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The potential of biosolid application for the phytostabilisation of metals

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ABSTRACT

The aim of this study was to determine the suitability of a biosolid originated from food industry for land remediation as well as the influence of soil heavy metal bioavailability on the Pb, Zn and Cd stabilisation/removal potential of five grass species to assess their potential use as phytoremediation agents. A column device assessment was conducted using contaminated soil from a site surrounding the zinc smelter. The application of sewage sludge from food industry decreased the bioavailability of metals (particularly of Pb and Cd). The shoot biomass of the investigated plant species increased in the following order: *Festuca rubra* L. < *Dactylis glomerata* L. < *Lolium perenne* L. < *Lolium westerwoldicum* L. < *Festuca arundinacea* Schreb. An analysis of the biomass confirmed that used amendment promoted plant growth and significantly increased plant yield. A significant decrease in the metals' uptake in plants was achieved, reflecting a decrease in bioavailability and stabilisation of heavy metals in the soil. Thus, the investigated grass species and biosolid from food industry can be used to induce the phytostabilisation of soils contaminated with heavy metals.

Keywords: Biosolid; Aided phytostabilisation; Heavy metals; Grass species; Contaminated soil

1. Introduction

Heavy metals are common environmental contaminants in industrialised regions and similar trends have been observed in Europe and other industrialised regions worldwide. Many remote and rural sites became contaminated as a result of the atmospheric deposition of heavy metal compounds derived from emissions associated with industrial activity, power generation, transport, agriculture or as effluents from municipal wastewater treatment plants [1]. Poorly regulated metal processing industries and inefficient industrial processes result in sites contaminated with metals (Zn, Cu, Pb, Cd, Ni, Cr, Hg and Sn) and metalloids (mainly As). Soil contamination is a widespread problem of varying intensity and significance. The European Environment Agency estimates that currently there are approximately 250,000 sites with contaminated soil requiring remediation, particularly in Poland, which has an estimated 8,000 km^2 of degraded terrain [2]. Long-term heavy metal deposition has led to the accumulation and migration of these compounds into the soil profile. Trace elements might be leached into exported groundwater by erosion into the surface of the

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water or entrance into the food chains [3]. Metal dynamics are most intense on the surface of the soil where a more abundant and diverse microbial community, higher organic matter content and higher cation exchange capacity (CEC) have been observed. The dynamics of trace elements in soil also depends on biological interactions associated with the microbial activity of the soil-plant system [4]. Traditional remediation options have been subjected to much criticism because of their high costs, energy intensiveness, site destructiveness and lack of public support [5]. *In situ* heavy metal immobilisation is a remediation technology that is capable of reducing cost and environmental impact and can be employed in conjunction with a plant application. The combined use of plants and waste organic fertilisers, such as sewage sludge, promotes plant water retention in the soil and reduces the volume of metal containing leachate [4]. Contaminated sites generally support little vegetation. As a result, barren soils experience erosion and leaching, which can lead to off-site migration of the pollutants into the environment [5,6]. Therefore, the induction of phytostabilisation methods is justified. Phytostabilisation is the precipitation or immobilisation of inorganic contaminants from the soil, root surfaces or within root tissues [7–9]. The species selected for these applications contain root systems that are fibrous and dense enough to maximise the volume of the rhizosphere, such as occurs in species of grass, including fescue (*Festuca* sp.), ryegrass (*Lolium* sp.), and wheatgrass (*Agropyron* sp.) and some herbaceous species [7,10]. Moreover, many grass species are also associated with mycorrhizal fungi, which enhance the phytoremediation effect [11]. Even if the plants remove little or no contaminants, they can be used as phytostabilisation agents as long as they tolerate and grow in contaminated conditions [5]. Growing plants have the potential to decrease the bioavailability of soil contaminants [12]. Furthermore, a rapid greening effect that occurs during the rapid growth of grass species prevents the leaching of N and P compounds from sludged soil [13,14]. Soil amendments have been added to promote plant growth and maximise the stabilisation process [5–7,15]. A number of studies have examined the potential value of soil amendments in immobilising metals in soil. Phosphate compounds, lime, coal ash and organic residues are commonly used as soil amendments [1,6]. The application of biosolid into the soil causes nutrient enrichment and improves the physical properties, water infiltration and water-holding capacity of the soil to enhance phytostabilisation. The term biosolid was introduced by the wastewater treatment industry in the early 1990s and has been recently adopted by the U.S. EPA to distinguish high quality, treated sewage sludge from sewage sludge containing large amounts of pollutants [16]. The most hazardous effect of sewage sludge soil application is the frequent contamination of soil with considerable amounts of heavy metals derived from sewage sludge [17,18]. This problem is usually observed in the case of the application of urban sewage sludge on land [19,20]. As a consequence of the European legislation (EC/91/692), a large amount of biodegradable wastes have been used for land applications and composting. For the reclamation of industrial-degraded areas, this practice seems to be an excellent method of recycling organic matter and nutrients [21]. In particular, land use and re-treatment of organic waste, such as sewage sludge from the food preparation process (with low metal concentration), should be implemented. Currently, the use of nonaccumulator plants in combination with the manipulation of soil conditions has been proposed as an alternative to hyperaccumulators.

