Environmental Earth Sciences (2018) 77:542
https://doi.org/10.1007/s12665-018-7714-x

THEMATIC ISSUE

Bioleaching potential of bacterial communities in historic mine waste areas

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Received: 13 February 2018 / Accepted: 6 July 2018

Abstract
Microbial activity has the potential to alter all cultural heritage in mining and metallurgy, due to metal mobilization by leaching. This communication shows the consequences of the bioleaching ability of two natural enrichments on copper slag samples from a historic ore smelting site in Sangerhausen (Mansfeld, Südharz, Saxony-Anhalt, Germany). Enrichment cultures gained from mine drainage were dominated by either the iron and sulfur-oxidizing *Acidithiobacillus ferrivorans*, or by the iron-oxidizing *Leptospirillum*. During 35 days of bioleaching in media containing copper slag pulp, inoculated with these enrichments, the change in pH and solubilized metal concentrations of the systems were monitored. Both bacterial strains were completely different from each other in their pattern of pH variation and rates of metal solubilization. The maximum removal of Cu (1725 mg/l) and Zn (715 mg/l) from copper slag substrate was achieved with enrichment culture of *A. ferrivorans* SCUT-1. However, maximum Mn (207 mg/l), Pb (86 mg/l), and Ni (75 mg/l) removal was observed with enrichment culture of *Leptospirillum* strain YQP-1. Implications for metal mobilization along with alteration of artifacts from not only historic mining areas but also aspects of decontamination and remediation are discussed.

Keywords Mine waste · Copper slag · Bioleaching · *Leptospirillum* · *Acidithiobacillus*

Introduction

Mining of transition metals has been considered as the trademark of civilization. However, the adverse effects of industrial effluents caused by mining since the earliest times of metallurgy have been highlighted as a serious threat to the environment (Cortizaz et al. 2016; Malik 2004; Donkor et al. 2005). Biodiversity sources and natural ecosystems are challenged or destroyed (Cooke and Johnson 2002; Getaneh and Alemayehu 2006). Contamination is not only limited to soil and surface water, but the contaminating metals may also infiltrate underground water resources and through plant material metals may enter the food chain and can pose serious effects on human health (Kim et al. 2001; Peter 2011). Severe environmental impacts date back to the chalcolithic and reached a first culmination during the Roman Empire (e.g., Leblanc et al. 2000; Mateus et al. 2011; Grattan et al. 2016).

On the other hand, mining activity is tightly connected to important items of our cultural heritage. Apart from the archaeological sites below ground, one important example is the Upper Harz Water Regale, which represents a system of channels and dams to funnel water to power water wheels for mining activities. It was (partly) active until the nineteenth century (Balck 1999). The Water Regale was declared a UNESCO World Heritage Site in 2010. Another example is the Zollverein Coal Mine Industrial Complex (Zeche Zollverein), World Heritage since 2001, and part of a European Network of Industrial Heritage, comprising numerous monuments and sites (including mining) throughout Europe (Douet 2013). Many other examples in Central Europe are of equal importance, like the Ore Mountain Mining Region (Czech Republic/Saxony; Wagenbreth et al. 1990), the Rhenish Massif ore mining areas (cf. Fig. 1; Reininghaus

This article is part of a Topical Collection in Environmental Earth Sciences on "Stone in the Architectural Heritage: from quarry to monuments—environment, exploitation, properties and durability", guest edited by Siegfried Siegesmund, Luís Sousa, and Rubén Alfonso López-Doncel.

