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Accumulation of Trace Metals in Grasses of Kazakhstan: Relevance to Phytostabilization of Mine Waste and Metal-smelting Areas

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ABSTRACT

The dominant grass species growing around metallurgic plants of Eastern Kazakhstan i.e. *Agropyron repens*, *Agrostis alba*, *Bromus inermis*, *Dactylis glomerata*, *Phleum pratense*, and *Setaria viridis* have shown to accumulate large amounts of trace metals both in field-grown and hydroponic conditions. *A. repens* and *S. viridis* were more tolerant to Pb and Zn as was observed from hydroponic experiments treated with extremely high concentrations of Pb and Zn, whereas *P. pratense* was more sensitive. The shoot/root Pb ratio was < 1 for all species, but the shoot/root ratio for Zn was > 1 for all species, except for *A. repens* and *A. alba.* In pot experiments these grass species accumulated trace metals mainly in the roots. From these investigations it is concluded that all these grass species can be used for phytostabilization of Pb and Zn in the soil.

Keywords: cadmium, copper, grasses, lead, phytostabilization, zinc

INTRODUCTION

Technogenic and anthropogenic sources of trace metals is a subject of importance not only to human health but also in general to the field of biogeochemistry, environment and medicine. Large areas in the Eastern part of Kazakhstan are contaminated with trace metals, e.g. lead (Pb) and zinc (Zn) since metal smelters and metallurgical enterprises are located in this part of the country. Smelting and mining processes are the point sources of trace metal contamination causing environmental contamination and pollution. Consequently, these pollutants get dispersed in natural resources (soil, water and air) and ultimately enter the food chain. Very high concentrations of Pb, Zn and cadmium (Cd) have already been reported in agricultural produce cultivated in the proximity of these metallurgic enterprises in Kazakhstan (Panin 1998). For instance, the concentrations of Pb in potatoes cultivated in contaminated soils exceeded 7 times the permissible levels (Panin 1998). The permissible limits as per local government standards for phytofoodstuffs by the Kazhakhstan Ministry of Health are copper (Cu) – 0.5-20; Pb – 0.1-0.5; Cd – 0.1; Zn – 5-20 (mg/kg) (Smagulov *et al*. 2002). Physical stabilization (covering metalliferrous waste with geotextiles or geomembranes, etc. and chemical stabilization (use of chemical binding agents) to reduce wind and water erosion is not a feasible proposition for large areas. However, phytostabilization (use of a specific type of vegetation is far more desirable than physical and chemical stabilization) (Tordoff *et al*. 2000; Prasad 2006).

Phytostabilization is an effective process of phytoremediation technology (**Fig. 1**). Grasses are tolerant to trace metals and have played a convincing role in phytostabilization (Shu *et al*. 2002; Lai and Chen 2006; Prasad 2006; Lai and Chen 2007; Néel *et al*. 2007; Vernay *et al*. 2007; Wang *et al*. 2007; Li *et al*. 2009; Zhang *et al*. 2010).

Abandoned mine soils and estuarine sediments are phytostabilized against erosion by grass species (Cambrollé *et al*. 2008; Comino *et al*. 2008; Mateos-Naranjo *et al*. 2008). Soil amendments and biosolids accelerate phytostabilization (Zhou *et al*. 2007; Santibañez *et al*. 2008). Grasses possess thickets of adventitious roots, unique root morphology (Li *et al*. 2009), high bioproductivity (Liu *et al*. 2009), and therefore have an added advantage for application in phytostabilization. Further, grasses are often associated with mycorrhizal and endophytic fungi (Deram *et al*. 2007; Chen *et al*. 2008; Deram *et al*. 2008; Kuldau and Bacon 2008; Ortega-Larroceae *et al*. 2010; Punamiya *et al*. 2010). Grasses, together with legume associations, have helped *in situ* stabilization of chemical waste (Hartley and Lepp 2008; Hartley *et al*. 2009). In climate-constrained and CO_2-enriched eras, grasses have a physiological advantage (the majority being C_4) of producing or increasing their biomass. Hence, grasses perform well in the phytostabilization process (Wu *et al*. 2009). In view of their advantageous metabolic processes, a hydroponic grass system based on plate or fabric can be considered for the treatment of aquacultural wastewater (Pan *et al*. 2007).

The aim of our research was to determine the ability of dominant grasses and their ability to accumulate trace metals. Based on preliminary phytophysiological and field work, the grass species that are widespread on contaminated soils in Eastern Kazakhstan around metallurgic enterprises have been identified and selected for experiments.

