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Speciation of arsenic under reducing conditions of organic-rich
soil

PhD thesis

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Summary

The PhD thesis deals with arsenic speciation under reducing conditions of soil with elevated content of organic matter. This issue has been studied in two wetland soils located in two geochemical As anomalies near the villages of Mokrsko and Smolotely (CZ). Speciation and mobility of As was studied in four experiments using a range of mineralogical (XRD, SEM/EDS, EMPA, Raman microspectroscopy) and geochemical (XRF, chemical extraction, pore water analysis) methods. Other methods used in this work included S isotopes and microbial community analysis. The results indicate that the formation of authigenic As sulfides (realgar and bonazziite) is a relatively common mechanism of As sequestration in reducing conditions of wetland soils. The sulfides are formed in highly reducing organic-rich microenvironments, where microbial production of dissolved sulfide is sufficiently fast compared to water flow velocity and related slow solute transport. In these microenvironments, Fe sulfides are the first precipitates; As sulfides precipitate after the depletion of Fe^{2+} in the pore solutions. This phenomenon was well evidenced in the long-term incubation experiment. Results of this experiment also showed that newly formed As sulfides can form in environments with variable concentrations of As and Fe in groundwater. Low reactivity of authigenic As sulfides under oxic conditions in combination with our previous findings indicate that precipitation of authigenic As sulfides is a promising remediation mechanism that can potentially be used in the constructed wetlands.

1. Introduction

Arsenic is highly toxic element that is ubiquitously found in soil, sediment, and groundwater. High concentration of As in groundwater is widely acknowledged environmental concern due to its widespread occurrence and threat to human health (Smedley and Kinniburgh, 2002; Nordstrom, 2002). The mobility of As is closely link to its speciation involving arsenate (As(V)) and more mobile and even more toxic arsenite (As(III)) species. Under well-aerated (oxic) conditions, oxyanions of As(V) are dominant and tend to be sequestered by metal (oxyhydr)oxides, especially by Fe(III) (oxyhydr)oxides. Under reducing condition, soluble arsenic mostly occurs as As(III) (e.g., arsenious acid) and its high concentrations in groundwater interact with buried natural organic matter (NOM) and other components of soils to form complexes and compounds that affect its subsequent mobility. This work focuses on one of the processes that can effectively immobilize arsenic under these conditions. It is microbial sulfidogenesis, which has recently been shown to be one of the main processes immobilizing arsenic in wetland (Langner et al., 2013; Drahota et al., 2017).

2. Aims

1. Provide information on the distribution and speciation of As in the Smolotely wetland soil and elucidate the role of NOM and soil microorganisms in Fe-As-S mineral formation (Experiments 1 and 2).
2. Quantitatively assess the *in situ* accumulation of As in three low-cost NOM incubated in three different natural geochemical subsurface systems (Experiment 3).
3. Clarify the short-term temporal dynamic of As and trace metals fractions and their coupling to other redox processes in the As-bearing sulfide-rich Mokrsko and Smolotely wetland soils (Experiment 4)

3. Materials and methods

3.1 Soil samples

The experiments 1 and 2 were performed in the natural geochemical arsenic anomaly at Smolotely-Horní Líšnice Au district (Fig. 1). Two soil types located at three sites (S1, S2, S3) plus the mining waste (S4) were sampled in four profiles (Fig. 1). Then we investigated in detail (Exp. 2) the wetland soil to provide more information on the distribution and speciation of As in the wetland soils by characterization of the other two profiles located at S3 (A and B). Solid samples were collected from excavation pits and composited along a profile for discrete depth intervals (5–20 cm) on the basis of morphological transitions (color, consistency). The samples intended for geochemical and mineralogical characterization were immediately placed in Ar-purged zip-lock plastic bags and stored at 4 °C and in anoxic conditions until their return to our laboratory. The soil material was freeze-dried, processed (sieving < 2 mm and milling < 50 µm) in an anoxic glove box ($pO_2 < 1$ ppm), and kept anoxic until the analyses.