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Published online: 19 July 2018

Fig. 1 Kilianstollen Marsberg (Rhenish Massif). **a** Adit with drainage channel at lower left. **b** View into a cross passage with apparent remineralizations of copper, iron and manganese at walls and the bottom. The foreground bottom line of the image is approximately 80 cm in width. **c** Copper precipitating on an iron plate (cement copper). The visible part of the copper plate measures approximately 1.5 m × 60 cm. **d** Mining drainage with ochre and greenish remineralization. The same drainage channel is visible in **a**

and Köhne 2008) and the Mansfeld and Sangerhausen mining districts. In the latter, mine waste piles with relevant amounts of transition metals are important elements of a cultural landscape (Fig. 2; Anonymous 1999). In all of these areas, sites are contaminated with metals and toxic byproducts of metallurgical processing. Hence, any kind of mine tailings threaten industrial heritage, and are part of it at the same time. Measures to prevent the environment against heavy metal contamination must be taken into account. Decontamination measures are also necessary to preserve mining areas as historic sites and to make them accessible to the public (cf. Conesa et al. 2008).

Many remediation techniques have been established and utilized in the past to remove metals from soil and waste water: (1) excavation for complete and fast removal of metals from contaminated sites (Wood 1997); (2) chemical precipitation (Matlock et al. 2002); (3) membrane filtration (Blöcher et al. 2003); (4) ion flotation (Polat and Erdogan 2007); (5) chemical reagents-based soil washing (Dermont et al. 2008); (6) chemical immobilization technique for heavy metal removal by decreasing their solubility (Fawzy 2008); (7) reverse osmosis (Bakalár et al. 2009); (8) phytoremediation (Nouri et al. 2009; Kord et al. 2010); (9) ion-exchange and electrokinetic technique to mobilize heavy metals ions (Pamukcu and Wittle 1992; Kang et al. 2007; Hubicki and Kołodyńska 2012; Sivapullaiah et al. 2015). Most of these heavy metal removal technologies are not widely used because of their high reagent requirements, limited cost effectiveness and unpredictable rate of metal ion removal at low-metal concentrations (Maini et al. 2000; Malik 2004). In addition, most of these techniques are not eligible, if complete or partial preservation of mining waste sites as cultural heritage is of importance.

In addition to physico-chemical methods, biological remediation ("bioleaching") by microorganisms is an efficient method of metal removal and recovery from ore and contaminated soil. Microorganisms have evolved a variety of mechanisms that enable them to bear metal stress and survive in

Fig. 2 Kupferhütte Sangerhausen. **a** Copperplate engraving depicting the Kupferhütte Sangerhausen and its surrounding area in the year 1835 (by F. Giebelhausen; regional history collections of Lutherstadt Eisleben https://www.museum-digital.de/st/index.php?t=objekt&oges=43906). **b** The neoclassical building of the Kupferhütte smelting plant in its current state. **c** The nearby copper slag pile in its current state. **d** Appearance of copper slag debris of the slag pile as depicted in **c. e** Copper slag showing apparent remineralizations as used for leaching experiments

heavy metal-contaminated sites. Detoxifying mechanisms involve metal solubilization, mobilization and immobilization (e.g., White et al. 1997; Gadd 2000), biotransformation of metals to non-toxic products (Verma et al. 2001), biosorption (Lin and Lin 2005), bioaccumulation (Umrania 2006; Rani and Goel 2009; Ahemad and Khan 2011) and oxidation and reduction reactions (Lovley and Phillips 1988; Sims et al. 1990; Lovley 1995; Nasrazadani et al. 2011). In many studies, the importance of bacterial metabolic pathways involved in bioleaching process have been highlighted (Lundgren and Silver 1980; Tyagi et al. 1988; Rulkens et al. 1995; Lombardi and Garcia 1999; Suzuki 2001; Sand et al. 2001). Some reports have shown the success of bioleaching technology on large scale for recovery of copper, zinc, nickel, cadmium and various other metals (Hutchins et al. 1986; Holmes 1991).

