



## Solubility of arsenic and its uptake by ryegrass from polluted soils amended with organic matter



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### ABSTRACT

The study focused on suitability of ryegrass (*Lolium perenne*) for phytostabilization of As-polluted soils as well as on the effects of soil amendment with organic matter on the solubility of As. Four soil samples, containing 505–6500 mg·kg<sup>-1</sup> As, were collected from the areas of historical ore mining and processing in Złoty Stok and Radzimowice (Poland). Soils were amended with different organic materials, i.e. cattle manure (M), three kinds of variously treated sewage sludge (fresh SS1, digested and limed SS2, and composted SS3), and two kinds of forest litter (representative of pine and alder stands FL1, FL2). In a three-month incubation experiment, the changes of As concentrations in water extracts and As extractability with 0.01 M CaCl<sub>2</sub> were measured. After that time, a pot experiment with ryegrass was established. Soil amendment with all organic materials caused an initial increase in As solubility and extractability that decreased during incubation, but in most cases remained higher compared to untreated samples. Plant growth and As concentrations in plant roots and shoots depended both on soil properties and on the kind of amendment. Sewage sludge SS2 and forest litters caused adverse effects on plant growth, except for the least polluted soil 4, while application of M, SS1 and SS3 posed beneficial effects. Unlike As extractability, its uptake by ryegrass was reduced in organic matter-amended soils, particularly those treated with SS2 and SS3, which was reflected by an amendment factor AF. In spite of that fact, the concentrations of As in shoots of ryegrass grown in soils 1 and 2 were assessed as relatively high and often exceeded 50 mg·kg<sup>-1</sup>. The effects of soil treatment with organic amendments on As phytoavailability to ryegrass turned out difficult to predict and therefore should be experimentally determined in each case of remediation.

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### 1. Introduction

Soil pollution with arsenic is not a common problem in Europe, but there are several sites, particularly those affected by historical mining and processing, where soils are highly enriched in this element. The environment in former gold and arsenic mining centers has inherited large burden of arsenic from ore mining and processing. Soils in the town in Złoty Stok (situated in the Sudeten Foreland, SW Poland, active from the 15th century until 1962) contain extremely high concentrations of As, over 1000 mg·kg<sup>-1</sup> (Krysiak and Karczewska, 2007; Karczewska et al., 2007, 2013a) whereas the natural concentrations of As in soils usually remain below 5 mg·kg<sup>-1</sup> (Kabata-Pendias, 2011). Similarly, in several smaller arsenic mining centers in Sudeten, such as Radzimowice (Karczewska et al., 2007), soils are highly enriched in As. Its concentrations in mine spoils spread in those areas

exceed not rarely 20,000 mg·kg<sup>-1</sup>. Locally, soils remain there almost barren and definitely need remediation. Moreover, a likely release of arsenic to ground water may become a major concern, therefore highly enriched lands should be controlled and remediated. Phytostabilization, a remediation technique based on sequestering contaminants in soils, and vegetating the land with excluding plants, that do not translocate contaminants to the shoots, should be recommended for such sites (Terry and Banuelos, 1999). Soil amendments should efficiently immobilize arsenic in soils. Various organic materials, such as peat, ground lignite, composts, sewage sludge as well as manure, are commonly used to improve soil physical properties, to increase soil sorption capacity, and to supply soils with nutrients necessary to introduce a plant cover. However, soil treatment with organic materials, particularly those reach in dissolved organic carbon (DOC), may cause adverse effects on soils and remobilize heavy metals in certain conditions (McBride, 2003; Mench et al., 2003; Singh and Agrawal, 2008; Bolan and Duraisamy, 2003; Cuske et al., 2016). The effects of organic amendments on the mobility and phytoavailability of arsenic in polluted soils

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are ambiguous (Carbonell-Barrachina et al., 1999; Bauer and Blodau, 2006; Hartley and Lepp, 2008; Karczewska et al., 2013b; Xie et al., 2015). Moreover, natural organic matter that accumulates in vegetated soils, may also cause remobilization of contaminants via various mechanisms, including chelation with low-molecular weight product of organic matter transformation (Kalbitz and Wennrich, 1998; Wang and Mulligan, 2006; Kabała et al., 2014).

Plant species planned to be used for coverage of lands extremely rich in arsenic should be examined on their As tolerance and roots to shoot translocation rates. The plants should be characterized by possibly low concentrations of contaminants in their aboveground parts, which is crucial from the standpoint of possible input of toxic elements into the food chain. In Europe, permissible limits for As in fodder (except for fish and aquatic animals) have been established at 2 and 4 mg of As per kg of fresh mass (moisture content 12%) that corresponds with 16–32 mg·kg<sup>-1</sup> related to dry mass (Directive, 2002). Normally, unlike phosphorus, arsenic shows low phytoavailability, and is poorly translocated from roots to shoots, except for hyperaccumulators. Its concentrations in plant biomass that range 5–20 mg·kg<sup>-1</sup> d.m. are critical for most of the non-accumulating plants (Vithanage et al., 2012). Simplified characteristics of contaminants' behaviour in soil-plant system, in terms of their phytoavailability, may be provided by calculated biogeochemical indices, of which the most commonly used are roots-to-shoots translocation (transfer) factor TF, soil-to-plant bioaccumulation factor BAF and bio-concentration factor BCF, the latter - determined in relation to concentrations in aqueous phase, such as nutrient solution in hydroponics, soil pore water, soil extracts, etc. (Vithanage et al., 2012). The processes involved in arsenic uptake from soils and its further distribution within plants have been partly recognized but many issues remain still unanswered and require examination in specific conditions (Gulz et al., 2005; Raab et al., 2007; Tripathi et al., 2007). This study was carried out to examine a suitability of ryegrass (*Lolium perenne*) for remediation of soils strongly enriched with arsenic, treated with organic amendments. This species, relatively fast growing and efficiently covering soil surface, is commonly used for phytostabilization purposes (Arienzo et al., 2004; Alvarenga et al., 2008), however, its usefulness for revegetation of some arsenic-contaminated soils was partly called in question in an introductory experiment (Karczewska et al., 2013b).