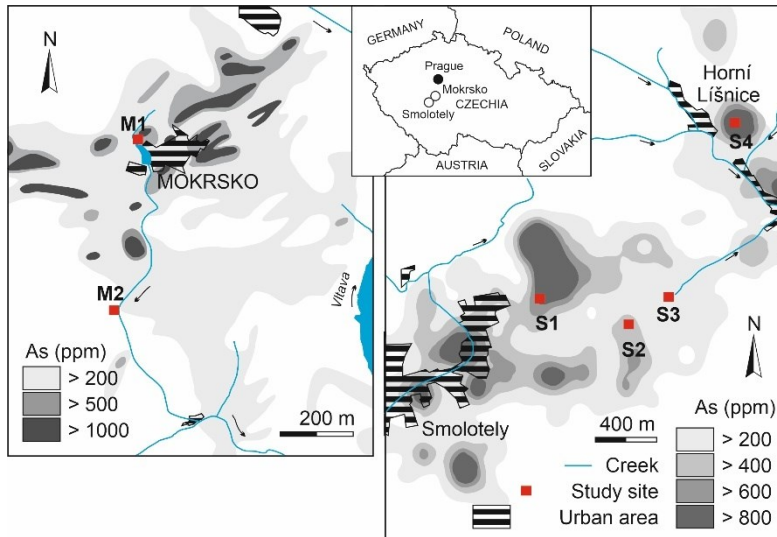


Fig. 1 Schematic maps of the wetland site locations and arsenic concentration in soils at the Mokrsko and Smolotely.

3.2 *In situ* experiment

The *in situ* incubation (Exp. 3) was examined in three naturally-contaminated wetland sites. Sites M1 and M2 are located at the Mokrsko geochemical anomaly (Fig. 1). The experimental site S3 is situated at the Smolotely geochemical anomaly (Fig. 1). Approximately one-meter-deep soil pits were excavated at each site and the bottom soil was sampled for geochemical and mineralogical characterization. The experimental bags with

different types of natural organic matter, were alternately inserted at the depth of 1 m and introduced ~10 cm from the soil pit front, in order to minimize the disturbance of the overlying soil structure. Subsequently, the soil pits were filled with excavated soil matrix. After 15 months, the bags were collected, immediately placed in Ar-purged zip-lock plastic bags and stored at 4°C under inert atmosphere until their return to the laboratory. The external nylon bag, containing the traces of the soil matrix was discarded, and the internal bag (IB) with NOM were freeze-dried and kept in anoxic conditions until the separate analyses.

3.3 Experimental drought

In May 2018, 1 m deep soil pits were excavated in both wetland sites (M1 and S3) to obtain soil samples from the saturated zone (60–100 cm) affected by the water table fluctuation. Approximately 17 kg of wet soil from this depth interval was sieved and moved to an experimental container, where the samples were thoroughly homogenized and saturated by pore water. The containers were then transported to the laboratory and kept in the open air at a laboratory temperature. To examine the As and metal fractionations during gradual drying of flooded soils, every 3 days of the first month and then once after the second (day 64) and third

month (day 96), 10 – 20 g of the triplicate soil sample was collected using a clean 50-mL plastic tube.

3.4 Analytical methods

The speciation and behavior of As in the collected samples were studied using a number of geochemical (bulk soil and pore water analyses, selective chemical extractions, S isotopes), mineralogical (X-ray diffraction (XRD), scanning electron microscopy and energy-dispersive X-ray spectroscopy (SEM/EDS), Raman microspectroscopy (RMS)) and DNA extraction analyses.