The aim of this study was to determine the potential of five grass species (*Festuca arundinacea* Schreb., *Lolium perenne* L., *Lolium westerwoldicum* L., *Dactylis glomerata* L. and *Festuca rubra* L.) for Pb, Zn and Cd stabilisation/removal through the application of sewage sludge derived from the food industry. This study was a part of a series of studies designed to confirm the use of grass species and biosolid amendment as credible phytoremediation agents of heavy metal-contaminated soils.

2. Material and methods

2.1. Experimental procedure

A column device assessment was conducted using contaminated soil from the site surrounding the zinc smelter in the Miasteczko Slaskie, Silesia region in Poland. The soil from this contaminated site was collected in the spring. One-metre high (15-cm diameter) columns were filled with soil to ensure unlimited root growth. The structure of the soil profile was preserved in each column (columns were filled with each layer of the soil and turned upside down). A column experiment was performed through the application of sewage sludge. Soil samples were obtained from the top of each column (30 cm deep), reweighed and mixed with sewage sludge (sewage sludge accounted for 3% of dry weight; according to the Polish Minister of Environmental Regulation, SS application for land reclamation cannot exceed 45 Mg dM/ha/3 years). The columns were subsequently refilled with soil mixed with SS. Control columns without any amendment were also prepared (30 cm deep soil sample was obtained and mixed without sewage sludge). The columns were prepared in four replicates for each grass species. After a two-week incubation under growth chamber conditions, the five grass species were seeded according to their remediation potential: *Festuca arundincea* Schreb. (Fa), *L. perenne* L. (Lp), *L. westerwoldicum* L. (Lw), *D. glomerata* L. (Dg) and *F. rubra* L. (Fr). The columns with and without amendment were sown with 4 g of grass seeds per column (with the exception of 2 g of seeds for Dg, because of the small seed size). The seeds were planted approximately 0.5 cm deep and watered daily with deionised water, using a spray bottle, until germination. Both vegetated and unvegetated columns without and with the application of sewage sludge were used as controls. A plant growth experiment was conducted in a growth chamber for five months. The plants were grown under artificial light (8,500 klx) and 12/24 photoperiod. The temperature was set to 20 and 14°C (day and night, respectively), and relative humidity was maintained at a level of 70%. During the growing season, fertiliser was not applied and irrigation with deionised water was applied as needed. The plants were harvested after five months. The aboveground biomass was measured by harvesting the plant within one centimetre above ground from each column. Subsequently, the samples were dried at 70°C for 48 h, reweighed and their weights were recorded to quantify the mass. The green plant biomass production was determined as the g of dry biomass/column. The roots were gently separated from the soil and rinsed under running water followed by a final rinse in deionised water. The dried shoot and root samples were ground in an electric grinder (<1 mm). Soil samples were obtained from each column from the rhizosphere of plants (from the depth not exceeding 30 cm). The samples were air dried and sieved (<0.5 mm).

2.2. Sample analysis

In the study area, the soil was contaminated with heavy metals as shown in Table 1. The top soil (0–20 cm) (properties included in Table 1) exhibited the following soil texture of 54% sand, 11% clay and 35% silt. An anaerobically digested sewage sludge (Table 1) was collected from an industrial wastewater treatment plant from a natural mineral water and juices manufacturer. The biosolid was dried and ground prior to analysis and a series of analyses were conducted for the purposes of characterisation. Prior to and after the plant growth experiment, soil subsamples were air dried and passed through a 2 mm mesh screen to remove the rocks and roots. Subsequently, the following parameters of the experimental soil were determined: pH in H_2O and KCl-deionised water suspension (soil-to-water ratio of 1:2.5) [22], CEC were determined according to Kappen's method, humid acids in accordance with the Stevenson's method, total N using Kjeldahl's method [23], total organic carbon using Multi N/C H1300 Analitykjena [24], available P using the Enger-Riehm's method and the total P [25], moisture content of each homogenised sample was also determined [26]. The soil samples were also analysed for bioavailable (0.01 M $CaCl_2$ extraction)