Many acidophilic, iron and sulfur-oxidizing heavy metal-resistant microorganisms have been isolated. Some prominent examples are *Acidithiobacillus thiooxidans* (Waksman and Joffe 1922; Kelly and Wood 2000), *Acidithiobacillus ferrooxidans* (Temple and Colmer 1951), *Thiobacillus albertis* (Bryant et al. 1983), *Acidithiobacillus caldus* (Hallberg and Lindström 1994), *Leptospirillum ferrooxidans* (Hippe 2000), and *Acidithiobacillus ferrivorans* (Hallberg et al. 2010). *Acidithiobacillus* species gain energy by oxidation of metal sulfides, Fe^{2+} or hydrogen and tolerates, besides low pH, high concentrations of transition metals (Kelly and Wood 2000). *Acidithiobacillus ferrooxidans* is the best studied member of the genus because of its role in metal sulfide ore bioprocessing (Rawlings and Johnson 2007). *Leptospirillum* is another important genus whose members are frequently used in industrial bioleaching processes. This genus was first described by Hippe (2000). *Leptospirillum* YQP-1 was isolated in 2013 from a volcanic lake (Wudalianchi volcano, NE China). Its draft genome was also recently published (Yan et al. 2015). The chemolithoautotrophic bacteria use Fe^{2+} ion as an electron donor and oxygen as an electron acceptor (Hippe 2000).

The present study aimed at the enrichment of indigenous heavy-metal-resistant, iron- and sulfur-oxidizing bacterial strains from Kilian adit ("Kilianstollen" copper mine; Marsberg, Germany; cf. Fig. 1) and evaluation of their metal-leaching abilities by monitoring of five different metals (Cu, Zn, Mn, Pb, and Ni). The enrichments were used for leaching samples from a historic slag pile (Kupferhütte Sangerhausen, Saxony Anhalt).

Materials and methods

Sampling sites

Samples for inoculating enrichment cultures were taken from mine drainages of the Kilian adit ("Kilianstollen" copper mine, abandoned in 1945, Marsberg, 51.453502°N, 8.861703°E). The mine is distinguished by a variety of secondary minerals and mine drainages. Sampled crusts were orange/brownish in color, indicative for formation of iron/manganese secondary minerals (Emmerich and Heydemann 1987; Fig. 1). For bioleaching assays crushed pieces of slag obtained from a former smelting plant (Kupferhütte Sangerhausen; 51.489302°N, 11.310185°E; Fig. 2) were used.

For enrichment cultures, 6.75 g of slushy, iron- and manganese-rich sediment as described in Emmerich and Heydemann (1987), was suspended in 50 ml of sterilized distilled water. 10 ml of the suspension was added to 90 ml of sterile 9K medium (containing per 1 l dist. water: 3 g $(NH_4)_2SO_4$, 0.1 g KCl, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \times 7\ H_2O$, 0.01 g $Ca(NO_3)_2$, 44.2 g $FeSO_4 \times 7\ H_2O$, pH adjusted to 2.5 with H_2SO_4; Silverman and Lundgren 1959). The enrichment cultures were incubated aerobically on a rotary shaker at 30 °C, 125 rpm agitation in Erlenmeyer flasks (Ki16-6c). Another culture in the same medium was incubated at room temperature, without agitation (Ki16-4b). Cultures were observed every other week to check for metal precipitation and change in color of the medium.

Adaptation experiments

Bacteria from enrichment cultures Ki16-6c and Ki16-4b were adapted to a substrate prepared from slag pile samples from the site Kupferhütte/Sangerhausen (SSP). For this, pieces of slag debris of different size (cf. Fig. 2d, e) with obvious signs of secondary mineral formation, were crushed to particles in the range of 1 mm or smaller. For adaptation experiments, a 10% inoculum from enrichment cultures in their stationary growth phase was transferred into fresh 9K medium (pH 2.4), containing 2% (w/v) sterile crushed slag substrate followed by incubation at 30 °C, 125 rpm agitation for Ki16-6c and 20 °C, without agitation for Ki16-4b.

The bacterial consortia were re-inoculated (10% v/v, inoculum) into fresh (2% w/v) SSP substrate containing 9K medium in intervals of either 3 or 7 days. After three such transfers to substrate containing 9K media, bacteria were used for bioleaching (see below: "Bioleaching assay").

