# Metalloid Contaminated Microhabitats and their Biodiversity at a Former Antimony Mining Site in Schlaining, Austria

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**Abstract:** This paper is on the biological impact of arsenic and antimony on the flora and microflora on a former Sb-mining site in Schlaining (Stadtschlaining, Burgenland, Austria). Several habitats were investigated with respect to biodiversity and metalloid contamination in soil. Although the overburden of the mining activity had been remediated less than ten years ago, metalloid concentrations occurred in soil up to 1.4‰ As and 3.6% Sb, respectively, in some microhabitats, as determined by Instrumental Neutron Activation Analysis. These metalloids were embedded into a nonuniform mineralogical background. Metalloid mobility could not be explained by common models, indicating that predictions on the mobility of geogenic metalloids require additional mineralogical data. The biological effects of this contamination were variable. We observed that metalloid resistant strands of microorganisms appeared in the contaminated soil. In cultivation experiments, Sb was found to be more toxic than As. Sulphur oxidising strand were more resistant than organotrophic ones and grew even better on cultivation media spiked with 10 ppm As than on the unspiked control. The flora was only partially influenced: the lowest biodiversity was found in metalloid richest soils, but moderate contamination resulted in enhanced species numbers. Only in one case, where the pH-buffering capacity of the soil was exceeded by consumption of the entire carbonate, no embryophytes occurred. This was probably due to extreme pH conditions as well as to metalloid concentrations. Our data support the hypothesis that higher plants are rather affected by extreme soil conditions, which often coincide with As contaminations, than by the contamination itself. A small rivulet in this area contained 26 µg/l and thus exceeded the WHO guideline value for As in drinking water by a factor of 2.6. Indeed we observed a diminished biodiversity in this rivulet.

**Keywords:** Soil antimony, soil arsenic, INAA, metalloid resistance, metallophytes, mine waste.

## 1. INTRODUCTION

### 1.1. The Antiomony Mining Site at Schlaining

Antimony (Sb) is a metalloid with various technological applications such as the production of flame-retardants and lead-acid batteries, as well as applications in the transportation, chemical, ceramics, and glass industries. In terms of its chemical properties, antimony is related to arsenic (As), its neighbour in the periodic table. Therefore both elements often occur simultaneously and geochemically often react in a similar way. From the environmental point of view, investigations on antimony are increasing because it has been identified as an important global pollutant [1].

In the mine in Schlaining (Stadtschlaining, Burgenland, Austria), antimony has been mined for more than 200 years. During that time, Austria was one of the main antimony producing countries worldwide due to the productivity of this mine. In the late 1990s, the ore has been depleted and consequently, the mine had to be abandoned in 1999. For more information on socioeconomic aspects of antimony mining in Stadtschlaining, compare Halisch [2].

The main antimony-bearing mineral mined in Schlaining was stibnite (antimonite, $Sb_2S_3$). In petrological investigations, however, also arsenopyrite (FeAsS) and pyrite ($FeS_2$) were found [3]. Some other ore minerals can be found to a minor extent, including chalcopyrite, cinnabar or sphalerite. The occurrence of the oxides of these ores has been reported in literature as well [4]. The geological setting of the mine has been classified into four lithological categories [5]: (1) Sb-mineralised limestone and calcareous slates; (2) Sb-ore-free limestone and mylonites; (3) Sb-ore-free calcareous phyllites and calcareous slates; (4) limestone. The origin of the Sb ore is not yet entirely clarified, but probably due to the deposition of hydrothermal, metalloid bearing solutions [4,6]. In the course of mining activity, substantial amounts of the waste rock have been deposited in the vicinity of the mine. These waste sites were remediated in the early 2000s [7] (see section *2.1*).

Cerny [5] provides chemical analyses of the rocks forming the geological setting in Schlaining: the Sb-ore bearing calcareous slates and limestone contain in average 4.4% per weight Sb, 6.1% Fe, and 3000 $mg \cdot kg^{-1}$ As (up to maximum

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values of 1.2% As). Sb-ore free limestone and mylonites contain 56 $mg \cdot kg^{-1}$ Sb, 185 $mg \cdot kg^{-1}$ As and 2.0% Fe; Sb-ore-free calcareous phyllites and calcareous slates contain 35 $mg \cdot kg^{-1}$ Sb, 42 $mg \cdot kg^{-1}$ As and 1.7% Fe; limestone from the bedrock in this site contain 107 $mg \cdot kg^{-1}$ Sb, 60 $mg \cdot kg^{-1}$ As and 0.8% Fe.

### 1.2. Toxicological Aspects of the Metalloids Arsenic and Antimony

To humans, antimony species are almost as poisonous as the respective arsenic compounds, when injected. When ingested, however, antimony is not taken up by the human gastrointestinal tract as efficiently as arsenic. This is also reflected by the guideline values of the WHO for potable water, which are 10 $\mu g \cdot l^{-1}$ for As and 50 $\mu g \cdot l^{-1}$ for Sb (provisional guideline value). According to the WHO, the term "provisional guideline value" is used for *constituents for which there is some evidence of a potential hazard but where the available information on health effects is limited* [8]. Indeed, many aspects of the toxicology, the metabolism, the bioinorganic chemistry and the geochemical cycle of antimony are not well understood yet [1,9]. Many As compounds are known to cause cancer, including the As species occuring in contaminated drinking water. The WHO guideline value for potable water mentioned above, reflects an excess skin cancer risk of $6 \cdot 10^{-4}$.

### 1.3. Aims of the Study

The former mining site at Schlaining was investigated with respect to metalloid contamination and its biological effects. In particular, the following topics were studied:

- The efficiency of the renaturation measures at Schlaining as well as occurrence and bioavailability of ore-derived As and Sb in several sites in the vicinity of the mine.
- Effects of As and Sb on growth and diversity of higher plants and soil microorganisms at the studied contaminated habitats as well as the resistance of microorganisms against toxic metalloids and their participation in the oxidation (and thus mobilisation) of sulphidic ores.
- Soil properties of selected sites in this area with respect to factors such as inorganic nutrients, metalloid concentrations, humus content, pH, clay content, mineralogical and petrological composition, and its consequences to metalloid mobility (The term "mobile" is defined as the water extractable percentage which is readily available to all organisms [10].). In general, heavy metal contaminated habitats are also affected by acidic soils [11].
- Some fundamental aspects of the role of streaming waters in the leaching of As and Sb from the spoil heaps of the former mine.

## 2. MATERIALS AND METHODOLOGY

### 2.1. Sampling Sites

A large number of sites was tested for As and Sb throughout the former mining area. Of these, four contaminated sites were selected for closer investigation. A map showing the sampling sites in the vicinity of Stadtschlaining (Burgenland/Austria) is shown in Fig. (**1**).

- **A**: Spoil heap, exposed to the west, located at 47° 20' 28,1" N; 16° 16' 48,9" E; 370 m a.s.l. In the course of remediation of the mining site, the mine's overburden was covered ("renaturated") with unpolluted soil in the early 2000s. The recent vegetation is a meadow with sporadic trees. At some areas, bold patches are found and the vegetation is partly chlorotic. Here, three microhabitats were sampled, two (A1 and A2) with a vegetation coverage of almost 100%, one (A3) including sporadic bold patches.
- **B**: On the same spoil heap, close to Site A. Here, the coverage of unpolluted soil was removed in the course of the construction of a street. The mine waste is open now, forming a steep slope exposed to the west. The site is shadowed by trees growing nearby, but only sparsely covered by plants (B1).
- **C**: Wild boars' muddy wallow in a forest, located at 47° 20' 52,3" N; 16° 16' 21,3" E; 326 m a.s.l. This side of the valley had been the dumpsite of the mine's overburden until around the beginning of the 20th century [7]. The wallow is filled with grey mud without any vegetation and obviously used by wild boars. The surrounding forest is dominated by *Picea abies*. The understorey is sparse. Both the mud of the wallow (C1) and the soil of the surrounding forest (C2) were sampled.
- **D**: Small rivulet, surrounded by a meadow, both shadowed by trees, located at 47° 20' 59,9" N; 16° 16' 00,5" E; 330 m a.s.l at the hamlet of Sauerbrunn. We sampled both the rivulet (D1; sediment and water) and the soil of the meadow (D2).

Plants were collected in two series, one in the beginning of May and the other one in the middle of June 2008, in order to consider seasonal changes of vegetation. The sampled sites were chosen after a geological introduction and explanation by Dr. Josef Hofer and after a first botanical inspection, looking for typical representatives of heavy metal resistant plants. The sizes of the sites varied from large meadows to small outcrops next to a street. Accordingly, the size of the sampled areas varied considerably and depended on the area covered by a uniform vegetation. In any case, the largest-possible area has been attempted to be sampled representatively. In both sampling events, the same areas were sampled. We focused on vascular plants and mosses. Lichens were absent at all sites. Data on fungi and soil algae will be topic of later publications.

### 2.2. Soil Chemistry

Soil samples were taken from a depth of 0 to 20 cm, corresponding to the depth of root penetration in the soil. Samples were kept a 4 °C until analysis. For analysis, fresh soil was sieved and the < 2 mm fraction was used in order to separate plants, roots and large rocks from the soil[1]. Dry

[1] In C1 and D2, the high stability of soil aggregates made it impossible to distinguish between the < 4 mm and the < 2 mm fraction.