## 2. Materials and methods

### 2.1. Characteristics of soils

Four soil samples (each of ca. 50 kg), that contained 505–6500 mg·kg<sup>-1</sup> As, were collected from the topsoil layer 0–20 cm in 4 various localities (Table 1). Sampling sites were situated in two former arsenic industry areas in Złoty Stok and Radzimowice (Karczewska et al., 2007). Soils were air-dried, homogenized, and sieved through a

10 mm sieve in order to remove stones, larger grains of gravel and artifacts. Then, the aggregates were crushed and soil was sieved to 2 mm prior to usage in the experiment. The subsamples, of 1 kg, were used for laboratory analyses. Basic soil properties, i.e. grain size distribution, organic carbon content (Corg.), pH, and cation exchange capacity CEC, were determined with commonly applied methods, described by Tan (2005). Soil organic carbon and the content of carbonates were determined on CS-MAT 5500 analyzer. Related data are presented in Table 1. For analysis of total As, soil samples were digested with concentrated perchloric acid HClO<sub>4</sub> (Merck, ACS 60%), in the open system with reflux. Concentrations of As in digests were measured using ICP-AES (Varian). Two certified soil reference materials were used to ensure good analytical quality: WEPAL RSM 2709 (San Joaquin Soil) and RTH 912 (Swiss Less Soil).

All soils had similar texture of sandy loam, but differed considerably in the content of Corg. and pH. Forest soil (3) was the most acidic and richest in organic matter, whereas soil 1 contained the lowest amount of Corg., and had the highest pH, which was determined by a certain content (1.26%) of carbonates. Arsenic concentrations in soils decreased in the order 1 to 4.

### 2.2. Organic amendments

Soils were amended with various kinds of organic materials commonly used in agriculture or land reclamation. The following materials were applied at the rate of 50 g d.m. per kg: dried cattle manure (M), three kinds of sewage sludge (fresh, dewatered – SS1, digested and freshly limed with CaO – SS2, and composted SS3), and additionally, two kinds of forest litter (collected from pine stand FL1 and alder stand F2) tested to examine a possible secondary effect that may be generated by litter fallout after successful land revegetation and forestation. The analysis of organic amendments involved determination of moisture, organic matter content OM (by loss on ignition), pH in water suspension, and dissolved organic carbon DOC extracted in cold water, according to the method by Gregorich et al. (2003) and determined on TOC 5000 Shimadzu analyzer. A spectroscopic index of organic matter humification and dispersion Q4/Q6 was measured in 0.5 M NaOH extracts as described by Zbytniewski and Buszewski (2005).

Basic properties of organic materials are presented in Table 2. All the materials except for forest litters were neutral or alkaline (SS2) in reaction. The alkaline sewage sludge and forest litters, in particular a pine forest litter FL1, contained relatively high amounts of DOC, above 10 g kg<sup>-1</sup>, whereas its concentration in manure was the lowest (1.52 g kg<sup>-1</sup>).

### 2.3. Incubation experiment

Soils amended with organic materials were incubated in the 1 kg pots at constant moisture corresponding with 70% of field water capacity. Incubated soils were not exposed to any additional treatment except for watering with distilled water, so that the experiment mimicked the processes that run in the field. Soil subsamples, each of 20 g, were collected after various time of incubation: 1, 7, 30 and 90 d, and extracted by overhead shaking with water at 10 g:25 ml (m:v) ratio, and with solution of 0.01 M CaCl<sub>2</sub> (1:10), m:v. The extracts were filtered through 0.45 μm syringe filters and analyzed on pH, DOC (TOC 5000 Shimadzu)

**Table 1**  
Basic soil properties.

| Feature, parameter     | Unit                  | Soils          |            |            |             |
|------------------------|-----------------------|----------------|------------|------------|-------------|
|                        |                       | 1              | 2          | 3          | 4           |
| Location               |                       | Złoty Stok     | Złoty Stok | Złoty Stok | Radzimowice |
| Usage                  |                       | Abandoned land | Meadow     | Forest     | Fallow land |
| Textural group (USDA)  |                       | SL             | SL         | SL         | SL          |
| Percentage of fraction | <0.02 mm              | 24             | 30         | 30         | 26          |
|                        | <0.002 mm             | 5              | 6          | 3          | 3           |
| C org.                 | g·kg <sup>-1</sup>    | 16.0           | 27.6       | 41.2       | 36.2        |
| pH (1 M KCl)           | –                     | 7.3            | 7.2        | 4.7        | 5.5         |
| CaCO <sub>3</sub>      | %                     | 1.26           | 0.24       | Absent     | Absent      |
| CEC                    | cmol·kg <sup>-1</sup> | 11.4           | 13.6       | 15.9       | 7.71        |
| Total As               | mg·kg <sup>-1</sup>   | 6500           | 1770       | 1690       | 505         |

**Table 2**  
Basic properties of organic amendments used in the experiment.

| Parameter             | Unit                | M    | SS1  | SS2  | SS3  | FL1  | FL2  |
|-----------------------|---------------------|------|------|------|------|------|------|
| OM                    | g·kg <sup>-1</sup>  | 315  | 261  | 358  | 325  | 560  | 450  |
| DOC                   | g·kg <sup>-1</sup>  | 1.52 | 4.59 | 13.6 | 2.20 | 11.2 | 17.1 |
| pH (H <sub>2</sub> O) | –                   | 7.4  | 7.8  | 8.9  | 7.4  | 4.2  | 5.6  |
| As                    | mg·kg <sup>-1</sup> | 0.40 | 1.3  | 0.2  | 8.1  | 1.9  | 2.7  |
| Q4/Q6                 | –                   | 4.19 | 4.91 | 1.57 | 4.47 | 5.91 | 5.63 |

