to improve plant establishment under extreme conditions (Entry et al. 2002), as mycorrhizal symbiosis provides plants with enhanced water and mineral supply (Smith and Read 1997). Small-scale experiments confirmed the potential role of arbuscular mycorrhizal fungi (AMF, Glomeromycota), in the establishment of grasses such as *Dactylis glomerata*, *Deschampsia caespitosa* and *Festuca rubra* in heavy metal polluted areas in Western Europe (Hetrick et al. 1994; Colpaert 1998; Schat and Verkleij 1998).

The main aims of the present research were to: (1) analyse the mycorrhizal status of grasses introduced under conventional phytoremediation programmes (including covering of the waste with a layer of non-contaminated soil and watering) and to monitor the differences on plots of different age; (2) to assess the effectiveness of mycorrhizal inoculation in the formation of a more stable vegetation cover without high input of soil and water; (3) to study mycorrhizal development of plants originating from natural succession, and finally (4) to check whether vegetatively multiplied grasses selected from plant specimens originating from natural succession would be a better solution for accelerating succession on the bare ground of industrial slopes.

Methods

Study area

The investigations were carried out on 1- to 30-year-old tailings of the ZG Trzebionka Mining Company located near Chrzanów (southern Poland, 30 km west of Kraków N 50° 09′, E 19° 25′). The tailing pond (Fig. 1) was used for the storage of waste produced during flotation of zinc/lead-rich dolomites and consequently elevated during subsequent filling with waste material. Investigations described in this study were carried out on the slopes of the pond, that were built using the solid fraction of post-flotation wastes containing up to 12,000 mg kg^{-1} of Zn, 2,400 mg kg^{-1} Pb, 100 mg kg^{-1} Cd (total values). The waste material had the mean pH value in H_2O of 7.4, and the following chemical characteristics: mean organic matter content – 1.12%, mean N content – 0.044%, mean P_2O_5 content – 74 mg kg^{-1}, mean K_2O content – 69 mg kg^{-1}, mean CaO content – 2,509 mg kg^{-1}. Analysis of the mycorrhizal status was carried out on six 10 × 15 m plots selected in: the youngest (T1), top part of the slopes, recently covered with a 10 cm thick layer of soil, where *Lolium perenne* was seeded and approximately 30 other species developed from the seed bank of the cover soil; a 5- to 10-year-old plot (T2) where patches the cover soil were still observed and where approximately 40 species of plants were recorded; a 15-year-old plot (T3) covered with milled dolomites and with a well developed vegetation cover composed of approximately 60 plant species; a 20-year-old plot (T4) showing no remnants of the soil cover and advanced erosion, where approximately 15 plant species were noted; a 20-year-old plot (T5) that was subjected to drastic damage due to accidental leakage the flotation pulp and where very scarce vegetation appeared

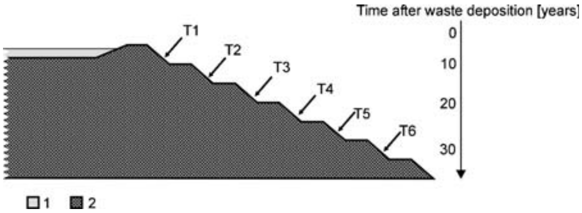

Fig. 1 Schematic drawing/cross-section of the ZG Trzebionka Mining Company tailings pond. *1* Liquid fraction of deposited post-flotation wastes, *2* solid fraction; *arrows* indicate plots located on the slopes

subsequently; a approximately 30-year-old plot (T6) with well developed vegetation cover composed of approximately 60 plant species. In addition four plots were selected outside the tailing in the closest vicinity as reference plots. The investigated plots differed in substratum moisture, which ranged from 0.06 (T5) to 0.15 $m^3 \times m^{-3}$ (T1) (Fig. 3).

