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# Comparison of phytoremediation potential of three grass species in soil contaminated with cadmium

## Porównanie potencjału fitoremediacyjnego trzech gatunków traw na glebach zanieczyszczonych kadmem

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**Keywords:** phytoremediation, cadmium, grass, soil contamination, tolerance, bioaccumulation, translocation
**Słowa kluczowe: fitoremediacja, kadm, trawa, zanieczyszczenie gleby, tolerancja, bioakumulacja, translokacja**

**Abstract**
The aim of the study was to compare the toleration of *Poa pratensis*, *Lolium perenne* and *Festuca rubra* to cadmium contamination as well as the phytoremediation potential of these three species of grass. The pot experiment was conducted in four replications in pots containing 2.0 kg of soil. The soil was contaminated with three doses of Cd – 30, 60 and 120 mg·kg$^{-1}$. After two months, the aerial parts of plants were harvested. The roots were dug up, brushed off from the remaining soil and washed with water. The biomass was defined and the cadmium concentration was determined in aerial parts and roots. The phytoremediation potential of grasses was evaluated using biomass of grasses, bioaccumulation factor (BF) and translocation factor (TF). All three tested species of grasses had TF < 1 and BF-root > 1. It indicates their suitability for phytostabilisation and makes them unsuitable for phytoextraction of Cd from the soil. Comparing the usefulness of the tested grasses for phytoremediation has shown that the phytostabilisation potential of *P. pratensis* was lower than that of *L. perenne* and *F. rubra*. *P. pratensis* was distinguished by higher TF, smaller root biomass and lower tolerance for Cd excess in the soil in comparison with the two other test grasses. At the same time, *L. perenne* was characterised by the smallest decrease in biomass and the largest Cd accumulation in roots at the lowest dose of Cd. It indicates good usefulness for phytostabilisation of soils characterised by a relatively small pollution by cadmium.



**Streszczenie**
Celem pracy było porównanie tolerancji *Poa pratensis*, *Lolium perenne* i *Festuca rubra* na zanieczyszczenie kadmem oraz potencjału fitoremediacyjnego tych trzech gatunków traw. Doświadczenie wazonowe przeprowadzone było w 4 powtórzeniach w doniczkach o pojemności 2 kg. Do gleby wprowadzono 3 dawki Cd - 30, 60 i 120 mg·kg$^{-1}$. Po upływie 2 miesięcy części nadziemne zostały ścięte, a korzenie usunięte z gleby i dokładnie wypłukane. Określono biomasę oraz oznaczono zawartość Cd w częściach nadziemnych i korzeniach. Potencjał fitoremediacyjny traw oceniono używając biomasy traw, współczynnika bioakumulacji (BF) i translokacji (TF). Wszystkie trzy badane gatunki traw charakteryzowały się TF<1 oraz BF-korzenie>1, co wskazuje na ich przydatność do fitostabilizacji, a czyni nieprzydatnymi do fitoekstrakcji Cd z gleby. Porównując przydatność badanych traw do fitoremediacji wykazano, że potencjał fitostastabilizacyjny *Poa pratensis* był niższy niż potencjał *Lolium perenne* i *Festuca rubra*. *Poa pratensis* odznaczała się wyższym TF, mniejszą biomasą korzeni oraz mniejszą tolerancją na nadmiar Cd w glebie w porównaniu z dwiema innymi badanymi trawami. Jednocześnie *Lolium perenne* charakteryzowała się najmniejszym spadkiem biomasy oraz największą akumulacją Cd w korzeniach przy najniższej dawce Cd. Świadczy to o dobrej przydatności do fitostabilizacji gleb charakteryzujących się względnie małym zanieczyszczeniem kadmem.

## 1. INTRODUCTION

Heavy metal contamination is a consequence of the expanding industry and urbanisation. This is one of the main environmental problems in the world. Accumulation of heavy metals in the soil is a serious threat because of their high toxicity and long biological half-life. These inorganic contaminants have a high capacity of entering the organisms of humans and animals through the food chain [Pinto et al. 2004].