4. Results and discussion

4.1 Speciation of As in the wetland soil (Exp. 1 and 2)

In the soils and mining waste at the Smolotely geochemical anomaly, the primary As ore (arsenopyrite) has almost completely oxidized to secondary As minerals such as arseniosiderite, bariopharmacosiderite, yukonite, and Fe (oxyhydr)oxides (ferrihydrite, goethite, and hematite). Arsenic distribution and speciation were found to vary with depth and soil type. Whereas the presence of multiple As hosting phases that occurred in the mining waste and cambisol developed over a granodiorite, bariopharmacosiderite was absent in the cambisol overlying gabbrodiorite. Poorly-crystalline phases such as yukonite and As-bearing ferrihydrite were not detected in the wetland soil. These differences in the secondary As mineralogy were attributed to the different redox conditions and variations in the prevailing chemical systems in the saprolites/soils. The variable solubility of the secondary As-bearing phases influences the mobility of As in shallow soils and near-surface mining wastes

The distribution and speciation As in the wetland soils were investigated in the second experiment. Total solid-phase analyses and selective extractions of the soil samples below 40 cm showed

that As (up to 1.16 g kg^{-1}) and Fe(II) are coupled to S and TOC, and had accumulated to a considerable extent in the exterior parts of NOM fragments (up to 19 wt.% As). Microscale imaging and RMS revealed that As speciation in the NOM exteriors is a combination of realgar ($\alpha\text{-As}_4\text{S}_4$), bonazziite ($\beta\text{-As}_4\text{S}_4$) and arsenian Fe sulfides, primarily greigite (Fe_3S_4) and framboidal pyrite (FeS_2). The sulfide phases were depleted in the ^{34}S isotope by 6.3–29.4‰ relative to pore water SO_4^{2-} ; thus implying their biologically induced formation. Microbial communities associated with sulfidogenic environments in NOM and bulk soil had variable compositions, although the dissimilative SO_4^{2-} reduction was usually the main metabolic trait. Relatively low isotopic fractionation in sulfide-rich NOM fragments (6.3–11.6‰) compared to bulk soil (down to 26.1‰) revealed a reservoir effect that developed probably at mm-scales. This indicates formation of sulfide phases in highly localized environments depleted in aqueous SO_4^{2-} due to strong microbial sulfidogenesis when compared to the transfer rate of the solutes. The very high proportion of fermenting microorganisms in sulfide-rich NOM fragments provided further evidence of strongly reducing conditions, which are a prerequisite for sulfide phase precipitation. We have shown that by the development of suitable conditions for

sulfidogenesis, NOM fragments play an active role in As immobilization in an As-enriched wetland soil. Regarding the effectiveness of As sulfidogenesis in shallow wetland soils to remediate groundwater, our findings imply that As contamination may either be limited by the low content of labile organic matter or by the fast transfer of solutes (groundwater flow).

4.2 Impact of organic matter on As sulfidation in wetlands (Exp. 3)

After 15 months of *in situ* incubation, all of the organic materials and their corresponding inner bags were covered by yellow-black mineral accumulations, dominantly composed of crystalline As_4S_4 polymorphs (realgar and bonazziite) and reactive Fe(II) sulfides (probably mackinawite); while the major fraction of As (~80%) was sequestered as AsS minerals. The amount of As accumulation in the experimental bags varied significantly (0.03–4.24 g As kg^{-1}) and corresponded with different levels of As (0.23–9.4 mg As L^{-1}) in the groundwater. Our findings suggest an authigenic formation of AsS minerals in strongly reducing conditions of experimental bags by a combination of reduced exchange of solutes through the pores of the bag and comparatively fast microbial production of dissolved sulfide. Arsenic sulfide

formation, as an effective treatment mechanism for natural and human-constructed wetlands, appears to be favored for As(III)-rich waters with a low Fe(II)/As(III) molar ratio. These conditions prevent the consumption of dissolved As and sulfide by their preferential incorporation into natural organic matter, and newly-formed Fe(II) sulfides, respectively.