Assessment of mycorrhizal status

Plant samples were collected from the investigated plots at flowering stage to allow identification (May to August, depending on the species). No differences in the phenological stage of vegetation between samples from the wastes and from the reference plots were observed. For the estimation of mycorrhizal colonisation, the roots were prepared according to the modified method of Phillips and Hayman (1970). After careful washing with tap water, the roots were softened in 10% KOH for 24 h, washed in water, acidified in 5% lactic acid in water for 12–24 h, stained with 0.01% aniline blue in 5% lactic acid for 24 h at room temperature and eventually stored in 5% lactic acid. The following parameters describing the intensity and effectiveness of the mycorrhization were recorded microscopically: relative mycorrhizal root length (*M*%), intensity of colonisation within individual mycorrhizal roots (*m*%), relative arbuscular richness (*A*%) and arbuscule richness in root fragments where the arbuscules were present (*a*%) (Trouvelot et al. 1986; http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html). In total 180 root samples collected from the mine tailings and 33 root samples from outside part of the tailing were analysed. Mycorrhizal colonisation and arbuscular richness were estimated microscopically in 6,000 1 cm-long pieces of roots.

Field experiment – influence of mycorrhizal inoculation on growth of grass cultivars

In an area similar to T5 two experimental plots, 20× 15 m were selected. Both were fertilized with 16 g of KNO_3 and 6.5 g $Ca_3(PO_4)_2$ per m^2. One was inoculated with *Glomus claroideum* (Symbio-M, Lanskroun, Czech Republic), selected previously as the most effective in mycorrhizal colonization of grasses (Orłowska et al. 2005); the second was left without inoculation. The inoculum was mixed with the top layer of substratum at a concentration of 500 ml per m^2. Each plot was divided into subplots, 20×1 m each and seeded with commercially available grasses (all the available cultivars from local suppliers): *L. perenne* cv. INKA and SOLEN, *Festuca ovina* cv SPARTAN, *F. rubra* cv. LEO and NIMBA, *Poa pratensis* cv. ALICJA, and the commercially available seed mixtures Eko mix and Uni mix. In addition, in order to improve soil moisture conditions, a part of plots (1×1 m) seeded with *L. perenne* cv. INKA were treated with AgroHydroGel (AgroIdea, Pobiednik Mały, Poland), according to producer's instructions.

Laboratory experiment – vegetative reproduction of grasses

Selected grasses (*Agrostis gigantea*, *Corynephorus canescens*, *Festuca trachyphylla*, *Molinia caerulea*) were tested for their ability to reproduce vegetatively. In the case of *A. gigantea* 3–4 cm long fragments of rhizomes were dissected; tussocks of *C. canescens* and *F. trachyphylla* were split into fragments 3 cm in diameter, whereas tussocks of *M. caerulea* were divided into parts containing two to three wintering internodes. Plants obtained in this way were planted in pots filled with substratum collected from the slopes of the tailings and cultivated under laboratory conditions under the following light regime: 100–110 $\mu mol \times m^{-2} \times s^{-1}$ PAR, 12/12 h.

Field experiment – *M. caerulea* cultivation on wastes

The field trial was carried out using vegetatively propagated *M. caerulea*. At the beginning of June and October 2004, individual plant specimens were divided into rhizome fragments containing two to three wintering internodes with developed fine roots and planted without any soil additives on the T5 part of tailing, leaving 20 cm space between plantlets. The total number of fragments introduced was 120. Their survival and flowering were recorded every month.

Data analysis

Statistical analysis of data was performed with the non-parametric ANOVA Kruskal–Wallis test using the STATISTICA (ver. 5.0) software ($P<0.05$).

Results

Grasses as colonizers of Zn tailings

In total, 25 species of grasses were recorded on the slopes of the ZG Trzebionka tailings pond. Other numerous plant families observed on the site included Asteraceae and Fabaceae. The family Poaceae dominated in terms of the percentage of substratum coverage. About 60% of grass species were observed on plots recently covered with a soil layer originating from non-polluted places. Most of them, including *L. perenne* seeded under the remediation programme, were not spreading into the non-restored plots and disappeared from the restored plots as soon as the surface soil layer was lost due to erosion. In such places, populations of *Arrhenatherum elatius*, *Bromus inermis*, and *F. rubra* showed increased stability, although their role in the remediation of the tailings was rather weak due to their slow expansion and weak underground and aboveground systems. Much more expansive and successful in the attenuation of substratum erosion were species that had not been introduced on the site intentionally, such as *Molinia caerulaea*, *A. gigantea*, *Festuca tenuifolia*, *F. trachyphylla* and *C. canescens*. The vegetation cover involving these plants was permanent and dense. Noteworthy, these plants reproduced vegetatively. The largest clusters with relatively high biomass were formed by *M. caerulea*, which allowed for organic matter accumulation in the substratum (Fig. 2). *D. glomerata* was present on most plots. However, this plant mostly reproduced by seeds and was not efficient in the formation of a vegetation cover on larger areas.

