

The effects of ageing process on the release of arsenic into soil pore water and related phytotoxicity assessed based on seed germination

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Abstract

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Phytotoxic effects caused by soil contaminants depend both on their total concentrations and also on their forms, in particular those that can be easily released into soil pore water. The contaminants introduced into soil in water soluble forms undergo various transformations referred to as “ageing” which causes reduction of their solubility and toxicity. In this study, we examined the dynamics of such changes under controlled conditions in an incubation experiment. Two relatively light soils, differing in texture (loamy sand and sandy loam), and brought to three various pH ranges, were spiked with water solution of sodium arsenate to obtain different soil concentrations of As, up to 1000 mg·kg⁻¹. The soils were incubated at constant moisture (80% of water holding capacity) for three months. The changes in water extractability of As over time were examined. The phytotoxicity of As was assessed based on the reduction of germination, using the seeds of two different plant species: white mustard and red fescue. We found that the process of As immobilization in light mineral soils, poor in organic matter, proceeded quickly, and the concentrations of water-soluble As in these soils dropped significantly within one week. The dynamics of those changes depended on soil properties and pH, and As immobilization was most efficient under acidic conditions. There were no significant differences between the toxicity of As to both plant species examined, as measured in the germination test. The effective concentration of soluble As in soil, resulting in a 50% reduction in the number of germinated seeds, was assessed at about 100 mg·kg⁻¹. The results provide a reference base for further experiments with spiked soils, and will be used in examination of As binding mechanisms in soils.

1. Introduction

Arsenic is a metalloid that can pose a serious threat to human health because of its high toxicity, in particular in inorganic form (ASTDR, 2007; WHO, 2010; Alloway, 2013; Roşca et al., 2020; Balali-Mood et al., 2021; Barea-Sepúlveda et al., 2022; Ozturk et al., 2022). The permissible concentration of As in drinking water has been set by the WHO at 0.01 mg·dm⁻³ (WHO, 2022). The enrichment of the environment with As can be caused by both natural sources (Mahimairaja et al., 2005; Kabata-Pendias, 2011; Hasanuzzaman et al., 2015; Ren et al., 2020) and anthropogenic ones, which include, among others, the historical and contemporary ore mining and processing, smelting of non-ferrous metals, as well as various kinds of industry (Krysiak and Karczewska, 2007; Karczewska et al., 2007; Hasanuzzaman et al., 2012; Karc-

zewska et al., 2013; Hasanuzzaman et al., 2015; Al-Makishah et al., 2020). The greatest threat to human health caused by arsenic is associated with lithogenically enriched groundwater in several Asian regions, in particular West Bengal, Bangladesh and Taiwan (an endemic area of As-caused “black-foot disease”), where tens of millions of people are considerably affected. A smaller scale of the problem occurs in several regions in South America and Australia, and in Europe, in Pannonian Basin (Hungary, Serbia, and Romania). European hotspots of human-made environmental contamination with arsenic are mainly related to historical and contemporary mining (of gold, arsenic, and other ores) in England, Spain, Czechia, and in the Sudetes in Poland, particularly in Złoty Stok (Karczewska et al., 2013; Medunić et al., 2020). Arsenic is considered to be poorly soluble in soils, and poorly bioavailable by plants and other biota (Mahimairaja et

al., 2005; Kabata-Pendias, 2011; Hasanuzzaman et al., 2015; Al-Makishah et al., 2020; Adlassnig et al., 2022). However, under certain conditions, such as strongly reducing environment, or at high concentrations of phosphates or dissolved organic carbon, it can be released into soil pore water and, as a consequence, leached from soil, transported with natural or underground water and finally included in food chains (Dradrach et al., 2019; Lewińska et al., 2019). Arsenic, when supplied to soils in the form dissolved in water, is usually relatively quickly bound to the soil solid phase, in particular in well aerated soils (Mahimairaja et al., 2005; Kabata-Pendias, 2011; Alloway, 2013; Wenzel, 2013).

In the soil, arsenic is strongly bound by iron, aluminium and manganese oxides and hydroxides (Kabata-Pendias, 2011; Wenzel, 2013; Komárek et al., 2013). Many researchers have reported that over time, the binding strength of arsenic introduced into the soil in an easily soluble form increases, and its susceptibility to release from soils decreases. This phenomenon, called ageing, applies not only to arsenic, but also to other contaminants, in particular toxic metals (Tang et al., 2007; Venegas et al., 2016; Lin et al., 2019; Lu et al., 2019). However, the literature does not provide information on the dynamics of the ageing process that takes place in soils with different properties. Such knowledge is important not only from a scientific standpoint, but also for the practical reasons, in particular when planning the experiments with soils spiked with water-soluble arsenic and other contaminants. Undoubtedly, the course of this process depends on soil composition and properties. Changing conditions of soil pH or redox potential can considerably affect As sorption and cause its release from (hydro)oxides. Introduction of phosphates or organic matter into the soil may also contribute to the desorption of As from the soil solid phase (Wenzel, 2013; Lewińska et al., 2017; Berg, 2017; Cuske et al., 2017; Karczewska et al., 2017; Dradrach et al., 2020a, 2020b). All these factors make As bioavailability strongly dependent on soil properties (Song et al., 2006; Wenzel, 2013).