4.3 Arsenic fractionation and mobility during experimental drying (Exp. 4)

During the initial stage of incubation (~20 days), the re-flooding of the soils triggered a microbially-mediated SO_4^{2-} reduction, which immobilized the Co, Cu and Ni. The reductive dissolution of As-bearing Fe (oxyhydr)oxides and the release of As were documented only in the Fe-rich/organic-low soil (S3). Over the next stage of incubation (~75 days), the exposure and drying of the soils led to the oxidation of the Fe and As sulfides. The arsenic and trace metals released via oxidation of the sulfide phases (particularly Fe sulfides) were almost entirely sequestered by the Fe(III) (oxyhydr)oxides, but acidification during the oxidation stage of the incubation resulted in the pH-dependent release of the As and trace metals (Co, Cu, Ni) (especially in the Fe-rich/organic-low soil of S3). These findings suggest that sulfidic soils in

wetlands can be considered as long-term sources of As during major drought events.

5. Conclusion

Microbial sulfidogenesis plays a potentially important role in As biogeochemistry within wetland soils, sediments, and aquifers. We identified extensive precipitation of realgar, bonazziite, and arsenian Fe sulfides in a naturally As-enriched wetland soils. Microbiological and S isotope results indicate that sulfide phases were generated by *in situ* microbial reduction of oxidized forms of S in the deeper (>40 cm), water-saturated soil. Sulfidation of As is mainly associated with buried NOM fragments, that represents highly localized environments with mass-transfer limitations on solute transport, where microbial sulfidogenesis leads to SO_4^{2-} depletion and an enrichment in isotopically heavy SO_4^{2-} , which in turn mutes the fractionation associated with sulfide formation.

The experimental bag technique was used to simulate the interaction of NOM with the indigenous sulfate-reducing microorganisms of three As-enriched wetland soils, with a special emphasis on As immobilization by the newly formed sulfide

phases. A 15 months *in situ* incubation of low-cost organic materials (sawdust, wood cubes, and hemp shives) in pH-neutral soils with contrasting As and Fe concentrations (As: 0.45–9.9 mg L⁻¹; Fe: 3.3–21 mg L⁻¹) in the groundwater resulted in precipitation of As and Fe sulfides in all types of organic materials and all of the studied wetland sites. This bioremediation process relying on the mechanism of AsS precipitation offers the potential to promote removal of As from many contaminated waters and effluents. However, further study of the optimal hydraulic properties of the organic-rich substrates providing the long-term AsS precipitation is important to better understand the feasibility of this process in treatment systems such as constructed wetlands.

6. Reference

- Drahota P., Falteisek L., Redlich A., Rohovec J., Matoušek T., Čepička I. (2013) Microbial effect on the release and attenuation of arsenic in the shallow subsurface of a natural geochemical anomaly. *Environ. Pollut.* **180**, 84–91.
- Langner P., Mikutta, C., Suess E., Marcus M.A., Kretzschmar R. (2013) Spatial distribution and speciation of arsenic in peat studied with microfocused X-ray fluorescence spectrometry and X-ray absorption spectroscopy. *Environ. Sci. Technol.* **47**, 9706–9714.
- Le Pape P., Battaglia-Brunet F., Parmentier M., Jouliau C., Gassaud C., Fernandez-Rojo L., Guigner J. M., Ikogou M., Stetten L., Olivi L., Casiot C., Morin G. (2017) Complete removal of arsenic and zinc from heavily contaminated acid mine drainage *via* an indigenous SRB consortium. *J. Hazard. Mater.* **321**, 764–772.
- Newman D.K., Beveridge T.J., Morel F.M.M. (1997) Precipitation of arsenic trisulfide by *Desulfotomaculum auripigmentum*. *Appl. Environ. Microbiol.* **63**, 2022–2028.
- Nordstrom D.K. (2002) Public health – worldwide occurrences of arsenic in groundwater, *Science* **296**, 2143–2145.