Preparation of samples for next-generation sequencing

DNA extraction was performed using PowerSoil DNA isolation kit (MO BIO, Carlsbad, Cal., USA) with slight modifications of the manufacturer's protocol. For DNA extraction, 6 ml of enrichment cultures were taken in sterile Eppendorf tubes and centrifuged at 10,000×*g* for 10 min. Supernatants were discarded, pellets were resuspended in remaining supernatant liquid and ~250 µl of these samples were then

added to the provided Power Bead Tubes. All other steps were performed according to the manufacturer's protocol.

Analysis of bacterial community composition of the samples from enrichment cultures Ki16-6c and Ki16-4b was performed by polymerase chain reaction (PCR) amplification and subsequent sequence analysis of V3 and V4 regions of the 16S rRNA genes. To make the amplicon suitable for Illumina MiSeq sequencing, the primers were additionally linked to the overhang adapter sequences (16S amplicon PCR forward primer = 5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′ ; 16S amplicon PCR reverse primer = 5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC-3′) (Klindworth et al. 2013).

PCR reaction mixture for amplification of bacterial DNA was according to Klindworth et al. (2013) with some modifications: a final volume of 50 µl in double-distilled nuclease-free water contained 5 × Phusion GC Buffer (10 µl), 10 µM forward primer (1.0 µl), 10 µM reverse primer (1.0 µl), 50 mM $MgCl_2$ (0.15 µl), 5% DMSO (2.5 µl), 10 mM dNTPs (1.0 µl), 2 U/µl Phusion HF DNA polymerase (0.5 µl; Thermo-Fisher Scientific, Waltham, MA, USA), 25 ng template DNA (2.0 µl). Quality of PCR products were controlled on a 0.8% agarose gel with 1× TAE buffer (Thermo-Fisher Scientific). PCR products were extracted and processed by using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA quantification was performed with a NanoDrop (Thermo-Fisher Scientific) according to the manufacturer's instructions.

Indexing of the PCR products was performed with Nextera XT DNA library prep kit (Illumina, San Diego, Cal, USA) according to manufacturer's instructions. Paired-end sequencing was performed in collaboration with Göttingen Genomics Laboratory with an Illumina MiSeq sequencer (Illumina).

Bioinformatical 16S rRNA gene processing

Demultiplexing of MiSeq sequences (2 × 300 bp, MiSeq Reagent Kit v3) was performed by CASAVA data analysis software (Illumina). Paired-end sequences were merged using PEAR v0.9.10 (64 bit) (Zhang et al. 2014) with default parameters. The resulting sequences that fulfilled at least one of the following criteria were removed with the *split_libraries_fastq.py* script from QIIME 1.9.1: average quality score lower than 20 and containing unresolved nucleotides (Caporaso et al. 2010). Additionally, unclipped reverse and forward primer sequences were removed by employing cutadapt v1.10 (Martin 2011) with default settings. For operational taxonomic unit (OTU) clustering, USEARCH (8.1.1861) with the UPARSE algorithm was used. All quality-filtered sequences were sorted by length, dereplicated, short sequences (below 400 bp) and singletons were removed. Subsequently, OTUs were clustered at 97% sequence identity using USEARCH. Chimeric sequences were removed using UCHIME in reference mode against RDP trainset15_092015.fasta (https://sourceforge.net/projects/rdp-classifier/files/RDP_Classifier_TrainingData/). All quality-filtered sequences were mapped to chimera-free OTUs and an OTU table was created using USEARCH. Taxonomic classification of OTUs was performed with *parallel_assign_taxonomy_blast.py* against the SILVA SSU database release 123.1. Extrinsic domain OTUs, chloroplasts, and unclassified OTUs were removed from the dataset by employing *filter_otu_table.py*. Sample comparisons were performed at the same surveying effort, utilizing the lowest number of sequences by random selection (13,400 reads). Species richness, alpha and beta diversity estimates and rarefaction curves were determined using the QIIME 1.9.1 script *alpha_rarefaction.py*.