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Original Research Paper

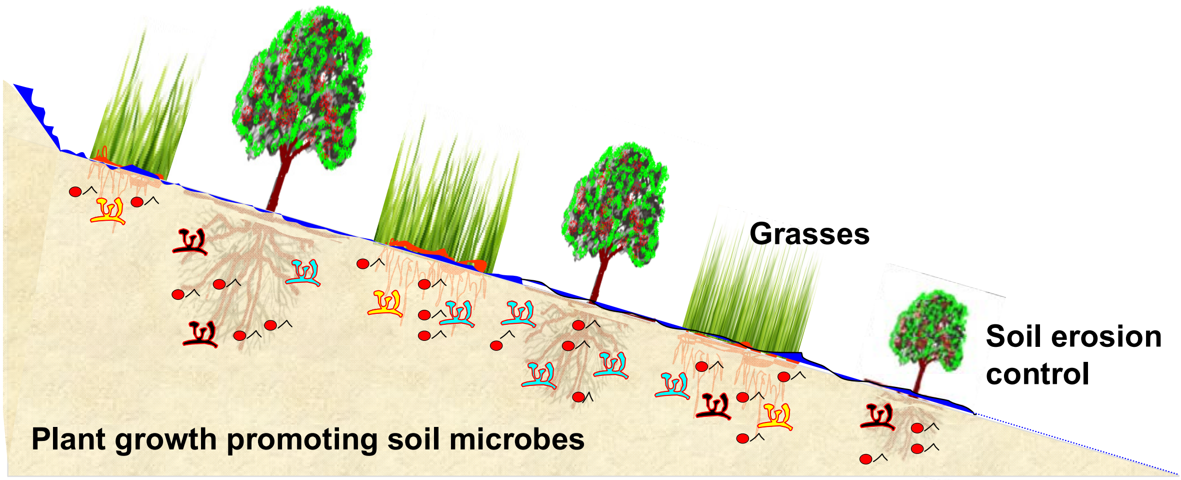

Fig. 1 Grasses accelerate the phytostabilization of soils contaminated with trace metals. Their unique adventitious root system coupled with plant growth microbes are implicated in this process.

MATERIALS AND METHODS

Trace metals budgets in grasses grown on soils spiked with trace metals

Grass species (*Agropyron repens*, *Dactylis glomerata*, *Phleum pratense* and *Setaria viridis*) widely spread around metallurgic plants in Eastern Kazakhstan were identified by local floras. The seeds of *Agrostis alba* were sown, but they did not germinate on these soils.

Seeds were collected in August from 25-30 plants. Seeds were sown in test pots 1 m × 1 m in size spiked with the following salts: $ZnSO_4 \cdot 7H_2O$, $Pb(NO_3)_2$, $(CuSO_4)_2 \cdot 5H_2O$, $CdSO_4$ in May. The final concentrations of the spiked soils were (in mg/kg): Zn – 1000, Pb – 1000, Cu – 100, Cd – 100.

These levels of heavy metals concentrations were selected as approximately middle concentrations which could be present in contaminated soils since, in previous research the range of concentrations of heavy metals from the middle of contaminated soil sites around metallurgic plants were determined to be, in mg/kg: Zn – 355.7-2898.3; Pb – 932.8-1645.3; Cu – 43.0-373.0; Cd – 35.8-120.0 (Atabayeva 2006).

There were three replicates for each treatment. The seeds were placed at a depth of 2-3 cm, with an inter-row distance of 5-7 cm.

Grass seedlings were collected in August with roots. Soil from roots was removed gently and mechanically without washing. Washing roots may result in uneven loss of certain trace metals (Dunn 1992). All samples were air dried for 4 days and divided into roots and shoots. Then they were then dried at 105°C for 3 h, ground and analysed.

Evaluation of selected grasses for trace metal accumulation using hydroponics

Seeds of *Agropyron repens* (*A. repens*), *Agrostis alba* (*A. alba*), *Bromus inermis* (*B. inermis*), *Setaria viridis* (*S. viridis*) and *Pleum pratense* (*P. pratense*) were collected in August from fields of the Altay Botanic Garden. The seeds of *Dactylis glomerata* (*D. glomerata*) were not viable and they were not used in hydroponic experiments. Seeds were stored in a dark room at 22-24°C; before sowing the seeds were stored for 20 days at 4-6°C (Ovcharov 1979). The seeds were sterilized with 16% H_2O_2 followed by 3 rinses in distilled water, 5 min for each rinse. Seeds were germinated on water-moistened filter paper at 25°C in a dark room for 7 days. Afterwards the seedlings were placed in plastic containers (20 × 30 cm) filled with Hoagland's 1/4 strength (macro- and microelements) medium: (Hoagland and Broyer 1940). After 7 days 30 seedlings were transferred to medium containing various concentrations of Pb (450, 900 mg/L) and Zn (350, 700 mg/L) in the forms of Pb $(NO_3)_2$ and $ZnSO_4$. Control plants were grown on 1/4-strength Hoagland's without metals. The experiment was carried out in a controlled environment room under the following conditions: 14-h photoperiod with a light intensity of 400 µmol photons m^{-2} s^{-1}; 22°C: 18°C day: night temperature; relative humidity, 60%. There were three replicates for each treatment. Plants were harvested 6 days after treatments. Shoots and roots were separated, oven dried at 80°C for 48 h and dry weights were recorded. The contents of trace metals in shoots and roots were determined as described next.

