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Use of *Poaceae f.* species to decontaminate soil from heavy metals

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Soil contamination with heavy metals is a problem of worldwide concern and is still unsolved. Phytoremediation is a new and prospective technology that applies plants for cleaning lightly contaminated soils. Three kinds of *Poaceae f.* Species – *Lolium perenne* L., *Poa pratensis* L. and *Festuca pratensis* Huds. – have been chosen in this work for decontaminating soil from heavy metals. These plants were grown under artificial laboratory conditions in soil that was once and periodically contaminated with heavy metals. It has been established that it is *Lolium perenne* L. that most efficiently removes heavy metals and cleans soil. It removed up to 94% of copper, up to 72% of lead, up to 70% of manganese, up to 90% of zinc, up to 70% of nickel, and up to 80% of chromium from the soil. The *Poa pratensis* L. and *Festuca pratensis* Huds. removed less heavy metals, however, all the three kinds of the species of grass vegetation are efficient enough to decontaminate soil from heavy metals.

Key words: *Lolium perenne* L., *Poa pratensis* L., *Festuca pratensis* Huds., phytoremediation, heavy metals

INTRODUCTION

Soil contamination by heavy metals is a problem of worldwide concern that is still unsolved. Contaminants occur in all spheres of human activities, such as mining, industry, transport, land use for the discharge of household and industrial waste, etc. All of these activities cause contamination which in one way or another gets into the environment in the form of gas, solid particles and solutions (Morel, 2002; Lin, 2005; Wu, 2006; Kuo, 2006; D'Ascoli, 2006). Contamination with heavy metals is one of the main environmental problems. Heavy metals is a general term for a group of elements with a density higher than 6 g/cm^3 (Gardea-Torresdey, 2005). These metals are found in agricultural lands and plants growing there, as well as in different food chains which finally lead to serious ecologic and human health problems (Malik, 2004; Zheljazkov, 2006). Ions of most metals are essential to animals and plants, but their excessive concentration is toxic (Yoshida, 2006). Therefore, many countries carry out ecogeochemical and geohygienic research into residential and natural environments. Usually it is an analysis of the surface layer of soil, showing the general technogenic load of contaminants, including atmospheric and geochemical analysis (Jankaitė, 2005).

Recently, scientists have suggested using various plants for local soil cleaning. Phytoremediation is a new approach to the removal of contaminants from the environment. It may be also called the removal of contaminants from the environment by applying plants (Glick, 2003). It is a new and promising technology for cleaning lightly contaminated soils due to its low cost and universal character. The technology is most efficient for decontaminating soil when contaminants are near plant roots (1–2 m deep) (Wilde, 2005; An, 2006; Khan, 2005). Plants are an essential component of ecosystems because they carry elements from the abiotic to the biotic environment (Chojnacka, 2005). Plants are more resistant than most microorganisms to high concentrations of contaminants; plants also absorb contaminants considerably faster and reduce their toxicity (Schnoor, 2005). Therefore, plants are called "the green liver" which removes environmental contaminants. While heavy metals concentrate in living organisms, plant ability to absorb fairly high concentrations of contaminants without any greater damage to growth encourages applying plants for cleaning not only soil but water as well (Raskin, 2001). Absorption and accumulation of contaminants depend on the properties of a plant and its kind (Singh, 2003). Plant population and selection of the appropriate plant are highly significant for developing the phytoremediation technology (Fischerova, 2006; Deng, 2006). Plants must also meet these requirements: the following must grow locally, have a sufficient level of tolerance for contaminants as well as high correlation ties between the level of contamination in the environment and plant tissues (Krolak, 2003). Currently, plant usage for cleaning contaminated soils is considered to be one of the most promising methods (Shann, 1995).

Therefore, the aim of this work is to analyse the possibilities of applying *Poaceae f.* species (*Lolium perenne* L., *Poa pratensis* L., and *Festuca pratensis* Huds.) for cleaning soils from heavy metals and to find out which one of the selected grass plants cleans soil most efficiently.

METHODS

The experiment was carried out under artificial laboratory conditions during the period of February through April 2006.