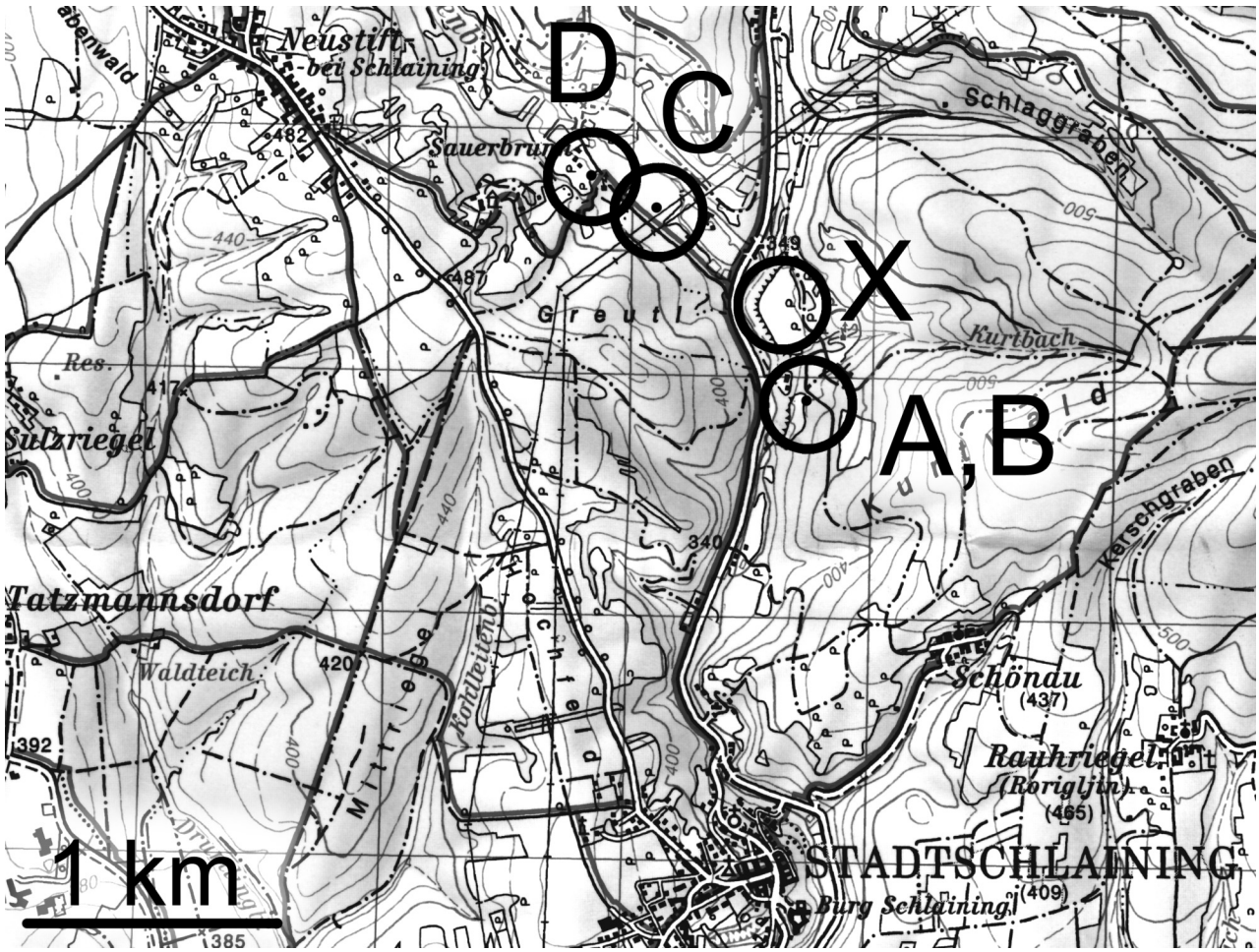


**Fig. (1).** Sampling sites **A** - **D** near the city of Schlaining. X indicates the former mining site (Based on [80]).

weight content was determined by drying samples of fine soil at 70 °C until constant weight.

Actual soil pH was determined using a Voltcraft PH-100ATC electrode in a 25% pure water extract; for potential pH, extracts with 0.01 M $CaCl_2$ and 1 M KCl were used.

Phosphate was extracted overnight using 200 ml 0.5 M $NaHCO_3$ (pH 8.5) per 10 g soil according to [12,13]. Dissolved phosphate was determined photometrically using Merck 1.14543.0001.

Sulphate, ammonium and nitrate were extracted from 75 g dry soil overnight using 300 ml of a 0.0125 M $CaCl_2$ solution [14,15]. Sulphate was quantified by densitometry using Merck 1.14548.0001, both ammonium (Merck 1.14739 .0001) and nitrate (Merck 1.14542.0001) by photometry. Iron was extracted overnight using 100 ml 0.1 M $Na_2S_2O_4$ + 0.57 M $Na_3$-citrate [16,17] and analysed by Instrumental Neutron Activation Analysis (INAA, see section 2.5). Sulphide, total carbon, hydrogen, and nitrogen content of the soil samples were determined using CHNS elemental analysis (EA 1108 CHNS-O by Carlo Erba/Thermo-Quest), after soluble sulphate removal by washing three times with an excess of distilled water with the help of an ultrasonic bath. It can be assumed that the majority of sulphate is present in the form of passably soluble salts (such as gypsum). After this washing procedure, the samples were well-ground and dried until constant weight at 70 °C.

Carbonates (as calcium carbonate) were quantified by decalcification of the samples by aqueous HCl and subsequent gravimetric determination. Clay content was determined by sedimentation according to Öhlinger [18]. Humus content was calculated from total C [19]. Humus quantification by wet oxidation [20] proved to be inapplicable since interferences with easily oxidisable sulphides were observed. Instead, the humus content was estimated from percentages of $C_{organic} = C_{total} - C_{inorganic}$ (based on the results of the elemental analysis and carbonate analysis) using Eq. (1) [19].

$$\text{Humus (\%)} = C_{organic} \cdot 1.72 \quad (1)$$

Coarse particles of the bedrock were gained by sieving. Their carbonate content was tested using aqueous HCl.

In selected samples, microscopic soil minerals were studied by X-Ray Microanalysis. Fine soil was dried at 70° C, aggregates were broken up in a mortar. Fine particles were fixed on carbon tape and imaged at 30 kV in a Philips LX 20; X-Ray Microanalysis was used for qualitative or semiquantitative element analysis.

### 2.3. Water Chemistry

Water from the rivulet in D2 was characterised by determination of pH (Voltcraft PH-100ATC), conductivity (Voltcraft WA-100ATC), carbonate hardness (Aquamerck 1.08048.0001), total hardness (Aquamerck 1.08033.0001), ammonium (Aquamerck 1.08024.0001), nitrite (Aquamerck

1.08025.0001) nitrate (Aquamerck 1.11170.0001), phosphate (Aquamerck 1.14661.0001) and oxygen content (Aquamerck 1.11152.0001). Oxygen saturation was calculated after Oehme and Schuler [21]. Metalloid (As and Sb) content was determined using INAA (see Section 2.5).

### 2.4. Microbiology

Two complementary techniques were used to evaluate the microbial diversity on the study sites in Schlaining: (1) Counting of cultivable microbes on selected media spiked with Sb and As and (2) determination of total microbe number *in situ* after fluorescent staining.

(1) For cultivation experiments, fresh soil with a dry weight of 10 g was extracted in 90 ml sterile phosphate buffer (0.2%, pH 7.0) for 45 minutes at 150 rpm [22]. After 20 minutes for sedimentation of coarse particles, the extracts were diluted 1 : 200 to 1 : 160,000 and 100 µl of each were inoculated on the following media: 1) Plate Count Medium (Merck Plate-Count-Agar 1.05463.0500) for the unspecific quantification of cultivable bacteria, 2) Kimmig Medium for the isolation of fungi [23], 3) a selective medium for sulphide oxidising bacteria (SOB; [24]). Furthermore, we tested a selective medium for actinobacteria as described by Papageorgiou [25], which proved to be too unspecific for our purposes. The plates were incubated for seven days at room temperature. Spiking was performed by adding 10 and 100 ppm As and Sb to the media. Antimony was applied as tartar emetic (potassium antimony(III) oxide tartrate hemihydrate, $K(SbO)C_4H_4O_6 \cdot 0.5\ H_2O$; Merck, Darmstadt, puriss.), whereas arsenic was used as disodium hydrogenarsenate ($Na_2HAsO_4 \cdot 7\ H_2O$; Riedel de Haën, Seelze, puriss.).

(2) For the determination of the total microbe number [22], the same soil extracts were diluted 1 : 1 with sterile water. After adding a 1% agarose solution, 40 µl of the solution were transferred to slides and stained with 0.0065% Acridine Orange. After washing, the stained bacteria were counted using an Olympus BX-41 epifluorescence microscope (4 parallel preparations and 20 counts per soil).

### 2.5. Determination of Metalloid Content

Quantification of metalloids and Fe was performed by Instrumental Neutron Activation Analysis (INAA). The content of arsenic and antimony was determined in the water of the rivulet at D2 as well as in the soil. In the latter case, we distinguished between the total metalloid content of the fine soil and mobile metalloids (10 g extracted with 67 ml of pure water). Furthermore, we tested the potential of 1 M $NH_4NO_3$ to extract exchangeable metalloids (extractable metalloids).

For the quantification of metalloids, INAA offers several advantages compared to other analytical methods, i.e. no matrix dependency as well as no dependency on the oxidation state or the chemical environment of the analyte. Furthermore, INAA requires only a minimum of sample preparation (*inter alia* avoiding chemical dissolution of the insoluble ores), which makes it the method of choice e.g. for geological samples [26,27]. Both elements, arsenic and antimony, can be determined with extreme sensitivity. The geological material was carefully dried at 70 °C until constant weight, homogenised in an agate mortar, weighed into polyethylene vials, sealed and irradiated in the TRIGA Mark II reactor at the Atominstitut for 4 minutes using the pneumatic sample transfer system. The neutron flux density in this position is approximately $3 \cdot 10^{12}\ cm^{-2} \cdot s^{-1}$. For quantification, the certified standard reference materials (NIST SRM 1633b Coal Fly Ash and NIST SRM 2702 Inorganics in Marine Sediments) were used.