Plant samples (0.5 g) were digested in a mixture of 5 ml of 50% HNO_3 and 0.5 ml HCl at 95 ± 5°C according to standards for operation procedures (LMN/SOP-06 2001). Samples were transferred to a digestion block (section) at ± 5°C, closed within glass and heated without bringing to the boil for 10-15 min. Then they were cooled and 5 ml of concentrated HNO_3 was added, placed into a digestion block at 95 ± 5°C, closed within glass and heated without bringing to the boil for 30 min until brown fumes disappeared. Then the samples were cooled and 2 ml of water and 3 ml of H_2O_2 were added; heating was continued until the volume was reduced to about 5 ml, removed from digestion blocks, allowed to cool, then filtered; the filter was washed and deionized water was added up to final volume to 50 ml. Samples were analyzed using the appropriate SOP.

The concentration of metals in plants and soils were measured by atomic absorption spectrophotometry using an installed Winlab AAnalyst 300 (Perkin Elmer, Germany) (LMN/SOP-08 (FLAA) 2001) with an installed and aligned HCL/EDL lamp. HCL lamps were stabilized/aligned for 25-min, EDL lamps for 45 minutes; operating pressure ~ 0.7 kgf/cm^2 for acetylene and 2.8-3.0 kgf/cm^2 for compressed air. Following calibration, samples were analysed.

Statistical analysis of data

In field experiments the samples for measurement of trace metal contents were taken from three test pots for each treatment. The data of pot experiments were analysed statistically using two-way ANOVA with species and treatments as main effects for shoot and root biomass and concentration of metals in plant parts. LSD was calculated using the following equation: $LSD_{0.05} = t_{0.05} \sqrt{(2 \cdot MSError/n)}$ was used to differentiate the means. All values were expressed as the mean of three measurements for each treatment. Values represent means ± standard error (SE).

In hydroponic experiments the samples for measurement of trace metal contents were taken separately from three plastic containers (3 replicates) for each treatment. All values are expressed as the mean of three measurements for each treatment. The data were analysed statistically using two-way ANOVA with species and treatments as main effects for shoot root biomass and concentration of metals in plant parts. LSD was calculated as above to differentiate the means. Values represent means ±SE.

RESULTS AND DISCUSSION

Trace metal budgeting in field-grown plant parts. Effects of heavy metals on plant biomass

A Zn hyperaccumulator is defined as a plant that contains > 10,000 mg/kg Zn dry wt, whereas a Pb hyperaccumulator contains > 1000 mg/kg Pb dry wt. The mean of Zn and Pb concentrations in non-accumulating plants growing on contaminated soils are expected to be < 1000 for Zn and < 100 for Pb (McGrath 1998).

Our previous studies showed that all collected and identified grass species growing around Zn and Pb manufacturing plants in Eastern Kazakhstan (*A. repens*, *A. alba*, *B. inermis*, *D. glomerata*, *P. pratense*) accumulated trace metals (Zn, Pb) in great amounts, mainly in the roots (Atabayeva 2006; Sarsenbayev 2006). Our data showed that the content of Pb and Zn in these species was much higher than the means defined for non-accumulators growing on contaminated soils. These plant species which were growing on highly contaminated soils (total soil Pb - 12672.4 mg/kg) around metallurgic plants in Eastern Kazakhstan accumulated Pb: *D. glomerata* – up to 3760.0 mg/kg in shoots and 6715.9 mg/kg in roots , *B. inermis* – up to 709.1 mg/kg in shoots and 6787.8 mg/kg in roots, *A. repens* – up to 287.0 3 mg/kg in shoots and 3982.8 mg/kg in roots, *A. alba* – up to 339,7 mg/kg in shoots and 2496.0 mg/kg in roots, *P. pratense* – up to 419.6 mg/kg in shoots and 4789.4 mg/kg in roots.

These species accumulated Zn in great amounts too: *D. glomerata* – up to 1498.2 mg/kg in shoots and 2300.0 mg/kg in roots, *B. inermis* – up to 1498.2 mg/kg in shoots and 2896.5 mg/kg in roots, *A. repens* – up to 2743.9 mg/kg in shoots and 29934.1 mg/kg in roots, *Ag. alba* – up to 4900.0 mg/kg in shoots and 14,820.3 mg/kg in roots, *P. pratense* – up to 997.6 mg/kg in shoots and 10383.3 mg/kg in roots (Atabayeva 2006; Sarsenbayev 2006).

