*minerals*

MDPI

*Article*

# Ecotoxicity of Pore Water in Meadow Soils Affected by Historical Spills of Arsenic-Rich Tailings

**Agnieszka Dradrach [1], Katarzyna Szopka [2] and Anna Karczewska [2,*]**

[1] Institute of Agroecology and Plant Production, Wrocław University of Environmental and Life Sciences, 50-363 Wrocław, Poland; agnieszka.dradrach@upwr.edu.pl
[2] Institute of Soil Science and Environmental Protection, Wrocław University of Environmental and Life Sciences, 50-357 Wrocław, Poland; katarzyna.szopka@upwr.edu.pl
* Correspondence: anna.karczewska@upwr.edu.pl

Received: 6 July 2020; Accepted: 24 August 2020; Published: 25 August 2020


**Abstract:** This study was carried out in Złoty Stok, a historical centre of gold and arsenic mining. Two kinds of soil material, containing 5020 and 8000 mg/kg As, represented a floodplain meadow flooded in the past by tailings spills and a dry meadow developed on the plateau built of pure tailings, respectively. The effects of soil treatment with a cattle manure and mineral fertilizers were examined in an incubation experiment. Soil pore water was collected after 2, 7, 21, 90, and 270 days, using MacroRhizon samplers and analyzed on As concentrations and toxicity, and assessed in three bioassays: Microtox, the Microbial Assay for Risk Assessment (MARA), and Phytotox, with *Sinapis alba* as a test plant. In all samples, As concentrations were above 4.5 mg/L. Fertilization with manure caused an intensive release of As, and its concentration in pore water of floodplain soil reached 81.8 mg/L. Mineral fertilization caused a release of As only from the pure tailings soil. The results of bioassays, particularly of Phytotox and MARA, correlated well with As concentrations, while Microtox indices depended additionally on other factors. Very high toxicity was associated with As > 20 mg/L. Despite an effect of "aging", pore water As remained at the level of several mg/L, causing a potential environmental risk.

**Keywords:** mining; arsenic; tailings; Złoty Stok; MacroRhizon; fertilization; manure; Microtox; MARA; Phytotox

## 1. Introduction

A historical mining and ore processing complex in Złoty Stok (formerly Reichenstein), situated at the foothill of Złote mountain range, for over two centuries, until 1962, was one of the largest producers of arsenic in Europe [1,2]. Old type facilities used for ore enrichment were modernized in the years 1930–1937 to apply an efficient flotation technology. The concentrates produced in the flotation process contained ca. 40% of As in mass, while As concentrations in tailings, disposed in the impoundments, were initially in the range 1.5–2.5%, and later, after the next improvement of technology, in the range 0.8–1.5%. After stormy rainfall events, relatively frequent in that area, tailings overflew the dams and were disposed in the valley of a stream Trująca, causing a considerable soil enrichment in arsenic within a distance of at least 2 km down the stream. The valley has been partly forested, but its large parts are abandoned or used as grasslands, and recently also as recreational areas. Some grasslands, situated within a floodplain of the stream, can be classified as fresh meadows. An almost 2 ha large area of the valley, at the foreland of tailings impoundments, is covered by a thick layer of pure tailings that form a plateau, with the surface elevated by 2–3 m over the neighboring terrain [1,3]. This area is covered by grassy vegetation, and can be classified as a dry meadow. Soils of both meadows were previously the targets of research that examined a spatial distribution of As and

its extractability [1]. Those works indicated that large areas are covered by soils with very high As content, of several thousand mg/kg. Despite such high total As concentrations in soils, its solubility and ammonium-sulphate extractability was in general very low owing to particularly strong binding of this metalloid by iron oxyhydroxides [4,5]. It was proved, however, that negatively charged particles present in soil pore water at high concentrations, in particular phosphates or dissolved organic compounds, under oxidized conditions, can cause desorption of arsenates from the solid phase. Numerous studies reported a release of As from soils upon treatment with phosphates [6–8]. Such effects were also described for soils of the Trująca stream valley [9]. Similarly, there is evidence that organic matter, particularly its easy soluble fractions, can act as the factors of temporary As release into pore water [4,10,11]. Such effects were reported from the field and laboratory experiments that examined As solubility and bioavailability in soils polluted with As and fertilized with pig slurry or manure [11,12], sewage sludge [13], or various organic waste materials [14]. It has also been proven that decomposition of forest litter can act as a trigger for mobilization of As from highly enriched mine soils [15]. A mechanism of As release from phosphate- and organic matter-treated soils is mainly based on anionic competition for sorption sites on iron (oxy)hydroxides. That effect can be accelerated under flooded conditions, both via reductive dissolution of iron oxyhydroxides and owing to microbial transformations [4,14,16]. The increase in As concentrations in pore water may not be necessarily associated with an increased uptake of As by plants [6,9,17,18], as the phytoavailability of As in soils depends on various factors, including the relationships between As and P concentrations in pore water, and the net effects of As and P competition for their uptake by plant roots [8,19].

Similarly, the reactions of microbes and other biota that live in soils to high As concentrations in soil solution are in fact very difficult to predict. Various authors proved that chemical extractability of As and other potentially toxic elements present in soils can only party explain their real bioavailability and toxicity [7,20]. Arsenic ecotoxicity depends on numerous factors, including its speciation in pore water, oxidation state As (III) versus As (V), water pH, co-existing components, and their concentrations [4,21,22]. In order to assess the environmental risk associated with the presence of toxic elements in real soil systems, it is necessary to examine both soil chemistry and the effects posed to biota.