Plastic pots of 7 × 25 × 11 cm with double bottoms were used for the experiment. 0.5 kg of uncontaminated soil was put into each of the pots. The composition of uncontaminated soil was:

- pH 5.0–6.0
- salt content 1.5 g/l
- nutritional substances included nitrogen (N) – 150–300 mg/l, phosphate (P_2O_5) – 150–300 mg/l, kali (K_2O) – 200–300 mg/l.

Each pot with soil contained 10 g of each kind of seeds. Uncontaminated soil was used for growing control plants. Seeds of each plant were seeded to uncontaminated soil, soil contaminated with heavy metals once, and soil which was periodically contaminated with heavy metals. Since humidity is the main factor ensuring the growth of plants and the necessary physiological processes, grass plants were watered every 5 days. Control plants and those grown in the soil contaminated once were watered with 200 ml of ordinary water while plants grown in the periodically contaminated soil were watered with 200 ml of a mixture of heavy metals. The maintained temperature was 22–24 °C and the lighting was natural. The experiment lasted three months. Every three weeks *Lolium perenne* L., *Poa pratensis* L. and *Festuca pratensis* Huds. were rooted, prepared for analysis and then analysed. The material was mineralised in the Sector of Chemical Analysis, Institute of Botany; the AAS analysis was carried out at the Department of Chemical Analysis of the Vilnius University Faculty of Chemistry. Heavy metals (Cu, Pb, Mn, Zn, Ni, Cr) were identified by a using Perkin-Elmer M403 atomic absorption spectrometer (USA) in a flame mode.

The concentration of heavy metals after the soil had been contaminated once was: Cu – 20 mg/kg; P – 5.0 mg/kg; Mn – 12.4 mg/kg; Zn – 11.5 mg/kg; Ni – 3.0 mg/kg; Cr – 2.3 mg/kg. The concentration of heavy metals after the soil had been contaminated periodically was: Cu – 84 mg/kg; Pb – 160 mg/kg; Mn – 162 mg/kg; Zn – 395 mg/kg; Ni – 26 mg/kg; Cr – 45 mg/kg.

RESULTS AND DISCUSSION

Three grass plant species were selected for the research:

- *Lolium perenne* L. is a grass species belonging to the *Poaceae* family. It grows up to 15–90 cm high and is an important perennial feeding plant.
- *Poa pratensis* L. is a grass species belonging to the *Poaceae* family. Usually it is a perennial plant, rarely annual. This plant is native to temperate and cool climate zones and mountains in the tropical zones. The *Poa pratensis* L. grows to 30–90 cm high, its leaves are flat and narrow, and the panicle is up to 20 cm long.
- *Festuca pratensis* Huds. is a perennial plant belonging to the *Poaceae* family. The stem is 50–100 cm high, leaves are flat and 3–5 mm in width with a roughish upside. The plant is resistant to cold weather; it is mostly found in humid and fertile areas (Jankaitė, 2006).

It was established that copper concentration in soil contaminated once reached 20 mg/kg. The highest concentration of this metal was found in *Lolium perenne* L. 13.75 mg/kg of copper was found in the above-ground part of this plant. Copper concentration amounted to 9.0 mg/kg in *Festuca pratensis* Huds. grown in the same soil. The lowest amount of copper (4.1 mg/kg) was absorbed by *Poa pratensis* L. After the period of soil cleaning had expired and *Poaceae f.* Species have been removed from the soil, we found that in soil where *Lolium perenne* L. grew copper concentration was reduced to 3.35 mg/kg, i. e. it was almost 6 times lower than it the introduced amount. Copper concentration decreased from 20 mg/kg to 4.85 mg/kg in soil where *Festuca pratensis* Huds. Grew, while the soil where *Poa pratensis* L. grew contained 5.4 mg/kg of copper. Under such copper concentration, it was *Lolium perenne* L. that was most efficient in cleaning soil from copper, and *Poa pratensis* L. was least efficient, but soil was cleaned with each of the study plants.