Table 1
Selected chemical and physical characteristics of the contaminated soil and sewage sludge

Parameter	Soil ± SD	Sewage sludge ± SD
Humidity, %	18.46 ± 0.97	90 ± 0.43
CEC (cmol(+) kg^{-1} dm)	3.60 ± 0.41	Nd
pH in H_2O	5.3 ± 0.4	7.01
pH in 1 M KCl	4.80 ± 0.63	6.85
Humic acid (%)	0.8. ± 0.04	Nd
C total (g kg^{-1} dm)	14.30 ± 1.1	314 ± 23.87
C total organic (g kg^{-1} dm)	13.21 ± 3.19	300.2 ± 14.76
N Kjeldhal (g kg^{-1} dm)	0.95 ± 21.78	15 ± 2.65
P available (mg kg^{-1} dm)	18.97 ± 4.76	2461.35 ± 54.17
P total (mg kg^{-1} dm)	71 ± 5.1	3479.73 ± 61.23
Total heavy metal concentrations (mg kg^{-1} dm)		
Cd	15.8 ± 0.5	1.2 ± 0.8
Zn	773 ± 7.63	270 ± 18
Pb	1290.7 ± 15.4	130 ± 9
Bioavailable ($CaCl_2$ extraction) heavy metal concentrations (mg kg^{-1} dm)		
Cd	0.98 ± 0.07	0.2 ± 0.01
Zn	11 ± 0.06	80 ± 5
Pb	39.7 ± 1.25	12 ± 0.7

heavy metal concentrations, referred to as the bioavailable fraction. Extractions were performed with shaking at a 1:10 (w/v) soil-to-solution ratio. Plant (0.3 g), sewage sludge (0.3 g) and soil (0.5 g) material was digested using hot aqua regia according to [27]. The digested experimental material and the soil solution were analysed for the presence of Cd, Pb and Zn using inductively coupled plasma atomic emission spectrometry (ICP OES apparatus). The calibration of the spectrometer was ensured by measurement of the prepared samples of known concentrations (5% deviation allowed, blank analysis were also performed). The soil samples were analysed for heavy metals contamination at the beginning and the end of the experiment. All measurements were obtained in triplicate. As an environmental matrix, the following reference materials were used (LGC standards): plants material ERM-CD281, soil material LGCQC3004 and sewage sludge material BCR-146R.

2.3. Statistical treatment of data

All statistical analyses were conducted with the Statistica 6.0 software (StatSoft, Inc., 2001). All data were subjected to one-way analysis of variance (ANOVA), and data not satisfying assumptions for ANOVA were analysed non-parametrically using Kruskal–Wallis ANOVA test. If significant differences were observed ($p < 0.05$), a *post hoc* Tukey's honest significant difference test was used to further elucidate differences among the means ($p < 0.05$). Pearson's correlation coefficients were calculated between the total and bioavailable concentrations of heavy metals in the soil and shoots and the relative plant growth.

3. Results and discussion

3.1. Effects of biosolid application on plant growth and Cd, Pb and Zn concentrations

3.1.1. Plants biomass

Plants grown on the amended soil were devoid of any macroscopic symptoms of metal toxicity or nutrient deficiency compared with plants grown on non-amended soil, which showed inhibited plant growth and phytotoxic effects. On the contrary, a dense root system in columns with sewage sludge application was achieved, particularly for Fa, Lw and Lp. Unfortunately, only Fa reached deeper than the layer of sewage sludge application (over 30 cm). An analysis of the plants grown on soil amended with sewage sludge revealed that the sewage sludge application increased the plant biomass and the results are shown in Fig. 1(d).