Fig. 2 *M. caerulea* on the zinc wastes. A dense stand on the oldest part of the wastes, withstanding erosion; *arrow* indicates organic matter accumulation within a tussock

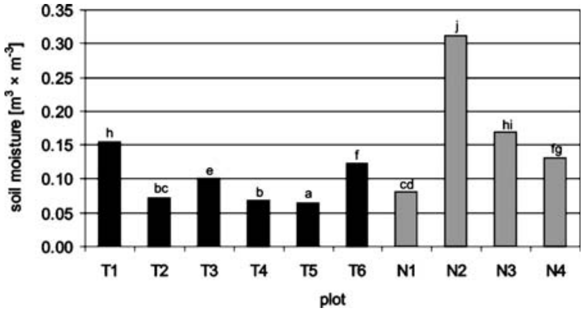

Fig. 3 Soil moisture on the investigated plots. *T1–T6* plots located on the zinc wastes; *N1–N4* plots located outside the wastes. *Different letters above bars* indicate statistically significant differences ($P<0.05$)

In older parts of the waste area, the liquid sedimentation pulp was often incidentally leaking from damaged pipes. In such cases, existing vegetation usually disappeared and new grass species were developing. Among the first spontaneously establishing plants were: *C. canescens*, *D. glomerata*, *Elymus caninus*, *F. tenuifolia* and *M. caerulea*.

The highest diversity of grasses was recorded either on the oldest (25- to 30-year-old) plots or on the approximately 10-year-old plots that were covered with soil transported from outside of the industrial wastes.

Experimental plots that were selected outside the waste heap showed a generally higher (approximately 70%) soil moisture level than the tailings (Fig. 3). They were inhabited by 14 grass species, among which *Festuca* spp. and *M. caerulea*, that were also present on zinc tailings, were dominating.

Mycorrhizal status of of grasses

Arbuscular mycorrhizal fungi were observed in the roots of all grass species analysed, with the exception of some samples of *Calamagrostis epigejos*, *M. caerulea* and *C. canescens* from the T2 plot and *L. perenne* from the youngest plot (T1) (8% of all samples analysed). *A. gigantea*, *A. elatius*, *Deschampsia caespitosa*, *Elymus caninus*, *Koeleria glauca* and *Phleum pratense* were usually highly mycorrhizal, similarly to species of the *Festuca* genus, although the richness of mycorrhizal structures varied among different stands. Mean values of mycorrhizal colonization parameters for the investigated species are listed in Table 1. The most common type of mycorrhiza,