Ecotoxicological examination of environmental samples should thus be an important complement to chemical analysis in a comprehensive assessment of environmental risk [23]. Numerous assays, which use various groups of biota, representative of different food chain levels, have been adopted as ISO or OECD norms [24–26]. Several bioassays are now available as easy in maintenance and standarizable commercial products, and have gained increasing acceptance and popularity. They are now commonly used in environmental research, particularly in the analysis of acute toxicity, determined usually in the first step of tiered environmental risk assessment procedures. Bacterial bioassays are used particularly extensively for the assessment of acute toxicity as they are rapid, simple, and highly reproducible [27,28]. A worldwide used Microtox® toxicity test is based on the inhibition of bioluminescence produced by bacteria *Vibrio fischeri* (reclassified recently as *Allivibrio fischeri*) when exposed to toxic compounds present in water [28–30]. It can be applied for the risk assessment associated with rock, soil, and water pollution in metal ore mining sites. A suitability of this test for the assessment of As toxicity in environmental samples has already been confirmed; however, the reactions of *A. fischeri* to different As species, that is, As (III) versus As(V), proved to be strongly dependent on water pH [21,22,28,31]. Among the bacterial assays, a test MARA (the Microbial Assay for Risk Assessment), designed particularly for the examination of pharmaceuthicals and biocides, has also gained a common acceptance in environmental research [32,33]. The MARA is a multispecies assay based on the measurement of dehydrogenase activity, visualized by a reduction of tetrazolium red. The set of diverse microbial strains involves ten species of bacteria and additionally one species of yeast [34]. Among the acute and subchronic toxicity tests, there are a few plant-based assays. The most commonly used one is a Phytotox assay that measures a decrease in seed germination and the inhibition of root elongation of plant seedlings supplied with contaminated water or exposed to contaminated

soil compared with the control based on pure water [35–37]. The test was recommended as a good tool for screening the environmental risk caused by metals and metalloids, including As, in mine sites [31,35].

Toxicological bioassays provide much more reliable information on the effects exerted by environmental pollution on biota than the data on soil chemistry alone, as they are based on reactions of living organisms to the complex sets of factors that can act synergistically or antagonistically. For instance, soil fertilization causes the better supply of all biota with nutrients, apart from a possible release of As into soil pore water. The reactions of biota to those co-concurring factors are highly site-specific, and thus have not been well recognized yet. It should be stressed that fertilization is particularly needed in the case of poor soils, such as those that developed on tailings. This study was aimed to determine the ecotoxicological effects caused by mineral and organic fertilization of two kinds of soils strongly enriched in As. The analysis was based on the results of three commercially available bioassays related to the data illustrating pore water chemical composition, in particular the concentrations of As.

## 2. Materials and Methods

### *2.1. Experimental Design*

A representative soil material was collected from two meadows: (1) a fresh hay meadow, frequently flooded in the past with As-rich tailings, after which soils were mixed with tailings by plowing; and (2) a dry one, situated on a 2–3 m elevated plateau, built of almost pure tailings (Figure 1).

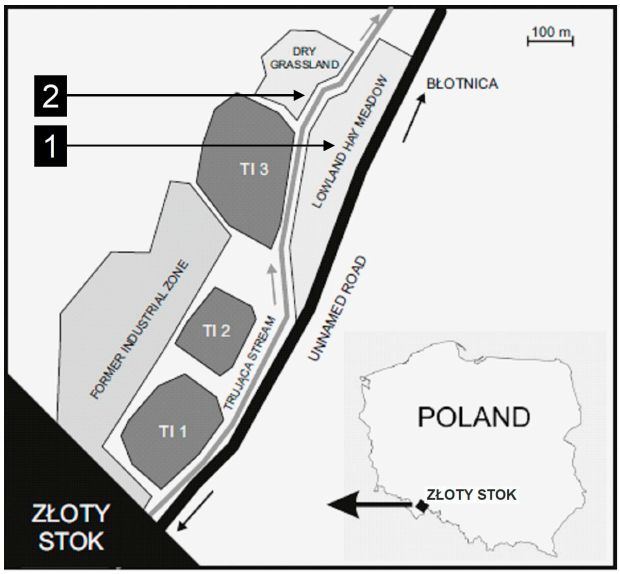


**Figure 1.** Location of the sites 1 and 2 under study. 1—Lowland hay meadow flooded by tailings. 2—Dry grassland on the plateau built of tailings. TI 1, TI 2, TI 3—tailings impoundments No 1, 2, 3, respectively. Błotnica—the name of a nearby village.

The material was crushed and sieved to <5 mm on site, and then transported to the laboratory, air-dried, and homogenized. A 270-day incubation experiment was carried out in 1 kg pots filled with untreated soils (0) or soils fertilized with inorganic fertilizers (F) or with a granulated cattle manure (M). Soil moisture was maintained at 70% of water holding capacity. Soil pore water was acquired after 2, 7, 21, 90, and 270 days of incubation, using MacroRhizon porous suction samplers, as described in previous papers [31,37]. Collected pore water samples were filtered (0.45 µm), examined on chemical properties (pH and As concentrations), and subjected to ecotoxicological assays. The experiment was carried out in three replicates. The data presented in diagrams illustrate the mean values and confidence intervals at $p = 0.95$.

### 2.2. Soil Properties

Basic soil properties were determined using representative aliquots of soils. Their texture was determined by a combined sieve and hydrometer method, and chemical analyses were performed with commonly used methods [38] after soil grinding to a fine powder (Table 1). Soil pH was measured in a suspension in 1 M KCl (1:2.5; *v*/*v*). Organic carbon (Corg) and carbonates were determined using CS-MAT 5500 analyzer (Strohlein), and dissolved organic carbon (DOC), extracted in cold and hot water [39], was measured on a TOC 5000 Shimadzu instrument. Total concentrations of metal(loid)s were determined after soil digestion with *aqua regia* in a microwave system. The concentrations of As, Cd, Cr, Cu, Mn, Ni, Pb and Zn in the digests were determined by ICP-AES (iCAP 7400, Thermo Fisher Scientific, Waltham, MA, USA). All analyses were made in triplicates. The accuracy of the results was checked with certified reference materials CRM 027 and CRM 059 supplied by Sigma-Aldrich. The mean recovery of As in CRMs was 98.3%, and 97.8%, respectively.