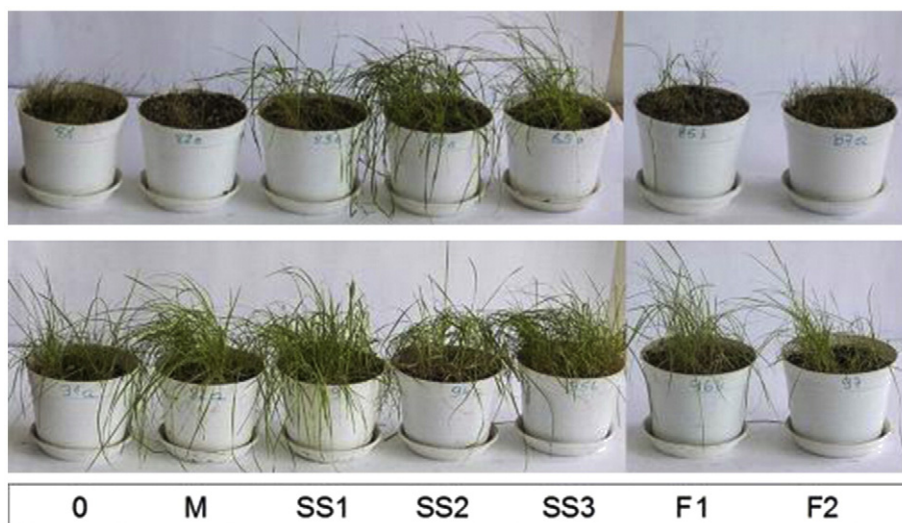


Fig. 1. Ryegrass growing in soil 1 (upper row) and 2 (lower row) amended with various organic materials in a pot experiment. More details – in the text.

and As concentrations (ICP-AES, iCAP 7400 Thermo Scientific). According to the series of preliminary tests (unpublished), the concentrations of some trace metals in water extracts obtained in this way, after a 16 h equilibration, are comparable with their concentrations in real soil pore water obtained at soil moisture of ca. 70%, therefore the results have been presented as concentrations in aqueous phase,  $\text{mg} \cdot \text{L}^{-1}$ . The results of  $\text{CaCl}_2$  extraction were recalculated to relate As extractability to soil mass ( $\text{mg} \cdot \text{kg}^{-1}$ ). For technical reasons,  $\text{CaCl}_2$  extraction was not performed after a 7-day incubation. The experiment was carried out in 3 replicates. The significance of changes in As extractability caused by various treatments was checked statistically.

#### 2.4. Pot experiment

After a 3-month incubation of soils mixed with amendments, the pots were placed in the greenhouse, and the seeds of ryegrass (*Lolium perenne*, var. Kinga) were sown (0.10 g per pot). The pot experiment (Fig. 1) was carried out in a randomized complete design, in 3 replicates, to examine reactions of ryegrass to soil contamination and to applied remediation measures. Grass shoots were harvested twice, after 1 and 3 months of plant growth (cut 1 and cut 2,

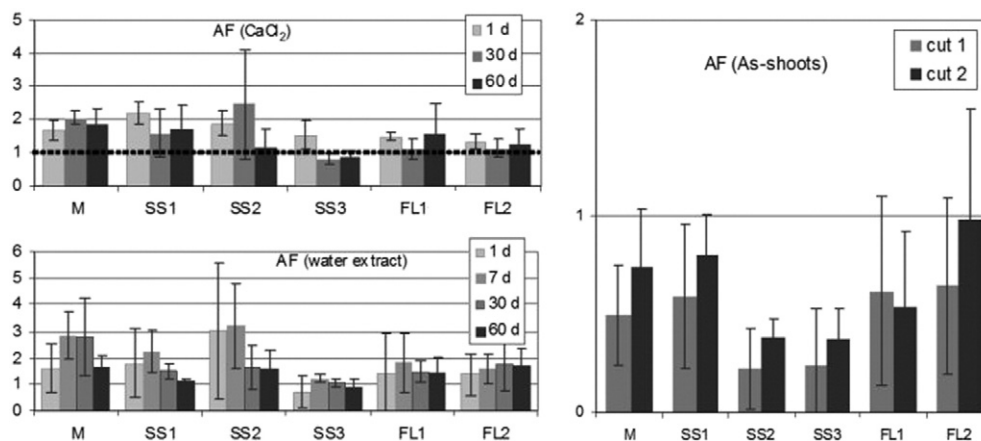
respectively). Harvested shoots were washed with deionised water, dried initially at room temperature and then in oven (60 °C, 12 h). Dried material was weighed, ground and analyzed on total arsenic. Plant material was digested with perchloric acid in the open system with reflux, and As concentrations in digests were determined by ICP-AES (iCAP 7400 Thermo Scientific). After the second harvest of grass shoots, soil material was removed from the pots, and the roots were then carefully separated from soils, washed, cleaned ultrasonically (Cuske et al., 2014), oven-dried for 24 h, digested and examined on As concentrations, in the same way as the shoots. NCS Certified Reference Material DC 73348 (Branches Bush and Leaves), as well as internal standards were used to validate analytical accuracy in determination of As in plant samples.

The results were worked out statistically in order to check the significance of effects caused by various amendments. Crucial biogeochemical indices TF, BAF and BCF were calculated. Translocation factor TF was defined as the ratio of As concentrations in shoots (cut 2) to roots. Bioaccumulation factor BAF was determined for plant shoots, as the ratio of As concentrations in grass shoots to soil concentrations. Bio-concentration factor BCF was determined in relation to As concentrations in water extracts at the end of incubation (90 d).