Rodriguez-Freire L., Sierra-Alvarez R., Root R., Chorover J., Field J.A. (2014) Biomineralization of arsenate to arsenic sulfides is greatly enhanced at mildly acidic conditions. *Water Res.* **66**, 242–253.

Rodriguez-Freire L., Moore S.E., Sierra-Alvarez R., Root R.A., Chorover J., Field J.A., (2016) Arsenic remediation by formation of arsenic sulfide minerals in a continuous anaerobic bioreactor. *Biotechnol. Bioeng.* **113**, 522–530.

Smedley P.L., Kinniburgh D.G. (2002) A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochem.* **17**, 517–568.

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Education

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Publications

Peřestá M., Drahota P., Culka A., Matoušek T., Mihaljevič M. (2022) Impact of organic matter on As sulfidation in wetlands: An in situ experiment. *Science of the Total Environment* **819**, 152008. (IF =10,753)

Dordevic T., Drahota P., Kolitsch U., Majzlan J., **Peřestá M.**, Kiefer S., Stöger-Pollach M., Tepe N., Hofmann T., Mikuš T., Tasev G., Serafimovski T., Boev I., Boev B. (2021) Synergetic Tl and As retention in secondary minerals: An example of extreme arsenic and thallium pollution. *Applied Geochemistry* **135**, 105114. (IF =3,61)

Drahota P., **Peřestá M.**, Trubač J., Mihaljevič M., Vaněk A. (2021) Arsenic fractionation and mobility in sulfidic wetland soils during experimental drying. *Chemosphere* **277**, 130306. (IF =7,086)

Knappová M., Drahota P., Falteisek L., Culka A., Penížek V., Trubač J., Mihaljevič M., Matoušek T. (2019) Microbial sulfidogenesis of arsenic in naturally contaminated wetland soil. *Geochimica et Cosmochimica Acta* **267**, 33–50. (IF = 4,258)

Drahota P., Kulakowski O., Culka A., **Knappová M.**, Rohovec J., Veselovský F. and Racek, F. (2018) Arsenic mineralogy of near-neutral soils and mining waste at the Smolotely-Lišnice historical gold district, Czech Republic. *Applied Geochemistry* **89**, 243–254. (IF = 2,581)

Drahota P., **Knappová M.**, Kindlová H., Culka A., Majzlan J., Mihaljevič M., Rohovec J., Veselovský F., Fridrichova M., Jehlička J. (2016) Mobility and attenuation of arsenic in sulfide-rich mining wastes from the Czech Republic. *Science of the Total Environment* **557**, 192–203. (IF = 4,42)

Conference abstracts and contribution

World Soil Day conference, 5th December, Prague, Czech republic, **Peřestá M.**, Drahota P., Falteisek L., Culka A., Penížek V., Trubač J., Matoušek T., Mihaljevič M. (2019) Vznik sekundárních sulfidů As v glejových půdách na lokalitě Smolotely (oral presentation)

Goldschmidt 2019, 18 – 23 August, Barcelona, **Knappová M.**, Drahota P., Falteisek F. (2019) Microbial sulfidogenesis of As in naturally contaminated wetland soil (poster).

UNCE/SCI/006 PhD conference, 4th April, Prague, The Czech Republic, **Knappová M.**, Drahota P., Falteisek L., Culka A., Penížek V., Trubač J., Matoušek T. (2019) Microbial sulfidogenesis of As in wetland (oral presentation).

CEMC (Central-European mineralogical conference), 26 – 30 June, Baňská Šťávnice, Slovakia, **Knappová M.**, Drahota P., Penížek V., Klementová M., Veselovský F., Racek M. (2018) Microbial sulfidogenesis of As in wetland (poster).

Geochémia, 6 – 7 December, Častá-Papiernička, Slovakia, **Knappová M.**, Drahota P., Kulakowski O. (2017) Vazba As v glejových pôdach prírodnej geochemickej anomálie Smolotely u Příbrami (poster).