The toxicity of As to plants depends on various factors, including the concentration of As and P in soil pore water and their ratio (Zhoa et al., 2009; Li et al., 2017; Dradrach et al., 2020b). An important point in the development of plants is the phase of seed germination that involves three stages: water imbibition and swelling, activation and intra-seed growth. During germination, seeds absorb large amounts of water and may be exposed to toxic substances dissolved in soil pore water (Parkpian et al., 2002; Pflugmacher et al., 2020). Research shows that the tolerance of seeds of different plant species, for instance *Brassica rapa* L., *Sinapis alba* L., *Amaranthus retroflexus* L., *Helianthus annuus* L., varies greatly both between species and between different genotypes of the same species, and seed germination followed by plant growth can be stimulated at low concentrations and inhibited at high concentrations of the toxic factor (Zhang et al., 2002; Han et al., 2003; Gvozdenac et al., 2013; Cozma et al., 2019; Dradrach et al., 2019; Roşca et al., 2020).

The measurement of toxic effects caused by the presence of contaminants dissolved in soil pore water, standardized in ecotoxicological bioassays, can be used as a handy tool in the assessment of environmental risk assessment and environmental monitoring. One of the most common endpoints used with

this purpose is the inhibition of seed germination. Standard test plants used for these purposes should be relatively tolerant to contaminants, as recommended by the OECD, US EPA and ISO (OECD, 2006; Visioli et al., 2014; Paustenbach, 2015; ISO 18763, 2016; Karczewska and Kabala, 2017). Their adaptation to the environment involves various defence mechanisms, including homeostatic cellular mechanisms regulating the concentration of metal ions inside the cell to minimize the potential damage that could result from the exposure to harmful ions, which was proved to work already at the stage of germination and early growth phase (Benavides et al., 2005; Tenea et al., 2021).

The aim of this study was to examine the dynamics of As ageing in soils to which it was introduced in a water soluble form. The ageing process results in decreasing in the amounts of As remaining in soil pore water. However, the specific data are not available in the literature on how the As immobilization process proceeds over time and whether its progress depends on soil texture and pH. Therefore, two different soils and different pH conditions were tested in this study. Another aim of the experiment was to assess the effects of water extracts, acquired from As-spiked soils, on seed germination of two different plant species, i.e. *Sinapis alba* L., that is traditionally used in ecotoxicological bioassays (OECD, 2006; ISO 18763, 2016), and the grass *Festuca rubra* L., which is often used for phytostabilization of contaminated sites (Radziemska et al., 2017; Dradrach et al., 2020c). It can be expected that the results of that assay should be well correlated with As concentrations in the extracts, however such relationships could also be influenced by other components of the extracts, therefore appropriate experiments were performed in this study. It was also interesting to check whether these dependencies are similar for the seeds of different plant species, with various germination times. The results provided a reference base for further experiments with As spiked soils, and will be used in examination of As binding mechanisms in soils.

2. Materials and methods

2.1. Soil properties

Uncontaminated soils were taken from a layer of 0-20 cm from organic farms (where chemical plant protection products were not used), assuming that they would differ in grain size distribution. The soil material was dried, sieved through a 2 mm sieve, mixed. Two kinds of soil material used in the experiment differed in texture, although both soils can be classified as light soils. Their basic properties were determined using the methods commonly used in soil science. Soil grain size composition was determined by a combined sieve and hydrometer method (Papuga et al., 2018), and textural classes were determined according to the commonly used classification by USDA (U.S. Department of Agriculture)(USDA, 2017). Chemical analyses were carried using ground aliquots of soils, with the common soil science methods (Tan, 2005). Soil pH was measured potentiometrically in a suspension in 1 M KCl (1:2.5; v/v). Organic carbon (Corg) was determined by a dry combustion method (Vario MacroCube, Elementar). All analyses were made in triplicates.