Table 3

The amounts of 0.01 M  $\text{CaCl}_2$ -extractable As in soils and concentrations of As in water extracts – the mean values of 3 replicates.

| Soil | Incubation time | $\text{CaCl}_2$ -extractable As, $\text{mg} \cdot \text{kg}^{-1}$ |      |      |      |      |      |      | As in water extract, $\text{mg} \cdot \text{L}^{-1}$ |      |      |      |      |      |      |
|------|-----------------|---|------|------|------|------|------|------|--|------|------|------|------|------|------|
|      |                 | Amendment   |      |      |      |      |      |      |  |      |      |      |      |      |      |
|      |                 | 0   | M    | SS1  | SS2  | SS3  | FL1  | FL2  | 0  | M    | SS1  | SS2  | SS3  | FL1  | FL2  |
| 1    | 1 d             | 16.0  | 19.2 | 27.5 | 21.5 | 19.4 | 26.3 | 17.3 | 3.90   | 7.28 | 5.48 | 2.18 | 5.97 | 4.16 | 4.11 |
|      | 7 d             | Not determined  |      |      |      |      |      |      | 3.75   | 14.3 | 7.19 | 6.11 | 4.82 | 6.22 | 4.88 |
|      | 30 d            | 44.0  | 88.4 | 29.2 | 59.8 | 29.5 | 48.3 | 32.4 | 3.61   | 14.6 | 5.24 | 4.40 | 4.34 | 4.05 | 5.18 |
|      | 90 d            | 33.8  | 49.3 | 33.6 | 31.5 | 28.7 | 30.1 | 29.2 | 3.82   | 6.84 | 4.11 | 4.92 | 3.75 | 4.06 | 5.07 |
| 2    | 1 d             | 6.32  | 11.9 | 15.3 | 12.5 | 9.11 | 8.97 | 9.13 | 1.36   | 0.71 | 1.99 | 2.45 | 0.95 | 4.90 | 2.31 |
|      | 7 d             | Not determined  |      |      |      |      |      |      | 1.24   | 3.89 | 2.84 | 2.78 | 1.65 | 4.27 | 2.75 |
|      | 30 d            | 11.2  | 21.7 | 24.4 | 19.2 | 8.35 | 15.3 | 14.1 | 0.79   | 3.01 | 1.22 | 0.90 | 0.96 | 1.21 | 0.98 |
|      | 90 d            | 10.6  | 16.3 | 16.0 | 6.3  | 6.5  | 10.8 | 8.5  | 0.82   | 1.75 | 0.98 | 0.99 | 0.39 | 1.12 | 1.37 |
| 3    | 1 d             | 2.2   | 3.89 | 5.35 | 4.9  | 4.72 | 3.28 | 2.54 | 0.09   | 0.25 | 0.34 | 0.59 | 0.05 | 0.03 | 0.21 |
|      | 7 d             | Not determined  |      |      |      |      |      |      | 0.14   | 0.38 | 0.46 | 0.73 | 0.19 | 0.13 | 0.26 |
|      | 30 d            | 5.1   | 9.7  | 11.2 | 24.6 | 5.21 | 6.65 | 5.9  | 0.13   | 0.33 | 0.25 | 0.38 | 0.14 | 0.27 | 0.45 |
|      | 90 d            | 0.86  | 2.00 | 2.21 | 1.59 | 0.87 | 2.52 | 1.53 | 0.11   | 0.18 | 0.13 | 0.29 | 0.13 | 0.25 | 0.28 |
| 4    | 1 d             | 0.60  | 1.02 | 1.25 | 1.17 | 0.75 | 0.82 | 0.96 | 0.25   | 0.35 | 0.17 | 0.79 | 0.03 | 0.17 | 0.11 |
|      | 7 d             | Not determined  |      |      |      |      |      |      | 0.27   | 0.47 | 0.39 | 1.02 | 0.27 | 0.35 | 0.24 |
|      | 30 d            | 0.96  | 2.24 | 1.29 | 1.80 | 0.68 | 0.63 | 1.33 | 0.22   | 0.19 | 0.25 | 0.29 | 0.19 | 0.27 | 0.25 |
|      | 90 d            | 0.42  | 0.89 | 0.79 | 0.54 | 0.41 | 0.59 | 0.65 | 0.18   | 0.20 | 0.21 | 0.20 | 0.16 | 0.21 | 0.23 |



**Fig. 2.** The values of amendment factors AF calculated for arsenic water- and CaCl<sub>2</sub>-extractability (on the left) and arsenic uptake by ryegrass (based on As concentrations in ryegrass shoots harvested in two cuts).

### 2.5. Statistical analysis

For each treatment, the mean values, standard deviations (SD) and 95% confidence intervals were calculated. The results are given as arithmetic means of data from three pots. Significance of differences between the means for soils and treatments were tested by separate one-way analyses of variance (ANOVA) followed by Dunnett's test ( $p < 0.05$ ) that does not imply the assumptions of equal variance between treatments. Significance of correlations between the results, in particular between the data on extractable As in soils, harvested biomass and As concentrations in plant shoots and roots was checked at the probability level  $p < 0.05$ . All statistical analyses were performed by Statistica 10 (Statsoft) software.