The shoot biomass ranged from 1.5 to 6 g dm in the first crop and 3–12 g dm per column. The plant biomass was influenced by soil fertility and low heavy metals' bioavailability. The shoot biomass of plant species increased in the following order: Fr < Dg < Lp < Lw < Fa. The presence of used biosolid did not negatively influence the plant growth, whereas Alvarenga et al. [28] observed phytotoxic effects using compost from the organic fraction of unsorted municipal solid waste at a similar ratio, which potentially reflected the nature of the biosolid used (concentration of heavy metals was three times higher than that of the biosolid used in this study). László [29] also observed that after municipal sewage sludge compost application, plants grew well, and the blades and tips of the leaves were green and healthy. There was little to no plant biomass production (Fig. 1(d)) on soil without sewage sludge addition despite favourable water and temperature conditions for all grass species. Moreover, for most grass species grown on soil without amendment, only a few centimetres seedling stage was achieved (one exception was Fr for which enough biomass was obtained to include it for analysis). For other grass species, growth was inhibited after three weeks of growth. Plants from non-amended columns were 1 cm high, with yellow shoots and underdeveloped roots. As shown in previous experiments, at least 1% dm (15 Mg/ha) of sewage sludge amendment is required to achieve a minimal crop on this heavy metal contaminated soil [30]. The plant biomass was probably influenced by soil fertility derived from sewage sludge rich in N and P. According to Tordoff et al. [31], there is no doubt that (bio)fertilisers containing N and P should be applied for the remediation of contaminated areas. It was observed that after five months, all five species of grass seeded on biosolids amended soil produced high quantities of aboveground biomass. The greatest increase (more than two times) was obtained in the second crop after five months compared with the first crop after 2.5 months. This result potentially reflects enhanced root development and decreasing heavy metal bioavailability after sewage sludge soil application. Moreover, significant differences between each grass species were noted. However, the result was expected given the growth characteristics of the plant species. At the end of the study, Fa and Lw obtained the highest quantities of biomass. The analysis of the biomass of plants grown on soil amended with sewage sludge, confirmed that sewage sludge amendment promoted plant growth and significantly increased plant yield (Fig. 1(d)). The biomass production was the highest for Fa