**Table 1.** Basic soil properties.

| **Site No** | | | | **1** | **2** |
|---|---|---|---|---|---|
| **Site description, settings** | | | | **Fresh meadow (4 ha) within the floodplain of the Trująca river, frequently flooded in the past by stormwater mixed with tailings (the distance from tailings impoundments: 0.2–1.0 km)** | **Foreland of tailings impoundment, an elevated plateau (1.6 ha) built of tailings** |
| Skeleton (>2 mm) | | | % | 21 | 0 |
| <0.002 mm | | | % | 6 | 3 |
| Textural group * (USDA) | | | - | SL | LS |
| Soil properties (fine soil) | Corg | | g/kg | 24.5 | 5.5 |
| | N total | | g/kg | 3.09 | 0.38 |
| | C:N | | - | 7.9 | 14.5 |
| | pH (1M KCl) | | - | 6.32 | 7.60 |
| | $CaCO_3$ | | % | absent | 2.1 |
| | As total | | mg/kg | 5020 | 8000 |
| | 1M $NH_4NO_3$ -extractable As | | mg/kg | 7.3 | 12.9 |
| | 0.43 M $HNO_3$ -extractable As | | mg/kg | 2720 | 5310 |
| | "Available" | P | mg/kg | 242 (very high) | 194 (very high) |
| | | K | mg/kg | 72 (low) | 131 (medium) |
| | | Mg | mg/kg | 245 (very high) | 76 (high) |

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

### 2.3. Fertilizers

Soil mineral fertilization (F) involved the application of soluble salts that contained macronutrients N, P, K, and Mg, at the amounts that corresponded to fertilization rates of 60, 60, 100, and 18 kg/ha, respectively (Table S1). In particular, the dose of P applied to soils in a soluble form was 50 mg/kg P. Granulated cattle manure (M) was applied at the rate 10 g/kg, corresponding to ca. 45 Mg/ha, expressed as a fresh matter. The manure had a neutral reaction (pH 7.3) and its dry matter contained 360 g/kg Corg, of which 38.2 and 75.3 g/kg was determined as cold-water- and hot-water-soluble Corg, respectively. Total concentrations of N, P, K, and Mg in manure dry matter were ca. 2, 4, 2, and 1%, respectively, and the related fertilization rates were 90, 180, 90, and 45 kg/ha, respectively. These amounts of nutrients were stepwise released from manure, thus they cannot be compared directly with the rates of mineral fertilization. Arsenic concentration in the manure was 10.7 mg/kg.

### 2.4. Chemistry of Soil Pore Water

Chemical analysis of soil pore water, acquired during the incubation experiment, involved potentiometrical determination of pH as well as the analysis of potentially toxic elements, including As and the following metals: Cd, Cu, Mn, Pb, and Zn (by ICP-AES, iCAP 7400, Thermo Scientific).

### *2.5. Bioassays*

Three bioassays, Microtox, MARA, and Phytotox, were applied to determine the ecotoxicity of pore water. All the measurements were performed based on a screening-mode of the tests, which means that undiluted pore water samples were analyzed directly after their collection. In parallel, the bioassays were performed with control solutions of As(III) and As(V) at various concentrations, prepared freshly of $NaAsO_2$ and $Na_2HAsO_4{\cdot}7H_2O$. More detailed description of bioassays was provided in a previous paper [31].

Briefly, a light output of the luminescent bacteria *Allivibrio fischeri* placed in soil pore water samples was compared with blank control samples. Luminescence inhibition was assessed after 5 and 15 min of exposure with a "81.9% basic test protocol" and the toxicity was expressed as percent effect (%). The measurements were carried out using a Microtox® M500 analyzer, and lyophilized bacteria, reagents, and consumables, produced by SDIX, were purchased from Tigret (Warszawa, Poland). All the measurements were performed in three replicates.

The Microbial Assay for Risk Assessment (MARA) was performed according to the standard protocol described by Wadhia [34]. The inhibition of the growth was determined in microplates for 11 lyophilized strains of microorganisms, that is, 10 species of bacteria and 1 yeast species: (11) *Pichia anomala*. The intensity of tetrazolium red, an indicator of microbial activity, was measured based on scanned images of microplates.

Subchronic phytoxicity of soil pore was determined using a Phytotoxkit assay, with *Sinapis alba* (L) as a test species. The inhibition of root elongation was measured after a 3-day incubation at 25 °C, in darkness, in comparison with the control based on pure water. Pictures of the test plates were taken by a digital camera, and the length of plant roots was measured manually.

### *2.6. Statistics*

The significance of differences between the treatments (0, F, M) and incubation periods (2, 7, 21, 90, and 270 days) was assessed with Fisher's least significant differences test ($p < 0.05$). Pearson correlation coefficients were calculated to examine the relationships between the pore water concentrations of elements and the results of bioassays. Where necessary, the data were normalized prior to analysis. Principal component analysis (PCA) was performed in order to examine the distribution pattern of results at a reduced number of variables. Only those principal components were considered that contributed to a total variance more than 10%. Statistical analyses were performed using a software Statistica, version 12.0 (Dell Inc., Round Rock, TX, USA).

## 3. Results

### *3.1. Soil Properties*

Soils 1 and 2 used in the experiment contained As in very high concentrations of 5020 and 8000 mg/kg, respectively. They had a neutral (soil 1) and alkaline (soil 2) pH, and differed considerably in other properties (Table 1). Soil 1 had a texture of sandy loam and contained a higher amount of clay fraction (6%) compared with soil 2 (loamy sand, 3% clay). It was also much richer in organic carbon (2.45% vs. 0.55%). Both soils were very rich in extractable, considered plant-available, P and Mg, but unlike the soil 2, which was collected from the floodplain (soil 1), were relatively poor in available K.

### *3.2. Pore Water Concentrations of As and Other Potentially Toxic Elements*

The concentrations of As in pore water collected from various treatments during the experiment were in the range of 4.5–81.8 mg/L (Figure 2), and were the highest within the first week of incubation. Generally, the maximum As concentrations in pore water of soil 2 were found after 2 days, while in the soil 1, they initially increased during the first week and reached the maximum after 7 days. Then, As concentrations in pore water started to consistently decrease; however, after 270 days, they still

remained two or three orders higher than 0.02 mg/L, a threshold value set by the Polish law for good quality underground water.