## 3. Results and discussion

### 3.1. Arsenic solubility in soils

Soil amendment with all organic materials caused an initial increase in As solubility and extractability that decreased during incubation, but in most cases remained higher compared to untreated samples. The concentrations of As in water extracts (1:2.5, m:v), supposed to mimic its concentrations in real soil solutions in pot experiments carried at 70% of water field capacity (assumption based on unpublished data), were in the ranges 3.61–14.6 mg·L<sup>-1</sup> in the case of soil 1, and 0.79–4.27; 0.09–0.73 and 0.03–1.02 mg·L<sup>-1</sup> in soils 2, 3 and 4, respectively. Those values are considerably higher than the limits of As concentrations in ground water of acceptable quality and drinking water standards, established in Poland at the level of 0.01 mg·L<sup>-1</sup>. The effect of increased water-extractability of arsenic after soil treatment with organic amendments was observed in some of combinations already in the first day of incubation, and was the strongest after 7 d. Then, the concentrations of As in water extracts tended to decrease, and were the lowest after 90 d, except for a few nonessential cases (Table 3). The highest increase of water extractability was caused in various soils by various amendments. Application of manure (M) caused an increase of As concentrations in water extracts

by a factor over 4 in soil 1, compared to untreated soil. Forest litter FL1 acted as the strongest As mobilizing amendment in soil 2 (increase of As water extractability by over 3-fold), and alkaline sewage sludge SS2 caused the highest increase of As water solubility (by a factor 4 and 5, respectively) in more acidic soils 3 and 4. The effects of increased As concentrations in 7 d and 30 d water extracts were least pronounced after application of composted sewage sludge SS3. In soil 2 treated with SS3, a relative reduction of As water extractability (compared to control) was observed after 90 d. Similarly, the amounts of As extractable with 0.01 M CaCl<sub>2</sub> were at the beginning of incubation considerably higher in all organically amended soils compared to control, and this effect was still observed after 90 d in most treatments, though, in some cases, a significant decrease in CaCl<sub>2</sub>-extractability of As was noted at the end of incubation: by 15% in soil 1 amended with SS3, and by 41–43% in soil 2 amended with SS2 and SS3. Attention should be paid, however, to the fact that the changes in water- and CaCl<sub>2</sub>-extractability of As were observed during incubation in non-amended soils as well. These effects, that might have been caused by local differentiation of redox conditions, did not show any constant trends and therefore made the interpretation of experimental results more difficult. In general, it should be stressed that application of organic amendments to the soils under study in most cases caused a considerable, by several-fold, though often temporal, increase of As solubility in soils. The mechanisms responsible for such effect, reviewed by Bauer and Blodau (2006), may involve i) desorption of arsenic from iron oxides by dissolved organic matter that forms stable complexes with mineral surface or ii) formation of aqueous arsenic/DOM complexes by positively charged amino groups in DOM, or iii) reductive dissolution of metal oxides and release of bound arsenic, or iv) the changes in arsenic speciation caused by redox active functional groups present in DOM. The increase of As mobility in soils induced by natural dissolved organic matter, reported by cited authors, was similar to that observed in our study, i.e. in a variety of natural samples, the concentrations of water-extractable arsenic increased by a factor of 1.5–3 and posed a risk to raise arsenic concentrations to the levels that exceed drinking water standards.

**Table 4**  
Correlation coefficients R between As and DOC in CaCl<sub>2</sub> extracts and water extracts of soils. The data in brackets stand for the correlations insignificant at  $p < 0.05$ . The data were normalized before calculating R coefficients.

| Group of results | Extracts in 0.01 M CaCl <sub>2</sub> |         |          | Water extracts |          |         |         |
|------------------|--------------------------------------|---------|----------|----------------|----------|---------|---------|
|                  | 1 d                                  | 30 d    | 90 d     | 1 d            | 7 d      | 30 d    | 90 d    |
| All soils        | (0.038)                              | 0.518   | (0.311)  | (0.067)        | (-0.016) | (0.266) | 0.382   |
| Soil no. 1       | (0.449)                              | (0.423) | (-0.183) | (-0.405)       | (-0.039) | (0.071) | (0.144) |

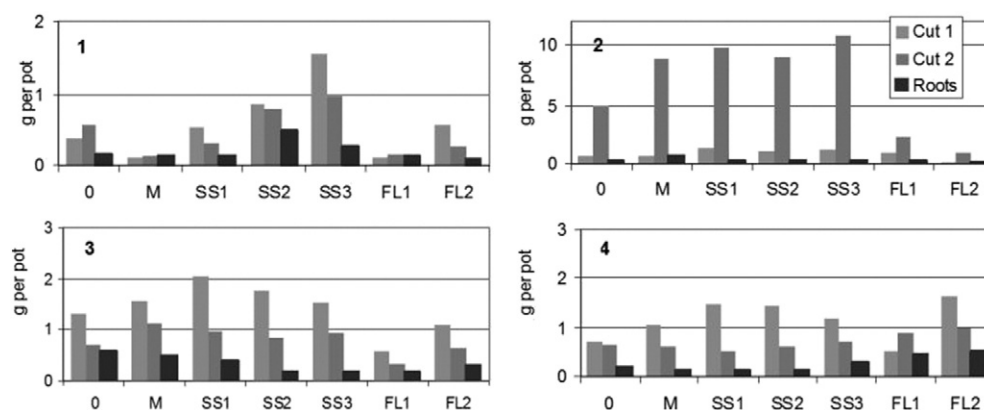


Fig. 3. The biomass of ryegrass roots and shoots (expressed in g of dry matter) collected from soils 1, 2, 3, and 4 in a pot experiment.