After a cooling time of 24 hours, the vials were decontaminated and a γ-spectrum was measured to obtain the activities of the relatively short-lived activation products $^{76}As$ and $^{122}Sb$ (see Table **1**). The measuring time was 1800 s. All samples were measured in a fixed position at a distance of 8 cm beside the detector. The γ-spectrometry was performed with a 222 $cm^3$ HPGe-detector (1.78 keV resolution at the 1332 keV $^{60}Co$ peak; 48.2% relative efficiency), connected to a PC-based multi-channel analyzer with a preloaded filter and a Loss-Free Counting system.

For quality control, solutions of $Na_2HAsO_4 \cdot 7\ H_2O$ and $K(SbO)C_4H_4O_6 \cdot 0.5\ H_2O$ with each 100 $mg \cdot l^{-1}$ As and Sb, respectively, were analysed in each analytical cycle. For the quantification of $^{76}As$ and $^{122}Sb$, the bromine content of the sample is a crucial factor: The main photo peaks of the three

**Table 1. Basic Nuclear Data that are Important for the INAA in this Study**

| Element | Nuclide Capable for Neutron Capture (Natural Abundance) | Cross Section for Thermal Neutron Capture, (n,γ)-Reaction[1] | Activation Product | Detectable After Short or Long-Term Activation | Half-Life | γ-Photon Energy [keV] (Branching Ratio) |
|---|---|---|---|---|---|---|
| Antimony | $^{121}Sb$ (57.21%) | 5.9 b | $^{122}Sb$ | short-term (1-4 min) | 2.7238 d | 564.24 (70.67%) |
| Antimony | $^{123}Sb$ (42.79%) | 4.06 b | $^{124}Sb$ | long-term (>8 h) | 60.11 d | 1690.975 (47.79%) |
| Arsenic | $^{75}As$ (100%) | 4.3 b | $^{76}As$ | short-term (1-4 min) | 1.0942 d | 559.10 (45.0%)<br>563.23 (1.20%) |
| Bromine | $^{81}Br$ (45.31%) | 2.64 b | $^{82}Br$ | short- and long-term | 35.282 h | 776.517 (83.4%)<br>554.348 (71.1%)<br>619.106 (43.5%) |
| Iron | $^{58}Fe$ (0.282%) | 1.3 b | $^{59}Fe$ | long term (>8 h) | 44.495 d | 1099.245 (56.5%) |

Data taken from [79].
[1] 1 b (barn) = $10^{-24}$ cm²; this value represents the total cross section (including neutron capture yielding short-lived metastable nuclear levels).

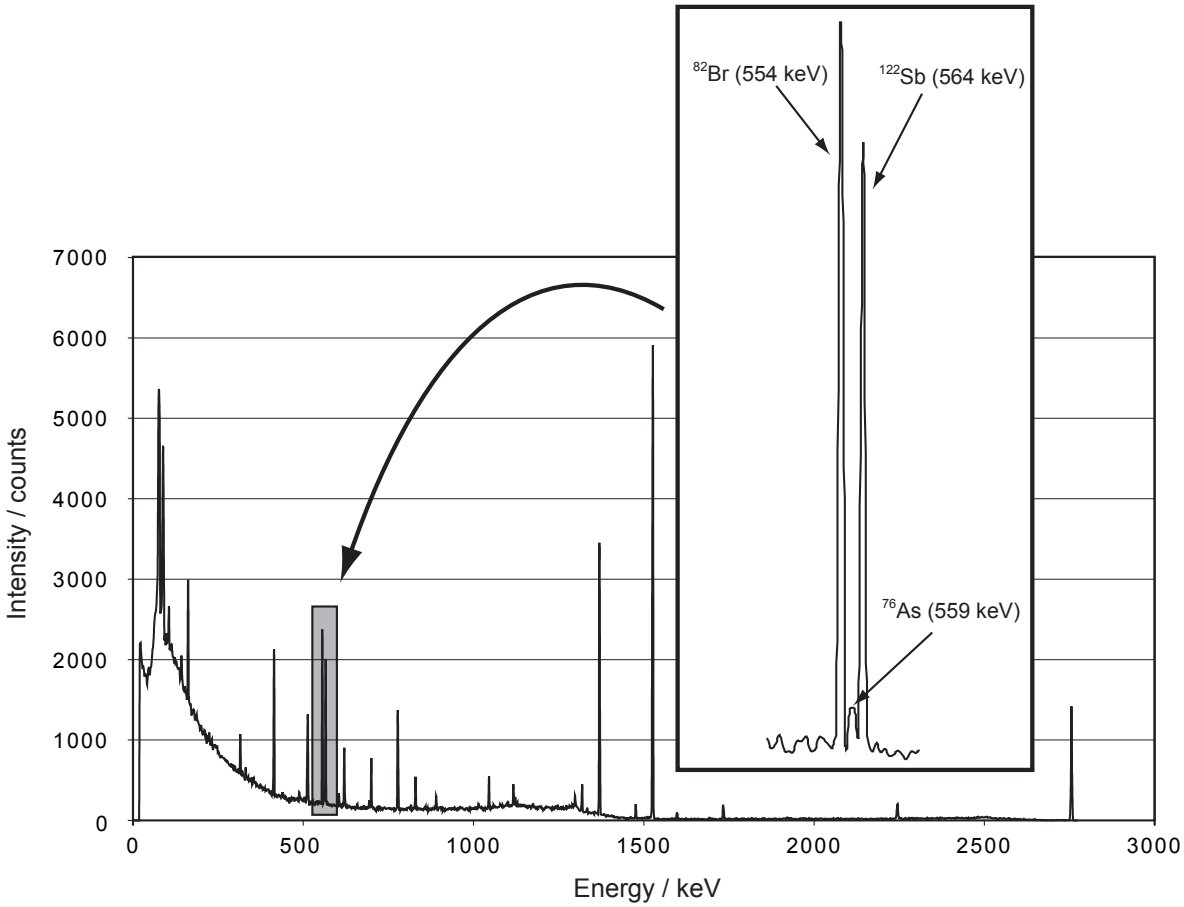


**Fig. (2).** Gamma-spectrum of a Br, As, and Sb-containing water sample from the rivulet (location D). The spectrum shows the potential interference of $^{82}$Br with the main photopeaks of $^{76}$As and $^{122}$Sb in bromide-rich matrices.

activation products are within a very small energy range in the γ-spectrum (see Table **1**). Thus a high content of bromine in the sample can cause the overlap of the 554 keV peak with the other two photopeaks and thwart a reliable peak evaluation. In our case, the Br content was low enough in every case to resolve these three peaks and provide a flawless analysis (Fig. **2**). Also, the 563 keV photo peak of $^{76}$As contributes only marginally to the 564 keV $^{122}$Sb-peak due to the small branching ratio (the percentage of disintegrations with the emission of a γ-photon) of this γ-photon (compare Table **1**). However, this overlap has been accounted for by evaluating a pure arsenic standard.

For quantification of the iron content in soil extracts, 1 ml of the extract was pipetted into Suprasil™ quartz glass vials and carefully evaporated at 80 °C. After complete evaporation, the quartz vials were sealed and irradiated together with standard samples in an irradiation tube (neutron flux density approximately $2 \cdot 10^{12}$ cm$^{-2} \cdot$ s$^{-1}$) of the reactor for approximately 35 hours. Since $^{59}$Fe is a long-lived activation product, the γ-measurement was performed two weeks after irradiation, i. e. after complete cooling of the remarkable $^{24}$Na background ($T_{1/2}$ = 15.0 h), which was a consequence of the utilisation of sodium dithionite and citrate as a reducing and coordinating agent in the course of extraction. After decontamination of the vials, they were packed into PE vials fitting the automatic sample changer device of the Atominstitut in Vienna and measured on the same γ-detection system as described before at a distance of 4 cm to the detector. Standard materials for iron were ferric-ammonium sulphate ($NH_4Fe(SO_4)_2 \cdot 12\ H_2O$; Merck, Darmstadt, p.a.) and iron powder (Merck, Darmstadt, p.a.). The analytical error of the INAA in this work is ≤5% rel. for Sb and <10% rel. for As and Fe.

### 2.6. Statistics

SPSS 16.0 was used for statistical analysis. The Kolmogorov-Smirnov test gave evidence that none of our data were normally distributed. Thus, the Mann-Whitney-U-test was used to test for differences. The Spearman test was used to test for correlations of soil parameters, where no cause-effect relations were evident. Biodiversity of embryophytes as well as the number of bacteria were regarded as effects of the soil composition. Therefore, linear regression was used in those cases.