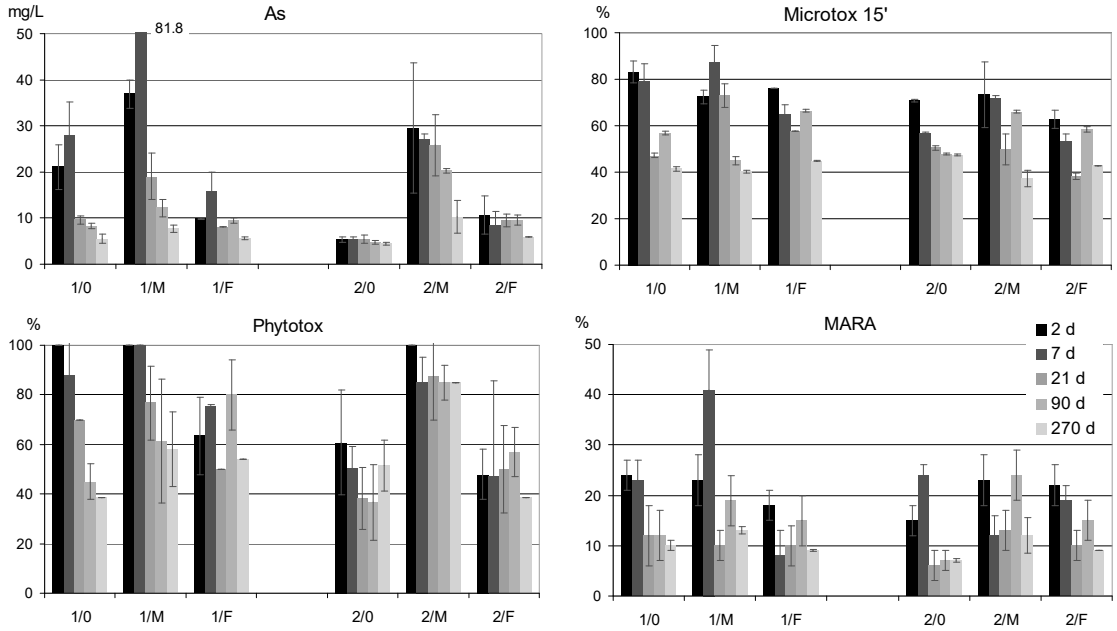


**Figure 2.** Arsenic concentrations in soil pore water and the results of bioassays, determined after 2, 7, 21, 90, and 270 days of incubation. 1, 2—soil identifiers; 0, M, F—treatments: 0—no addition, M—manure, F—mineral fertilization. The data are mean values of three replicates. Error bars stand for confidence intervals, $p = 0.95$.

The concentrations of As in pore water of untreated soil 1 (1/0) were, particularly at the beginning of incubation, much higher than those in pore water of untreated soil 2 (2/0), despite its lower total and 1M $NH_4NO_3$-extractable As, as well as lower pH value. The difference between the As level in two untreated soils tended to decrease with time. We can hypothesize that the factor responsible for much more intensive release of As from the solid phase of soil 1, the one that originated from the habitat of a fresh lowland meadow, was its higher biological activity and more intensive processes of organic matter transformation that started once soil became moistened. Discharge of mineral compounds, including phosphates, as well as low molecular organic compounds, might have stimulated a release of As from the solid phase. Soil treatment with manure (M) caused, in both soils, an intensive release of As in the first week, and its concentrations in pore water remained significantly higher compared with non-amended soils (0) until the end of the experiment. An extremely high As concentration in pore water, 81.8 mg/L, was reported from M-treated soil 1 after the 7-day incubation. This value was much higher than the concentrations of As in pore water of mine dumps [31]. The effects caused by mineral fertilization (F) were ambiguous. Rather unexpectedly, As concentrations in pore water of fertilized soil 1 at the beginning of incubation were significantly lower compared with 0 treatment, whereas an opposite effect was observed in soil 2, in which the application of fertilizers caused a considerable release of As into pore water, and its concentrations throughout the whole experiment were significantly higher in F treatment than those in untreated soil.

The release of potentially toxic metals (Cd, Cu, Pb, Zn, Mn, Fe) into pore water was negligible, and their concentrations remained below the threshold values of good quality underground water, except for a temporally enhanced Mn level in soil pore water of manure-treated soil 1, which might have been associated with a local drop in redox potential [31]. Pore water concentrations of both Cd and Pb remained below detection limits of ICP-AES (0.005 and 0.008 mg/L, respectively) during the whole incubation. Soil treatment with manure caused a detectable release of Cu into pore water, likely owing to the formation of soluble organic complexes [37]. In all cases when the concentrations

of Cu in pore water were measureable, they in fact remained very low, below 0.2 mg/L (Table S2). An apparent decrease in concentrations of potentially toxic elements during a prolonged incubation can be explained by an effect of their aging [37,40].

The mechanisms of As aging in soils were extensively reported in the literature, mainly based on the experiments with spiked soil material. Wang et al. [41,42] proved that the content of amorphous and free Fe oxides and soil pH were the main factors controlling the speed of As aging. The main mechanisms of aging processes involve the formation of inner-sphere complexes with amorphous and free Fe oxy-hydroxides through the ligand exchange to surface hydroxyl groups, diffusion within micropores, or surface precipitates [41–43]. These processes depend on soil pH and redox conditions, and run particularly quickly in oxidized acidic soils. Neutral pH of soils in this experiment should be considered as a factor responsible for the long-lasting presence of relatively high As concentrations in soil pore water.

### *3.3. Bioassays*

The assessment of pore water ecotoxicity, determined with Microtox and Phytotox assays, proved very strong adverse effects to both bacteria and germinating plants. These two assays indicated a very high toxicity of all the samples of pore water examined in the experiment. At the beginning of incubation, the toxicity of pore water in untreated soil 1 was much higher compared with that in soil 2, according to higher As concentrations. The results obtained with Microtox procedure after two standardized times, 5′ and 15′, were highly correlated, with R = 0.988 (Table 2), which indicates that the main factor of toxicity had an inorganic nature [24].