For a simplified comparison of the effects caused by various kinds of soil amendments in various soils, a parameter “Amendment Factor” (AF) may be established, defined as the ratio between As mobility in amended and corresponding non-amended soils. The values of AF for  $\text{CaCl}_2$ -extractable arsenic were in the experiment in the range 0.6–4.8, and in most cases considerably exceeded the value of 1.0 (Fig. 2). The corresponding AF values calculated for As concentrations in water extracts fell in the range 0.6–6.8, and their mean values determined for various amendments ranged 0.7–3.2 (Fig. 2). Composted sewage sludge SS3 was the only kind of amendment that – based on average values – happened to reduce As concentrations in water extracts, with the mean AF values (at various stages of incubation): 0.7–1.1. Generally, most values of AF determined for  $\text{CaCl}_2$ - and water-extractable As considerably exceeded the value of 1.0, which illustrates arsenic mobilization by organic amendments, except for SS3 the application of which caused various effects in various soils. It should be stressed, however, that there was no simple dependence between the concentrations of arsenic and DOC in soil extracts, either if considering all the soils together or each of them separately. Significant correlations ( $p < 0.05$ ) were found for  $\text{CaCl}_2$  extracts after 30 d, and for water extracts – after 90 d (Table 4), but their poor strength and transiency indicate a possibly accidental character.

### 3.2. Plant growth

The germination of ryegrass seeds, appearance of plants and their growth depended both on soil properties and the kind of amendment, as illustrated in the photo (Fig. 1) and in the graph (Fig. 3). Generally, pine forest litter (FL1) caused clearly adverse effects on plant growth, and composted sewage sludge SS3 affected positively ryegrass growth and its yields. The effects of other amendments applied to As-enriched soils were ambiguous, and the ryegrass grown

in various soils responded differently to the same kind of amendment. For instance, the application of cattle manure M to the soil 1 resulted in much poorer growth of the grass compared to untreated soil, while the same manure had a beneficial effect on the growth of grass in the soil 3 (Fig. 3). Similarly, application of alder forest litter F2 improved the growth of grass in soil 4, and was definitely disadvantageous for the grass grown in soil 2. The shoot biomass of ryegrass grown in soil 2 – both that non-amended and amended with manure M or any kind of sewage sludge SS – was, particularly in the cut 2, strikingly higher than the biomass of grass grown in all other soils under study. The analysis of correlation between the biomass of grass shoots (cut 1, cut 2 and the sum of both) and the concentrations of extractable As in soils (at the time of grass seeding, i.e. 90 d of incubation) indicated that the biomass of shoots in the cut 1 was significantly ( $p < 0.05$ ) negatively correlated with As in extracts ( $R = 0.56$ ), but similar correlations were not found for the biomass of cut 2 (Table 5). Several other soil factors beside arsenic toxicity and solubility were apparently crucial for the condition of ryegrass grown in our experiment, particularly in the later stages of its growth, however, those factors were not easy to determine and examine.

### 3.3. Arsenic uptake by plants

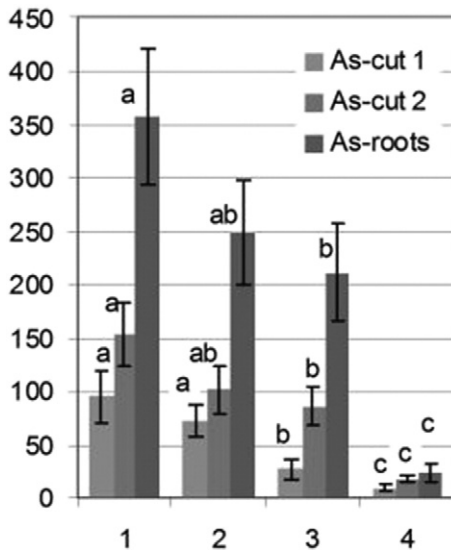
The concentrations of As in the shoots and roots of ryegrass varied strongly within soils and treatments, as shown in Figs. 4 and 5. In general, the shoots harvested in cut 1 contained much lower concentrations of As (range:  $5.1\text{--}189\text{ mg}\cdot\text{kg}^{-1}$ ) than those harvested in cut 2 (range:  $8.7\text{--}256\text{ mg}\cdot\text{kg}^{-1}$ ). Those differences were particularly well expressed and statistically significant ( $p < 0.05$ ) in the case of ryegrass grown in soils 1 and 3. The content of As in roots was higher than in the shoots (except for some insignificant cases in soil 4), and varied in the range  $10.3\text{--}774\text{ mg}\cdot\text{kg}^{-1}$ . In fact, the differences between the mean concentrations of As in shoots and roots of grass did not differ significantly

Table 5

Significant correlation coefficients R between the parameters of plant growth, As concentrations in plants and extractable As (0.01 M  $\text{CaCl}_2$  extracts and water extracts of soils). The data were normalized before calculating R coefficients.

| Parameters                                | Biomass of shoots |       |                | As in dry mass |                |       |
|---|-------------------|-------|----------------|----------------|----------------|-------|
|   | Cut 1             | Cut 2 | Sum: cut 1 + 2 | Shoots - cut 1 | Shoots - cut 2 | Roots |
| Biomass of shoots – cut 2                 | -                 | x     | 0.897          | n.d.           | -              | -     |
| Biomass of shoots – sum: cut 1 + 2        | -                 | 0.897 | x              | -              | -              | -     |
| Biomass of roots                          | -                 | -     | -              | -              | -              | -     |
| As concentrations in dry mass – cut 1     | -0.655            | n.d.  | -              | x              | 0.867          | 0.496 |
| As concentrations in dry mass – cut 2     | n.d.              | -     | -              | 0.867          | x              | 0.467 |
| $\text{CaCl}_2$ -extractable As – 90 d    | -0.562            | -     | -              | 0.590          | 0.653          | 0.548 |
| As concentrations in water extract – 90 d | -0.560            | -     | -              | 0.519          | 0.593          | 0.466 |