## 3. RESULTS

### 3.1. Soil

The soils of the various sampling sites proved to be very different. The majority of samples, however, showed to be moderately rich in nutrients, rich in carbonates and with a neutral pH. A survey of chemical soil parameters is given in Table **2**. With the exception of C1, all soils were well aer-

**Table 2. Soil Composition at the Test Sites**

| Sampling Sites | A | | | B | C | | D | |
|---|---|---|---|---|---|---|---|---|
| **Microhabitats** | **A1** | **A3** | **A2** | **B1** | **C2** | **C1** | **D2** | **D1*** |
| **Vegetation** | **Meadow Over a Renaturated Spoil Heap** | | | **Spoil Heap with Sparse Vegetation** | ***Picea abies* Forest** | **Mud without Vegetation** | **Meadow at the Bank of D1** | **Rivulet without Aquatic Macrophytes** |
| Coarse Particles (>4 mm) [%] | 53.0 | 53.8 | 38.9 | 55.7 | 37.8 | 10.5 | 28.9 | |
| Clay [%] | 7.5 | 5.5 | 5.5 | 5.5 | 2.0 | 4.0 | 3.0 | |
| Humus [%] | 5.1 | 2.5 | 1.2 | 3.2 | 5.1 | 1.3 | 2.6 | |
| Water Content [%] | 23.9 | 17.3 | 18.5 | 9.2 | 9.9 | 23.6 | 24.4 | |
| $pH_{H2O}$ | 7.9 | 8.4 | 6.9 | 8.0 | 7.9 | 2.4 | 7.1 | 7.8 |
| $pH_{KCl}$ | 7.0 | 7.9 | 4.8 | 8.0 | 7.6 | 2.3 | 6.2 | |
| $pH_{CaCl2}$ | 7.1 | 7.4 | 6.3 | 7.3 | 7.1 | 1.9 | 6.5 | |
| Carbonate [mg · $kg^{-1}$] | 2.1 | 25.0 | 1.5 | 29.8 | 4.6 | 2.5 | 4.0 | 2.9 mM · $l^{-1}$ (carbonate hardness) 5.7 mM · $l^{-1}$ (total hardness) |
| Ammonium [mg · $kg^{-1}$] | 14.1 | 24.9 | 19.4 | 36.0 | 17.5 | 18.0 | 12.8 | < 0.1 |
| Nitrate [mg · $kg^{-1}$] | 7.2 | 8.6 | 9.1 | 8.2 | 4.4 | 15.5 | 11.7 | 5 |
| Total Nitrogen [mg · $kg^{-1}$] | 3120 | 1740 | 1160 | 1790 | 2040 | 1440 | 1840 | |
| Phosphate [mg · $kg^{-1}$] | 17.2 | 25.0 | 10.1 | Not detected | Not detected | 11.6 | 24.5 | Not detected |
| Sulphide [mg · $kg^{-1}$] | 550 | 3040 | < 200 | 1780 | 2820 | $4.28 \cdot 10^4$ | 3880 | |
| Sulphate [mg · $kg^{-1}$] | 29.3 | 83.4 | 25.1 | 331 | 46.9 | 7880 | 35.6 | 60.5 |
| Extractable Fe [mg · $kg^{-1}$] | 2800 | 3700 | 1400 | 8000 | 5300 | 3700 | 3700 | |
| Total As [mg · $kg^{-1}$] | 49 | 370 | Not detected | 470 | 32 | 1400 | 810 | 4500 (sediment) |
| Mobile As [mg · $kg^{-1}$] | 0.070 | 0.88 | Not detected | 0.34 | Not detected | 0.51 | 1.0 | 0.026 (water) |
| Mobile As [% of total As] | 0.14% | 0.24% | | 0.07% | 0.00% | 0.04% | 0.13% | |
| Extractable As [mg · $kg^{-1}$] | 0.00 | 0.93 | 0.00 | 0.69 | 0.00 | 11.3 | 0.25 | |
| Extractable As [% of total As] | 0.00% | 0.25% | | 0.17% | 0.00% | 0.80% | 0.03% | |
| Total Sb [mg · $kg^{-1}$] | 135 | 1000 | Not detected | 439 | 32.0 | 246 | $3.64 \cdot 10^4$ | 6380 (sediment) |
| Mobile Sb [mg · $kg^{-1}$] | 0.45 | 3.05 | Not detected | 1.70 | Not detected | 0.060 | 53.6 | 0.027 (water) |
| Mobile Sb [% of total Sb] | 0.34% | 0.31% | | 0.39% | 0.00% | 0.02% | 0.15% | |
| Extractable Sb [mg · $kg^{-1}$] | 0.34 | 3.64 | 0.00 | 2.58 | 0.00 | 0.65 | 24.8 | |
| Extractable Sb [% of total Sb] | 0.26% | 0.37% | | 0.59% | 0.00% | 0.26% | 0.07% | |

*Indicates a water sample; thus no extracts but untreated water was used for analysis.

ated. No black sulphide layers were found that might indicate anaerobic conditions [28].

At site A, three samples were taken. In A1, the uppermost 10 cm were densely penetrated by roots. Below, large rocks were found; the majority of these was rich in carbonates (calcareous slates), only a minor amount was serpentinite. The fine soil was moderately calcareous and exhibited a neutral pH. Contamination with metalloids was low, but well above the detection limit. At A3, only sparse roots were

found in the uppermost 6 cm. The rocky mineral below primarily consisted of calcareous phyllite (graphitic slate) with minor amounts of green slate with sulphide inclusions (Fig. **3**). The soil at this site was calcareous and slightly alkaline. The contamination with metalloids was about seven times higher than in A1. In A2, the rooted soil layer was deeper than 20 cm. Rocks (green slate with quartz inclusions; phyllite) were evenly distributed through the soil; none of them was calcareous. Soil pH was slightly acidic. No detectable amounts of metalloids were found in the fine soil fraction.

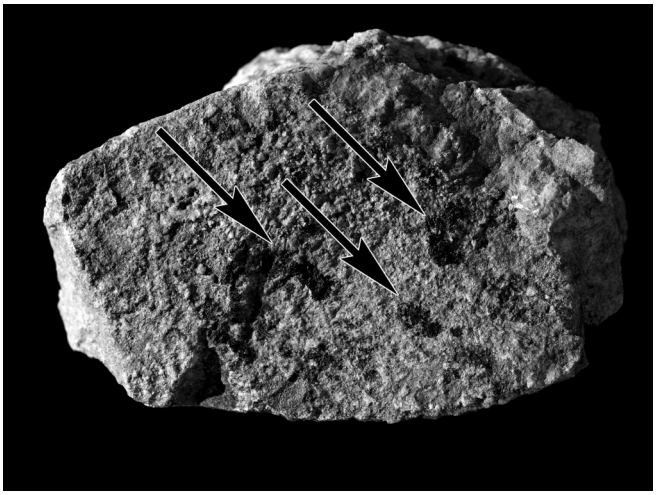

**Fig. (3).** Green slate with several ore inclusions (arrow) diameter ~8 cm.

At site B, the soil was rich in large particles and exhibited no distinct soil layers. All rocks were calcareous phyllites (graphitic slates); accordingly, soil pH was slightly alkaline. The fine soil contained even more As, but less Sb than A3. Furthermore, large amounts of sulphate were found.

At site C, mud in a wallow (C1) and forest soil (C2) were distinguished. At C2, soil was covered by a thin layer of spruce needles and duff. Below, an unstratified soil was found. All rocky stones were calcareous phyllites (graphitic slates); soil pH was > 7. Only very small amounts of As and Sb were detected. C1 exhibited completely different properties than all other samples: The mud was wet; no roots and no soil layers were detected. Spruce needles, fir cones and other organic particles were obviously conserved for a longer time period. The soil was extremely acidic (pH 2.38 in water, 1.88 in $CaCl_2$). Accordingly, little carbonate was found in the fine soil nor in the sparse rock particles. The mud contained 7.9‰ sulphate and even 4% sulphide. In the X-Ray Microanalysis, numerous crystals of gypsum were found (Fig. **4**). Furthermore, 1.4‰ As, but comparatively little Sb were found.

Site D consisted of two microhabitats as well, a rivulet and a meadow. The bedrock material of rivulet D1 was covered by a flocculent red iron ore mineral, probably goethite (Fig. **5**). In the rivulet's water, the maximum permissible value in drinking water (World Health Organization, WHO [8]) for As (10 $\mu g \cdot l^{-1}$) was clearly exceeded. The value for Sb was in the same range but yet below the limit which is 50 $\mu g \cdot l^{-1}$ in this case. The sediment, especially the iron hydroxide, contained up to 4.5‰ As. The sorption of As species onto iron hydroxide has been object of intense investigation (e.g. [29]). The meadow D2 along the bank is probably affected by flooding. The soil is loamy, poor in large rocks and only sparsely penetrated by roots. About half of the rocks were calcareous phyllites; the other half was serpentinite and partly anthropogenic smelting residues; soil pH was neutral. Furthermore, small scoria particles (< 4 mm) were abundant. The soil was very rich in As and especially in Sb; the percentage of mobile metalloids were relatively high as well.

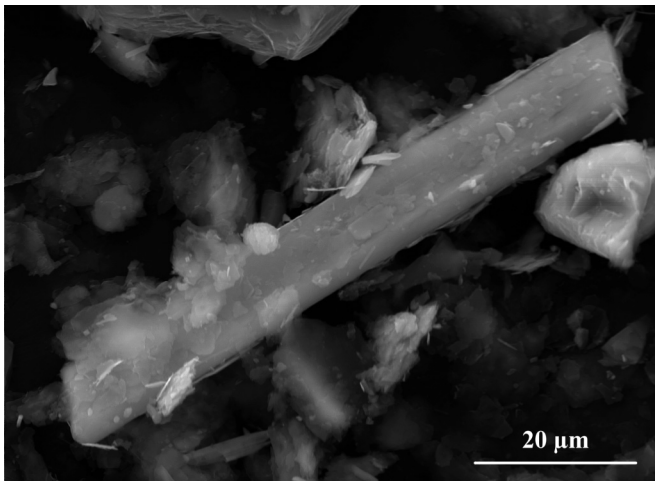


**Fig. (4).** Gypsum crystal from the mud of C1 (X-Ray Microanalysis).

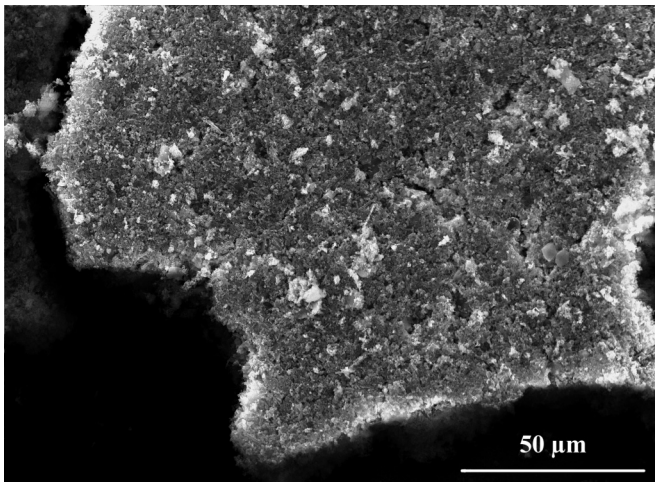


**Fig. (5).** Iron ore particle (goethite) from rivulet D1. This specific particle contained 0.36% adsorbed As (X-Ray Microanalysis).