2.2. Soil spiking and incubation

Soil material was moistened to 80% of water holding capacity (WHC), using distilled water or diluted solutions of HCl acid or KOH, so as to adjust soil pH to three different pH ranges: 4.0–5.0 (acidic), 5.5–6.5 (neutral), and 7.0–8.0 (alkaline). Soil material was then incubated for 2 weeks to equilibrate pH. After this time, soils were spiked with the solutions of disodium hydrogen arsenate $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ (p.a., Merck) to obtain soil As concentrations: 10, 20, 50, 100, 200, 500 and 1000 $\text{mg}\cdot\text{kg}^{-1}$. The soils treated with distilled water were used as control. Spiked soils were incubated (aged) in 1 kg pots for 1, 2 and 12 weeks. The experiment was carried out in triplicates. Total concentrations of As in soils were determined randomly after the end of the experiment, in 6 replicates for each of the variants: soil x As concentration x pH. For this purpose, digestion in aqua regia followed by As determination by ICP-AES was applied. A reference material CRM 027 (Fluka), certified for aqua regia-extracted elements, that contained 152 $\text{mg}\cdot\text{kg}^{-1}$ As, was used for validation of the method. An average As recovery from that CRM was 97%. The average As recoveries from the spiked samples were in various experimental variants in the range 97–105%, with individual results of recovery in a broader range of 86–117%. Such a considerable variance should be attributed to the heterogeneity of As distribution in spiked soils, despite the fact that the treatment of spiking was done as carefully as possible.

2.3. Analysis of soil pore water and water extracts

Before starting the experiment, preliminary series of analyses were carried out on soils spiked with As to determine the method of water extraction of soil samples, so that the obtained extracts had a composition similar to real soil pore water at 80% of WHC. It should be stressed that the composition of soil solution is in fact not a constant feature for a given soil, and depends, among other factors, on soil moisture. Therefore, numerous authors, as well as we in our previous ones, analyzed real soil pore water, collected with MacroRhizon samplers (Pongratz, 1998; Dradrach et al., 2019, 2020b). However, embedding of these devices in soil often poses some technical problems and the results do not show good reproducibility. Therefore, we decided to examine aqueous extracts with appropriate extraction parameters instead of collecting real soil pore water. For this purpose, water extracts can be obtained either from saturated paste or by soil shaking with water at the appropriate m/v ratio, followed by centrifugation. Based on the literature (Pongratz, 1998; Száková et al., 2009) and preliminary tests, the optimal method for our experiment was chosen that involved shaking for 2 hours with distilled water at a ratio of 1:5; m/v, centrifugation at 3200 rpm for 15 min. In that way, water extracts similar in composition to soil pore water were obtained after each period of ageing. The concentrations of As in extracts were measured by ICP-AES (iCAP 7400, Thermo Fisher Scientific) after filtering the supernatants through a 0.45 μm filter. A determination limit for As in extracts was 0.005 $\text{mg}\cdot\text{dm}^{-3}$. The correctness of the analyses was verified using internal standards, and selectively by the standard addition. The recoveries of As added to the samples as

standards (0.1 $\text{mg}\cdot\text{dm}^{-3}$) were in the range 90–110% and did not indicate any systematic errors that would come from the matrix of the analyzed extracts.

2.4. Germination test

Germination test was performed according to OECD/ISO procedure (OECD, 2006; Visioli et al., 2014; Paustenbach, 2015; ISO 18763, 2016; Karczewska and Kabała, 2017), using the seeds of two different plant species: white mustard *Sinapis alba* L. and red fescue *Festuca rubra* L. The ecotoxicological endpoint was the percentage of germinated seeds compared to control. The test was performed under controlled laboratory conditions in a phytotron with a day cycle of 16 hours at 24°C and a night cycle of 8 hours at 14°C. Following previous studies (Pruchniewicz and Halarewicz, 2019), filter papers with 10 cm diameter were put into sealed containers with a volume of 500 cm^3 . The use of sealed containers minimized the problem of water loss by evaporation during the test. After sterilization with a UV lamp, that eliminated seed damage by pathogens or insects, 50 seeds of each species were placed on filter paper, in 4 replicates. Then, each paper filter was moistened with 5 ml of soil pore water obtained from As-spiked soils and from the control soils, without As. The experiment was terminated after 3 days for *Sinapis alba* and after 7 days for *Festuca rubra*. After this time, the number of germinated seeds was determined.

2.5. Data analysis

To assess the significance of differences between the means obtained for different soils, different pH values and various ageing times, a one-way analysis of variance (ANOVA) was applied followed by Tukey's test, at $P < 0.05$. For each set of results, a confidence interval at $p=95\%$ was determined. After normalizing the data, correlation coefficients were calculated between As concentrations in soil pore water and seed germination. Additionally, PCA analysis was performed to examine multiple relationships between variables. Statistical analyses were performed using the tools of Excel (Microsoft Office) and Statistica 13.0 software (StatSoft).

3. Results

3.1. Basic soil properties

Soils differed in texture, although both belonged to light soils, with clay content (<0.002 mm) of 4 and 11%, respectively. Sand fraction (0.05–2.0 mm) was a dominant one in both soils, with the share over 70%, while the content of silt fraction (0.002–0.05 mm) did not exceed 20% (Table 1). Soil 1 was classified as loamy sand (LS) and soil 2 – as sandy loam (SL). Further in the text, they are referred to as sandy and loamy soils. Soil 1 was originally acidic (pH: 4.35), while soil 2 had a slightly acidic pH of 5.65. Both soils were relatively poor in organic matter and contained low concentrations of N, lower than in typical surface levels of arable soils developed of sands and loams.