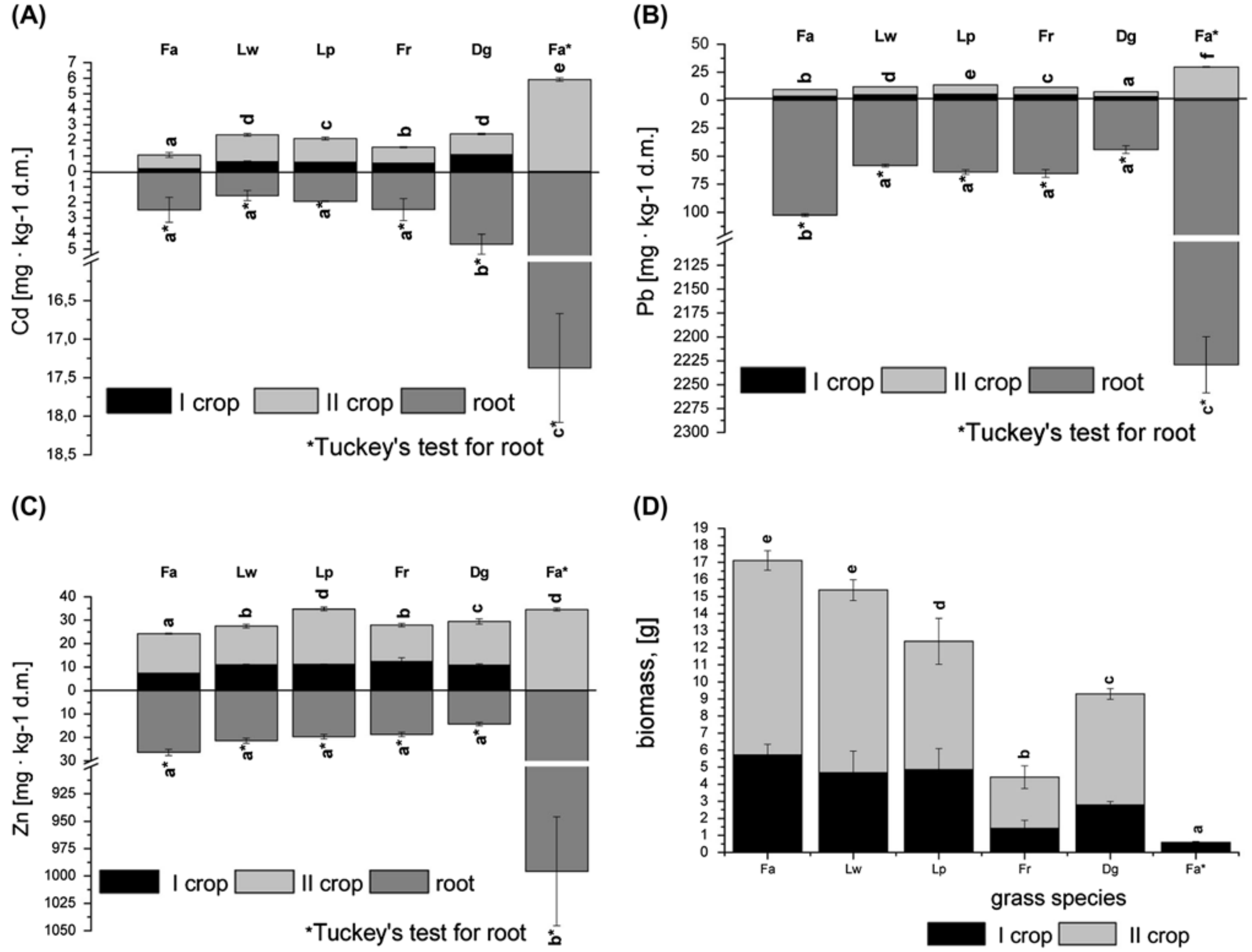

Fig. 1. Heavy metals concentration Cd (A), Pb (B) and Zn (C) in plants tissues and plant biomass (D); Fa—*F. arundinacea* Schreb., Lw—*L. westrwoldicum* L., Lp—*L. perenne* L., Fr—*F. rubra* L., Dg—*D. glomerata* L. and Fa—*F. arundinacea* Schreb. on soil without sewage sludge. Results are averages ($n = 3$) ± SD. Averages, where the same letters occur are not significantly different in statistics (Tuckey's test, $p < 0.05$).

compared with the other four grass species. Nevertheless, the rapid growth of Fa and its capability of producing substantial biomass makes it a potential phytoremediation candidate. Thus, biosolid was capable of supplying two essential macronutrients (N, P) for the promotion of plant growth. However, Zhang et al. [9] demonstrated that despite the favourable fertiliser, the conditions increasing heavy metal contamination significantly decreased plant yield. The plant biomass, as it was often shown [32], is potentially influenced by soil fertility, the interaction of organic amendment with the rhizosphere, decreased metal bioavailability in soil with sewage sludge application and improved soil properties. In the present study, most of these results occurred after biosolid application.