Cadmium is one of the most toxic heavy metals. It has a negative impact on people, animals and plants [Kabata-Pendias, 2010]. According to the Regulation of the Minister of the Environment, the concentration of cadmium in Polish soils should not exceed 4 mg·kg$^{-1}$ [Rozporządzenie … 2002]. Despite the fact that soil contamination with cadmium in Poland concerns only 1.8% of the soils [Maliszewska-Kordybach et al. 2013], there occur small areas highly contaminated with this element (the so-called hot spots). In the studies of Dziubanek et al. [2012], which were carried out on the territory of the Upper Silesia, Poland, some soil samples collected from vegetable gardens contained about 40 mg·kg$^{-1}$ Cd, whilst that from residential areas contained more than 60 mg·kg$^{-1}$ Cd. Siuta [2004] found that sludge lagoons at Hajdówka Wastewater Treatment Plant, which required remediation, contained from 36 to 171 mg·kg$^{-1}$ of cadmium. These levels of cadmium concentrations occur also in the soils in other countries [Davies et al. 2003, Lin et al. 2015].

Cadmium has the highest mobility amongst all heavy metals [Clemens 2006] and, therefore, enters plants easily. The intensity of cadmium accumulation by plants depends on their species and cultivar. Cadmium uptake by plants also depends on soil properties, mainly pH, texture and organic matter content [Karczewska 2008]. The presence of cadmium in a plant results in the disturbance of numerous metabolic and physiological processes [Ekmekci et al. 2008, Rizzardo et al. 2012]. Cadmium also contributes to reducing the uptake and distribution of minerals and water [Di Toppi and Gabbrielli, Gomes et al. 2013]. It leads to the occurrence of many diseases of plants, inhibition of their growth or even their death [Kaznina and Titov 2014, Wahid and Ghani 2008].

Remediation techniques are used to exclude heavy metals from the soil. Using natural or genetically modified plants for this purpose is called phytoremediation. Phytoextraction and phytostabilisation are the two most popular phytoremediation techniques. Phytoextraction involves the absorbing of heavy metals from the soil and translocating them to the harvestable aboveground plant tissues, which are then removed from the contaminated area. The plants used for this process should have a fast growth rate, large biomass and a high tolerance to heavy metals [Marques 2009]. Phytostabilisation involves immobilising or reducing the mobility of heavy metals. Metals can be accumulated by the roots, absorbed on their surface or precipitated in the rhizosphere. Plants ideal for phytostabilisation should have a well-developed root system and exhibit tolerance to high levels of heavy metals. They also need to have a high metal accumulation in the roots and a low metal translocation from roots to shoots [Karczewska et al. 2013, Korzeniowska et al. 2011, Stanisławska-Glubiak et al. 2012].

Grasses are potential plants for phytoremediation [Abaga et al. 2014, Xia 2004, Spiak and Gediga 2012]. They are distinguished by a fast growth rate, well-developed root system, large biomass and a long-term growth cycle. In addition, they show a high tolerance to soil contamination with heavy metals [Chen et al. 2004]. Grasses can accumulate large quantities of heavy metals in both roots and shoots [Aibibu et al. 2010, Xu and Wang 2013, Zhang et al. 2014].

The aim of the study was to compare the tolerance of *Poa pratensis, Lolium perenne* and *Festuca rubra* to soil contamination with cadmium and investigate the suitability of these three species for phytoremediation.

## 2. MATERIALS AND METHODS

### 2.1. Pot experiment

The pot experiment was conducted in the greenhouse of the Department of Weed Science and Tillage Systems in Wrocław, Poland. The pots with the 2-kg capacity were filled with soil, the characteristics of which were shown in Table 1. Three grass species: *P. pratensis L.*, *L. perenne L.* and *F. rubra L.* were used as test plants. Before sowing, the soil was contaminated with three different doses of cadmium in the form of $CdCl_2$: 30, 60 and 120 mg·kg$^{-1}$ Cd. The experiment was carried out in four replicates. The plants were sown one week after the introduction of Cd into the soil. Three weeks after sowing, fertilisation was carried out with 50 mg of N, 8.5 mg of P and 27.5 mg of K per pot.