At none of the sites, antimony ore particles could be found in the fine soil using X-Ray Microanalysis. Instead, quartz, alumosilicates, mica, clinopyroxene, hornblende and other microscopic mineral particles could be identified which contained up to 1% Sb and 0.2% As.

### 3.2. Flora

At site A, 51 species of Embryophytes were found (Table **3**). At A1 (26 species) and A2 (16 species), the vegetation was very dense and dominated by grasses and herbs. At A3 (29 species), shrubs and mosses were found as well. Vegetation density, however, was lower and spots of open soil occurred.

At site B, 15 species were found (Table **4**). As this habitat was located at the edge of the forest, meadow and forest

**Table 3. Vascular Plants and Bryophytes Found at Site A**

| A1 | A3 | A2 |
|---|---|---|
| **30 m² Sampled** | **50 m² Sampled** | **50 m² Sampled** |
| *Achillea millefolium agg.* (Asteraceae) | *Acer campestre* (Aceraceae) | *Aegopodium podagraria* (Apiaceae) |
| *Aegopodium podagraria* (Apiaceae) | *Alopecurus pratensis* (Poaceae) | *Arrhenaterum elatius* (Poaceae) |
| *Arrhenaterum elatius* (Poaceae) | *Arrhenaterum elatius* (Poaceae) | *Aster lanceolata* (Asteraceae) |
| *Aster lanceolata* (Asteraceae) | *Aster lanceolata* (Asteraceae) | *Calamogrostis epigeios* (Poaceae) |
| *Avenula pubescens* (Poaceae) | *Avenula pubescens* (Poaceae) | *Dactylis glomerata* (Poaceae) |
| *Carex hirta* (Cyperaceae) | *Barbula unguiculata* (Pottiaceae) | *Festuca pratensis* (Poaceae) |
| *Cirsium sp.* (Asteraceae) | *Brachythecium oedipodium* (Brachytheciaceae) | *Galium mollugo* (Rubiaceae) |
| *Dactylis glomerata* (Poaceae) | *Campanula patula* (Campanulaceae) | *Holcus sp.* (Poaceae) |
| *Festuca pratensis* (Poaceae) | *Carpinus betulus* (Betaluceae) | *Knautia arvensis* (Scabiosaceae) |
| *Hypericum perforatum* (Hypericaceae) | *Carex hirsuta* (Cyperaceae) | *Lamium album* (Lamiaceae) |
| *Knautia arvensis* (Scabiosaceae) | *Clematis vitalba* (Ranunculaceae) | *Leucanthemum sp.* (Asteraceae) |
| *Lathyrus pratensis* (Fabaceae) | *Festuca pratensis* (Poaceae) | *Lotus corniculatus* (Fabaceae) |
| *Lotus corniculatus* (Fabaceae) | *Galium aparine* (Rubiaceae) | *Myosotis arvensis* (Boraginaceae) |
| *Mentha sp.* (Lamiaceae) | *Galium mollugo* (Rubiaceae) | *Potentilla sp.* (Rosaceae) |
| *Potentilla sp.* (Rosaceae) | *Knautia maxima* (Scabiosaceae) | *Solidago* cf. *canadensis* (Asteraceae) |
| *Pulmonaria officinalis* (Boraginaceae) | *Lamium album* (Lamiaceae) | *Urtica dioica* (Urticaceae) |
| *Ranunculus sp.* (Ranunculaceae) | *Lysimachia nummularia* (Primulaceae) | |
| *Rubus sp.* (Rosaceae) | *Lysimachia punctata (Primulaceae)* | |
| *Rumex crispus* (Polygonaceae) | *Mnium marginatum* (Mniaceae) | |
| *Scutellaria hastifolia* (Lamiaceae) | *Myosotis arvensis* (Boraginaceae) | |
| *Silene vulgaris* (Caryophyllaceae) | *Origanum vulgare* (Lamiaceae) | |
| *Solidago* cf. *canadensis* (Asteraceae) | *Potentilla sp.* (Rosaceae) | |
| *Vicia cracca* (Fabaceae) | *Pulmonaria officinalis* (Boraginaceae) | |
| *Vicia grandiflora* (Fabaceae) | *Pyrus pyraster* (Rosaceae) | |
| *Vicia hirsuta* (Fabaceae) | *Robinia pseudacacia* (Fabaceae) | |
| *Vicia sepium* (Fabaceae) | *Salvia glutinosa* (Lamiaceae) | |
| | *Solidago* cf. *canadensis* (Asteraceae) | |
| | *Thlaspi sp.* (Brassicaceae) | |
| | *Trisetum flavescens* (Poaceae) | |
| **26 species** | **29 species** | **16 species** |

species intermixed. The vegetation consisted of shrubs, young trees and herbs; mosses and grasses were missing.

Site C was dominated by *Picea abies* which were planted by man. In the forest, 24 additional species were found. Besides sparse shrubs and young trees[2], we found mainly the fern *Dryopteris filix-mas* and abundant mosses (9 species). The wallow C1 was found without any vegetation. The only organisms were bacteria occasionally forming orange biofilms.

At site D, rivulet D1 proved to have similar characteristics to the wallow. No aquatic mosses, vascular plants or algae were found; however, *Thiothrix*-like bacteria (Fig. **6**) formed filaments between particles of iron hydroxide. D2, the meadow at the bank of the rivulet, was very poor in species as well. Only six species of vascular plants were found. Two bryophytes, *Pellia* cf. *epiphylla* and *Plagiomnium undulatum*, covered most of the area.

Although the highest diversity of embryophytes was found in a contaminated habitat (A3), comparison of all habitats showed that total soil As content led to a significant decrease in biodiversity ($R^2 = 0.62$; $P < 0.05$). Mobile and extractable As or Sb had no significant effect on the biodi-

[2]Without *Picea abies*, which does not reproduce in Burgenland.

**Table 4. Embryophytes Found at the Spoil Heap B1, the *Picea abies* Forest C2 and the Meadow D2**

| B1 | C2 | D2 |
|---|---|---|
| **10 m² Sampled** | **15 m² Sampled** | **5 m² Sampled** |
| *Cardaminopsis arenosa* (Brassicaceae) | *Asarum europaeum* (Aristolochiaceae) | *Geranium sp.* (Geraniaceae) |
| *Cornus sanguinea* (Cornaceae) | *Betula sp.* (Betulaceae) | *Hedera helix* (Araliaceae) |
| *Corylus avellana* (Betulaceae) | *Brachythecium velutinum* (Brachytheciaceae) | *Oxalis sp.* (Oxalidaceae) |
| *Eupatorium cannabinum* (Asteraceae) | *Carpinus betulus* (Betulaceae) | *Pellia* cf. *epiphylla* (Pelliaceae) |
| *Fagus sylvatica* (Fagaceae) | *Cornus sanguineus* (Cornaceae) | *Plagiomnium undulatum* (Mniaceae) |
| *Fragaria vesca* (Rosaceae) | *Dryopteris filix-mas* (Dryopteridaceae) | *Viola sp.* (Violaceae) |
| *Galium aparine* (Rubiaceae) | *Epilobium sp.* (Onagraceae) | |
| *Geum sp.* (Rosaceae) | *Eupatorium cannabinum* (Asteraceae) | |
| *Impatiens noli-tangere* (Balsaminaceae) | *Eurhynchium angustirete* (Brachytheciaceae) | |
| *Knautia maxima* (Scabiosaceae) | *Fragaria vesca* (Rosaceae) | |
| *Melica nutans* (Poaceae) | *Galium aparine* (Rubiaceae) | |
| *Rosa sp.* (Rosaceae) | *Geum sp.* (Rosaceae) | |
| *Rubus sp.* (Rosaceae) | *Hypercicum perforatum* (Hyperciaceae) | |
| *Solidago* cf. *canadensis* (Asteraceae) | *Hypnum cupressiforme* (Hypnaceae) | |
| *Tilia platyphylla* (Tiliaceae) | *Impatiens noli-tangere* (Balsaminaceae) | |
| | *Isopterygium muellerianum* (Brachytheciaceae) | |
| | *Lophocolea heterophylla* (Geocalycaeae) | |
| | *Picea abies* (Pinaceae) | |
| | *Plagiochila asplenoides* (Plagiochilaceae) | |
| | *Plagiothecium curvifolium* (Plagiotheciaceae) | |
| | *Polytrichum formosum* (Polytrichaceae) | |
| | *Pottia sp.* (Pottiaceae) | |
| | *Rubus sp.* (Rosaceae) | |
| | *Sambucus nigra* (Sambucaceae) | |
| | *Viburnum sp.* (Adoxaceae) | |
| **15 species** | **25 species** | **6 species** |

versity. Other factors reducing the number of species included lacking soil skeleton ($R^2 = 0.60$; $P < 0.05$) and low soil pH, especially $pH_{H2O}$ ($R^2 = 0.55$; $P < 0.05$). Due to the limited number of samples analysed, statistical interpretation in this work should be regarded as preliminary information in the first place.