3.1.2. Heavy metals content in plant tissues

The Cd concentration in grass shoots (Fig. 1(a)) was significantly lower after biosolid amendment (ranged between 0.25 and 1.5 mg/kg), compared with the soil control without biosolid, with Cd concentration of more than 6 mg/kg dm Generally, the Cd concentration in roots was higher than in the shoots. The lowest Cd concentrations were observed for the fescue species (Fa and Fr). Cd was greatly accumulated in the shoots of Lw, Lp, Dg and Fa. For Lp, Park et al. [33] reported no significant differences in the concentrations of Cu, Pb and Zn in the shoots after sewage sludge application as a result of extra sludge-born heavy metal contamination. In the present study, the heavy metal contamination of sewage sludge was low. Heavy metal accumulation in plant tissues is influenced by many factors affecting metal dynamics in the soil [32]. The application of organic amendments was reported to decrease the bioavailability of metals [34,35]. The bioavailable fractions of heavy metals decreased in amended soil, and the same was true for the heavy metal concentrations in the plant tissues. The Cd concentration in the roots was similar to that in the shoots for Lw and Lp, which suggests a limited use of both species in the phytostabilisation of Cd contaminated soils. For Fa grown on soil without biosolid, the Cd concentration in the roots exceeded the value in the soil. For Dg the concentration in the roots was threefold higher than the one in the shoots, and for fescue grass, a twofold higher concentration was observed. In the first crop obtained after 2.5 months, the Cd concentration was lower compared with the second crop obtained after five months. The only exception was Dg, for which there were no significant differences between the two terms of crop collection, which is an interesting phenomenon that requires more research but potentially reflects the characteristics of the species. At the beginning of the experiment, in the second crop, a higher Cd concentration can be explained by accessible Cd transfer to aboveground biomass without "first step" accumulation in the root tissues, as observed with the Dg species. The lowest Cd concentration was noted for Fa both in the first and the second crops. The Pb concentration in the aboveground biomass was low compared with the total Pb concentration in the soil. For all species derived from sludge soil, the concentration ranged between 3–9 mg/kg dm and in plants derived from soil without amendment, this concentration was significantly higher, reaching 30 mg/kg dm for Fa. The Pb concentration in the aboveground tissues of all grass species grown on the amended soil was similar for particular grass species in the first and the second crops; however, higher values were obtained in the second one (Fig. 1(b)). In the present study, the Pb concentration in Fa grown on non-amended soil was 30 mg/kg dm, whereas the total concentration in soil was 1,200 mg/kg dm Alvarenga et al. [28] observed that in experiments with Lp, the concentration of Pb in the shoot biomass was 140 mg/kg dm, whereas the total concentration in the soil was 5,500 mg/kg. Despite large differences in the Pb concentration in the soil and shoots in both experiments, 2.5% of the total Pb concentration in the soil was transferred to the shoots and accumulated in the aboveground plant tissues. Additionally, the mobile metal fraction reported by Alvarenga et al. [28] contained 1.8% of the total Pb concentration in the soil. Pb primarily accumulated in the roots, which is quite common for plants. However, some significant differences in the Pb concentration between grass species were observed. The highest values were noted for Fa (100 mg/kg dw) and the lowest for Dg (75 mg/kg d.w.). For Fa grown on soil without biosolid, the concentration of Pb in the roots exceeded the value in the soil by twofold (2,225 mg/kg dw). In the present study, the lowest Pb concentration in the shoots was noted for Dg and Fa in the biomass of the first and second crops. The smallest differences in metal concentration between the two terms of biomass collection were also observed for Dg. In the present study, the Pb concentration in aboveground shoots was low compared with the concentration in the soil (1% of total concentration); therefore, the risk of Pb transfer to the food chain was quite low. Zn concentrations in shoots (Fig. 1(c)) did not exceed the critical concentration for plants (100–400 mg/kg dm) [36]. For plants grown on non-amended soil, the Zn concentrations were lower (34 mg/kg dm) than the values reported by Alvarenga et al. [28] (59 mg/kg dm). The Zn concentration in the shoot biomass depends on many factors, and it is not directly correlated with the total concentration of Zn in the contaminated soil. Alvarega et al. [28] reported higher values, whereas the concentration in soil was lower (253 mg/kg dm) compared with the values obtained in the present study (773 mg/kg). Previous studies have shown that Zn is a highly mobile metal. For Fa grown on non-amended soil, 34 mg/kg dm of Zn was observed, while on amended soil 17 mg/kg dm was obtained from both crop collections. Zhang et al. [9] observed almost 900 mg/kg dm of Zn uptake in the aboveground shoots for *Pennisetum* sp. (Gramineae) with 220 mg/kg dm of total Zn concentration in the soil (no amendment). In the present study, the least Zn concentration was observed in Fa; for this grass grown on soil without sewage sludge, the concentration of Zn in roots was similar to that in the soil and much higher than in the shoots, but in this case, the limited growth of plants must be considered. Plants grown on amended soil accumulated low values of Zn in the roots and a similar or slightly lower accumulation in the shoots. In the roots of Dg, the concentration of Zn was the lowest among the species tested. Rizzi et al. [37] also observed a 20-fold higher concentration of Zn in the shoots of Dg compared with the roots of plants examined in the field study (soil was spiked with Zn sulphate to a final concentration of 1,000 mg/kg Zn). Moreover, Atabayeva et al. [15] reported that Dg grown near Zn and Pb manufacturing plants in Kazakhstan accumulated Zn mainly in the roots (6715.9 mg/kg in roots, 3,760 mg/kg in shoots). Similar results were achieved in the present study for Fa grown on soil without biosolid. In contrast with other metals, a significant difference in the Zn concentration between the two terms of crop collection was noted in Dg. Moreover, lower concentrations of Pb and Zn in the leaves and stems were reported after adding organic biosolids to some grass species and crop plants [37,38]. We observed that the metals accumulated primarily in the root tissues after biosolid application. A significant reduction in the uptake of metals in plants was achieved after biosolid application, which reflects the decreased bioavailability and stabilisation of heavy metals in soil.

3.2. Bioavailability of Cd, Pb and Zn

Concentration of Cd, Pb and Zn in tested soil did not change significantly with the application of sewage sludge compared with the control soil. The soil used in the present study was characterised by high heavy metal bioavailability. After six months, the sewage sludge amendment decreased the bioavailability (extractable with 0.01 M $CaCl_2$) of Cd, Pb and Zn (Fig. 2(a)–(c)).