**Table 2.** Pearson correlation coefficients illustrating the relationships between As concentrations in soil pore water and the results of ecotoxicity assays. MARA, the Microbial Assay for Risk Assessment.

| Parameter | Microtox 5′ | Microtox 15′ | Phytotox | MARA, Toxicity | MARA, Growth of Strain 11 |
|---|---|---|---|---|---|
| As in soil pore water (mg/L) | 0.593 | 0.632 | 0.716 | 0.719 | −0.447 |
| Microtox 5′ | x | 0.988 | 0.586 | 0.563 | −0.484 |
| Microtox 15′ | x | x | 0.648 | 0.599 | −0.522 |
| Phytotox | x | x | x | 0.541 | −0.552 |
| MARA, toxicity | x | x | x | x | −0.591 |

Soil toxicity assessed in the light of the Phytotox assay (considering both seed germination and root elongation indices) reached 100% in all the samples with As concentrations above 20 mg/L (Figure 2). This effect stays in agreement with our previous observations that As concentrations of 20 mg/L posed a strong phytotoxic effect on the early growth of *Sinapis alba* [31]. It is also consistent with the results of several other studies that found this level of As in aqueous solutions is strongly toxic to the majority of aquatic and soil biota [4,28,44]. The relationships between As concentrations in pore water and its toxicity determined by Microtox and Phytotox corresponded well with the curves obtained for pure As(V) solutions (Figure 3). As stressed in the previous paper [31], the toxicity of pure As(V) solution turned out to be higher than that of As(III), which may seem inconsistent with general knowledge on As toxicity. In fact, similar observations were also described by Mamindy-Pajany [45], Rubinos [28], and Fulladosa [21], who compared toxic effects of As(V) and As(III), and found their dependence on pH and the ranges of concentrations.

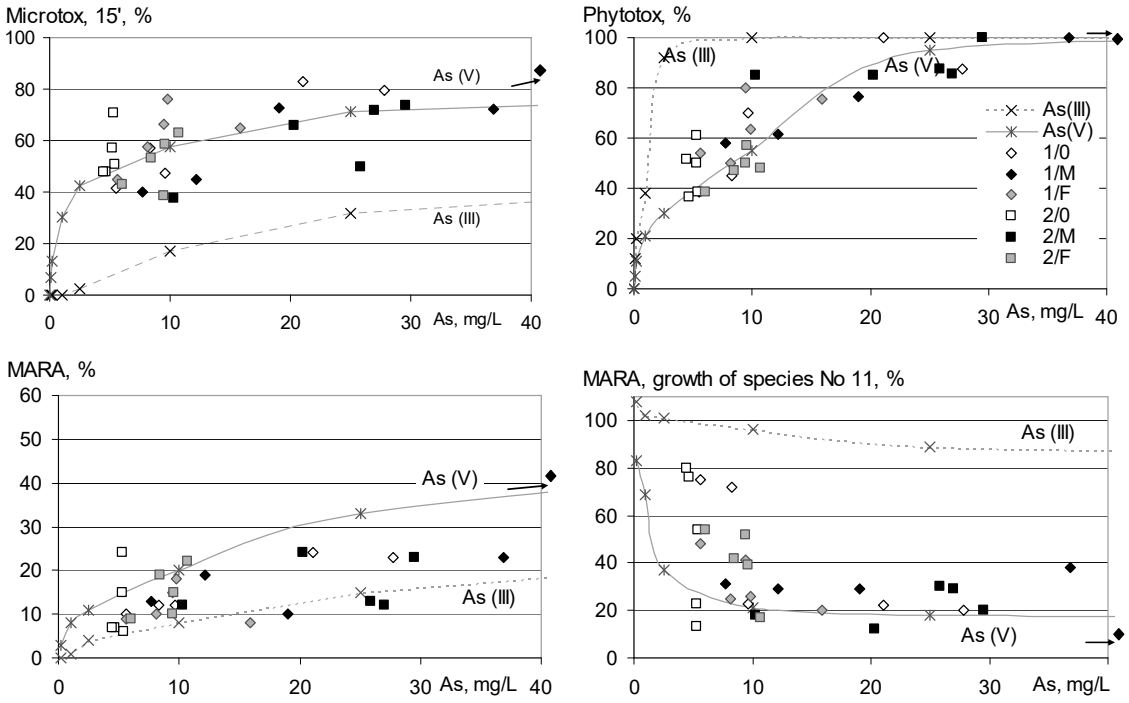


**Figure 3.** Results of bioassays related to the concentrations of As in soil pore water. The data obtained for two soils (1 and 2), all treatments (0, M, F), and five incubation times. The curves illustrate control results obtained for standard solutions of As(III) and As(V). All data are the means of three replicates.

The indices of pore water toxicity, determined with the bioassay MARA, were in general much lower than those measured with two other bioassays. In no cases were the indices of toxicity produced by MARA higher than 50%, and they usually remained below 20% (Figure 2). Although being in line with the results of Microtox and Phytotox, the toxicity indices produced by MARA were the highest within the first week of incubation and tended to considerably decrease along with increasing incubation time. Moreover, contrary to our previous study, a statistical analysis of all the results indicated that the results of MARA correlated better with As concentrations in pore water than the results of Microtox bioassay (Table 2). Basically, graphically illustrated relationships between all the toxicity indices and As concentrations in pore water followed the standard curves obtained for As(V), as shown in Figure 3. In the case of MARA, however, particularly within a range of higher As concentrations, our experimental results were considerably lower compared with the standard curve. Apparently, some protective effects caused by the components of pore water were involved that reduced As toxicity to the strains of microbes used in the assay. At the scarcity of related bibliographic data on this issue, a systematic series of experiments with standard As solutions amended with various additives would be necessary to more closely determine those effects. Unlike in the study on mine waste dump material [31], in the present research, the growth of a yeast strain *Pichia anomala* (No 11 in the MARA assay) rather poorly corresponded with the curve obtained for the pure standard As (V) (Figure 3), and a visible effect of *Pichia anomala* growth stimulation by pore water components occurred, particularly at lower As concentrations. A closer analysis of this effect might be interesting, and should be the matter of a separate study.