The concentration of manganese was one of the highest both in soil contaminated once and periodically. In soil contaminated once it was 12.4 mg/kg. *Lolium perenne* L. contained 13.5 mg/kg of manganese which might be related to a high accumulation of manganese in grass when the major part of this metal is steadily absorbed by grass. The amount of manganese decreased to 8.4 mg/kg in soil where *Lolium perenne* L. grew. The level of absorption of manganese by *Poa pratensis* L. was higher than by *Festuca pratensis* Huds. The concentration of manganese in *Poa pratensis* L. grown in soil contaminated once amounted to 12.2 mg/kg, while soil where this grass grew contained 10.0 mg/kg of manganese. The amount of manganese found in *Festuca pratensis* Huds. was 9.4 mg/kg, i. e. 1.4 times bigger in soil where *Festuca pratensis* Huds. grew compared with soil where *Lolium perenne* L. grew.

The concentration of zinc in soil contaminated once was 11.5 mg/kg. The largest amount of zinc (4.7 mg/kg) was found in *Lolium perenne* L. The concentration of zinc in soil where this grass grew was 4.05 mg/kg, i. e. 2.8 times less than in contaminated soil. *Poa pratensis* L. contained 3.81 mg/kg of zinc, i. e. 1.2 times less than the concentration found in *Lolium perenne* L. 8.0 mg/kg of zinc was found in soil where *Poa pratensis* L. grew. The concentration of zinc reached 3.38 mg/kg in *Festuca pratensis* Huds. and 7.25 mg/kg in soil where this plant grew. Thus, *Lolium perenne* L. showed the highest ability to absorb zinc from soil, while the lowest amount of zinc was absorbed by *Festuca pratensis* Huds. (Fig. 1).

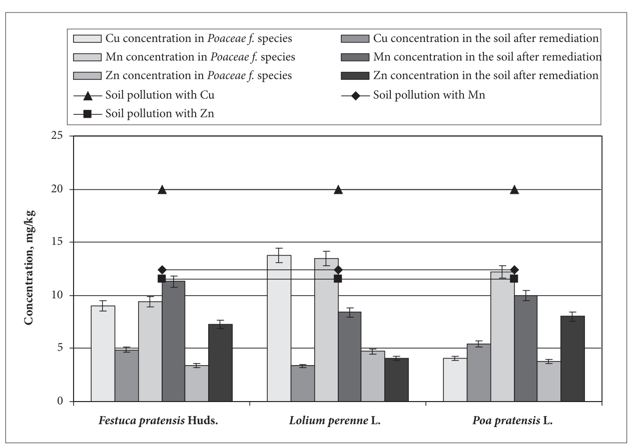

Fig. 1. Soil cleaning from heavy metals (Cu, Mn, Zn) with *Poaceae f.* species (soil contaminated once)

The estimated concentration of lead amounted to 5.0 mg/kg in soil contaminated once and planted with *Poaceae f.* species. The highest concentration of this metal was found in *Lolium perenne* L. (2.5 mg/k) and the lowest in *Festuca pratensis* Huds. (1.38 mg/kg). Lead concentration decreased from 5.0 mg/kg to 1.4 mg/kg in soil where *Lolium perenne* L. grew – it was by 72% lower than in the primary contaminated soil. 1.8 mg/kg of lead was found in *Poa pratensis* L., its concentration was reduced by 4% (to 4.8 mg/kg) in soil where this grass grew. The lowest lead concentration was found in *Festuca pratensis* Huds., however, the amount of lead decreased to 4.3 mg/kg in soil where *Festuca pratensis* Huds. grew which is 1.2 times more than in soil after *Poa pratensis* L.

The concentration of nickel in soil contaminated both once and periodically was among the lowest concentrations of heavy metals in this experiment. Soil contaminated once contained 3.0 mg/kg of this metal. The amount of nickel was 2.1 mg/kg in *Lolium perenne* L. which grew in this soil, 2.7 mg/kg in *Festuca pratensis* Huds. and 2.0 mg/kg in *Poa pratensis* L. After decontamination of soil in which *Festuca pratensis* Huds. grew the concentration of nickel was reduced to 0.88 mg/kg which was 3.4 times less than in contaminated soil. *Poa pratensis* L. removed up to 1.9 mg/kg and *Lolium perenne* L. up to 0.93 mg/kg of nickel from soil. *Lolium perenne* L. removed up to 68%, *Festuca pratensis* Huds. up to 70% and *Poa pratensis* L. up to 37 % of nickel compared to contaminated soil.