Bioleaching assay

In 90 ml of 9K medium, SSP substrate was added as 5% (w/v) and 10% (w/v) pulp density in separate Erlenmeyer flasks. After sterilization by autoclaving at 121 °C for 3 h and cooling to room temperature, 10 ml (10% v/v) of active adapted bacterial culture (see above: "Adaptation experiments") were inoculated into the flasks. For negative controls, 1 ml of 37% formaldehyde solution was added in cultures as bactericidal agent. The flasks inoculated with enrichment cultures of iron and sulfur-oxidizing bacteria (*A. ferrivorans* SCUT-1) were incubated at 30 °C, 125 rpm agitation. However, the flasks inoculated with enrichment cultures of iron-oxidizing bacteria (*Leptospirillum* sp. YQP-1) were left at 20 °C without agitation.

Measurement of the solubilized metal concentrations with photometric standard analysis

The leaching solutions were sampled in 7 days of intervals and processed for the measurement of pH and concentrations of soluble metal ions (Cu, Zn, Mn, Pb, and Ni). Metal ion concentrations were determined with standard assay kits using a pHotoFlex® Series, PROG. V2.05/2.05W Photometer (WTW Xylem Analytics, Germany). Metal ion detection tests were performed by following manufacturer's instruction (Photometry Analysis Manual ba75509e21 07/2014). For each test, appropriate dilutions of the leaching solutions were prepared. For Cu, Zn, Mn and Ni tests, pH of the leaching solutions was adjusted to at least 5 using 5% diluted sodium hydroxide solution or 5% conc. sulfuric acid. For Pb tests, pH of the leaching solutions was adjusted to ~ 5 using

5% ammonia solution or 5% diluted nitric acid as necessary. After pH adjustment, leaching solutions were centrifuged at 10,000×*g* for 10 min and then supernatant was processed to measure solubilized metals concentration with the standard assay kits.

Results and discussion

Bacterial community composition in iron-oxidizing enrichment cultures

When samples from iron- and manganese-rich sediment (Kilianstollen, Marsberg; Emmerich and Heydemann 1987) were inoculated in 9K medium, after 14–18 days a change in color of the medium from yellowish to dark reddish-brown as well as formation of a precipitate was observed, presumably due to oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) (Maeda et al. (1999); see also Fig. 3). This feature was observed for cultures grown aerobically under continuous agitation of the flask ("aerated"), as well as semi-anaerobically grown cultures without agitation ("dysoxic"). Samples were processed for bacterial community analysis by 16S rRNA gene sequencing to get insights into their indigenous iron-oxidizing bacterial communities. Significant differences between the microbial communities were observed. In 9K-6c culture sample (9K medium inoculated with Ki16-6c sample), *Proteobacteria* (76.86%) was the dominant phylum with *Gammaproteobacteria* and *Betaproteobacteria* as the dominant classes. However, in 9K-4b culture sample (9K medium inoculated with Ki16-4b sample), the dominant phylum was *Nitrospirae* (92.21%; Fig. 4).

At higher taxonomic resolution in sample 9K-6c 116 genera were detected, however, three of them, *Acidithiobacillus* (47.84%), *Acidovorax* (14.14%) and *Rhodanobacter* (6.16%) were the most abundant. In 9K-4b sample in total 24 genera were observed but only one genus, *Leptospirillum* (92.15%), was highly abundant in that sample (Fig. 4). We observed that during further enrichment (either aerated or dysoxic, as explained above) of these two positive samples, it was possible to retrieve 99.31% of *Acidithiobacillus* genus in 9K-6c-1E sample and 99.85% of *Leptospirillum* genus in 9K-4b-1E sample (Fig. 4). The OTUs were identified as closest relatives of *A. ferrivorans* SCUT-1 (100% sequence identity; accession no. KU987584.1) and *Leptospirillum* strain YQP-1 (100% sequence identity; accession no. KJ573504.1).