Table 1

Basic properties of soils used in the experiment. Means \pm standard deviation values of 3 replicates are provided

Parameter	Unit	Soil 1	Soil 2
Soil textural class (USDA)*		LS	SL
Sand (0.05–2.0 mm)	%	78 \pm 2	73 \pm 2
Silt (0.002–0.05 mm)	%	18 \pm 1	16 \pm 1
Clay (<0.002 mm)	%	4 \pm 1	11 \pm 1
Water holding capacity	% (v/v)	35.0 \pm 1.8	39.3 \pm 1.6
Corg.	g·kg ⁻¹	15.2 \pm 0.4	14.2 \pm 0.2
N	g·kg ⁻¹	0.70 \pm 0.05	0.83 \pm 0.05
pH (1M KCl)	–	4.35 \pm 0.03	5.65 \pm 0.05

Note: * – Textural groups according to soil particle size classification developed by the U.S. Department of Agriculture (USDA): LS – loamy sand, SL – sandy loam.

3.2. Concentrations of As in soil pore water

Water solubility of As tended to decrease rapidly, and the dynamics of that decrease depended on soil properties and total concentrations of As added to soils. It should be stressed, however, that the extracts obtained by centrifugation were usually not completely clear and contained considerable amounts of highly dispersed particles of colloidal size, that were removed finally by filtering the extracts through a 0.45 μm filter prior to analysis. Figure 1 shows the changes of As concentration in water extracts for the examples of soil As concentrations 20 and 200 $\text{mg}\cdot\text{kg}^{-1}$, and acidic and alkaline reaction. These graphs show that the fastest decrease in As concentrations in water extracts occurred shortly after peaking, so after 1 week the concentration of As in the extracts decreased to between a dozen

and 25% of the initial concentration. This decrease was much greater in the case of low amounts of As introduced into the soils (Fig. 1). It should also be emphasized that the final solubility of As in acidic soils was clearly lower than that in alkaline soils, while there were no statistically significant differences between the sandy and loamy soils.

A closer analysis of the results, performed for a wide spectrum of As concentrations and considering also the conditions of neutral pH, confirmed that the course of the ageing process did not show significant differences between the sandy and loamy soils (Figure 2). At the same time, it was confirmed that the solubility of As at individual times, i.e. after 1, 2 and 12 weeks (1W, 2W, 12W) clearly decreased, and the differences between those time points were usually statistically significant ($P < 0.05$).

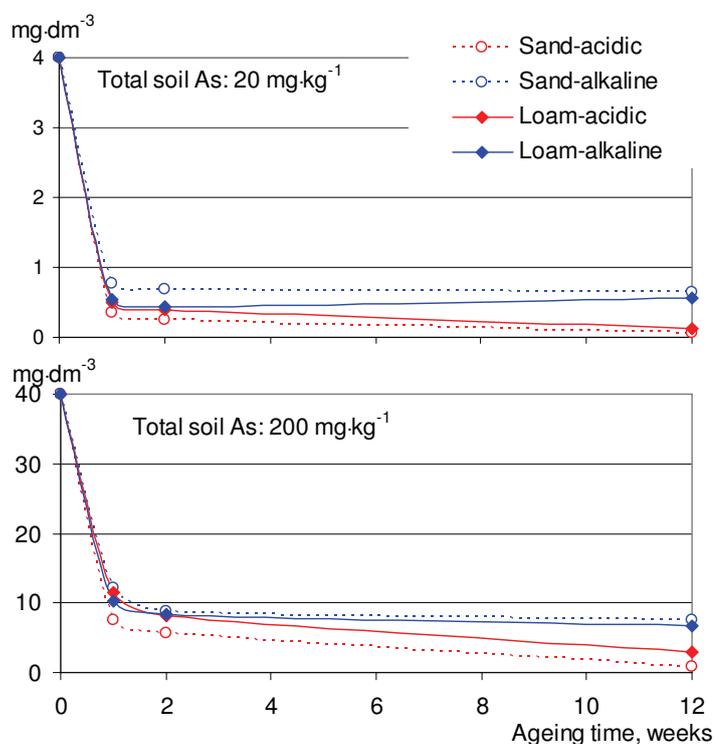


Fig. 1. Ageing-related changes of As concentrations in water extracts from soils containing 20 and 200 $\text{mg}\cdot\text{kg}^{-1}$ of As, under acidic (red lines) and alkaline (blue lines) conditions. The initial As concentration in the extracts corresponds to 100% water-soluble arsenic.