The mean Pb concentration in plant parts of *A. alba*, *B. inermis*, and *D. glomerata* was much higher than 100 mg/kg and the mean Zn concentration for all species on highly contaminated soils was > 1000 mg/kg. Therefore, these species were chosen for further studies, including the screening of these species for their ability to accumulate heavy metals in hydroponic conditions and their applicability for removal of trace metals from soils spiked with metals. The ability of wild grass species to accumulate trace metals in field conditions was studied by sowing their seed on artificially contaminated soils.

The shoot and root biomass of these wild grass species was compared to assess the abundance of trace metals in the soils. The root biomass of plant species from 1 m^2 of each heavy metal treatment increased in the following order (**Table 1**):

Zn - *A. repens* > *S. viridis* > *D. glomerata* > *P. pratense* ($P < 0.05$)

Pb - *A. repens* > *S. viridis* > *P. pratense* > *D. glomerata* ($P < 0.01$);

Cu - *A. repens* > *P. pratense* > *D. glomerata* > *S. viridis* ($P < 0.05$);

Cd - *A. repens* > *S. viridis* > *D. glomerata* ≥ *P. pratense*; in this treatment the differences between species were significant ($P < 0.01$), with the exception of *D. glomerata* vs. *S. viridis* ($P > 0.05$).

Shoot biomass of plant species from 1 m^2 for each treatment increased in the following order:

Zn - *D. glomerata* ≥ *A. repens* > *S. viridis* > *P. pratense*; the differences between species were significant ($P < 0.01$) with the exception of *D. glomerata* vs. *A. repens* ($P > 0.05$).

Pb - *P. pratense* > *A. repens* ≥ *D. glomerata* > *S. viridis* ($P < 0.05$); the difference between *A. repens* and *D. glomerata* was not significant ($P > 0.05$);

Cu - *P. pratense* > *A. repens* ≥ *D. glomerata* > *S. viridis*; $P < 0.01$ between all species with the exception of *A. repens* vs. *D. glomerata* ($P > 0.05$);

Cd - *P. pratense* > *A. repens* > *D. glomerata* > *S. viridis* ($P < 0.05$ between all species).

Table 1 Trace metals in root and shoot biomass of wild grass species, g/м^2.

Species	*Agropyron repens*	*Dactylis glomerata*	*Phleum pratense*	*Setaria viridis*
Shoots*				
Zn	305.0 ± 13.1	345.0 ± 15.8	0	150.0 ± 5.5
Pb	395.0 ± 15.5	350.0 ± 13.1	800.0 ± 41.2	280.0 ± 1.0
Cu	425.0 ± 17.2	340.0 ± 16.0	655.0 ± 30.2	115.0 ± 4.0
Cd	440.0 ± 20.2	320.0 ± 11.0	570.0 ± 21.1	245.0 ± 9.8
Roots**				
Zn	605.0 ± 19.2	16.0 ± 0.55	0	40.0 ± 1.2
Pb	1060.0 ± 4.2	39.0 ± 1.3	50.0 ± 2.2	90.0 ± 3.0
Cu	840.0 ± 4.3	40.0 ± 1.9	45.0 ± 1.9	25.0 ± 0.7
Cd	1050.0 ± 38.0	30.0 ± 1.4	27.0 ± 0.8	45.0 ± 1.8

*The differences across species and metals are not significant: $P > 0.05$ ($P = 0.22$, $P = 0$ 14 for shoots and roots, respectively);
**The differences across species are significant $P < 0.05$ (P=1.46E-06), across metals are not significant – $P > 0.05$ ($P = 0.26$). Two-way ANOVA was used. LSD for roots – 108.3, for shoots – 178.3 at $P = 0.95$.

The mean shoot biomass of *P. pratense* was highest among all species in the presence of Cu, Cd and Pb, and the shoot and root biomass of *S. viridis* was the lowest among all grasses. In the presence of Zn *D. glomerata* had the highest biomass, *P. pratense* the lowest. *A. repens* accumulated the highest root biomass in all treatments compared with other species.

Thus, *D. glomerata* and *A. repens* were tolerant to all four metals. The shoot biomass of these species was relatively high (**Table 1**). *S. viridis* was tolerant to Pb and sensitive to Zn, Cu and Cd. *P. pratense* was relatively sensitive to all metals.

Assessment on a species-by-species basis (Table 1)

A. repens

A comparison of shoot biomass in all treatments showed that among metals Zn was distinguishable by a stronger negative effect on biomass (305 g/m^2). In the presence of other metals (Pb, Cu, Cd) this species produced an approximately equal biomass (395, 425, 440 g/m^2, respectively, $P > 0.05$). The greatest root biomass was observed in the treatments with Pb and Cd and the lowest in the treatment with Zn.

P. pratense

Zn fully inhibited the growth of *P. pratense*. In the presence of Pb the mean shoot biomass was highest, and in the presence of Cu and Cd the biomass was almost equal. The mean shoot biomass in different treatments decreased in the following order (g/m^2): Pb (800.0) > Cu (655.0) ≥ Cd (570.0); root biomass decreased in the following order (g/m^2): Pb (50.0) ≥ Cu (45.0) > Cd (27.0) ($P < 0.01$) with the exception of Pb vs. Cu ($P > 0.05$).