The content of chromium in soil contaminated once was 2.3 mg/kg. The largest amount of this metal was found in *Lolium perenne* L. (2.2 mg/kg), whereas. *Poa pratensis* L. absorbed the smallest amount of chromium (1.68 mg/kg). *Festuca pratensis* Huds. contained 1.9 mg/kg of nickel. After soil was cleaned, the concentration of chromium both in soils after *Lolium perenne* L. and *Festuca pratensis* Huds. reached 1.08 mg/kg. Thus, both *Lolium perenne* L. and *Festuca* pratensis Huds. removed up to 47% of chromium. 1.93 mg/kg of chromium was found in soil where *Poa pratensis* L. grew. This concentration was 1.2 times lower than in soil contaminated with chromium (Fig. 2).

Copper concentration reached 84 mg/kg in soil which had been contaminated periodically. *Lolium perenne* L. absorbed most of copper. 78.75 mg/kg of copper was found in *Lolium perenne* L. and its amount decreased to 11.9 mg/kg in soil where *Lolium perenne* L. grew. *Festuca pratensis* Huds. absorbed less copper: the estimated content of copper in this grass reached 51.25 mg/kg. *Poa pratensis* L. absorbed the least part of copper compared to the other *Poaceae f.* species: the concentration of copper in this plant was 40 mg/kg. 22.5 mg/kg of copper was found after the experiment in soil where *Festuca pratensis* Huds. grew. The amount of copper decreased to 58 mg/kg in soil after *Poa pratensis* L. *Lolium perenne* L. absorbed most of the copper in soils contaminated both once and periodically. Copper concentration in soil was reduced to almost 94% by this plant. *Festuca pratensis* Huds. reduced copper in soil to 74%. The lowest level of soil cleaning was shown by *Poa pratensis* L. which removed up to 31% of the primary copper concentration. However, all of the analysed *Poaceae f.* species efficiently absorbed copper from soil.

The periodical contamination of soil increased the concentration of manganese to 162 mg/kg. The level of absorption of this metal was similar in all *Poaceae f.* species. A slightly higher concentration of manganese was found in *Lolium perenne* L. (156.25 mg/kg). The content of manganese in *Festuca pratensis* Huds. and *Poa pratensis* L. was almost the same – 152.5 mg/kg and 152.3 mg/kg, respectively. The lowest content of manganese was found in soil after *Lolium perenne* L. (51.3 mg/kg). Soil where *Festuca pratensis* Huds. grew contained 58 mg/kg of manganese which is only 1.13 times more than in soil here *Lolium perenne* L. grew. *Poa pratensis* L. reduced manganese to 97 mg/kg, which is 1.9 times less compared to *Lolium perenne* L. and 1.7 times less than in soil where *Festuca pratensis* Huds. grew. All the *Poaceae f.* species studied showed rather good absorptive properties for manganese.

The concentration of zinc in periodically contaminated soil was 395 mg/kg, which was the highest concentration in this experiment. *Lolium perenne* L. which grew in this soil contained 200 mg/kg of zinc which was the highest concentration among the analysed plants. *Poa pratensis* L. contained 156.25 mg/kg and *Festuca pratensis* Huds. 125 mg/kg of zinc. According to these findings, all the three analysed *Poaceae f.* species have rather good abilities to absorb zinc. The concentration of zinc in soil where *Lolium perenne* L. grew was 34 mg/kg. *Poa pratensis* L. removed up to 43.5 of zinc from soil, which is 1.4 times less compared *to*