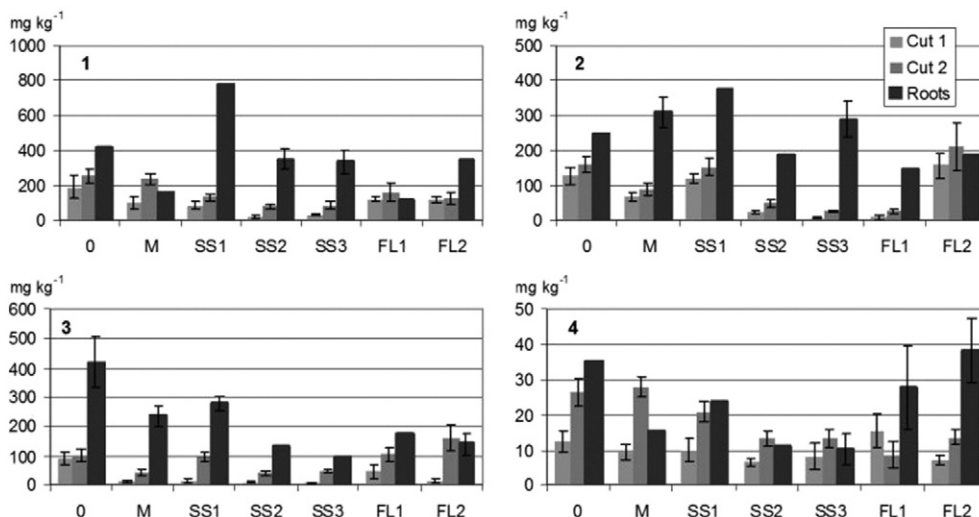
n.d. – not determined (non-reasonable), “-” stands for lack of significant correlation ( $p = 0.05$ ).



**Fig. 4.** Comparison of mean concentrations of As in the shoots (cut 1 and 2) and roots of ryegrass grown in four tested soils. Error bars indicate the intervals of 95% confidence. Same letters indicate univariate groups in which As concentrations in plants do not differ significantly ( $p < 0.05$ ) among the soils (in the light of ANOVA).

between soils 1 and 2 (Fig. 4), in spite of a large discrepancy between total soil As content. On the contrary, the mean values of As concentrations in the cut 1 of shoots, differed significantly between soils 2 and 3 ( $73.4$  and  $27.5 \text{ mg} \cdot \text{kg}^{-1}$ , respectively) despite similar As concentrations in soils. The grass grown in soil 4 contained far lower concentrations of As compared to soils 1, 2 and 3.

Very high concentrations of As in plant shoots, exceeding  $100 \text{ mg} \cdot \text{kg}^{-1}$ , were noted in plants grown in soils 1, 2 and 3 (Fig. 5) and should be considered as potentially dangerous for consumers, in particular grazing animals. Such high values of As concentrations in plant shoots were reported from mining areas or the experiments with enhanced As concentrations in soil/solutions (Mench et al., 2003; Gulz et al., 2005; Raab et al., 2007; Tripathi et al., 2007; Vithanage et al., 2012), but they always raise the concern in terms of ecological risk related with the input into the food chain. The concentrations of As in the shoots of ryegrass grown in soils 1, 2 and 3 should be assessed as relatively high compared with recommendation for grass species to be used in phytostabilization of metal-contaminated soils (Terry and Banuelos, 1999).



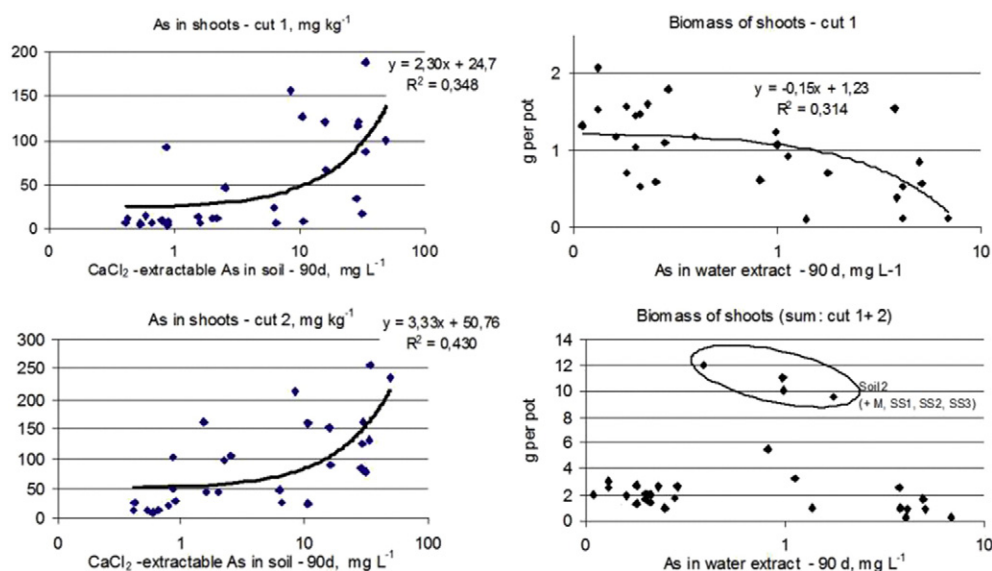
**Fig. 5.** Mean concentrations of As in the shoots (cuts 1 and 2) and roots of ryegrass as related to the kind of amendments. Error bars indicate the intervals of 95% confidence.

Soil amendment with several organic materials examined in the study turned out to be effective in reducing As concentrations in ryegrass shoots. Arsenic uptake by plants was considerably (and significantly) reduced after application of sewage sludge SS3, and - unlike As extractability in soils - after application of SS2 (Fig. 5). The effects of other amendments (M and SS1), and forest litter (FL1, FL2) differed among the soils. In general, the mean values of amendment factor AF, calculated for As concentrations in ryegrass shoots, remained for all treatments below 1, quite differently to the values of AF calculated for soil extractable As (Fig. 2). This observation lets us to conclude that soil amendment with organic materials, as well as the input of forest litter, will usually, but not always (which depends on soil properties), reduce As uptake by ryegrass. Application of composted sewage sludge seems to be a safe measure to improve the effects of remediation for As-polluted soils.