Table 1 Mycorrhizal colonisation of grasses on zinc wastes

Plant species	Plot	*F*%	*M*%	*m*%	*A*%	*a*%	Mycorrhizal type
Agrostis gigantea Roth	T4	79.4a	49.8a	63.1a	19.5a	37.0a	*Paris*
Agrostis gigantea Roth	T6	80.0a	56.5a	70.3a	35.9a	62.7a	*Paris*
Apera spica-venti (L.) P. Beauv.	T2	8.3	3.2	36.7	1.2	35.4	Intermediate
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	T2	33.0a	11.7a	36.2a	7.2a	60.4ab	*Paris*
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	T3	100.0c	79.2d	79.2b	41.4c	51.4ab	*Paris*
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	T4	59.9b	38.9c	64.2b	14.0b	38.0a	*Paris*
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	T6	98.4c	73.7d	74.9b	46.3cb	62.8b	*Paris*
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	N1	54.3b	21.8b	39.9a	12.9ab	57.1ab	*Paris*
Avenula pubescens (Huds.) Dumort.	T4	22.0b	11.8b	53.4b	4.2b	35.5b	*Arum*
Avenula pubescens (Huds.) Dumort.	T6	53.7a	36.8a	70.3a	27.8a	74.2a	*Paris*
Bromus hordeaceus L.	T2	28.4	13.9	49.0	11.8	83.1	*Paris*
Bromus inermis Leyss.	T3	0.0b	0.0b	0.0b	0.0a	0.0b	None
Bromus inermis Leyss.	T4	66.7a	30.8a	47.0a	6.1a	20.8a	*Paris*
Bromus inermis Leyss.	N4	57.0a	35.3a	59.4a	25.7b	70.2c	*Paris*
Calamagrostis epigejos (L.) Roth	T2	0.0a	0.0b	0.0b	0.0a	0.0a	None
Calamagrostis epigejos (L.) Roth	T4	53.2b	41.1b	66.5a	15.7b	48. 7b	Intermediate
Calamagrostis epigejos (L.) Roth	T6	1. 7a	1.2a	35.0a	0.1a	5.0a	Intermediate
Corynephorus canescens (L.) P. Beauv.	T2	12.1a	9.0a	48.2cd	8.2cd	61.4a	Intermediate
Corynephorus canescens (L.) P. Beauv.	T5	46. 7b	7.8b	16.8b	3.0b	37.9b	Intermediate
Corynephorus canescens (L.) P. Beauv.	T6	56.7c	35.5c	63.7d	25.6bd	70.6b	Intermediate
Corynephorus canescens (L.) P. Beauv.	N1	0.0a	0.0a	0.0ac	0.0ac	0.0a	None
Dactylis glomerata L.	T2	5.0a	7.3a	5.2ac	0.0a	0.0a	Intermediate
Dactylis glomerata L.	T3	74.9c	58.0c	76.9d	31.0c	53.0c	Intermediate
Dactylis glomerata L.	T4	83.3d	43.0b	51.6bcd	20.3abc	47.2abc	Intermediate
Dactylis glomerata L.	T5	55.5bd	30.3b	54.9b	13.9b	46.3bc	Intermediate
Dactylis glomerata L.	T6	70.7bcde	33.6bc	49.0bd	11.6b	35.2b	Intermediate
Dactylis glomerata L.	N4	93.3e	55.3c	59.5b	20.0b	35.8bc	Intermediate
Deschampsia cespitosa (L.) P. Beauv.	T2	59.9a	27.2a	45.8a	13.2a	50.9a	*Paris*
Deschampsia cespitosa (L.) P. Beauv.	N4	66.5a	34.2a	49.5a	11.0a	30.7b	Intermediate
Echinochloa crus-galli (L.) P. Beauv.	T1	53.0b	21.3a	43.1a	14.1a	65.7a	*Arum*
Echinochloa crus-galli (L.) P. Beauv.	T2	10.0a	2.0a	40.1a	0.9a	44.6a	*Arum*
Elymus caninus (L.) L.	T5	62.0	40.4	65.1	29.3	72.7	Intermediate
Festuca arundinacea Schreb.	T2	75.9b	51.3b	67.3b	31.0b	61.6b	*Paris*
Festuca arundinacea Schreb.	T3	71.1b	47.2b	64.7b	26.6b	58.2b	*Paris*
Festuca arundinacea Schreb.	T4	5.0a	0.7a	11.2a	0.0a	5.7a	*Paris*
Festuca rubra L. s. str.	T6	93.3	58.9	63.0	34.6	56.1	*Arum*/*Paris*
Festuca tenuifolia Sibth.	T4	73.0a	39.1ab	52.2b	22.7a	56.3ab	*Paris*
Festuca tenuifolia Sibth.	T5	77.4a	32. 8a	41.5ab	21.2a	59.7b	*Paris*
Festuca tenuifolia Sibth.	T6	89.2a	61.1b	68.2c	48.1b	77.8b	*Paris*
Festuca tenuifolia Sibth.	N1	56.9a	17.2a	29.5a	7.5a	43.0a	*Paris*
Festuca trachyphylla (Hack.) Krajina	T4	63.3b	31.8a	50.4a	8.3a	26.3b	*Paris*
Festuca trachyphylla (Hack.) Krajina	T6	86.5a	50.5a	57.1a	35.7a	65.8a	*Paris*
Festuca trachyphylla (Hack.) Krajina	N4	75.8ab	39.9a	51.5a	21.8a	49.4ab	*Paris*
Koeleria glauca (Spreng.) Dc.	T4	48. 9b	20.7b	41.8b	6.47b	25.0a	*Arum*
Koeleria glauca (Spreng.) Dc.	T6	86.1a	70.5a	81.7a	40.0a	56.6a	*Arum*/*Paris*
Lolium multiflorum Lam.	T1	11.7b	5.3b	49.8a	2.6b	46.5b	Intermediate
Lolium multiflorum Lam.	T2	76.4a	61.7a	78.2a	37.7a	60.7a	Intermediate