### *3.4. General Considerations*

Pearson correlation coefficients R were calculated to illustrate the relationships between the (normalized) results of all three toxicity tests—Microtox, Phytotox, and MARA—and As concentrations in soil pore water. The relationships were significant at $p < 0.001$, and the R ranged from 0.541 to 0.719 (Table 2), which means that the results of ecotoxicity assays, with a probability of 29–52% ($R^2$), were determined by As pore water concentrations. In the case of *Pichia anomalia*, an absolute value

of corresponding R was relatively low: 0.407. A principal component analysis (PCA) confirmed close relationships between the indices of toxicity obtained in various assays and As concentrations. A related graph (Figure 4) illustrates a particular interdependence between the result of MARA, Phytotox, and As in pore water, which were similarly determined by two main principal components 1 (67.8%) and 2 (11.9%). The latter, in fact of relatively small importance, was apparently the factor responsible for different responses of Microtox (both after 5 and 15 min) and two other assays to the same level of As in pore water. It was probably a set of pore water components, including the concentrations of phosphates and low weight organic compounds, which were not directly determined in this study, that modified the toxicity of As. Rubinos et al. (2014) proved, for instance, that P presence in water could protect aquatic microorganisms against As(V) toxicity. Similar conclusions were drawn from the studies related to higher plants [3,6,9].

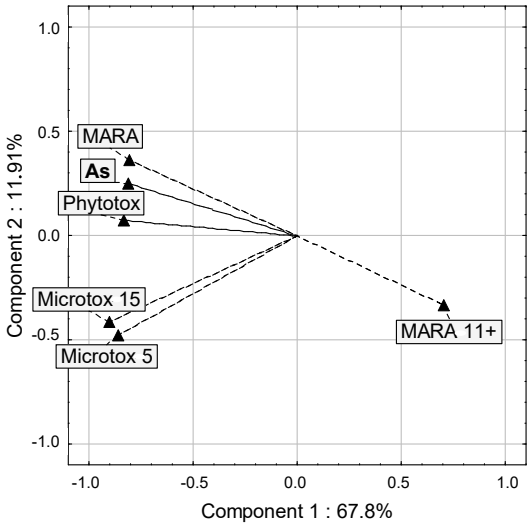


**Figure 4.** The results of principal component analysis (PCA) performed for soils 1 and 2.

In general, however, our experiment proved a very high ecotoxicity of soils developed of pure tailings (soil 2), as well as those flooded by tailings and plowed thereafter (soil 1). A high concentration of As in soil pore water was confirmed to be the main factor of toxicity to both bacteria and germinating plants. The toxicity was particularly strong in the first week after soil treatment with manure or—in the case of soil 2—also with inorganic fertilizers. This fact should be considered when planning remediation measures for the areas affected by the tailings produced in the past by arsenic ore mining in Złoty Stok.

## 4. Conclusions

Pore water of dry meadow soil, developed of almost pure tailings, contains high concentrations of As that never drop below 4.5 mg/L. Fertilization of that soil, particularly with manure, as well as mineral fertilization, causes an additional increase in As level in soil pore water.

Pore water in soil of lowland meadow, formerly flooded with tailings, contains even higher concentrations of As and can be even more toxic compared with soil developed of tailings. Its toxicity to bacteria and plant seedlings, particularly after soil treatment with manure, can increase to 100%. The hypothesis that this effect may be caused by a higher biological activity of soil needs closer examination when considering phytostabilization as a way of land remediation.

Very high phytotoxicity, above 80%, assessed by the Phytotox assay, occurs when pore water concentrations of As exceed 20 mg/L. This level of As in soil pore water can also strongly inhibit the activity of various bacteria strains.

In the absence of high concentrations of other toxic components in soil solutions, the results of all bioassays, particularly those of Phytotox and MARA, correlate very well with the concentrations of As.

The differences in toxicity between As(III) and As(V), which, in this study, were only partly in line with common toxicological knowledge, indicate the importance of As speciation when determining the risk associated with the presence of As in the environment.

An effect of As "aging" develops after long lasting soil incubation at moderate moisture (70%). It should be stressed, however, that As concentrations in pore water of both soils examined remained very high, at the level of several mg/L, which is much higher than that considered environmentally safe.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2075-163X/10/9/751/s1, Table S1: The components of solution applied to soils for mineral fertilization (F), and corresponding fertilization rates; Table S2: Chemistry of soil pore water. The values of pore water pH and the concentrations of As and potentially toxic metals.

**Author Contributions:** Conceptualization, A.D. and A.K.; methodology, A.D.; software, A.D. and A.K.; validation, A.K. and K.S.; formal analysis, A.D. and K.S.; investigation, A.D., K.S. and A.K.; resources, A.D.; data curation, A.D. and K.S.; writing—original draft preparation, A.D.; writing—review and editing, A.K. and K.S.; visualization, A.D. and K.S.; supervision, A.K.; project administration, A.K. and A.D.; funding acquisition, A.K., K.S. and A.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by National Science Centre Of Poland, grant number 2016/21/B/ST10/02221. The APC was financed from the same project.