### 3.4. Final remarks

A closer analysis of interrelationships between As extractability in soils, the growth (biomass) of ryegrass and As uptake and translocation to shoots, confirms that the mechanisms governing both the behaviour of As in soil/soil pore water, and in plants, are quite complex and cannot be described by simple correlations. Application of organic matter rich in DOC makes those mechanisms even more complex. This research did not involve a closer insight into plant physiology, but indicates several issues that should be explained in further studies. The analysis of relationships between extractable As in soils and As concentrations in plant shoots and roots (Table 5) confirmed that these parameters were correlated ( $p < 0.05$ ), however the correlations were not strong ( $R: 0.466\text{--}0.653$ ) and explained only 22–43% of total data variability, as shown in the Fig. 6.

The values of biogeochemical factors differed broadly between soils and treatments. Arsenic bioaccumulation factor BAF was, as expected, very low, i.e. in the range:  $0.012\text{--}0.096$ , which should be considered as a beneficial feature of ryegrass that indicated a typical behaviour of arsenic excluders, suitable for phytostabilization of highly enriched soils. However, the values of As translocation factor TF (calculated for the cut 2), that varied in a range:  $0.17\text{--}1.81$ , show clearly that in certain conditions, such as for instance application of manure or forest litter, As concentrations in ryegrass shoots may exceed those in roots, which may be disadvantageous for phytostabilization. This observation stays in agreement with findings by Raab et al. (2007) who reported higher TF values for organic As species compared to mineral arsenic present in solution. Large variability of bioconcentration factors BCF, calculated



**Fig. 6.** Selected significant relationships between plant parameters and As solubility in soils. On the left: As concentrations in ryegrass shoots (cut 1 – upper, cut 2 – lower) as related to  $\text{CaCl}_2$ -extractable As in soil. On the right: biomass of shoots (cut 1 – upper, sum of cut 1 and 2 – lower) as related to As concentrations in water extracts. Exposed are the cases with extraordinarily high biomass.

in relation to  $\text{CaCl}_2$ - and water-extractable As (Table 6), were in the broad ranges: 2.5–119 and 16–932, respectively, and differed by the factors of 48 and 58. Such a variability confirmed that As uptake by ryegrass from contaminated soils cannot be easily predicted based on analysis of extractable As in soils, and in each case requires close investigation to be carried out as greenhouse experiments followed by field trials.

#### 4. Conclusions

In the light of this study, ryegrass should be assessed as quite tolerant to high concentrations of As in soils, and suitable for soil phytostabilization purposes due to low values of bioaccumulation factor BAF. Relatively high values of translocation factor TF, as well as considerably high absolute values of As concentrations in shoots, raise certain concern related to possible input of As into the food chain.

Amendment of As-rich soils with organic materials, in particular those rich in DOC, may affect considerably solubility of As in soils and therefore pose increased risk related to ground water contamination.

Unlike solubility of As in soils, its uptake by ryegrass is usually reduced after application of organic amendments. There are, however, exceptions from this rule, and therefore a closer examination is required in each particular case.

Forest litter may affect solubility and phytoavailability of As in contaminated soils. It is difficult to predict whether those changes will have adverse or beneficial character, therefore afforested sites will

require regular control, and the issue itself needs closer examination and further studies.

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**Table 6**

Biogeochemical indices of As uptake by *Lolium perenne*. Translocation factor TF, bioaccumulation factor BAF, and bioconcentration factor BCF. Definitions – in the text.

| Soil | TF   |      |      |      | BAF   |       |       |       | BCF ( $\text{CaCl}_2$ -90d) |      |      |      | BCF (Water-90d) |     |     |     |
|------|------|------|------|------|-------|-------|-------|-------|-----------------------------|------|------|------|-----------------|-----|-----|-----|
|      | 1    | 2    | 3    | 4    | 1     | 2     | 3     | 4     | 1                           | 2    | 3    | 4    | 1               | 2   | 3   | 4   |
| 0    | 0,61 | 0,65 | 0,24 | 0,75 | 0,039 | 0,090 | 0,061 | 0,053 | 7,6                         | 15,1 | 119  | 63,3 | 67              | 195 | 932 | 147 |
| M    | 1,49 | 0,29 | 0,18 | 1,82 | 0,036 | 0,050 | 0,026 | 0,056 | 4,8                         | 5,4  | 21,8 | 31,4 | 34              | 50  | 242 | 140 |
| SS1  | 0,17 | 0,41 | 0,35 | 0,87 | 0,020 | 0,086 | 0,057 | 0,041 | 3,9                         | 9,5  | 43,8 | 26,4 | 32              | 155 | 745 | 99  |
| SS2  | 0,22 | 0,26 | 0,32 | 1,19 | 0,012 | 0,027 | 0,025 | 0,026 | 2,5                         | 7,6  | 27,0 | 25,0 | 16              | 48  | 148 | 67  |
| SS3  | 0,25 | 0,09 | 0,51 | 1,29 | 0,013 | 0,015 | 0,029 | 0,026 | 2,9                         | 4,1  | 56,5 | 32,6 | 22              | 67  | 377 | 83  |
| FL1  | 1,40 | 0,17 | 0,59 | 0,31 | 0,025 | 0,014 | 0,062 | 0,017 | 5,4                         | 2,3  | 41,7 | 14,7 | 40              | 22  | 421 | 41  |
| FL2  | 0,36 | 1,13 | 1,15 | 0,35 | 0,019 | 0,120 | 0,096 | 0,027 | 4,3                         | 25,0 | 106  | 20,9 | 25              | 155 | 580 | 59  |

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