Table 1 (continued)

Plant species	Plot	*F*%	*M*%	*m*%	*A*%	*a*%	Mycorrhizal type
Lolium perenne L. s. str.	T1	0.0b	0.0b	0.0b	0.0b	0.0b	None
Lolium perenne Lolium perenne L. s. str.	T2	68.2a	57.6a	85.3a	45.5a	78.9a	Intermediate
Molinia caerulea (L.) Moench s. str.	T2	2.2a	0.4a	5.8a	0.4a	33.3ab	*Paris*
Molinia caerulea (L.) Moench s. str.	T4	71.8b	40.7cd	52.0c	17.3cd	42.4a	*Paris*
Molinia caerulea (L.) Moench s. str.	T5	52.5b	14.8b	29.6abcd	6.9bc	44.4ab	*Paris*
Molinia caerulea (L.) Moench s. str.	T6	80.1b	40.4c	50.7c	34.6de	56.4b	*Paris*
Molinia caerulea (L.) Moench s. str.	N2	77.2b	60.8d	79.2d	42.6e	70.2b	*Paris*
Molinia caerulea (L.) Moench s. str.	N3	4.5a	1.5a	32.5b	1.0ab	51.1ab	*Paris*
Phleum pratense L.	T2	56.3a	42.4a	75.0a	21.3a	50.9a	*Arum*
Phleum pratense L.	T3	68.8a	42.1a	59.5a	23.6a	57.2a	*Arum*/*Paris* /intermediate
Phragmites australis (Cav.) Trin. ex Steud.	T6	40.6	5.3	13.6	1.8	41.9	*Paris*
Poa compressa L.	T3	53.4	41.6	75.3	33.9	80.4	Intermediate/*Paris*
Poa pratensis L. s. str.	T6	44.4	15.8	33.4	3.1	28.7	Intermediate
Setaria pumila (Poir.) Roem. & Schult.	T4	13.3	5.0	56.8	1.0	15.0	*Arum*

Plant names are given according to Mirek et al. (2002). Different letters beside the values indicate statistically significant difference ($P<0.05$). *T1–T6*: plots located on zinc waste slopes, *N1–N4*: plots located outside the waste (reference plots), *F*%: mycorrhizal colonisation frequency, *M*%: relative mycorrhizal root length, *m*%: intensity of colonisation within individual mycorrhizal roots, *A*%: relative arbuscular richness, *a*%: arbuscule richness in root fragments where the arbuscules were present.

observed in over 50% of samples, was of the *Paris* type. The *Arum* type was observed in 18% of samples and 32% of samples were classified as intermediate. The *Arum* type was found only in samples from the slopes of the waste. The plots differed in mean values of mycorrhizal colonization parameters. The highest values were noted on one plot located outside the waste area, which was a typical habitat for *M. caerulea*, the only grass species occurring there. Among the plots on slopes, the most abundant mycorrhiza formation was observed on plots T6, T3 and T4, while the youngest plot, T1, showed the lowest values of mycorrhizal colonization parameters. In the case of seven species (*Avenula pubescens*, *B. inermis*, *F. trachyphylla*, *K. glauca*, *Lolium multiflorum* and *L. perenne*), the values of all mycorrhizal parameters increased with the age of depositions, while the reverse tendency was found only in *Festuca arundinacea*.