D. glomerata

In all treatments the shoot biomass was approximately equal (g/m^2): Pb (350.0) ≥ Zn (345.0) ≥ Cu (340.0) ≥ Cd (320.0) ($P > 0.05$). The root biomass had the highest means in the presence of Pb and Cu. The mean root biomass decreased in the following order (g/m^2): Cu (40.0) ≥ Pb (39.0) > Cd (30.0) > Zn (16.0) ($P < 0.01$), with the exception of Cu vs. Pb ($P > 0.05$).

S. viridis

The shoot biomass decreased in the following order (g/m^2): Pb (280.0) > Cd (245.0) > Zn (150.0) > Cu (115.0) ($P < 0.01$); root biomass (g/m^2): Pb (90.0) > Cd (45.0) ≥ Zn

Table 2 Shoot/root ratio of trace metals content.

Species	*Agropyron repens*	*Dactylis glomerata*	*Phleum pratense*	*Setaria viridis*
Zn	0.87	0.39	0	0.48
Pb	0.28	0.1	0.03	0.08
Cu	0.53	0.16	0.17	0.12
Cd	0.45	0.22	0.07	0.01

The differences across species and metals are significant – $P < 0.05$ ($P = 0.046$, $P = 0.005$ for shoots and roots, respectively). Two-way ANOVA was used. LSD = 0.143 at $P = 0.95$.

(40.0) > Cu (25.0) ($P < 0.001$) with the exception of Zn vs. Cd ($P > 0.05$).

Content of heavy metals in plant parts of wild grass species

The content of heavy metals in plant parts was determined. Different plant parts accumulated high concentrations of metals. The shoot/root ratio was < 1 for all species (**Table 2**).

S. viridis accumulated the highest amount of Zn in shoots and roots, and Pb in shoots (**Fig. 2**). Cu was most accumulated in the roots. Cd was least accumulated by this species than by other species (**Fig. 3**). The shoot/root ratio was < 1 for all metals. The lowest shoot/root ratio was for Pb. The concentration of metals in the shoots decreased in the following order (mg/kg): Zn (299.1) > Pb (76.9) > Cu (14.3) > Cd (0.2); in the roots: Pb (971.2) > Zn (627.4) > Cu (119.1) > Cd (17.3).

P. pratense was the most sensitive grass to the presence of Zn in the soil. This plant did not germinate in Zn-contaminated soil. *P. pratense* accumulated Pb in the roots > 1000 ppm, i.e. 1330 mg/kg. Cd accumulated by the roots of *P. pratense* was the highest among all the grass species assessed (59.4 mg/kg) (**Fig. 2**). The shoots of *P. pratense* accumulated the least Cu compared with the other heavy metals (**Fig. 3**). The shoot/root ratio for all metals was < 1 and was the lowest among all species. The concentration of metals in the shoots decreased in the following order (mg/kg): Pb (45.3) > Cu (7.5) > Cd (4.2); in the roots: Pb (1330.0) > Cd (59.4) > Cu (44.9).

D. glomerata accumulated a considerable amount of Pb in the roots. In the shoots the concentrations of Zn was lowest among all species (**Fig. 2**). The concentration of metals in the shoots of *D. glomerata* decreased in the following order (mg/kg): Zn (118.4) > Pb (79.3) > Cu (13.0) > Cd (8.7); in the roots: Pb (770.4) > Zn (304.1) > Cd (39.4) > Cu (17.5) (**Figs. 2, 3**).

A. repens accumulated the least amount of Pb in the shoots and Cd in the roots. The shoot/root ratio was < 1. The concentration of metals in the shoots of *A. repens* decreased in the following order (mg/kg): Zn (159.7) > Pb (38.2) > Cu (13.7) > Cd (3.4); in the roots: Zn (184.1) > Pb (135.2) > Cu (25.8) > Cd (7.5) (**Figs. 2, 3**).

Thus, the highest Zn concentration was observed in the roots and shoots of *S. viridis*. Pb was greatly accumulated in the roots of *P. pratense*, *S. viridis* and *D. glomerata*. The shoot/root ratio was < 1 for all species.

The removal efficiency of trace metals by roots and shoots of plants was calculated using the means of plant part biomass and the concentration of trace metals in plant parts according to equation: g/ha = Concentrations of metals (g/kg) × yield (kg/m^2) × 10,000; preliminarily the mean of metal concentrations in mg/kg was converted into g/kg, yield in g/m^2 into kg/m^2 and the coefficient 10,000 is necessary to convert 1 m^2 into 1 ha.