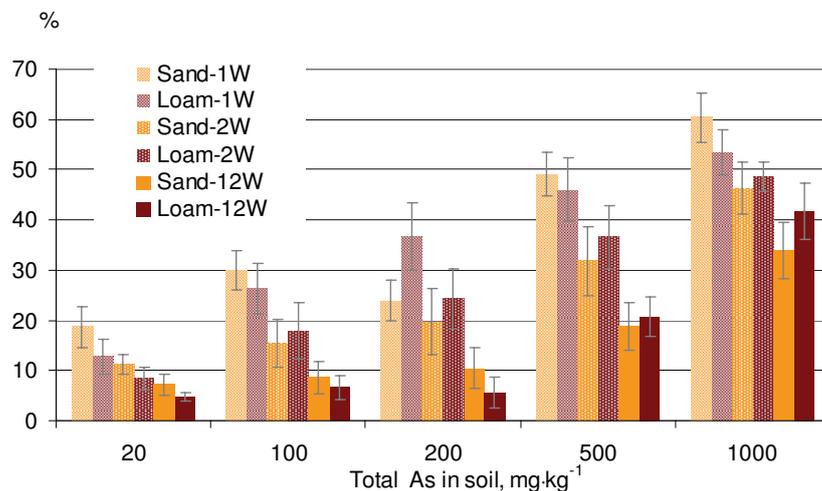


Fig. 2. The percentage of total As released into the soil water (aqueous extract) as related to total As concentrations in soils (20–1000 mg·kg⁻¹). The graph shows the results obtained for sandy and loamy soils at the pH close to neutral (pH: 5.5–6.5), after the ageing time of 1 week (1W), 2 weeks (2W) and 12 weeks (12W). Error bars indicate confidence intervals at P=95%.

The percentage of soluble As in relation to its total content in the soil was the lowest at the lowest As total concentration in soil (20 mg·kg⁻¹) and increased with increasing As concentrations. A very large part (>80–98%) of As added to the soils in the amount of 20 mg·kg⁻¹ was adsorbed, and the percentage of immobilized As dropped to 20–60% (depending on soil, pH and time) at the highest total As concentrations. Such an effect should be explained by the limited capacity of soils to directly adsorb high amounts of As. The percentage of adsorbed As showed a clear (and statistically proven) tendency to increase

with time, which is illustrated by the graphs. This observation applied to both neutral pH (Figure 2) and acidic and alkaline pH (Figure 3), and the described trend was observed throughout the entire incubation period. Moreover, under alkaline pH conditions, the share of soluble As did not fall below 10%, even at low total concentrations of As in the soil and despite ageing. Under acidic conditions, the ageing process effectively reduced As solubility at total As concentrations up to 200 mg·kg⁻¹, in particular in the sandy soil.