Field experiment – influence of mycorrhiza inoculation on grass cultivars

In the experiment aimed to show the influence of mycorrhizal inoculation on the growth and establishment of grass cultivars introduced into the plots as seeds, during the first 6 weeks a continuous growth of plants was recorded. Plants developing in inoculated plots showed a slightly more intensive colour of leaves and faster growth rate than plants from the non-inoculated plots. However, after this period, plant growth was slowed down, no new shoots were formed and symptoms of drying were observed. This was accompanied by increased erosion processes, caused by rainfalls, especially after longer periods of drought. The seeds and fungal inoculum introduced during initial stages of the experiment were subjected to erosion and washed away to lower parts of plots. Only a few specimens of the introduced grasses were recorded in the autumn. In the following vegetation season, the establishment of grass species other than the originally introduced was observed (i.e. *F. trachyphylla*). These plants showed a high degree of mycorrhizal colonization (over 90%).

The application of AgroHydroGel significantly improved plant growth and vigour, even during the relatively dry summer of the first vegetation season. A dense plant cover of young plants was observed and the soil bore only few signs of erosion. Plots enriched with the gel showed an about twofold higher soil moisture level than other parts of the experimental plots. Statistically significant differences were found among all parts of plots. The plots did not survive the following seasons.

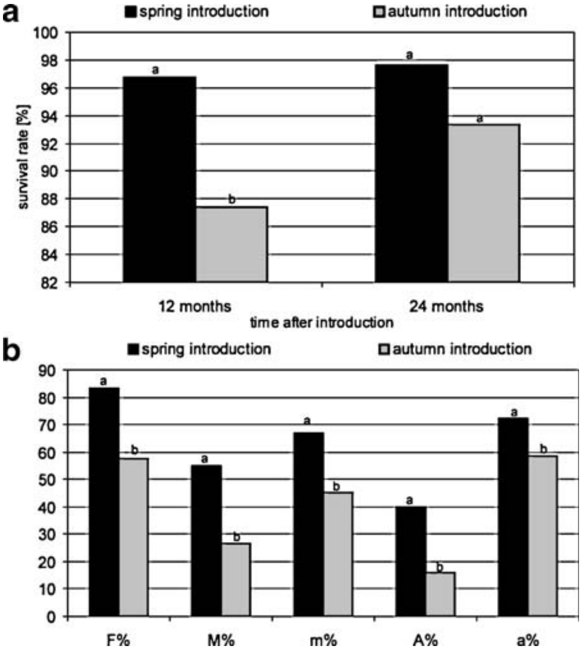

Fig. 4 Performance of introduced *M. caerulea*. **a** Survival and flowering rates. **b** Mycorrhizal colonisation evaluated in September 2005. *F%* mycorrhizal roots frequency, *M%* relative mycorrhizal root length, *m%* intensity of colonisation within individual mycorrhizal roots, *A%* relative arbuscular richness, *a%* arbuscule richness in root fragments where the arbuscules were present. *Different letters above bars* indicate statistically significant differences ($P<0.05$)

Laboratory experiment – vegetative reproduction of grasses

Plants vegetatively multiplied and cultivated under laboratory conditions were able to grow and develop further. In all investigated species, the formation of new leaves and roots was observed. Moreover, *A. gigantea*, *C. canescens* and *M. caerulea* formed inflorescences. The highest survival rate was noted in *M. caerulea* (93%), while in *A. gigantea* and *F. trachyphylla* it was 83% and 80%, respectively. *C. canescens* showed the lowest survival rate of approximately 40%.

Field experiment – *M. caerulea* multiplication and cultivation on wastes

Plantlets of *M. caerulea*, introduced on the waste in June 2004, showed initial symptoms of drying of the above-ground parts, caused by low substratum moisture and high insolation. However, 1 month after the introduction, a massive formation of new leaves occurred in 95% plants. During the next months, 98% of plants showed increased growth, and the formation of inflorescences was observed in 58% of plants. In the area where *M. caerulea* was introduced, no erosion processes were observed. In the following vegetation season, June 2005, 98% of the introduced plants showed regular growth, and flowering was observed in 95% of plants.