### 3.3. Microbiology

The number of soil bacteria ranged between $7.3 \cdot 10^8$ per gram for the wallow C1 and $7.2 \cdot 10^9$ for the meadow A1. A weak negative correlation was found between the total number of bacteria and total As ($R^2 = 0.27$; $P < 0.01$), mobile As ($R^2 = 0.17$; $P < 0.01$), total Sb ($R^2 = 0.03$; $P < 0.05$) and mobile Sb ($R^2 = 0.03$; $P < 0.05$). Other factors were found to inhibit bacterial growth as well, including low $pH_{CaCl2}$ ($R^2 = 0.15$; $P < 0.01$), and even shadowing ($R^2 = 0.55$; $P < 0.01$). On the other hand, the number of soil bacteria was positively influenced by clay content ($R^2 = 0.59$; $P < 0.01$), total soil N ($R^2 = 0.41$; $P < 0.01$) or total soil H ($R^2 = 0.40$; $P < 0.01$).

The number of cultivable units on PC medium showed no correlation with the total number of bacteria as well. Cultivable sulphur oxidising bacteria were found in all samples except C1. Microbe numbers are given in Table **5**.

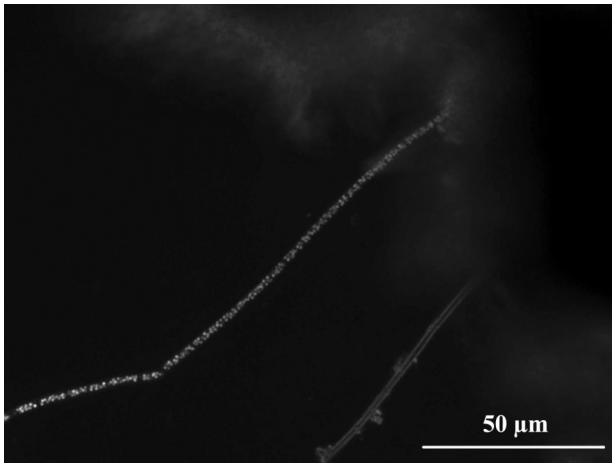


**Fig. (6).** Filamentous, *Thiothrix nivea*-like bacterium attached to an iron hydroxide particle. This organism is by far dominant in rivulet D1. Note the white sulphur globules inside the cells (dark field).

**Table 5. Microbe Numbers, as Quantified by Fluorescent Microscopy and Two Different Culture Media (Values per g)**

| Sampling Site | A1 | A3 | A2 | B1 | C2 | C1 | D2 | D1 |
|---|---|---|---|---|---|---|---|---|
| Total Bacteria | $7.16 \pm 1.26 \cdot 10^9$ | $3.54 \pm 0.82 \cdot 10^9$ | $3.25 \pm 0.84 \cdot 10^9$ | $1.49 \pm 0.18 \cdot 10^9$ | $1.18 \pm 0.27 \cdot 10^9$ | $7.25 \pm 1.3 \cdot 10^8$ | $1.60 \pm 0.30 \cdot 10^9$ | |
| Cultivable Units on PC medium | $3.20 \pm 7.12 \cdot 10^8$ | $6.54 \pm 13.8 \cdot 10^8$ | $2.70 \pm 7.84 \cdot 10^8$ | $3.53 \pm 7.84 \cdot 10^8$ | $4.01 \pm 4.31 \cdot 10^8$ | $1.95 \pm 0.01 \cdot 10^8$ | $5.36 \pm 11.0 \cdot 10^8$ | $0.56 \pm 10.3 \cdot 10^8$ |
| Cultivable Units on SOB medium | $1.80 \pm 2.83 \cdot 10^5$ | $3.06 \pm 1.41 \cdot 10^5$ | $3.96 \pm 6.22 \cdot 10^5$ | $1.28 \pm 2.01 \cdot 10^5$ | $5.40 \pm 8.49 \cdot 10^4$ | Not detected | $1.35 \pm 0.14 \cdot 10^5$ | $1.80 \pm 2.55 \cdot 10^4$ |

Bacteria cultivated on PC and SOB medium exhibited completely different reactions on As and Sb spiking of the medium: on PC medium the number of colonies decreased with As and Sb contamination; here, Sb proved to be more toxic than As. On a medium spiked with 100 ppm As less than 9%, on 100 ppm Sb less than 0.12% were able to grow, compared to the unspiked control (Figs. **7** and **8**). The majority of sulphur oxidising bacteria, on the other hand, exhibited most abundant growth on media spiked with 10 ppm As. Neither 100 ppm As, nor Sb-spiking led to a significant reduction in this case (Figs. **9** and **10**).