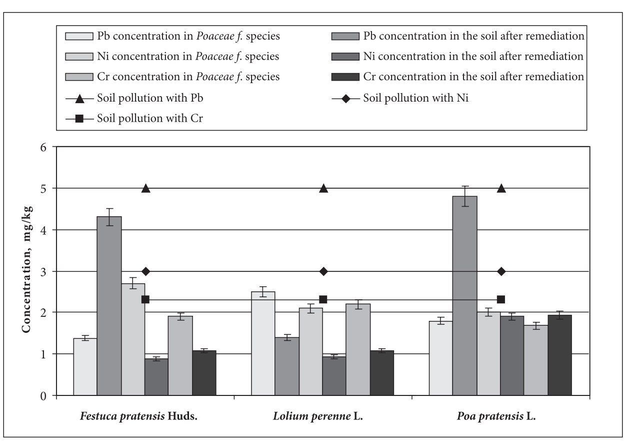

Fig. 2. Soil cleaning from heavy metals (Pb, Ni, Cr) with *Poaceae f.* species (soil contaminated once)

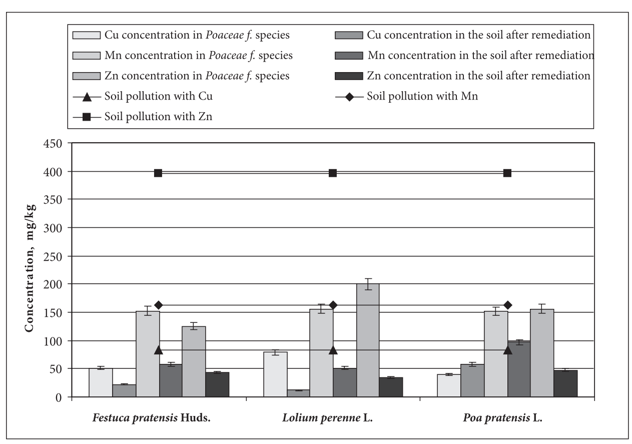

Fig. 3. Soil cleaning from heavy metals (Cu, Mn, Zn) with *Poaceae f.* species (periodically contaminated soil)

Lolium perenne L. The amount of zinc decreased to 42.5 mg/kg in soil where *Festuca pratensis* Huds. grew. According to these findings, *Lolium perenne* L. removed almost up to 90% of zinc from soil, while *Poa pratensis* L. and *Festuca pratensis* Huds. removed up to 87% and 86% respectively, compared to the amount of zinc in contaminated soil (Fig. 3).

Lead concentration in soil contaminated periodically was 160 mg/kg. The largest amount of lead was absorbed by *Lolium perenne* L. The amount of lead found in *Lolium perenne* L. was 132.5 mg/kg, whereas in soil where *Lolium perenne* L. grew it was 64.5 mg/kg. The amount of this heavy metal was reduced by 40% compared with primary soil contamination when *Lolium perenne* L. was used for cleaning soil. *Festuca pratensis* Huds. absorbed more lead than *Poa pratensis* L. The amount of lead found in *Festuca pratensis* Huds. was 118 mg/kg, i. e. 1.4 times lower than in *Lolium perenne* L. Lead concentration in *Poa pratensis* L. was 60 mg/kg, i. e. the lowest among the analysed plants. A comparison of the amount of lead remaining in soil showed that soil after *Lolium perenne* L. contained 1.8 times less lead than *Poa pratensis* L. and was 1.7 times less than in soil, where *Festuca pratensis* Huds. grew. In the periodically contaminated soil the concentration of nickel amounted to 26 mg/kg. The highest amount of nickel was found in *Festuca pratensis* Huds. (24.5 mg/kg). After the experiment was ended, the concentration of nickel in soil where *Festuca pratensis* Huds. grew was 4.1 mg/kg, i. e. 6.3 times less than in contaminated soil. *Poa pratensis* L. contained 17.38 mg/kg of nickel, which was 1.1 times less than in *Lolium perenne* L., whereas *Lolium perenne* L. contained 18.25 mg/kg. The amount of nickel in soil where *Poa pratensis* L. grew was 11.7 mg/kg, i. e. 2.8 times more than in soil cleaned by *Festuca pratensis* Huds. *Lolium perenne* L. cleaned up to 10.2 mg/kg of nickel. Thus, *Lolium perenne* L. has the best ability to remove nickel from soil.