Regardless of the time point of the introduction, the plants developed regular root systems. New roots penetrated surrounding soil in all directions, forming an expanded structure. The introduced plants were also intensively colonised by ants. Observations carried out in September 2005 revealed larger differences in survival rates between plants introduced at different times. Autumn plantlets showed statistically significantly lower survival rates and lower percentage of flowering plants (Fig. 4). However, in the next vegetation season (2006), the differences between plantlets were weaker and not statistically significant. Similar tendencies were observed in the case of mycorrhizal colonization in September 2005: plants introduced in the spring showed a better development of arbuscular mycorrhiza (Fig. 4b).

Discussion

The investigated grasses showed a variable ability to grow and form a stable vegetation cover. The present work clearly demonstrated the necessity of proper selection of plant species used in reclamation practices. The commercially available grasses used so far are able to grow only when a rich soil layer and additional watering are supplied, both practices being very expensive on large scale. During the exploitation of the heap, water is artificially supplied in amounts equal to the quantity of water originating from rainfall. This will be different after the company stops its activity, which is planned to happen in the nearest future. Already now, in areas where the substratum is not properly irrigated or in places where the liquid substratum leakage took place, the inadequacy of the restoration technology is clearly visible: patches of scarce vegetation containing no effective colonizers, able to form a good plant cover, are formed. There is a time gap between the disappearance of the introduced plants and the natural succession of plants that are resistant to dryness and toxicity and able to grow even on bare substratum, but relatively slowly expanding. There are two possible reasons for the slow rate of the establishment of the vegetation on waste areas. These are: the lack of appropriate microbiota such as mycorrhizal fungi, and the inhibition of the generative reproduction in favour of the vegetative, which is much slower. The field experiment with commercially available seeds intended for the restoration of the wastes and the inoculum that was selected on the basis of previous laboratory investigations (Orłowska et al. 2005) showed clearly that the use of these grasses is not appropriate. Although the plants start to grow relatively well, they are not able to survive longer periods of dryness; at the same time, erosion of the substratum leads to the disappearance of the inoculum. Eventually, the only places where inoculum was left were local depressions, and there it was used by other grass species that were introduced within the seed material, or were transported by wind from the surroundings. The use of the AgroHydroGel on a part of experimental plots allowed for longer survival of the vegetation, but eventually also failed. This experiment directed our attention towards grasses that were able to establish on the wastes by natural succession. Some of these plants, such as *M. caerulea* and *Phragmites australis* belong to species that usually occur in wetlands (Zarzycki et al. 2002). At the same time, also species typical for sandy grasslands were recorded. Grass species included *C. canescens*, *K. glauca* and *Poa compressa.* Both types of habitats were present in the close surroundings of the waste. Another possibility is that the seeds, or even more probably, the belowground parts of plants belonging to these species, were transported within the uncontaminated soil that was supplied during conventional restoration.

The first step of the present research was the analysis of the mycorrhizal status of these plants. Roots of all the investigated grass species were colonized by arbuscular mycorrhizal fungi (AMF) and the values of all mycorrhizal parameters increased with the age of the depositions. AMF, being obligate symbionts, are known to act as plant-growth promoters and were already shown to be an important component of successful remediation strategies (Jeffries et al. 2003; Khan 2005; Turnau et al. 2006b). Most of the grass species developed mycorrhiza of the *Paris* type or an intermediate type between the *Paris* and the *Arum* types. Research on the mycorrhizal status of grasses was carried out by many investigators, both in Poland (Dominik 1953; Strzemska 1975) and in other countries (Endrigkeit 1937; Johnston 1949; Nicolson 1959; Gerdemann 1965; Steltz 1968). The results were often contradictory. In 1973 Greny published slightly more precise data assigning the mycorrhiza of *Zea*, *Avena*, *Triticum*, *Hordeum*, *Lolium* and *Holcus* to the *Arum* type and the mycorrhiza of *Phleum*, *Alopecurus* and *Agrostis* to the *Paris* type (Greny 1973). These data were not confirmed in the present study on wastes, as *A. gigantea* and *Lolium* spp. formed the *Paris* or the intermediate type. In the case of *F. rubra*, *K. glauca* and *P. pratense* both the *Paris* and the *Arum* types were observed. According to the literature this might result from associations with different mycorrhizal fungi colonizing the roots (Cavagnaro et al. 2001). It is not possible to exclude the influence of the extreme conditions of the wastes on both symbionts.