Table 1. Soil characteristics

| pH in KCl | Soil fraction (%) | | $C_{org}$ | $P_2O_5$* | $K_2O$* | Mg |
|---|---|---|---|---|---|---|
| | 0.05–0.002 mm | <0.002 mm | mg·kg$^{-1}$ | | | |
| 5.4 | 23.47 | 2.59 | 0.66 | 237 | 159 | 65 |

$C_{org}$ – organic carbon

*According to the Enger–Riehm method.

### 2.2. The collection and chemical analysis of samples

The plants were harvested two months after sowing. The aboveground parts of grasses were cut 5 mm above the ground. The roots were removed from the pots, purified from the soil, first washed with tap water and next rinsed in distilled water for two hours using a rotary mixer. After that, the aboveground parts and roots were dried (24 hours at 50 ° C and 3 h at 100 °C), weighed and finely ground. After harvesting the grasses, soil samples were collected. They were dried at room temperature, ground in a mortar and passed through a sieve with a diameter of 2 mm.

All the chemical analyses were performed at the Main Laboratory of the Institute of Soil Science and Plant Cultivation, accredited by the Polish Centre for Accreditation (certificate number AB 339, based on PN-EN ISO/IEC 17025 standard). The content of cadmium in the soil was determined by flame atomic absorption spectrometry (FAAS) method, after digestion in aqua regia (PN-ISO 11047:2001). Organic carbon in soil ($C_{org}$) was determined by Tiurin's method using potassium dichromate (PN-ISO14235:2003), pH was established potentiometrically in KCl solution (ISO10390: 2005), phosphorus and potassium were determined using the Enger–Riehm method (PN-R-04023:1996 and PN-R-04022:1996 adequately), Mg by the Schachtschabel method (PN-R-04020:1994) and texture was evaluated by the aerometric method (PN-R-04032:1998). The concentration of cadmium in aboveground parts and roots was determined with inductively coupled plasma atomic emission spectroscopy (ICP-OES) method (PB 112.1-edition I-10.04.2013) after prior digestion in 20% nitric acid.

### 2.3. Calculation of the bioaccumulation and translocation factor

In order to compare the accumulation of metals in plants, bioaccumulation factor (BF) of the aboveground parts and roots was calculated according to the following formulas (Malik et al. 2010):

$$\text{BF root} = \frac{\text{Cd concentration in roots } (\text{mg} \cdot \text{kg}^{-1})}{\text{Cd concentration in soil } (\text{mg} \cdot \text{kg}^{-1})}$$

$$\text{BF shoot} = \frac{\text{Cd concentration in shoots } (\text{mg} \cdot \text{kg}^{-1})}{\text{Cd concentration in soil } (\text{mg} \cdot \text{kg}^{-1})}$$

The transfer of metals from the roots to the aboveground parts was determined based on the translocation factor (TF) expressed by the formula (Malik et al. 2010):

$$\text{TF} = \frac{\text{Cd concentration in shoots } (\text{mg} \cdot \text{kg}^{-1})}{\text{Cd concentration in roots } (\text{mg} \cdot \text{kg}^{-1})}$$

### 2.4. Statistical calculations

For biomass, one-way analysis of variance (ANOVA) was conducted. The evaluation of the significance of the data between the groups of the tested parameters was done using Tukey's test ($P < 0.05$). The calculations were performed using AWAR program developed at the Institute of Soil Science and Plant Cultivation [Filipiak and Wilkos 1995].