The concentration of chromium in periodically contaminated soil was 45 mg/kg. The highest content of this metal was found in *Lolium perenne* L. (40 mg/kg). *Festuca pratensis* Huds. contained 31.12 mg/kg and *Poa pratensis* L. 17.25 mg/kg of chromium. The largest amount of nickel was removed by *Lolium perenne* L. The concentration of this metal decreased 5 times, i. e. to 8.7 mg/kg. The concentration of chromium in soil

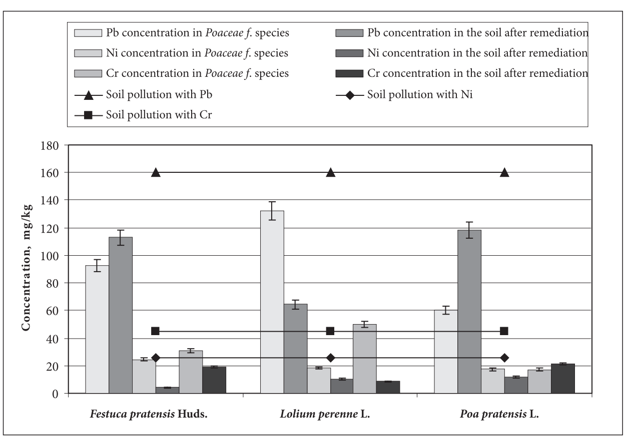

Fig. 4. Soil cleaning from heavy metals (Pb, Ni, Cr) with *Poaceae f.* species (periodically contaminated soil)

where *Festuca pratensis* Huds. grew decreased by 57% (19.1 mg/kg). The smallest amount of chromium(21.3 mg/kg) was removed by *Poa pratensis* L. – two times less than its content in soil (Fig. 4).

No thorough research has been carried out in Lithuania on plants' (i. e. *Poa pratensis* L., *Lolium perenne* L. and *Festuca pratensis* Huds.) ability to absorb heavy metals, however, the accumulation of heavy metals in *Poaceae f.* species may be compared to researches on clovers growing by the roadsides. T. Adomaitis and A. Antanaitis carried out a study by the Vilnius–Kaunas trunk-road near Rumšiškės, 5 m away from the roadside where the concentration of heavy metals is the highest. It has been established that clover plants most efficiently absorb zinc (28.3 mg/kg versus 58.3 mg/kg of zinc in the soil). High concentrations of this metal were found also in all the analysed *Poaceae f.* species. The concentration of copper in roadside soil was 12.6 mg/kg. The content of this metal reached 6.5 mg/kg in clovers. According to the results of our research, soil contained 84 mg/kg of copper. The highest content of this metal was found in *Lolium perenne* L. (78.75 mg/kg). The content of lead reached 17.3 mg/kg in roadside soil beside the Vilnius–Kaunas trunk-road, whereas clover contained 0.56 mg/kg of this metal. Experimental studies show that the analysed *Poaceae f.* species have better absorptive abilities of this metal compared to clover. The concentration of nickel found in soil near Rumšiškės was 14.3 mg/kg, versus 0.53 mg/kg in clovers. In our experiment, the concentration of nickel in soil was 26 mg/kg. The analysed *Poaceae f.* species contained significantly higher levels of this metal compared to clovers. The roadside soil 5 m away from the road edge contained 15.3 mg/kg of nickel, while the concentration of this metal was 1.2 mg/kg in clover plants growing in this place. According to our research, nickel is efficiently absorbed by *Lolium perenne* L., *Poa pratensis* L. and *Festuca pratensis* Huds.

A comparison of results obtained by T. Adomaitis and A. Antanaitis and the data of our experiments shows that all the analysed heavy metals are most efficiently absorbed by *Lolium perenne* L. However, application of this grassy plant for soil cleaning needs additional researches.

CONCLUSIONS

1. *Lolium perenne* L. most efficiently removes heavy metals from soil, while *Festuca pratensis Huds.* absorbs the least amount compared to *Lolium perenne* L. and *Poa pratensis* L.