Among plants inhabiting the zinc wastes, the C_4 grasses (Watson and Dallwitz 1999) were represented only by two species, *Echinochloa crus-galli* and *Setaria pumila*. Both formed mycorrhiza of the *Arum* type. It is usually believed that these plants are more abundantly colonized by mycorrhizal fungi (Hetrick et al. 1988) and are more dependent on this symbiosis because of a less developed root system and the necessity to compensate for the less developed absorption surface of the roots by the development of the absorptive mycelium (Newsham et al. 1995; Hetrick et al. 1988). This type of relations was not observed in the present research. Generally high levels of mycorrhizal colonization of grasses (including C_3) were also reported by other researchers from wastes rich in heavy metals (Pawłowska et al. 1996), and in grasses from soils polluted with toxic metals (Weissenhorn and Leyval 1995; Noyd et al. 1996; Khan et al. 2000).

Although there were numerous studies concerning spontaneous revegetation of the industrial wastes in Poland (Gucwa-Przepióra and Turnau 2001; Patrzałek 2003) little attention was paid to the possibility of using these plants in phytostabilisation of the wastes. Some of the plants were efficient in promoting organic matter accumulation, thus leading to the development of a proper soil layer, a crucial factor for the establishment of other plants. *M. caerulea* is a good example of such grasses, as it forms an abundant root system and also a significant quantity of above-ground biomass. This species showed high rates of survival on bare tailings both under laboratory and field conditions and should be considered a good candidate for potential practical applications in such areas. It can be easily replanted without additional treatment and watering, which is very advantageous from the economical point of view. Similarly to other grasses important in revegetation, such as *C. canescens*, *Festuca tenuifolia*, *F. trachyphylla*, *M. caerulea* is a perennial species able to form a dense vegetation cover by means of vegetative reproduction. The well developed root system is very efficient in the stabilization of the substratum, what is clearly visible on older parts of the waste. *M. caerulea* is especially valuable, as this species stores reserve compounds within the lower intercalar nodes (Jefferies 1915; Thornton and Bausenwein 2000; Taylor et al. 2001; van Heerwaarden et al 2005), that can be activated in the following vegetation seasons. This species is also known to host nitrogen-fixing bacteria (diazotrophs, Reinhold-Hurek and Hurek 1998) within its root system (Hamelin et al. 2002), which may also fertilize the substratum. The plantlets introduced into the experimental plots were not supported by any watering or supplementation with AgroHydroGel. All of the root samples collected from these plants were highly mycorrhizal, similarly to the mother plants that they originated from. This means that the plantlets can be used as a more effective inoculation in such areas. Further investigations are being carried out to assess whether this plant can be used as a source of inoculum for other plant species that could develop in between the *M. caerulea* individuals, thus leading to a higher diversity of the stable vegetation.

In conclusion, the results from our work allow to draw a few pieces of practical information. First, the design of the revegetation programme for such areas should include preliminary studies on naturally occurring vegetation on the wastes and the surrounding areas. The selection of plants suitable for phytoremediation should be done on the basis of their ecological properties and should focus on native species, in spite of them being more expensive to cultivate or breed/multiply. It might be advisable to introduce plantlets obtained by means of vegetative propagation instead of seeds, especially when substratum properties favour erosive processes. The introduced plants need to be accompanied by their microbial symbionts, i.e. mycorrhizal fungi, to ensure their proper establishment. These microsymbionts also play an important role in the formation and stability of the soil. Finally, the success of the phytoremediation programme can be assessed only by means of long-term monitoring spanning over several vegetation seasons.

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