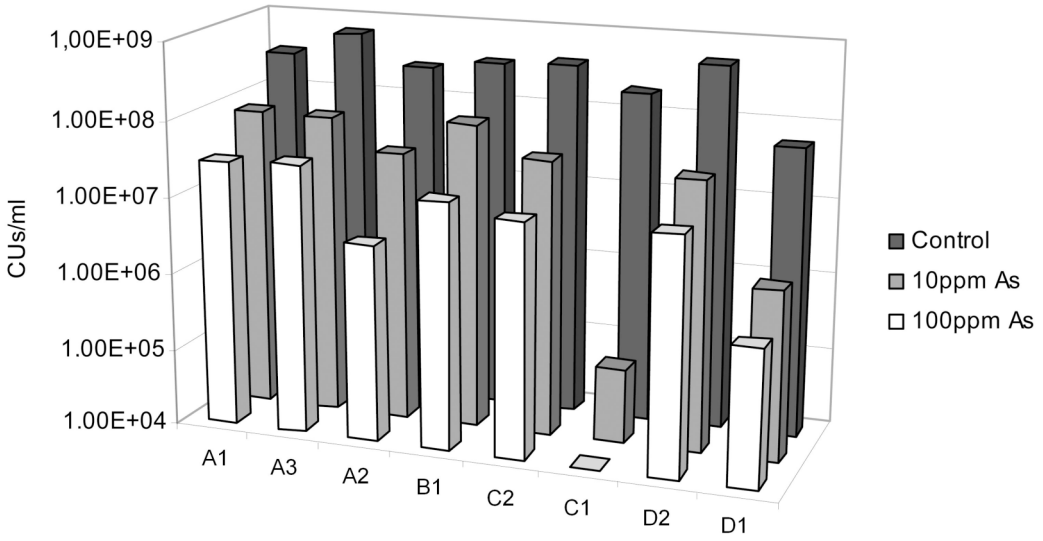


**Fig. (7).** Decrease of cultivable microbes on PC medium spiked with arsenate.

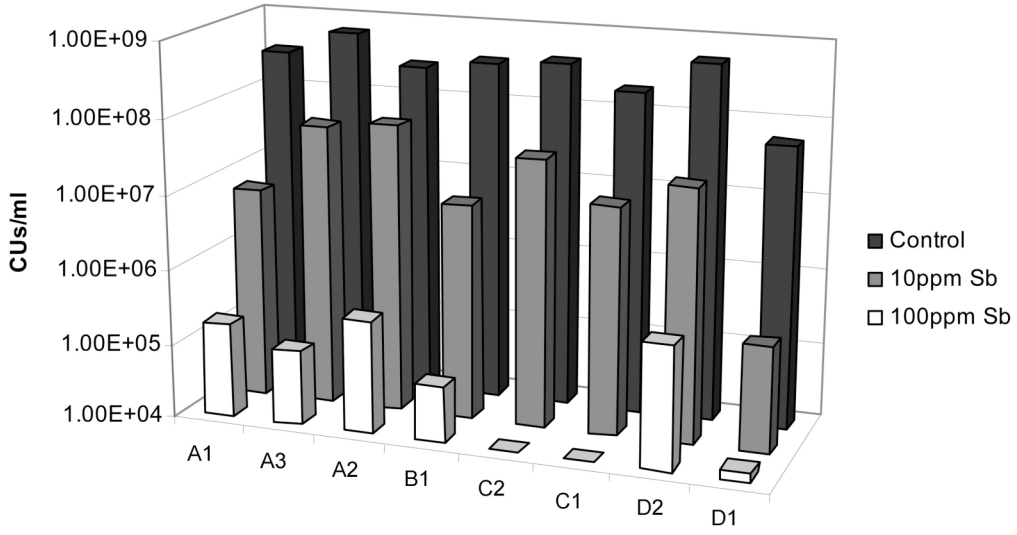


**Fig. (8).** Decrease of cultivable microbes on PC medium spiked with tartaric emetic.

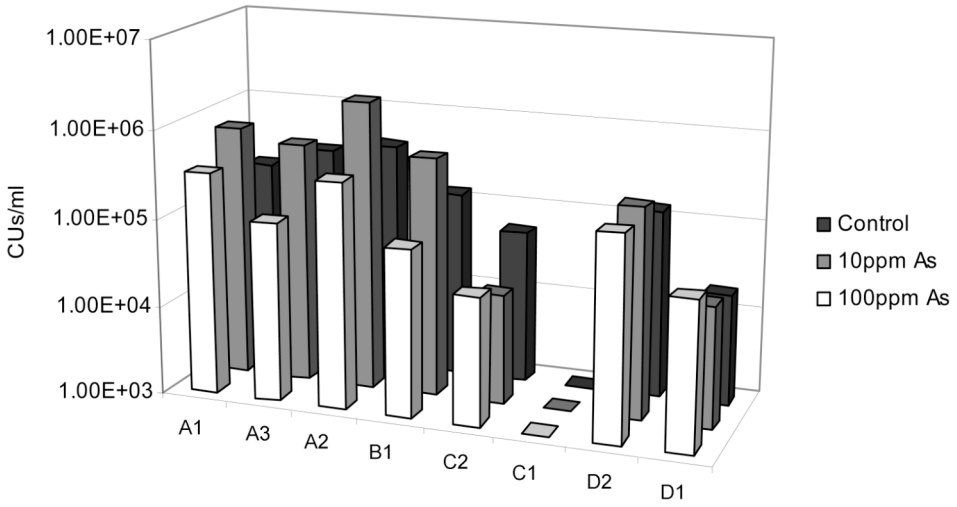


**Fig. (9).** Increase of cultivable microbes on SOB medium spiked with arsenate.

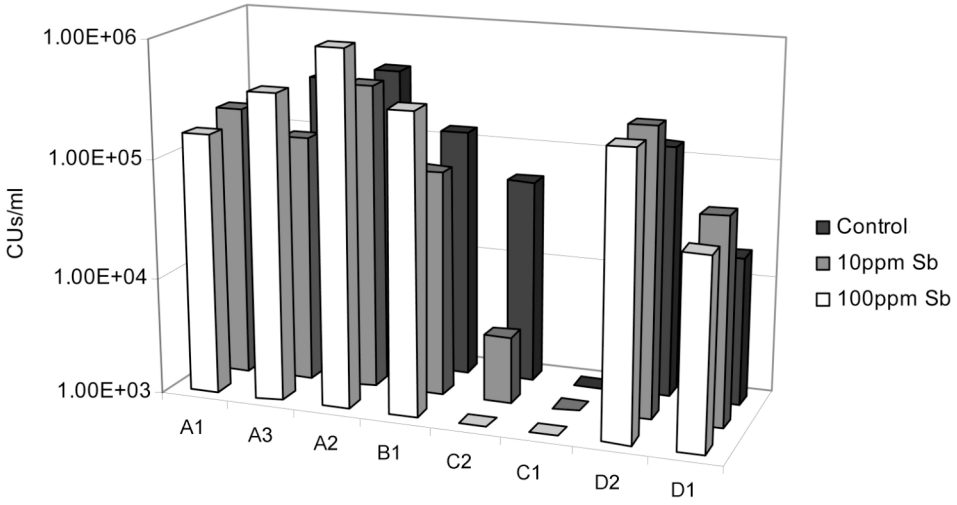


**Fig. (10).** Inrease of cultivable microbes on SOB medium spiked with tartaric emetic.

## 4. DISCUSSION

In recent years, many studies focused on poisonous metalloids in the environment. The most important example is arsenic-contaminated groundwater in the Bangladesh region that caused widespread diseases. Possible mechanisms of the As release into the aquifer have been discussed in recent papers [30,31]. It is appearant that reducing conditions in the fluvial sediments mobilize As. Other studies deal with As and Sb contamination as a consequence of atmospheric transport or import by herbicides [32-34] or As contamination as a result of metal processing [35]. However, much less is known on habitats with a bedrock rich in metalloids.

This may be due to the comparatively small number of habitats with edaphic metalloids, compared to sites contaminated with heavy metals. In his review of heavy metal habitats in the Eastern Alps, Punz [36] lists 21 habitats contaminated with Cu, 48 with Cr, but only three with As or Sb, including Schlaining.

### 4.1. Soil Chemistry

Many heavy metal contaminated habitats exhibit rocky and little developed soils or developed soils secondarily degenerated due to heavy metal stress [37]. In such habitats, effects of extreme pH, lacking nutrients, low water holding capacity etc. combine with heavy metal stress to suppress plant growth. This is not the case in the metalloid contaminated habitats in Schlaining. The pH values were neutral at most sites, in spite of the high abundance of extractable sulphate. The bedrock rich in limestone [4] was and still is able to buffer all sulphuric acid set free by oxidation of sulphides. The same is true for the rivulet D1, containing carbonates in the range of 2.9 mM $\cdot$ $l^{-1}$. Carbonate buffering of sulphide derived acids on mining sites is well known, in soil [38] as well as in drainage rivulets [39,40], but has experienced little attention so far [41]. In the vast majority of abandoned mining sites, soil and drainage waters are extremely acidic (e. g. [11]). In Schlaining, this was only the case in C1. Here, the buffer capacity of the soil was obviously overstrained by the

occurrence of sulphuric acid, causing a pH decrease down to 1.88. After the consumption of lime particles, calcium was precipitated in the form of gypsum. The high concentration in insoluble sulphur – supposingly sulphide – indicates that the process of acidification is far from being finished. To a minor extent, however, soil pH is influenced by sulphate at all sites: As expected, the sulphate, carbonate and humus content together completely explain $pH_{H2O}$ ($R^2 = 0.99$; $P < 0.05$) and $pH_{CaCl2}$ ($R^2 = 0.99$; $P < 0.01$).

The concentrations of plant nutrients were moderate at all sites. The high abundance of $NH_4^+$ found at B1 may indicate disturbed nitrification. The humus content is rather low, compared to typical grassland or woodland habitats and resembles agricultural soils [20]. Three hypotheses may explain the comparatively low effect of high concentrations of toxic metalloids on the soil: (1) In the majority of habitats investigated in this study, the soil is not autochthonous, but was heaped up in recent years during the remediation of the spoil heaps. Long term monitoring will give evidence if metalloid contamination will lead to soil degeneration. (2) In spite of the high concentrations of metalloids, soil microorganisms are still abundant and have partly adapted to metalloid stress (see 4.3). These resistant bacteria and fungi may be able to maintain "normal" soil metabolism. (3) The comparatively low mobility of metalloids (see 4.2) may reduce the toxic effect on soil metabolism.

### 4.2. Occurrence and Availability of Metalloids

Concentrations with toxic metalloids differed strongly between the investitated sites. At A2, no metalloids could be detected, at A1 and C2 the levels were very low. At A3, B1, C1 and D2, however, metalloids were abundant and exceeded by far the maximal permissible concentrations in soil [42]. At these polluted sites, the concentrations of metalloids were in the same range (370 - 1410 mg · $kg^{-1}$) as reported by Armienta *et al.* [43] for soils next to mine tailings in Mexico (290 - 2580 mg · $kg^{-1}$). However, mobility of As was much lower (< 1.7 mg · $kg^{-1}$ versus 1.0 - 8.4 mg · $kg^{-1}$) compared to mining sites in Mexico [43] and Australia [44]. The mobility rates at Schlaining are rather similar to gossans [44] or historic mining sites [10,45].

In any case, As and Sb mobility cannot be explained by single parameters. We tested several parameters discussed in earlier studies [17, 46] including pH, citrate-dithionite extractable Fe or clay content, but could not find significant correlations. A more complex model published by Jiang *et al.* [17] including not only extractable Fe and clay but also organic matter content and dissolved organic matter gave only weak and insignificant correlations as well. Unlike Jiang *et al*. [17], we found a significant correlation between the percentage of mobile As (supposingly $AsO_4^{3-}$) and extractable $PO_4^{3-}$ ($R^2 = 0.84$; $P < 0.05$), which probably reflects the competition of these anions for bonding partners (precipitation reactions) [47]. The correlation between mobile As and mobile Sb ($R^2 = 0.89$; $P < 0.01$) may be due to a similar effect. Mobile As also correlated highly significantly with the total Sb concentration ($R^2 = 0.96$; $P < 0.01$); however a definite explanation cannot be offered yet.

Besides the similar behaviour of phosphate and arsenate, the mobility of metalloids is difficult to explain. This is probably due to the mineralogical heterogeneity of the sites. Goldberg [46] provides adsorption data for As(III) and As(V) on various soil minerals, and describes very different adsorption rates, depending on As species, pH and respective minerals.

Antimony (and arsenic) ore occurs as intrusions in macroscopic slate or phyllite stones (see Fig. **3**). In the fine soil fraction, however, ore particles are rare which is probably due to sulphide oxidation. In X-Ray microanalytical investigations, no ore particles could be found (N = approx. 60). This may be the consequence of sulphur oxidising bacteria action ([48], see also 4.3) or sulphide dissolution by root exudates [49]. Both factors set soluble As species free.

In spite of the dissolution of sulphides, metalloid mobility was low as shown in Table **2**. The majority of metalloids was adsorbed or embedded in particles of other minerals like aluminosilicate, mica, hornblende etc., according to X-Ray microanalytical investigation. The sorption of As to aluminosilicates is known to be rather loose [50]. The relation between soil mineralogy and metalloid mobility recently became a key issue [16,51,52], but the recent knowledge is too limited for exact predictions for heterogeneous matrices.

In the rivulet D1, a remarkable amount of As was found in the water, and exorbitant As and Sb concentrations were found in the sediment. A similar situation was found by Morillo *et al.* [53] in the Spanish Odiel river (up to 791 mg/kg in the sediment). Compared to drainage waters from As rich spoil heaps in New Zealand [54] (up to 50 mg/l), As contamination in the Schlaining rivulet was rather low. At D1, the sediment was formed by goethite-like particles of iron hydroxide that contained up to 0.3% As. The incorporation of As into iron oxyhydroxyde exhibits a chemical selection, since arsenate is rather preferred than arsenite [55]. Surface water rich in As is a less observed phenomenon than As-calamities like ground- or well-water in Bengal/Bangladesh with As concentrations up to a milligram per litre range, see e.g. [56,57]. However, even rivers and surface waters have been reported to exceed the guideline value of the WHO for As. The As value found in the water of D1 (26 $\mu g \cdot l^{-1}$) is well above both, the permissible As value in potable water (10 $\mu g \cdot l^{-1}$) and the global average value of 1.7 $\mu g \cdot l^{-1}$ for dissolved As in stream water [58]. In comparison to a study on the water and sediments from the Okavango Delta (Botswana) [59], the As concentration in D1 water was approximately one order of magnitude higher than in surface water from there. Interestingly, the sediments from the Okavango river were relatively poor in As, in the range of 0.2-7 $mg \cdot kg^{-1}$, compared to the sediments of D1 (4.5‰ As). Several groundwater samples from the Okavango Delta region proved to have much higher As concentrations than the respective surface water samples (up to 177 $\mu g \cdot l^{-1}$ As), suggesting that groundwater from the Schlaining site could have even higher As concentrations, as the sediments are much richer in metalloids there.