Table 2. Concentration of cadmium in soil

| Species | Treatment | Cd concentration $mg \cdot kg^{-1}$ |
|---|---|---|
| *Poa pratensis* | Cd0 | 0.8 |
| | Cd30 | 30.1 |
| | Cd60 | 62.7 |
| | Cd120 | 124 |
| *Lolium perenne* | Cd0 | 0.5 |
| | Cd30 | 27.8 |
| | Cd60 | 69.2 |
| | Cd120 | 121 |
| *Festuca rubra* | Cd0 | 1.2 |
| | Cd30 | 30.5 |
| | Cd60 | 64.3 |
| | Cd120 | 128 |

## 3. RESULTS AND DISCUSSION

### 3.1. Cd content in the soil

Cd content in the soil increased steadily under the influence of the applied doses and ranged from 0.5 to1.2 $mg \cdot kg^{-1}$ in control treatments to 121–128 $mg \cdot kg^{-1}$ in the treatments with the highest dose (Table 2). In Cd120 treatments, Cd concentration was approximately 30-fold higher than the Polish standards for soil contamination with this element [Rozporządzenie… 2002]. Such high concentrations may occur in small areas of the Upper Silesia region or in sludge lagoons of wastewater treatment plants [Dziubanek et al. 2012, Siuta 2004].

### 3.2. The impact of Cd on biomass

The usefulness of plants for phytoremediation is assessed based on not only their tolerance to contamination but also the possibility of obtaining a suitable amount of biomass. Low amount of biomass of the aboveground parts is unfavourable for phytoextraction, whilst that of roots is unfavourable for phytostabilisation. Amongst the three tested grasses, the highest amount of biomass of shoots and roots was recorded for *L. perenne*, whilst the lowest for *P. pratensis*. It concerned both the plants in the control and the plants growing in Cd treatments.

The study showed a significant decrease in the biomass of the roots and shoots of all the tested plants along the increase in Cd in the soil (Table 3). Amongst the three tested grasses, the least biomass decrease was reported for *L. perenne* at the first cadmium dose (relatively small pollution). The shoots were most sensitive to the contamination than roots. Even the lowest Cd dose caused the losses in the shoot biomass by 19.3–40.2%, whilst only by 16.4–27.7% in the root biomass. The biomass decrease was also observed by Zhang et al. [2014] with *Pennisetum americanum* and *Pennisetum purpureum* at the cadmium doses of 15–100 $mg \cdot kg^{-1}$. The contamination higher than 60 and 90 $mg \cdot kg^{-1}$ caused the decrease in biomass of the shoots and roots of *Sigesbeckia orientalis* [Zhang et al. 2013]

Although cadmium is generally considered a toxic element that inhibits plant growth and development [Nazar et al. 2012], some authors found that excess of this metal in soil increased the biomass of grasses instead of decreasing it. In the studies of Xu and Wang [2013], *P. pratensis* and *F. arundinacea* reacted with shoot biomass increase to the doses 50 and 100 $mg \cdot kg^{-1}$ Cd. Zhang et al. [2010] recorded the increase in total biomass with *P. americanum* and *P. purpureum* at the doses from 1 to 8 $mg \cdot kg^{-1}$ Cd.

In order to compare the reduction of biomass, the average decrease from the three doses was calculated (fig. 1). The analysis of the data in Figure 1 showed that *P. pratensis* had a slightly higher average decline in the biomass of shoots than the other two grasses. It was about 59%, whilst for *F. rubra* and *L. perenne,* it did not exceed 53%. Higher differences amongst the grasses were recorded in terms of the decrease in root biomass compared to shoot biomass. The largest average decrease in the root biomass was recorded for *L. perenne* (53%), whilst the least for *F. rubra* (42%).

### 3.3. The concentration of Cd in roots and shoots

Increasing the dose of the Cd caused its increase in both the roots and shoots of the tested grasses (Table 4). In the case of the roots, this increase was almost linear. Xu and Wang [2013] obtained similar results for *P. pratensis* and *Festuca arundinacea*. They observed that the concentration of the Cd in roots ranged from 951 to 7441, whilst that in shoots from 40.9 to 341 $mg \cdot kg^{-1}$. According to Brooks [1998], the ability to accumulate more than 100 $mg \cdot kg^{-1}$ of Cd in shoots is one of the conditions the plant to be considered hyperaccumulators. This criterion was met by *P. pratensis* and *F. rubra*, which accumulated 148–341 and 135–266 $mg \cdot kg^{-1}$ of Cd under soil contamination at least 60 $mg \cdot kg^{-1}$.