2. The amount of copper in soil contaminated once was reduced 6 times by *Lolium perenne* L., 4 times by *Festuca pratensis* Huds. and 3.7 times by *Poa pratensis* L. The concentration of this metal in periodically contaminated soil was decreased to nearly 94% by *Lolium perenne* L., 74% by *Festuca pratensis* Huds. and 31% by *Poa pratensis* L. compared to the initial concentration of copper in soil.

3. The concentration of lead in soil contaminated once was reduced by 72% in soil where *Lolium perenne* L. grew, by 4% in soil with *Poa pratensis* L. and by 14% in soil with *Festuca pratensis* Huds. Compared to the amount of lead in the periodically contaminated soil, it was soil where *Lolium perenne* L. grew contained 2.4 times less lead than contaminated soil.

4. The concentration of manganese in soil contaminated once was reduced 1.48 times by *Lolium perenne* L., 1.1 times by *Poa pratensis* L. and 1.24 times by *Festuca pratensis* Huds. *Lolium perenne* L. removed up to 70% of manganese, *Poa pratensis* L. and *Festuca pratensis* Huds. removed up to 60% and 65% of manganese respectively from the periodically contaminated soil compared to the primary contaminated soil.

5. *Lolium perenne* L. reduced the concentration of zinc from soil contaminated once 2.8 times, *Poa pratensis* L. 1.4 times and *Festuca pratensis* Huds. 1.6 times. *Lolium perenne* L. removed 90%, *Poa pratensis* L. and *Festuca pratensis* Huds. up to 87%, 86% of zinc from periodically contaminated soil, respectively.

6. *Festuca pratensis* Huds. removed up to 85% of nickel from soil contaminated once, *Festuca pratensis* Huds. and *Poa pratensis* L. up to 61% 55% of nickel, respectively. The periodically contaminated soil contained 6.3 times less nickel where *Lolium perenne* L. grew, 2.5 times less where *Festuca pratensis* Huds. grew and 2.2 times less zinc where *Poa pratensis* L. grew.

7. Both *Lolium perenne* L. and *Festuca pratensis Huds*. removed reduced chromium by half in soil contaminated once. *Poa pratensis* L. reduced the content of chromium 2 times. *Lolium perenne* L. removed up to 80%, *Festuca pratensis* Huds. – 58% and *Poa pratensis* L. – 53% of chromium from periodically contaminated soil.

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DIRVOŽEMIO VALYMAS NUO SUNKIŲJŲ METALŲ NAUDOJANT ŽOLINĘ AUGALIJĄ

S a n t r a u k a

Dirvožemio tarša sunkiaisiais metalais visame pasaulyje aktuali problema iki šiol neturinti universalaus sprendimo. Fitoremediacija – atsirandanti ir perspektyvi technologija nestipriai užterštiems dirvožemiams valyti naudojant augalus. Šiame darbe dirvožemio valymui nuo sunkiųjų metalų pasirinktos trys *Poaceae f.* rūšys – *Polium perenne* L., *Poa pratinsis* L. ir *Festuca pratinsis* Huds. – augintos modelinėmis laboratorinėmis sąlygomis vieną kartą ir periodiškai sunkiaisiais metalais teršiamuose dirvožemiuose. Nustatyta, kad geriausiai sunkiuosius metalus iš dirvožemio sorbuoja ir didžiausias dirvožemio valymo efektyvumas pasiekiamas naudojant *Polium perenne* L. Ji dirvožemį nuo vario išvalo beveik iki 94%, nuo švino – iki 72%, nuo mangano – iki 70%, nuo cinko – 90%, nuo nikelio – iki 70%, nuo chromo – iki 80%. *Poa pratinsis* L. ir *Festuca pratinsis* Huds. sunkiuosius metalus iš dirvožemio šalina silpniau, tačiau visų tirtų žolinės augalijos rūšių atveju dirvožemio valymas nuo sunkiųjų metalų pakankamai efektyvus.

Raktažodžiai: *Lolium perenne* L., *Poa pratensis* L., *Festuca pratensis* Huds., fitoremediacija, sunkieji metalai