Plant availability of As and Sb is more difficult to estimate. The Whitney-Man-U-Test gave evidence that extraction with $NH_4NO_3$ (as suggested in [10]) did not yield significantly different metalloid concentrations than pure water extracts. In the literature, some other extraction procedures have been described, including gastric solution (Simplified Bioaccessibility Extraction Test (SBET) for soil) [51], KCl [60], $Na_2HPO_4$ [47] or EDTA [61], but no standard extrac-

tion procedure has been developed so far. Further investigations in this direction will be included in the ongoing research in our laboratories.

### 4.3. Microorganisms

Arsenic and antimony have toxic effects on microorganisms in general, but resistant strains evolve readily at contaminated sites [62]. Therefore, the total microbe numbers were weakly, though significantly, reduced at As polluted sites. Antimony had no detectable effect. Only at C1, where high concentrations of these metalloids were combined with low pH, microbe numbers were strongly diminished.

Concerning cultivable bacteria, the situation was slightly different. Bacteria growing on PC medium proved to be sensitive to enhanced metalloid concentrations, especially to Sb. At the natural habitat, they probable benefit from the spare solubility of metalloids. On media spiked with 100 ppm As, e.g. up to 9% of the total cultivable bacteria were able to grow, whereas less than 0.1% were found on a 100 ppm Sb medium. This result is of specific interest, since the effects of soil Sb were weaker and less significant. These results may reflect the difficulties to estimate the availability of metalloids for microorganisms.

Different results were found on media for sulphur oxidising bacteria. Though only $10^4$ to $10^5$ CUs/ml (Cultivable Units per ml) could be detected, sulphur oxidisers proved to be much more resistant against toxic metalloids. The highest numbers of cultivable units were found not on the unspiked control, but on 10 ppm As. The effect of 100 ppm As was more moderate than on PC medium as well. Concerning Sb spiking, no general trend could be found. However, a significant decrease of cultivable units was only detected on the virtually uncontaminated site C2. Since the metalloids occur as sulphidic ores at Schlaining [3], our results indicate that these bacteria are specialised in the utilisation of the noxious ore sulphides. In C1, no cultivable sulphur oxidising bacteria were found; obviously, they are not able to cope with the low pH of this habitat, though sulphide was available.

Metalloid resistant bacteria were found not only at polluted sites, but also on the uncontaminated habitats A2 and C2. Jackson *et al.* [63] already described that up to 50% of the total cultivable bacteria from arsenic free soils may be resistant against arsenate.

Besides the oxidation of sulphide ore particles, other bacteria may affect metalloid mobility by oxidising As(III) to As(V) [64] or *vice versa* [65], dissolution of pyrite crystals by bacterial ligands [49] or by the precipitation of colloidial ferric arsenate in running water [66]. Due to the lack of anaerobic soils, As release by bacterial Fe reduction [67] can be excluded for Schlaining.

### 4.4. Flora of Contaminated Microhabitats

In Site A, the slightly inclined, west exposed meadow was superficially very uniform. Soil composition, on the other hand, differed largely between the three sampled microhabitats. These differences, however, were not reflected by the floristic composition. Only little effects of As and Sb contamination in A3 were found. Several species, e. g. *Arrhenaterum elatius*, *Aster lanceolata*, *Festuca pratensis* or *Solidago canadensis* were found all over the meadow. The number of species was largest in the heavy contaminated A3 and smallest in A2 without detectable As and Sb. However, open patches were only found at A3. This was also the only part of the whole meadow where three species of mosses were able to grow between the vascular plants. Virtually all species, vascular plants as well as mosses, were typical for more or less nutrient rich meadows. With the exception of *Silene vulgaris* [68] and *Thlaspi sp.* (e. g. [69]), no species or genera typical for habitats contaminated with heavy metals could be detected. A more or less limited resistance to heavy metals was also described for *Festuca*, *Viola*, *Rumex* and *Lotus* [70], which were all found in the Schlaining mining site as well. *Holcus* was described to be As resistant [71], but occurred in Schlaining only at the uncontaminated microsite A2.

At the heavily contaminated site B1, meadow and forest species were intermixed. The number of species and the coverage were low. However, no typical metallophytes were found.

At Site C, conditions were far from being natural, since *Picea abies* forests do not naturally occur in Burgenland. Accordingly, only deciduous trees and shrubs were found in the understorey. Due to the low level of contamination, depletion of the flora was neither expected nor found at C2. C1, however, proved to be the most extreme habitat found at Schlaining. Here, neither vascular plants nor mosses were found. The only organisms were bacteria forming an orange biofilm at the surface of the mud.

D2, the meadow at rivulet D1, exhibited a very low biodiversity. Only six species of embryophytes could be detected. It is tempting to ascribe this to the very high amounts of total and mobile As and Sb (3.6%!). However, shadowing by neighbouring trees or destructive flooding by the nearby creek may contribute to lower biodiversity as well. Accordingly, shading resistant genera like *Viola*, *Hedera*, *Plagiomnium* or *Oxalis* were dominant. No floristic overlapping with other sites – contaminated or not – was found.

Rivulet D1 was even poorer in species than the neighbouring meadow D2, though, except for metalloids, no other extreme parameters were found. Thus it is highly probable that the As (and Sb) content is responsible for the lack of species. The flora was dominated by bacteria only (e.g. as shown in Fig. **6**).

As a summary, no evidence for the formation of a specific As-Sb-flora could be found, as it is well known for heavy metal contaminated habitats [72]. Even very high amounts of total and mobile As and Sb are tolerated by a closed though species poor vegetation (D2). Besides metalloid contamination, soil conditions were favourable at most investigated sites. A significant decrease in species numbers was found to be exclusively a function of the total As content.

Thus, our results support the hypothesis of Craw *et al.* [54] that colonisation of As rich habitats is often limited rather by the lack of nutrients than by the occurrence of toxic metalloids. In Schlaining, bold patches are only formed if high concentrations of As and Sb occur in combination with low pH (C1) or intense shadowing (C1, D2); the influence of acidic soils is significant. More subtle effects of As and Sb stress on the vegetations are probable, but will be difficult to

detect, since many other soil parameters vary between the sampled sites at the Schlaining mining site.

## 5. CONCLUSIONS AND FUTURE ASPECTS

This multidisciplinary study of the former mining site in Schlaining allows the following insights:

- In spite of extensive measures for renaturation, considerable amounts of the metalloids As and Sb (in the range of weight %) are found at several microsites in the former mining area. In most of the area, however, the metalloid content of the soil is negligible. Though the mobility of metalloids is relatively low, constant leaching of metalloids occurs through at least one rivulet and most probably into the ground water.
- The metalloids investigated in this study are derived from Sb-bearing sulphidic ore minerals. Though the mine waste is derived from only one orebody, the petrological composition of the bedrock differs between the microsites. Also, the ratios between As and Sb are only weakly correlated. This may be due to a different geochemical differentiation during the formation of the orebody.
- Consequently, soil properties differ significantly between various microsites. Differences include pH, nutrient and carbonate content and mineralogical composition. With the exception of metalloid contamination, soil parameters are moderate in most cases. Arsenic (supposedly in the form of $AsO_4^{3-}$) and $PO_4^{3-}$ mobility were correlated, reflecting the chemical similarity of these anions.
- Plant biodiversity is significantly affected by As, but not by Sb contamination. At the most polluted sites, a drastic decrease in species or bold patches were observed. However, no specific, metalloid resistant plant communities seem to exist at Schlaining. Our results support the hypothesis that floristic effects of metalloid contamination are significantly tempered by the presence of a thick soil layer rich in nutrients, carbonate and humus. In contrast to this, the aquatic environment of a small rivulet (D1) in the vicinity of the overburden deposits seems to be affected by the metalloid concentrations. The permissible values for As in drinking water were increased by a factor of 2.6. The fact that the biodiversity in the rivulet was obviously impacted cannot be due to other hydrological parameters, which were, as far as we know, all in the normal range. Thus it is very likely that the metalloid content caused these adverse effects to the aquatic biosystem.
- The recent and established models to predict metalloid mobility are also insufficient for habitats with a heterogeneous mineralogical background.
- The number of soil bacteria is not affected by metalloid contamination. At all sites, at least some strains were resistant to 100 ppm As and Sb. Organotrophic bacteria cultivated on PC medium proved to be more sensitive than sulphur oxidising strains. The latter even seem to benefit from moderate As spiking and are possibly adapted to the degradation of sulphidic metalloid ores.

Due to its profound geological and mineralogical characterisation and the diversity of contaminated habitats, the mining site of Schlaining can be used as a model ecosystem to study the availability and mobility of metalloids. Future studies will include speciation of As using Synchrotron-Radiation-Induced Total Reflection X-Ray Analysis [73]. Furthermore, microbial biodiversity will be characterised using molecular techniques; special attention will be laid on microbial transformations of As and Sb [64,74]. Futhermore, native species from Schlaining may be suitable for phytoremediation of other metalloid contaminated sites [75].

Possible endangerment of man and environment deserves specific investigation. Arsenic contamination of the soil surface is always regarded as dangerous, due to the possibility of incidental ingestion from hand-to-mouth activity, especially for children [44]. Though toxic metalloids basically may endanger humans *via* the food-chain [76], this can be excluded for Schlaining, since no food plants are grown at the contaminated sites. A nearby pasture proved to be uncontaminated. However, water of rivulet D1 enters the river Tauchenbach, passing through the city of Stadtschlaining. Long range transport and precipitation of As will be influenced by the incorporation into Fe oxyhydroxides with very different mobility [66,77] or by polymerisation [78], both depending on metalloid speciation and bacterial activity. Thus, it is yet impossible to make any prediction of As and Sb contamination in settled areas which should be investigated in the next future.

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