The removal of Zn by shoots extrapolated into 1 ha increased in the following order (g/ha): *A. repens* (487.1) > *S. viridis* (448.7) > *D. glomerata* (408.5); by the roots: *A. repens* (1113.8) > *S. viridis* (251.4) > *D. glomerata* (48.7) (**Fig. 4**).

Thus, the shoots and roots of *A. repens* removed the most Zn whereas the shoots and roots of *D. glomerata* the least. The roots of *A. repens* removed the most Zn and the roots of *D. glomerata* the least.

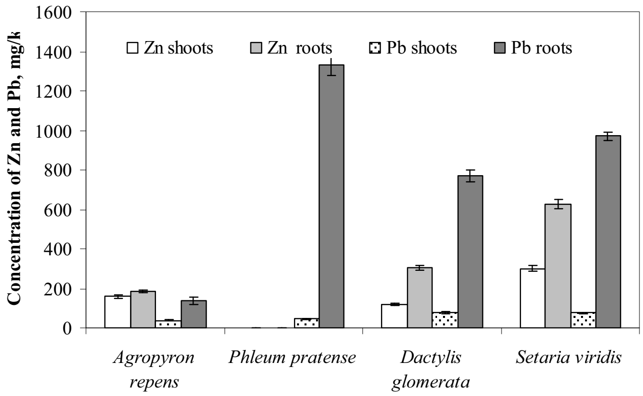

Fig. 2 Zinc and lead content in the plant parts of wild grass species. Differences between species according two-way ANOVA test are significant at $P < 0.05$ (P = 1.98E-32; 7.63E-24 for shoots and roots, respectively). LSD for roots – 1067 and for shoots – 210 at $P = 0.95$. Values represent mean ± Standard Error (SE).

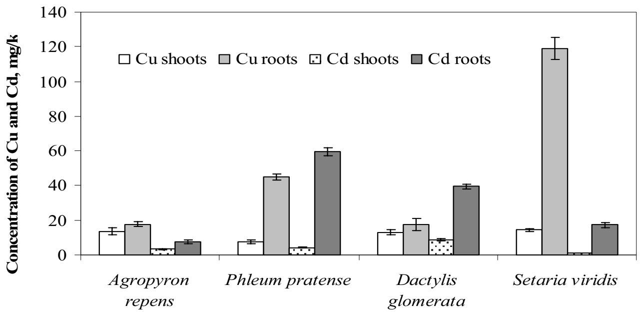

Fig. 3 Copper and cadmium content in plant parts of wild grass species. Differences between species according two-way ANOVA test are significant at $P < 0.01$ (P = 6.6E-35; 2.09E-37 for shoots and roots, respectively). LSD for roots – 105 and for shoots – 9 at $P = 0.95$. Values represent mean ± Standard Error (SE).

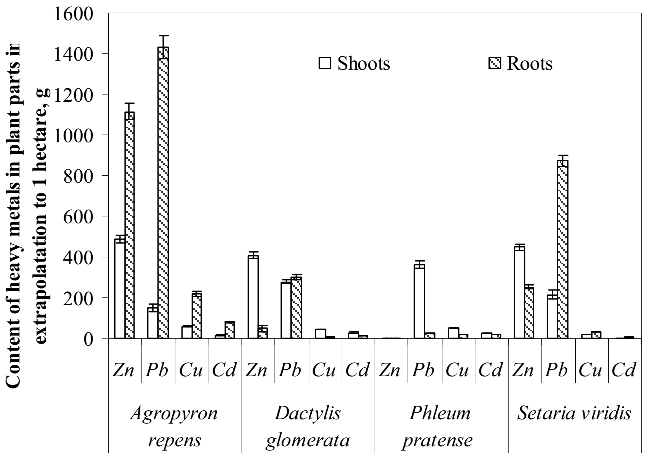

Fig. 4 Content of trace metals in plant biomass in extrapolation to 1 ha. Differences between species and metals are according two-way ANOVA test significant at $P < 0.05$ (P = 8.63E-16; 5.14E-19 for shoots and roots, respectively). LSD for Pb – 1314 and for Zn – 1608 at $P = 0.95$. Values represent mean ± Standard Error (SE).

The removal of Pb decreased in the following order (g/ha): by shoots: *P. pratense* (362.4) > *S. viridis* (215.3) > *D. glomerata* (277.6) > *A. repens* (150.1); by roots: *A. repens* (1433.0) > *S. viridis* (874.1) > *P. pratense* (665.0) > *D. glomerata* (300.5) (**Fig. 4**). *Ph. pratense* was removed the most amounts of Pb by the shoots, *A. repens* – the lowest one. *A. repens* removed the most amounts of Pb by the roots, *D. glomerata* – the lowest ones.