**Acknowledgments:** We express special thanks to Bernard Gałka for his engagement and technical help in all the field and laboratory works, as well to Bogna Kosyk for her assistance in performing bioassays.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Karczewska, A.; Krysiak, A.; Mokrzycka, D.; Jezierski, P.; Szopka, K. Arsenic distribution in soils of former as mining area and processing. *Pol. J. Environ. St.* **2013**, *22*, 175–181.
2. Kaźmierczak, U.; Strzałkowski, P.; Lorenc, M.W.; Szumska, E.; Sánchez, A.A.P.; Baker, K.A. Post-mining Remnants and Revitalization. *Geoheritage* **2019**, *11*, 2025–2044. [CrossRef]
3. Dradrach, A.; Karczewska, A.; Szopka, K.; Lewińska, K. Accumulation of arsenic by plants growing in the sites strongly contaminated by historical mining in the Sudetes region of Poland. *Int. J. Environ. Res. Public Health* **2020**, *17*, 3342. [CrossRef]
4. Wenzel, W.W. Arsenic. In *Heavy Metals in Soils. Trace Metals and Metalloids in Soils and Their Bioavailability*, 3rd ed.; Alloway, B.J., Ed.; Springer: Berlin, Germany, 2013; pp. 241–282.
5. Lewińska, K.; Karczewska, A.; Siepak, M.; Gałka, B. Potential of Fe-Mn wastes produced by a water treatment plant for arsenic immobilization in contaminated soils. *J. Geochem. Explor.* **2018**, *184*, 226–231. [CrossRef]
6. Bolan, N.S.; Mahimairaja, S.; Kunhikrishnan, A.; Choppala, G. Phosphorus–arsenic interactions in variable-charge soils in relation to arsenic mobility and bioavailability. *Sci. Total Environ.* **2013**, *463*, 1154–1162. [CrossRef]
7. Anawar, H.M.; Rengel, Z.; Damon, P.; Tibbett, M. Arsenic-phosphorus interactions in the soil-plant-microbe system: Dynamics of uptake, suppression and toxicity to plants. *Environ. Pollut.* **2018**, *233*, 1003–1012. [CrossRef]
8. Li, S.W.; Liu, X.; Sun, H.J.; Li, M.Y.; Zhao, D.; Luo, J.; Li, H.B.; Ma, L.Q. Effect of phosphate amendment on relative bioavailability and bioaccessibility of lead and arsenic in contaminated soils. *J. Hazard. Mater.* **2017**, *339*, 256–263. [CrossRef]
9. Lewińska, K.; Karczewska, A. Influence of soil properties and phosphate addition on arsenic uptake from polluted soils by velvetgrass (*Holcus lanatus*). *Int. J. Phytoremediat.* **2013**, *15*, 91–104. [CrossRef]
10. Wang, J.; Zeng, X.; Zhang, H.; Li, Y.; Zhao, S.; Su, S.; Zhang, T. Effect of exogenous phosphate on the lability and phytoavailability of arsenic in soils. *Chemosphere* **2018**, *196*, 540–547. [CrossRef]
11. Beesley, L.; Inneh, O.S.; Norton, G.J.; Moreno-Jimenez, E.; Pardo, T.; Clemente, R.; Dawson, J.J. Assessing the influence of compost and biochar amendments on the mobility and toxicity of metals and arsenic in a naturally contaminated mine soil. *Environ. Pollut.* **2014**, *186*, 195–202. [CrossRef]
12. Xie, H.; Han, D.; Cheng, J.; Zhou, P.; Wang, W. Fate and risk assessment of arsenic compounds in soil amended with poultry litter under aerobic and anaerobic circumstances. *Water Air Soil Pollut.* **2015**, *226*, 1–11. [CrossRef]

13. Karczewska, A.; Gałka, B.; Dradrach, A.; Lewińska, K.; Mołczan, M.; Cuske, M.; Gersztyn, L.; Litak, K. Solubility of arsenic and its uptake by ryegrass from polluted soils amended with organic matter. *J. Geochem. Explor.* **2017**, *182*, 193–200. [CrossRef]
14. Moreno-Jiménez, E.; Clemente, R.; Mestrot, A.; Meharg, A.A. Arsenic and selenium mobilisation from organic matter treated mine spoil with and without inorganic fertilization. *Environ. Pollut.* **2013**, *173*, 238–244. [CrossRef]
15. Karczewska, A.; Lewińska, K.; Siepak, M.; Gałka, B.; Dradrach, A.; Szopka, K. Transformation of beech forest litter as a factor that triggers arsenic solubility in soils developed on historical mine dumps. *J. Soils Sed.* **2018**, *18*, 2749–2758. [CrossRef]
16. Lewińska, K.; Karczewska, A.; Siepak, M.; Szopka, K.; Gałka, B.; Iqbal, M. Effects of waterlogging on the solubility of antimony and arsenic in variously treated shooting range soils. *Appl. Geochem.* **2019**, *105*, 7–16. [CrossRef]
17. Pigna, M.; Cozzolino, V.; Violante, A.; Meharg, A.A. Influence of phosphate on the arsenic uptake by wheat (*Triticum durum* L.) irrigated with arsenic solutions at three different concentrations. *Water Air Soil Pollut.* **2009**, *197*, 371–380. [CrossRef]
18. Wang, S.; Mulligan, C.N. Effect of natural organic matter on arsenic release from soil and sediments into groundwater. *Environ. Geochem. Health* **2006**, *28*, 197–214. [CrossRef]
19. Zhao, F.J.; Ma, J.F.; Meharg, A.A.; McGrath, S.P. Arsenic uptake and metabolism in plants. *New Phytol.* **2009**, *181*, 777–794. [CrossRef]
20. Rocha, L.; Rodrigues, S.M.; Lopes, I.; Soares, A.M.V.M.; Duarte, A.C.; Pereira, E. The water-soluble fraction of potentially toxic elements in contaminated soils: Relationships between ecotoxicity, solubility and geochemical reactivity. *Chemosphere* **2011**, *84*, 1495–1505. [CrossRef]
21. Fulladosa, E.; Murat, J.C.; Martínez, M.; Villaescusa, I. Patterns of metals and arsenic poisoning in *Vibrio fischeri* bacteria. *Chemosphere* **2005**, *60*, 43–48. [CrossRef]
22. Villaescusa, I.; Bollinger, J.C. Arsenic in drinking water: Sources, occurrence and health effects (a review). *Rev. Environ. Sci. Bio/Technol.* **2008**, *7*, 307–323. [CrossRef]
23. Paustenbach, D. (Ed.) *Human and Ecological Risk Assessment: Theory and Practice (Wiley Classics Library)*; Wiley: Hoboken, NY, USA, 2015.
24. Doherty, F. A review of the Microtox® toxicity test system for assessing the toxicity of sediments and soils. *Water Qual. Res. J.* **2001**, *36*, 475–518. [CrossRef]
25. Kokkali, V.; van Delft, W. Overview of commercially available bioassays for assessing chemical toxicity in aqueous samples. *TrAC Trends Anal. Chem.* **2014**, *61*, 133–155. [CrossRef]
26. Karczewska, A.; Kabała, C. Environmental risk assessment as a new basis for evaluation of soil contamination in Polish law. *Soil Sci. Ann.* **2017**, *68*, 67–80. [CrossRef]
27. Parvez, S.; Venkataraman, C.; Mukherji, S. A review on advantages of implementing luminescence inhibition test (*Vibrio fischeri*) for acute toxicity prediction of chemicals. *Environ. Int.* **2006**, *32*, 265–268. [CrossRef]
28. Rubinos, D.A.; Calvo, V.; Iglesias, L.; Barral, M.T. Acute toxicity of arsenic to *Aliivibrio fischeri* (Microtox® bioassay) as influenced by potential competitive–protective agents. *Environ. Sci. Pollut. Res.* **2014**, *21*, 8631–8644. [CrossRef]
29. Abbas, M.; Adil, M.; Ehtisham-ul-Haque, S.; Munir, B.; Yameen, M.; Ghaffar, A.; Iqbal, M. *Vibrio fischeri* bioluminescence inhibition assay for ecotoxicity assessment: A review. *Sci. Total Environ.* **2018**, *626*, 1295–1309. [CrossRef]
30. Urbanczyk, H.; Ast, J.C.; Higgins, M.J.; Carson, J.; Dunlap, P.V. Reclassification of *Vibrio fischeri*, *Vibrio logei*, *Vibrio salmonicida* and *Vibrio wodanis* as *Aliivibrio fischeri* gen. nov., comb. nov., *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2823–2829. [CrossRef]
31. Dradrach, A.; Szopka, K.; Karczewska, A. Ecotoxicity of soil pore water on historical arsenic mine dumps—The effects of forest litter. *Ecotoxicol. Environ. Saf.* **2019**, *181*, 202–213. [CrossRef]
32. Fai, P.B.; Grant, A. An assessment of the potential of the microbial assay for risk assessment (MARA) for ecotoxicological testing. *Ecotoxicology* **2010**, *19*, 1626–1633. [CrossRef]
33. Oleszczuk, P.; Jośko, I.; Kuśmierz, M.; Futa, B.; Wielgosz, E.; Ligęza, S.; Pranagal, J. Microbiological, biochemical and ecotoxicological evaluation of soils in the area of biochar production in relation to polycyclic aromatic hydrocarbon content. *Geoderma* **2014**, *213*, 502–511. [CrossRef]