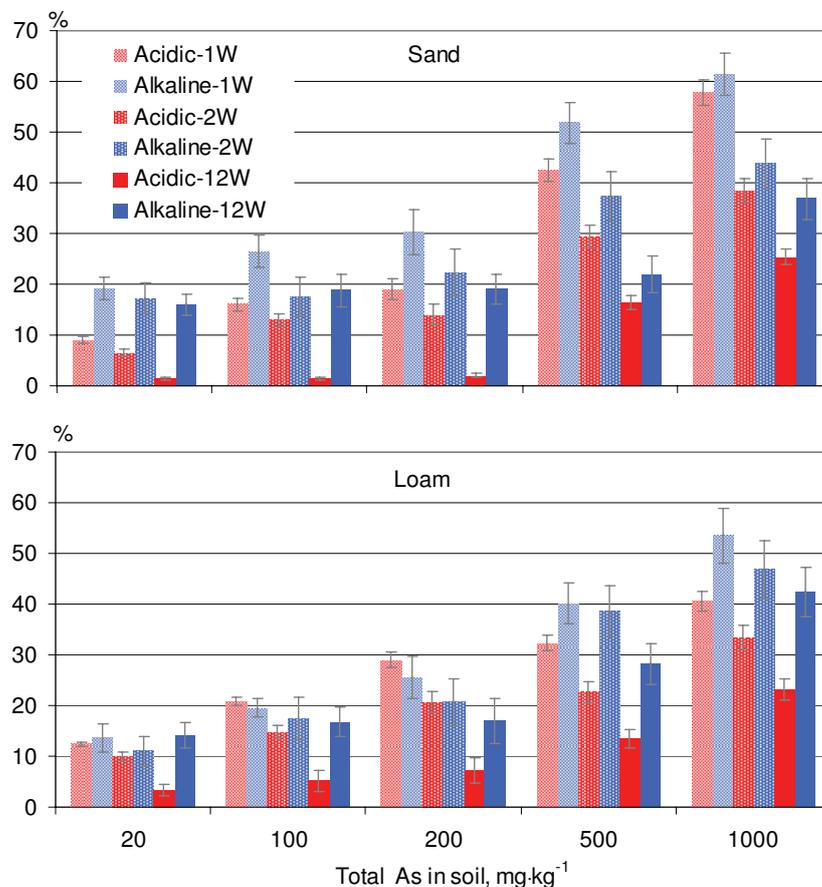


Fig. 3. The percentage of total As released into the soil water (aqueous extract) from the sandy soil (upper graph) and loamy one (lower graph), as related to total As concentrations in soils (20–1000 mg·kg⁻¹). The graph shows the results obtained in the conditions of acidic pH (pH: 4.0–5.0), presented in red color, and alkaline pH (pH: 7.0–8.0), in blue color, after the ageing time of 1 week (1W), 2 weeks (2W) and 12 weeks (12W). Error bars indicate confidence intervals at P=95%.

3.1. Seed germination

The results of germination assay indicated that within the range of lower total As concentrations in both soils, up to 100 mg·kg⁻¹, the percentage of germinated seeds of both species was high, above 85%, with minor exceptions after 1 week of ageing in loamy soil, under alkaline conditions (Table 2). In some cases, we observed a visible stimulatory effect of low concentrations of added arsenate, that corresponded to As concentration of 20 mg·kg⁻¹. At those concentrations of As, the percentage of germinated seeds was sometimes slightly higher than that in control samples, without As addition. However, the related differences were often statistically insignificant, as this test, in general, showed a relatively high variance among replicates, and the standard deviation (SD) values usually remained in the range of 5–10% (not shown in the table). The strong reduction in the percentage of germinated seeds or a complete lack of germination were observed at total As concentrations in the soils of 500 and 1000 mg·kg⁻¹ (Table 2), especially at the shortest ageing time. At such high As concentrations *Festuca rubra* turned out to be more resistant to As toxicity, despite the fact that the contact time of its seeds with water extract was longer than in the case of *Sinapis alba*.

Undoubtedly, the two key factors that might have a decisive impact on seed germination were the real As concentrations in water extracts and their pH values, and not the total As concen-

trations in soils. In the pH range in which the experiment was conducted, the pH values themselves did not turn out to be a significant factor influencing seed germination. There were no statistically significant differences ($p < 0.05$) between germination of *Sinapis alba* or *Festuca rubra* seeds in the extracts of control (non-spiked) soils under different pH conditions. (Related statistics are not presented). Figure 4 illustrates the dependence of seed germination of *Sinapis alba* and *Festuca rubra* on the concentrations of water-soluble pools of As in soils under acidic and alkaline pH conditions. This graph shows that up to a concentration of soluble As in soil of 10 mg·kg⁻¹ (that corresponded to 2 mg·dm⁻³ As in water extracts), plant germination was not substantially reduced, and even a stimulating effect was visible at very low concentrations. A considerable decrease in germination occurred above this concentration. Due to the high variance of the results, it was not possible to precisely determine the EC50 values for the tests, but they were estimated at about 100 mg·kg⁻¹ of soil-soluble arsenic, which corresponded to As concentrations of 20 mg·dm⁻³ in water extracts.

When the concentrations of As in water extracts exceeded 100 mg·dm⁻³, germination was no longer observed, which is consistent with the data reported by Piršelova (2011). The strong negative dependence of the percentage of germination on the concentration of soluble As in the soil is confirmed by the high absolute values of Pearson's correlation coefficients (Table 3). These correlations were significant at $P < 0.001$.

Table 2

Percentage of germinated seeds of *Sinapis alba* (*S.a.*) and *Festuca rubra* (*F.r.*) in soils after various time of ageing: 1, 2 and 12 weeks. Mean values of four replicates.

Ageing time, weeks	Total As, mg·kg ⁻¹	Sandy soil						Loamy soil					
		Acidic		Neutral		Alkaline		Acidic		Neutral		Alkaline	
		<i>S.a.</i>	<i>F.r.</i>										
1	0	98	90	93	83	85	93	93	93	78	80	78	90
	20	95	80	90	100	90	87	88	100	80	87	83	80
	100	98	100	88	83	83	97	90	97	90	80	68	57
	200	95	83	90	83	93	93	93	77	80	100	88	73
	500	15	0	5	0	0	0	13	0	0	0	0	23
	1000	8	3	0	0	0	0	0	10	0	0	0	0
2	0	90	90	95	70	95	93	95	77	90	90	90	83
	20	95	63	100	73	92	90	90	80	90	67	88	87
	100	100	97	100	90	93	87	100	93	95	100	88	97
	200	98	77	93	90	90	80	90	77	93	80	90	73
	500	23	40	13	73	0	30	28	50	8	20	3	13
	1000	0	7	3	7	0	0	3	33	3	0	0	0
12	0	93	87	90	85	98	93	100	90	95	83	93	83
	20	98	83	93	87	90	85	100	90	98	80	85	83
	100	98	98	95	83	93	90	98	90	95	85	95	87
	200	95	100	95	85	85	90	95	67	95	73	85	80
	500	70	60	83	80	65	77	75	47	70	73	45	93
	1000	20	37	5	7	3	17	8	17	5	63	5	67