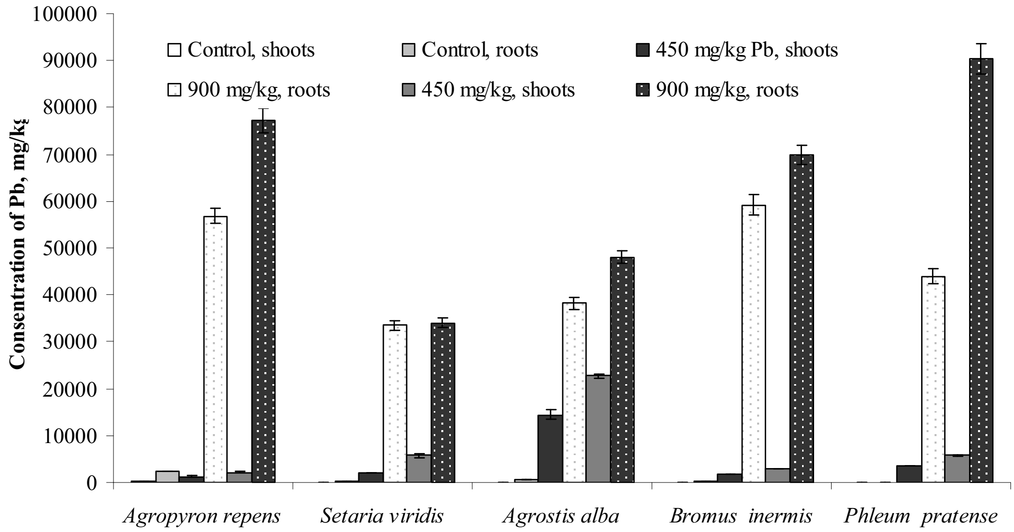

Fig. 5 Lead content in plant parts. Differences between treatments according two-way ANOVA test are significant at $P < 0.05$, (P = 5.33E-12; 5.15E-19 for shoots and roots, respectively). LSD for roots – 14425 and for shoots – 5000 at P = 0.95. Values represent mean ± Standard Error (SE).

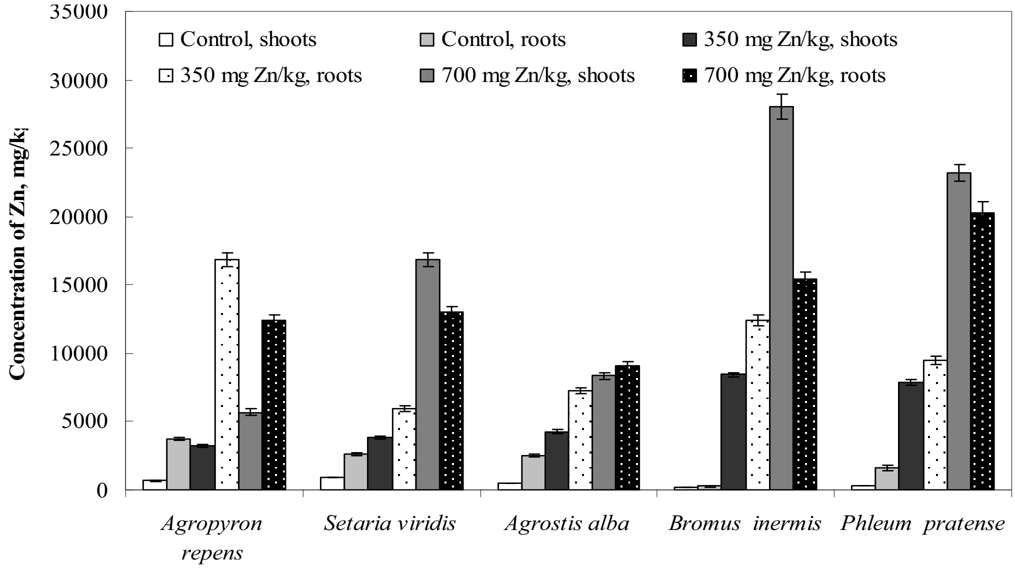

Fig. 6 Zinc content in plant parts. Differences between treatments according two-way ANOVA test are significant at $P < 0.05$ (P = 1.261E-37; 2.03E-34 for shoots and roots, respectively). LSD for roots – 4477 and for shoots – 6469 at P = 0.95. Values represent mean ± Standard Error (SE).

As for removal rates of Cu by the shoots the species were disposed in the following order (g/ha): *A. repens* (58.2) > *P. pratense* (49.1) > *D. glomerata* (44.2) > *S. viridis* (16.5); by the roots – *A. repens* (216.7) > *S. viridis* (29.8) > *P. pratense* (20.2) > *D. glomerata* (7.0) (**Fig. 4**).

The shoots of *A. repens* had the most removal rate of Cu, and the shoots of *S. viridis* – the lowest one. *A. repens* had the highest removal rate by roots and *D. glomerata* – the lowest one.