Acidithiobacillus ferrivorans is a novel species of *Acidithiobacillus* genus proposed by Hallberg et al. (2010) and described as straight rod-shaped, iron and sulfur-oxidizing, facultative anaerobic, diazotrophic, chemolithoautotrophic, psychrotolerant acidophilic bacterium. The specific bacterial strain, *A. ferrivorans* SCUT-1 was isolated in 2016 from acid mine drainage in China (Fan et al. unpublished).

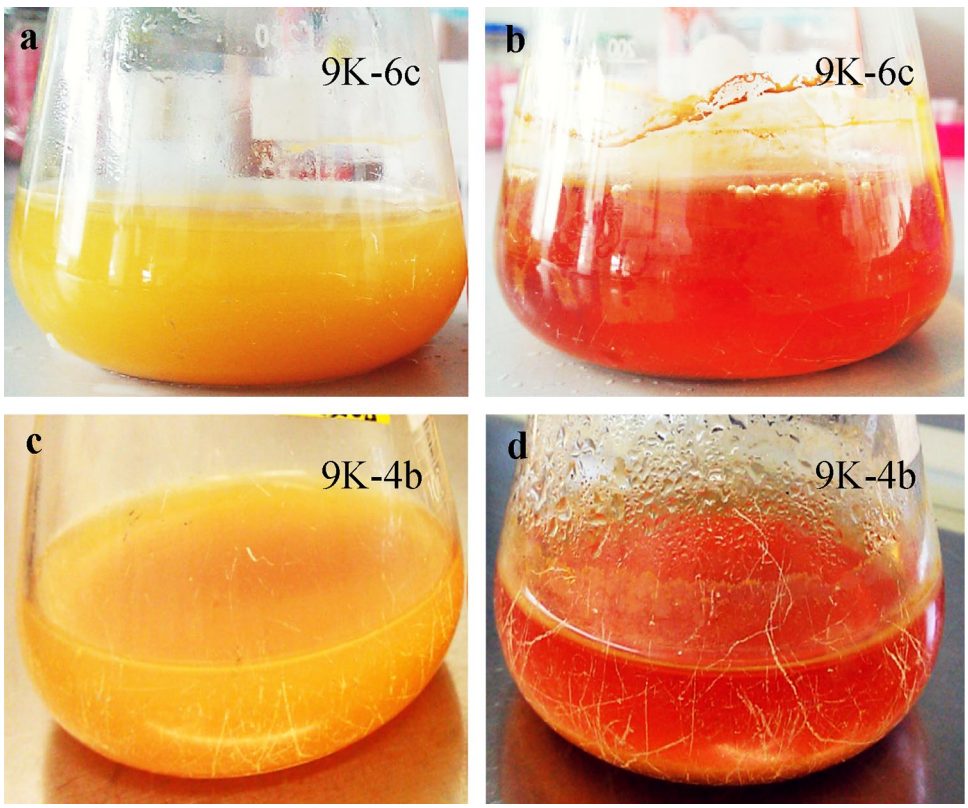

Fig. 3 **a–d** Apparent color change from yellowish to reddish-brown in cultures 9K-6c and 9K-4b inoculated with samples taken from Kilianstollen, Marsberg (cf. Fig. 1b) in 9K medium within 14–18 days of incubation