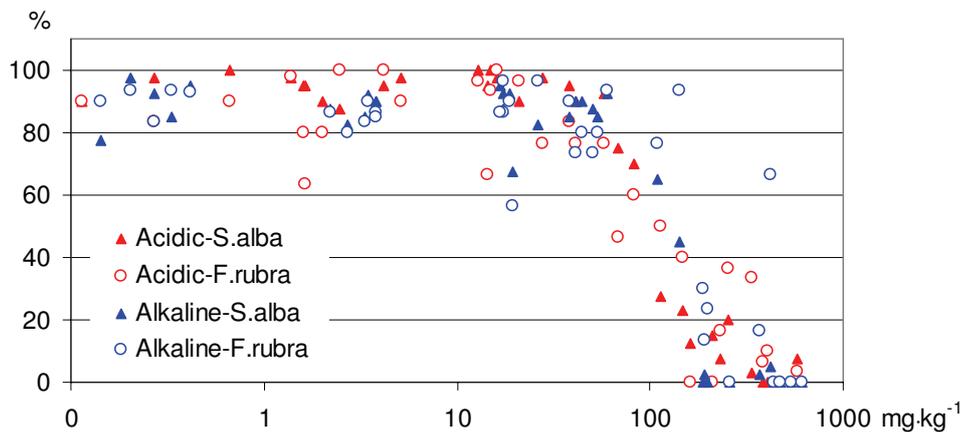


Fig. 4. Relationships between the concentrations of soluble As in soils and the germination of *Sinapis alba* and *Festuca rubra* under the conditions of acidic pH (red) and alkaline pH (blue).

Table 3

Pearson correlation coefficients between the concentrations of soluble As in soils and the percentage of germination of *Sinapis alba* (S.a.) and *Festuca rubra* (F.r.), under different pH conditions

Acidic		Neutral		Alkaline	
S.a.	F.r.	S.a.	F.r.	S.a.	F.r.
-0.894***	-0.854***	-0.900***	-0.852***	-0.886***	-0.858***

*** Significant at P<0.001

4. Discussion

The results of the experiments indicate that As introduced into soil undergoes relatively rapid immobilization, therefore in fact the first days, not weeks, are crucial for reducing its solubility in water. Of course, these results apply to the soils that were the subject of our research, i.e. light soils with grain size of loamy sand and sandy loam. Other authors suggested a period of 4 weeks of incubation following soil spiking (Romero-Freire et al., 2015), or even 3 months (Song et al., 2006), in order to stabilize the solubility of As, however, such periods were adopted a priori based on the literature. In fact, those authors did not analyze the dynamics of As binding in the soil solid phase. Some authors pointed to a very slow decrease and even an increase in the bioavailability of As with the prolongation of ageing period (Meunier et al., 2011; Zanget al., 2021), however, they formulated their conclusions based on extractions with stronger extractants and not with water. Moreover, the most likely factor that caused the release of As from the soils examined by those authors was the high content of organic matter, while in our soils the content of Corg was low (14.2 and 15.2 g.kg⁻¹). Therefore, our research should be extended to soils rich in organic matter, including those that developed from peat, as numerous studies emphasize the effect of organic matter on increasing the mobility of As in soils (Bauer and Blodau, 2006; Arco-Lázaro et al., 2016; Karczevska et al., 2018; Szopka et al. 2021).

What should be emphasized in our results, is the lack of significant differences between the two tested soils, as well as the similarity of the germination responses of seeds of both tested plant species. These similarities were confirmed by PCA (principal component analysis) performed after log-transforming the concentrations to obtain distributions close to normal

(Figure 5). The illustration of the projection of results onto the plane of principal components 1 and 2, which together determined nearly 70% of the variance in analyzed data, confirms the similarity of the results for both species and their strong negative correlations with the concentrations of total and water-extractable As in soils, as well as much poorer relationships with incubation time and pH, and practical no effect of the kind of soil.