Table 3. Biomass of the roots and shoots of three grass species

| Species | Treatment | Biomass of grasses ($g \cdot pot^{-1}$) | | Decrease of biomass[1] (%) | |
|---|---|---|---|---|---|
| | | Roots | Shoots | Roots | Shoots |
| *Poa pratensis* | Cd0 | 0.30 b | 2.84 c | - | - |
| | Cd30 | 0.23 b | 1.70 b | 24.7 | 40.2 |
| | Cd60 | 0.18 ab | 1.25 b | 40.9 | 56.0 |
| | Cd120 | 0.06 a | 0.56 a | 79.0 | 80.4 |
| *Lolium perenne* | Cd0 | 1.18 c | 4.88 d | - | - |
| | Cd30 | 0.99 c | 3.94 c | 16.4 | 19.3 |
| | Cd60 | 0.53 b | 2.27 b | 55.1 | 53.5 |
| | Cd120 | 0.15 a | 0.83 a | 87.0 | 83.1 |
| *Festuca rubra* | Cd0 | 0.47 b | 2.56 c | - | - |
| | Cd30 | 0.34 b | 1.77 b | 27.7 | 31.0 |
| | Cd60 | 0.33 b | 1.46 b | 29.8 | 43.1 |
| | Cd120 | 0.15 a | 0.58 a | 68.8 | 77.5 |

[1]The decrease compared to the control. Values followed by the same letters in the same column for the same species of grass indicate no significant difference according to Tukey's test ($P<0.05$)

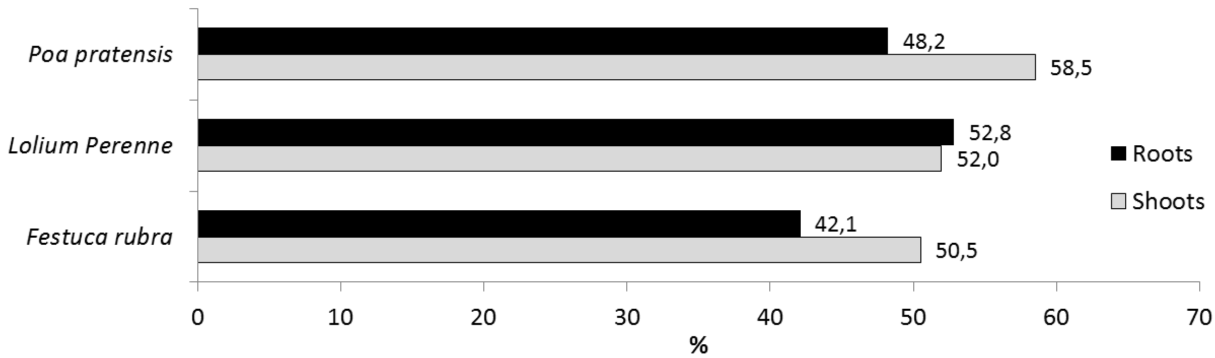


Fig. 1. A relative decrease in grass biomass – the mean of three doses of Cd

Cadmium concentration in the roots was higher than that in the shoot by about 10- to 15-fold for *P. pratensis*, 34- to 54-fold for *L. perenne* and 12- to 20-fold for *F. rubra*. The largest concentration of Cd was reported under its highest dose (Cd120) in the roots of *L. perenne*. Many authors have reported the accumulation of much larger quantities of cadmium in the roots than that in the aboveground parts of plants, including grasses growing on the contaminated soil [Deram et al. 2006, Guo et al. 2014, Nan et al. 2002, Stanislawska-Glubiak et al. 2015, Quezada-Hinojosa et al. 2015, Xia 2004, Zhang et al. 2014]. A much higher Cd concentration in the roots than that in the shoots was also recorded in the hydroponic experiment for *Vetiveria zizanioides* [Aibiby et al. 2010] and for *P. pratensis* [Xu and Wang 2013]. Reverse results were obtained by Xia [2004] for *Pennisetum glaucum* and *P. purpureum* and by Zhang et al. [2013] for *Siegesbeckia orientalis*. A larger concentration of Cd in the shoots than in the roots was also observed by Xu and Wang [2013] for *F. arundinacea* under soil contamination of more than 200 $mg \cdot kg^{1}$.