The removal of Cd by the shoots extrapolated to 1 ha decreased in the following order (g/ha): *D. glomerata* (27.8) > *P. pratense* (23.9) > *A. repens* (15.0) > *S. viridis* (0.5); roots – *A. repens* (78.8) > *P. pratense* (16.0) > *D. glomerata* (11.8) > *S. viridis* (7.8) (**Fig. 4**).

Thus, the most removal rate of cadmium was observed in the shoots of *D. glomerata* and in the roots of *A. repens*.

In general, all analysed species were tolerant to trace metals. They accumulated varied amounts of trace metals mainly in the roots. All these species can be used for phytoremediation of contaminated soils, particularly for phytostabilisation, due their ability to accumulate trace metals in the roots.

The following species as *D. glomerata*, *B. inermis*, *A. repens*, *A. alba* and *P. pratense* accumulated Zn and Pb in great amounts mainly in the roots from heavily contaminated soils. Therefore, these species can be the candidates for using them in *phytostabilization*.

Hydroponic experiments

The metal accumulation ability of grass species in hydroponic conditions at extremely high concentrations of zinc and lead (450, 900 mg/L of Pb and 350, 700 mg/L of Zn) was studied.

The analysis of trace metals in plant parts has shown the highest level of lead concentration in the roots (**Fig. 5**). The highest level of lead at 900 mg/L was accumulated in the roots of the species: *P. pratense* (90 281.9 mg/kg), *A. repens* (77 137.9 mg/kg) and *B. inermis* (69 991.8 mg/kg). The lowest level of lead was found in the roots of *S. viridis* (33 974.6 mg/kg).

The shoot/root ratio for all species at the concentration 900 mg Pb/L was < 1 and decreased in the following order: *A. alba* (0.47) > *S. viridis* (0.17) > *P. pratense* (0.06) > *B. inermis* (0.04) > *A. repens* (0.027).

At concentration 900 mg Pb/L the content of lead in shoots decreased in the following order: *A. alba* > *P. pratense* > *S. viridis* > *B. inermis* > *A. repens*; in roots - *P. pratense* > *A. repens* > *B. inermis* > *A. alba*.

The highest level of Pb was detected in the shoots of *A. alba* (22 670.0 mg/kg) and the lowest in the shoots of *A. repens* (2 091.3 mg/kg).

The study of Zn content in plant parts has shown that all species except of *A. repens* accumulated Zn mainly in the shoots (700 mg Zn/L). Shoot/root ratio of Zn for all species except of *A. alba* and *A. repens* was > 1. *A. alba* accumulated approximately equal amount of Zn in both roots and shoots, whereas *A. repens* accumulated mainly in the roots (**Fig. 6**).

The shoot/root ratio decreased in the following order: *B. inermis* (1.8) > *S. viridis* (1.3) > *P. pratense* (1.14) > *A. alba* (0.92) > *A. repens* (0.46).

The highest level of Zn detected at 700 mg Zn/L concentration in shoots and roots of *B. inermis* and *P. pratense*, the lowest in *A. repens* and *A. alba*. Concentration of Zn in shoots was decreased in the following order: *B. inermis* > *P. pratense* > *S. viridis* > *A. alba* > *A. repens*; in roots: *P. pratense* > *B. inermis* > *S. viridis* ≥ *A. repens* > *A. alba*.

P. pratense and *B. inermis* were distinguishable by high accumulation of Zn in the shoots and roots.

Thus, in hydroponic conditions Pb at high concentrations was accumulated mainly by the roots for all species and the shoot/root ratio was < 1. At a high concentration of Zn, it was accumulated mainly by the shoots except those of *A. repens*, whereas *A. alba* accumulated Zn in approximately equal amounts in both roots and shoots. *P. pratense* and *B. inermis* were distinguishable from the other species by accumulating Zn in their shoots to a high level. These species also accumulated a large amount of Pb in the roots.

CONCLUSION

Phytoremediation is an economically effective and ecologically safe technology of decontamination of trace metals from polluted soils. Phytostabilization is a process that prevents the movement and transport of dissolved contaminants using plants to decrease the mobility of pollutants in soils. The screening of grass species widely spread around metallurgic plants of Eastern Kazakhstan in hydroponic and field conditions has shown that almost all studied grass species accumulate trace metals mainly in the roots in great amounts. This work reports the ability of widely spread grass species in Eastern Kazakhstan to accumulate trace metals in great amounts mainly in the roots. The following wild grass species such as *D. glomerata*, *B. inermis*, *A. repens*, *A. alba* and *P. pratense* can be used for phytostabilization.

It is possible to use "induced" phytoremediation for heavy metal-contaminated soils around Zn and Pb manufacturing plants of East Kazakhstan with application of different chelators of trace metals, like EDTA, humic acids, etc., to enhance the removal efficiency of metals by plants from soils (Koopmans *et al.* 2008; Saifullah *et al.* 2009; Borggaard *et al.* 2009; Vamerali *et al.* 2010; Purakayastha and Chhonkar 2010).

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