Park et al. [33], Farrell and Jones [39] and Ociepa et al. [40] achieved similar results after the application of treatment with different biosolids. The application of organic amendment decreased the availability of metals (especially of Pb and Cd), which made the soil appropriate for plant growth. For Cd, the bioavailable fraction ranged between 2.5–3.75 mg/kg dm for untreated columns and 0.15–0.3 mg/kg dm for amended columns (Fig. 2(a)). The bioavailability of Pb decreased significantly after biosolid application at a range of 0.5–1.5 mg/kg dm compared with the untreated soil at a range of 10–20 mg/kg dm (Fig. 2(b)). This result probably reflects the application of sewage sludge, which contains a large portion of humified organic matter and stable chelates [41]. The application of such material allows the process of immobilisation of metals through adsorption reactions (increase in surface change and presence of metal binding compounds) to occur [33]. The highest decrease in bioavailability was observed for Pb, which is considered the least mobile (Fig. 2(b)). It has been proposed that the presence of phosphates in sewage sludge is primarily responsible for increasing Pb sorption. Balik et al. [42] observed that the long-term application of biosolid significantly increased phosphatase activity in the rhizosphere of plants. The changes in the bioavailability of Zn were not as promising as those observed for Cd and Pb, except for the fescue species, where an immobilisation effect was more clearly observed (Fig. 2(c)). László [29] reported that the lowest "plant available" fraction of Zn can be obtained after mineral amendment such as (calcium carbonate) application. In the present study, the effect of sewage sludge application was more important than the impact of each grass species on heavy metal bioavailability. In amendment control columns with-

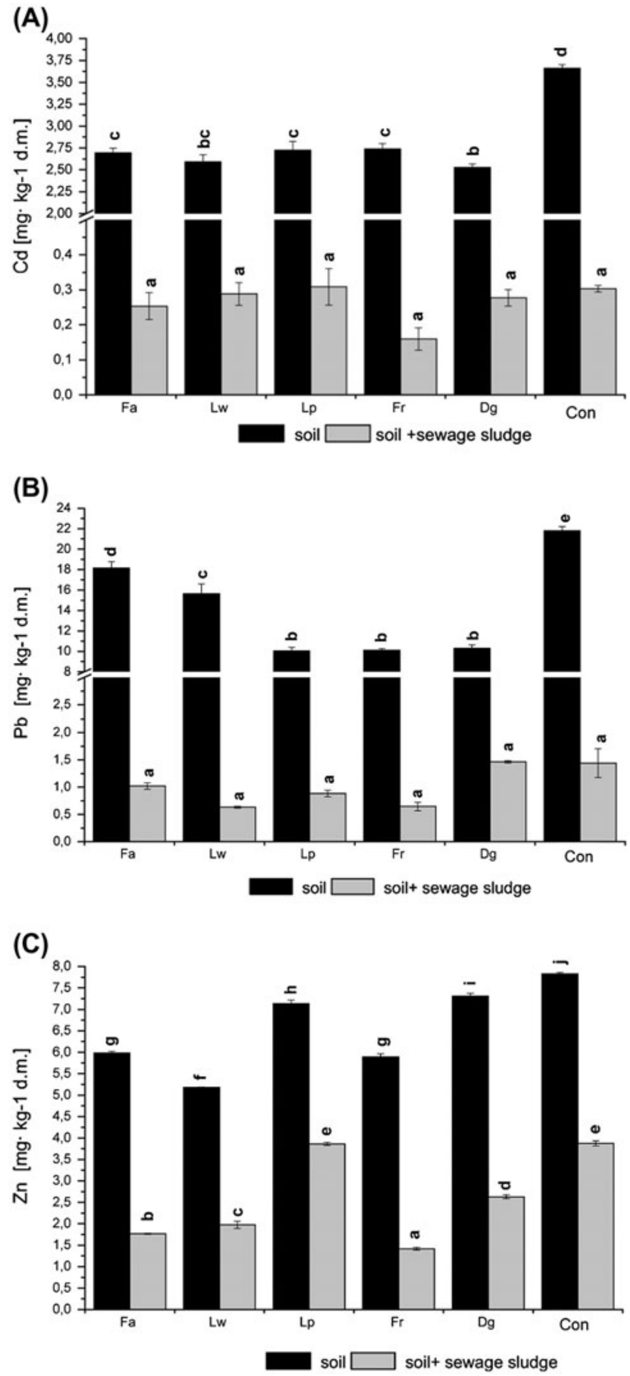

Fig. 2. The bioavailable (extractable with 0.01 M $CaCl_2$) Cd (a), Pb (b) and Zn (c) concentrations; Fa—*F. arundinacea* Schreb., Lw—*L. westervoldicum* L., Lp—*L. perenne* L., Fr—*F. rubra* L., Dg—*D. glomerata* L. and Con—control without plants. Results are averages ($n = 3$) ± SD. Averages, where the same letters occur are not significantly different in statistics (Tuckey's test, $p < 0.05$).

out plants, the bioavailability of heavy metals was similar to planted columns, except for *F. arundinacea* Schreb., *L. westervoldicum* L. and Fr L., where the