The highest quantities of cadmium were accumulated in the roots of *L. perenne*. The average Cd concentration in the roots of this grass was by about twofold higher than that in *P. pratensis* and *F. rubra* (fig. 2). The highest accumulation of Cd in the shoots was recorded for *P. pratensis*. The average Cd concentration was in this case 1.2- to 1.6-fold higher than that in *F. rubra* and *L. perenne.*

It is noteworthy that *L. perenne* had the highest average Cd accumulation in the roots and at the same time, the smallest in the shoots amongst all the tested grasses (Fig. 2). *P. pratensis* showed the opposite relation – it accumulated the least of Cd in the roots and the most in the shoots. The obtained data drew attention to the relationship between the Cd concentration in the root and shoots and grasses biomass (Fig. 1, 2). *L. perenne*, a grass that contained the most of the cadmium in the roots, had the largest decrease in the root biomass. The largest losses in the shoot biomass were obtained for *P. pratensis*, which accumulated most of the cadmium in these organs

## 3.4. Factor of bioaccumulation and translocation

BF in all the cases was higher than 1 (Table 5). BF-root amounted from 25.6 to 80.7, and BF-shoot from 1.2 to 2.7. A high BF-root indicates the accumulation of a significant amount of cadmium in

Table 4. The concentration of Cd in the roots and shoots of grasses

| Species | Treatment | Roots | Shoots |
|---|---|---|---|
| | | mg·kg-1 | |
| *Poa pratensis* | Cd0 | 5.4 | 1.6 |
| | Cd30 | 951 | 63.2 |
| | Cd60 | 1954 | 148 |
| | Cd120 | 3296 | 341 |
| *Lolium perenne* | Cd0 | 8.2 | 0.9 |
| | Cd30 | 2244 | 40.9 |
| | Cd60 | 4714 | 83.9 |
| | Cd120 | 7441 | 216 |
| *Festuca rubra* | Cd0 | 22.0 | 2.1 |
| | Cd30 | 1532 | 76.3 |
| | Cd60 | 2351 | 135 |
| | Cd120 | 3277 | 266 |

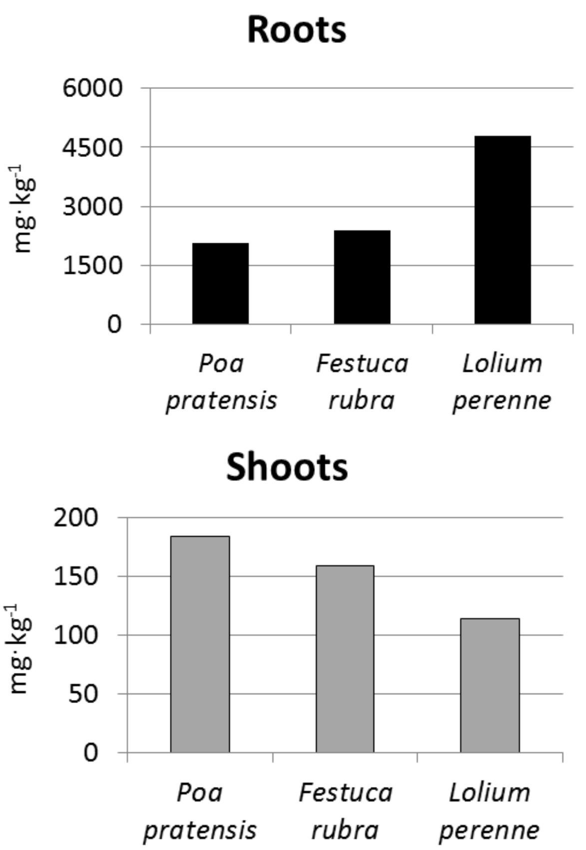


Fig. 2. The Cd concentration in roots and shoots of grasses– the mean of three doses of Cd

these organs. Low BF-shoot confirms a higher metal accumulation in the roots compared to the shoots. BF-root was the highest for *L. perenne*, ranging from 61.5 to 80.7, whilst the lowest for *P. pratensis*, from 26.6 to 31.6. It indicates that *L. perenne* has a higher ability of cadmium uptake from the soil than the other