34. Wadhia, K.; Dando, T.; Clive-Thompson, K. Intra-laboratory evaluation of Microbial Assay for Risk Assessment (MARA) for potential application in the implementation of the Water Framework Directive (WFD). *J. Environ. Monit.* **2007**, *9*, 953–958. [CrossRef] [PubMed]
35. García-Lorenzo, M.; Martinez-Sanchez, M.; Perez-Sirvent, C.; Molina, J. Ecotoxicological evaluation for the screening of areas polluted by mining activities. *Ecotoxicology* **2009**, *18*, 1077–1086. [CrossRef] [PubMed]
36. Baran, A.; Antonkiewicz, J. Phytotoxicity and extractability of heavy metals from industrial wastes. *Environ. Prot. Eng.* **2017**, *43*, 143–155. [CrossRef]
37. Cuske, M.; Karczewska, A.; Matyja, K.; Gałka, B. Ecotoxicity and phytotoxicity of soil solutions extracted from Cu-contaminated soils amended with organic waste materials. *Fresenius Environ. Bull.* **2017**, *26*, 1163–1173.
38. Tan, K. *Soil Sampling, Preparation, and Analysis*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2005.
39. Gregorich, E.G.; Beare, M.H.; Stoklas, U.; St-Georges, P. Biodegradability of soluble organic matter in maize-cropped soils. *Geoderma* **2003**, *113*, 237–252. [CrossRef]
40. Smolders, E.; Oorts, K.; Van Sprang, P.; Schoeters, I.; Janssen, C.R.; Mcgrath, S.P.; Mclaughlin, M.J. Toxicity of trace metals in soil as affected by soil type and aging after contamination: Using 295 calibrated bioavailability models to set ecological soil standards. *Environ. Toxicol. Chem.* **2009**, *28*, 1633. [CrossRef]
41. Wang, Y.N.; Zeng, X.B.; Lu, Y.H.; Su, S.M.; Bai, L.Y.; Li, L.F.; Wu, C.X. Effect of aging on the bioavailability and fractionation of arsenic in soils derived from five parent materials in a red soil region of Southern China. *Environ. Pollut.* **2015**, *207*, 79–87. [CrossRef]
42. Wang, Y.N.; Zeng, X.B.; Lu, Y.H.; Bai, L.Y.; Su, S.M.; Wu, C.X. Dynamic arsenic aging processes and their mechanisms in nine types of Chinese soils. *Chemosphere* **2017**, *187*, 404–412. [CrossRef]
43. Juhasz, A.L.; Smith, E.; Weber, J.; Naidu, R.; Rees, M.; Rofe, A.; Sansom, L. Effect of soil ageing on in vivo arsenic bioavailability in two dissimilar soils. *Chemosphere* **2008**, *71*, 2180–2186. [CrossRef]
44. Abedin, M.J.; Meharg, A.A. Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L.). *Plant Soil* **2002**, *243*, 57–66. [CrossRef]
45. Mamindy-Pajany, Y.; Hurel, C.; Géret, F.; Galgani, F.; Battaglia-Brunet, F.; Marmier, N.; Roméo, M. Arsenic in marine sediments from French Mediterranean ports: Geochemical partitioning, bioavailability and ecotoxicology. *Chemosphere* **2013**, *90*, 2730–2736. [CrossRef] [PubMed]