Some differences in the reaction of both species to very high concentrations of As in the soil solution, mentioned above, would require a closer analysis. They may result from different seed tolerance of these species, as *Festuca rubra* is considered

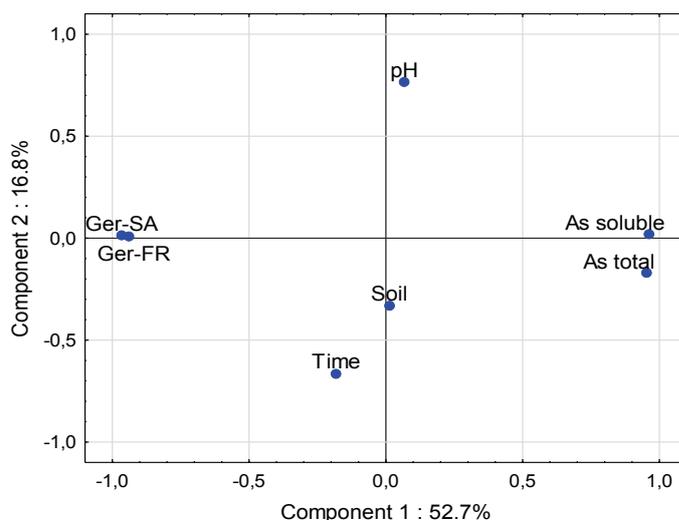


Fig. 5. The results of PCA analysis that illustrate multiple relationships between the crucial factors considered in the experiment.

to be relatively resistant in this respect (Vazquez et al., 2014; Viciano et al., 2021), but also from secondary transformations, for instance precipitation, that As may undergo in the soil solution during a longer time necessary to make *Festuca rubra* germinate. The latter effect will require closer examination.

5. Conclusions

This research allowed to conclude that the process of As immobilization in light mineral soils, poor in organic matter, proceeds quickly, and the concentrations of soluble As in these soils drop significantly within one week. The effectiveness of As sorption depends on its total content in the soil, and soil pH. The concentrations of remaining As soluble in water were significantly higher in alkaline compared to acidic conditions. There were no significant differences between the toxicity of As to both plant species examined, as measured in the germination test. The EC50 value, i.e. effective concentration of As, resulting in a 50% reduction in the number of germinated seeds, was assessed to be about 100 mg·kg⁻¹ of soluble As in the soil, which corresponds to about 20 mg·dm⁻³ in extracting water. These results may constitute a reference base for conducting experiments with soils spiked with As, however, in further research, the spectrum of the analyzed soils should be broadened, in particular taking into account soils rich in organic matter.

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Wpływ procesu starzenia (ageing) na uwalnianie arsenu do roztworu glebowego i związaną z tym ekotoksyczność ocenianą w oparciu o test kiełkowania nasion

Słowa kluczowe

Arsen
Gleba
Rozpuszczalność
Ekstrakcja
Fitotoksyczność
Kiełkowanie nasion

Streszczenie

Efekty fitotoksyczności powodowane przez substancje stanowiące zanieczyszczenia gleb zależą zarówno od ich całkowitych stężeń jak też od ich form, zwłaszcza tych, które mogą być łatwo uwalniane do roztworu glebowego. Zanieczyszczenia wprowadzone do gleby w formach łatwo rozpuszczalnych w wodzie ulegają różnym przemianom określanym terminem “starzenie się”, co skutkuje zmniejszeniem się ich rozpuszczalności i toksyczności. W niniejszej pracy badaliśmy dynamikę takich zmian w kontrolowanych w warunkach, w doświadczeniu inkubacyjnym. Do dwóch stosunkowo lekkich gleb, o różnym uziarnieniu (piasku gliniastego i gliny piaszczystej), i trzech różnych przedziałach pH, wprowadzony został roztwór wodny arsenianu sodu w różnych ilościach, tak że uzyskano stężenia As w glebach do 1000 mg·kg⁻¹. Gleby inkubowano w warunkach stałej wilgotności (odpowiadającej 80% polowej pojemności wodnej) przez trzy miesiące. Badano dynamikę zmian podatności As na ekstrakcję wodą. Fitotoksyczność As oceniano w oparciu o ograniczenie kiełkowania nasion dwóch różnych gatunków roślin: gorczyca białej i kostrzewy czarnej. Wykazano, że proces immobilizacji As w lekkich glebach mineralnych, ubogich w materię organiczną, przebiegał szybko, a stężenia rozpuszczalnego As w tych glebach zmniejszyły się znacząco w ciągu pierwszego tygodnia inkubacji. Dynamika tych zmian zależała od właściwości gleb i pH, a immobilizacja As była najskuteczniejsza w warunkach odczynu kwaśnego. Nie stwierdzono istotnych różnic między toksycznością As w stosunku do obu testowanych gatunków roślin, ocenianą w oparciu o test kiełkowania. Efektywne stężenie łatwo rozpuszczalnego As w glebach, które powodowało spadek liczby kiełkujących nasion o 50%, oszacowano na poziomie około 100 mg·kg⁻¹. Uzyskane wyniki stanowią bazę odniesienia dla dalszych doświadczeń z glebami sztucznie zanieczyszczonymi i będą wykorzystane do badania mechanizmów wiązania As w glebach.