Table 5. Factor of translocation and bioaccumulation of the tested grasses

| Species | Treatment | BF | | |
|---|---|---|---|---|
| | | Roots | Shoots | TF |
| *Poa pratensis* | Cd30 | 31.6 | 2.1 | 0.07 |
| | Cd60 | 31.2 | 2.4 | 0.08 |
| | Cd120 | 26.6 | 2.7 | 0.10 |
| *Lolium perenne* | Cd30 | 80.7 | 1.5 | 0.02 |
| | Cd60 | 68.1 | 1.2 | 0.02 |
| | Cd120 | 61.5 | 1.8 | 0.03 |
| *Festuca rubra* | Cd30 | 50.2 | 2.5 | 0.05 |
| | Cd60 | 36.6 | 2.1 | 0.06 |
| | Cd120 | 25.6 | 2.1 | 0.08 |

tested grasses (Fig. 2). It could be observed that increase in Cd doses, which caused the growth of Cd concentration in the roots, were accompanied by the decrease in BF-root values. (Tables 4 and 5). The same relation for other plants was found by Sun et al. [2003] and Yang et al. [2011].

TF, showing the plant ability to translocate metals from roots to aboveground parts of plants, was below 1 for all three grasses, ranging from 0.02 to 0.10 (Table 5). A similar TF values was obtained by Zhang et al. [2014] for *V. zizanioides*. It indicates a weak Cd translocation from the roots to shoots. In our study, the lowest TF (0.02–0.03) was obtained for *L. perenne*, and the largest for *P. pratensis* (0.07–0.10). These results indicate a higher Cd mobility in *P. pratensis* than that in the other grasses. This is confirmed by Xu and Wang studies with *P. pratensis* and *F. arundinacea* [Xu, Wang 2013]. The reduction of metal translocation from the roots to the shoots can be considered as one of the defence mechanisms that protect aboveground parts of plants from the harmful effects of heavy metals [Aibibu et al. 2010]. It has been confirmed by our research that *P. pratensis* had the highest TF values and at the same time, the lowest tolerance to soil contamination with Cd amongst the tested grasses. The study showed an upward trend of TF values with an increased soil contamination. It can suggest a weakening of defence mechanisms of plants at high soil pollution.

Plants that show an appropriate ability to take up metals may be used for phytoremediation. According to Mendez and Maier [2008], TF is one of the important factors to evaluate the phytoremediation potential of plants. It is assumed that plants with a high BF in the roots (BF-root > 1) and a simultaneous low TF (<1) are suitable for phytostabilisation of heavy metals in the soil [Cheraghi et al. 2011, Korzeniowska and Stanisławska-Glubiak 2015, Malik et al. 2010, Yoon et al. 2006]. Plants that are distinguished by both BF-shoots and TF values above 1 can be used for phytoextraction. [McGrath and Zhao 2003, Deram et al. 2007]. The tested grasses met the conditions for the plants to be used in phytostabilisation. It can, therefore, be assumed that *P. pratensis*, *F. rubra* and *L. perenne* have the potential for this type of phytoremediation.

## 4. CONCLUSIONS

The conducted research showed the suitability of the tested grasses for phytostabilisation and excluded usefulness of grasses for phytoextraction. All three tested grass species were characterised by TF of less than 1 (TF < 1) and BF for roots higher than 1 (BF-root > 1), which proves a high accumulation of cadmium in their roots and a low translocation from roots to shoots.

The comparison of the phytostabilisation potential of the tested grasses showed that it was lower for *P. pratensis* than that for *L. perenne* and *F. rubra*. *P. pratensis* had a higher TF value, lower root biomass and lower tolerance for Cd excess in the soil in comparison with the two other test grasses. At the same time, *L. perenne* was characterised by the smallest decrease in biomass and the largest Cd accumulation in roots at the lowest dose of Cd. It indicates good usefulness for phytostabilisation of soils characterised by a relatively small pollution by cadmium.

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