Table 2
Pearson's correlation coefficients between mobile metal content (extracted by 0.01 M $CaCl_2$) in soil and content of heavy metals in plant parts of grass species

	1	2	3	4	5	6	7	8	9
2	$.9445^{a***}$								
3	$.9239^{a***}$	$.8310^{a**}$							
4	$.5125^{b}$	$.6226^{b}$	$.2234^{b}$						
5	$-.4432^{b}$	$-.4948^{b}$	$-.1044^{b}$	$-.7018^{b**}$					
6	$-.5129^{b}$	$-.4400^{b}$	$-.2546^{b}$	$-.6738^{b**}$	$.9236^{b**}$				
7	$-.0799^{b}$	$-.1406^{b}$	$.0460^{b}$	$-.5556^{b**}$	$.6803^{b}$	$.6529^{b}$			
8	$-.3527^{b}$	$-.3885^{b}$	$.0202^{b}$	$-.7429^{b**}$	$.9502^{b**}$	$.9086^{b**}$	$.5499^{b}$		
9	$-.4332^{b}$	$-.4095^{b}$	$-.1006^{b}$	$-.7004^{b**}$	$.9476^{b**}$	$.9646^{b**}$	$.5532^{b}$	$.9824^{b***}$	
10	$-.4375^{b}$	$-.4168^{b}$	$-.1040^{b}$	$-.7036^{b**}$	$.9517^{b**}$	$.9651^{b**}$	$.5589^{b}$	$.9829^{b***}$	$.9999^{b***}$

Note: 1—Cd mobile in soil material; 2—Pb mobile in soil material; 3—Zn mobile in soil material; 4—plant biomass; 5—Cd in shoots; 6—Pb in shoots; 7—Zn in shoots; 8—Cd in roots; 9—Pb in roots; 10—Zn in roots missing data removed in pairs.

[a]($n = 12$).

[b]($n = 6$).

*$p < 0.05$.

**$p < 0.01$.

***$p < 0.001$.

bioavailability of metal was lower in comparison with other grass species. The soil from the contaminated area was characterised by a low pH, moisture content and carbon level, as well as a low content of biogenic compounds. The sewage sludge used in the present study was characterised by a high content of organic matter, and the generally low metal content. The application of biosolid increased the soil CEC (6.1 ± 0.6), pH (6.8 ± 0.02), P (127.52 ± 7.14), N (1.81 ± 0.06) and C (27.5 ± 0.35). The results of soil characteristics improvement are consistent with those obtained by other authors [34].

3.3. Pearson's correlations

The analysis of the results obtained in this study using Pearson's correlation coefficients (Table 2) showed a strong positive linear relationship between the metals in the shoots and those in the roots (in both cases, except for the correlation of Pb and Zn in the soil $r_{xy} > 0.9$ at $p < 0.001$). There was also a statistically significant negative correlation between the total metal content in the biomass and the metal concentration in the aboveground biomass in columns fertilised with sludge.

4. Conclusions

This work reports the ability of grass species to accumulate trace metals in large amounts, primarily in the roots. Thus, grass species, such as *F. arundinacea* Schreb., Lp L., Lw L., Dg L. and Fr L., can be used for the phytostabilisation (with biosolid amendment) of heavy metal contaminated soil. It is not possible to apply phytostabilisation effectively (including the proper growth of plants) using the grass species without soil amendment for tested soil. The use of high quality sewage sludge (biosolid) may be used as an alternative to relatively expensive chemical amendments. Biosolids from the food industry could be considered to be a beneficial soil amendment whose land application recycles soil-enhancing constituents such as plant nutrients and organic matter. The use of plants and certain selected sewage sludge is justified when the contaminated area requires nutrient input to recover the vegetation that has been degraded by metal influence. Grass species are appropriate for phytostabilisation because of the perennial growth cycle and a dense root system enabling the soil to be stabilised. Grass species are also advantageous as phytostabilisation plants because they tend to maintain soil-forming ability.

The shoot and root concentrations of metals were significantly higher for all grass species grown on soil without any sewage sludge application. The addition of sewage sludge decreased the bioavailable concentration of all investigated heavy metals in the soil. Our results demonstrated that the application of biosolid promoted heavy metal immobilisation in those soils compared with the ones without application. In conclusion, the application of used biosolid to strongly contaminated soil decreased the toxic effects on plant growth, modified the metal availability and improved the physical, chemical and biological fertility of soils. However, the application of organic amendments can increase metal (especially Zn and Cd) leaching losses potentially as a consequence of pH decrease during the organic matter mineralisation process [43,44]. Obtaining confirmation of this process requires some further study.

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