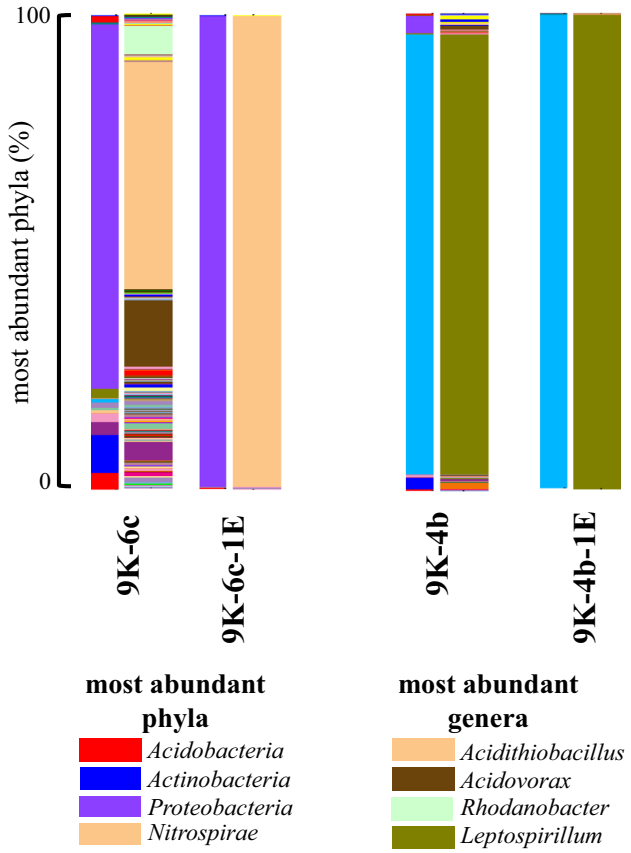

Fig. 4 Composition of bacterial community in iron-oxidizing cultures (9K-6c and 9K-4b) and iron-oxidizing enrichment cultures (9K-6c-1E and 9K-4b-1E) of Kilianstollen, Marsberg (cf. Fig. 1b), based on 16S rRNA gene amplicon sequencing at phylum (left) and genus (right) level; depicted in stacked bar charts are the relative abundances

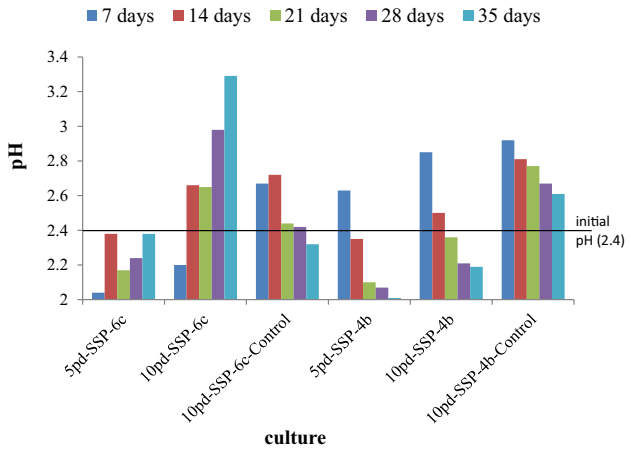

Fig. 5 Variation in pH of the leaching systems with time. SSP-6c: cultures with Sangerhausen slag pile (SSP) as a substrate and *A. ferrivorans* SCUT-1 enrichments as inoculum. SSP-4b: cultures with SSP substrate inoculated with iron-oxidizing bacteria *Leptospirillum* sp. YQP-1 enrichments. Control: formaldehyde treated cultures. Culturing conditions: 30 °C, 125 rpm for SSP-6c and 20 °C, no agitation for SSP-4b, substrates at 5% and 10% pulp density

Members of the genus *Leptospirillum* are frequently used in industrial bioleaching processes (Sand et al. 2001). This first valid description of the genus was given by Hippe (2000). Metabolically, these bacteria are chemolithoautotrophic using Fe^{2+} as an electron donor with oxygen as electron acceptor (Hippe 2000). *Leptospirillum* YQP-1 was isolated from a volcanic lake (Wudalianchi volcanic field, NE China). The draft genome of this *Leptospirillum* sp. YQP-1 was recently published (Yan et al. 2015). The bioleaching efficiency of both strains with respect to copper slag has not yet been addressed.

Bioleaching assay

Variation in pH of the leaching system with time

It was observed that pH of cultures with Sangerhausen slag pile substrate that were inoculated with enrichment cultures of *A. ferrivorans* SCUT-1 (SSP-6c) decreased during the first week and then increased till the end of the leaching experiments (Fig. 5). However, in cultures inoculated with enrichments of *Leptospirillum* sp. YQP-1 (SSP-4b), pH increased during the first week and then decreased after the 7th day till the end of the leaching experiments. In case of all abiotic (formaldehyde-treated) controls, pH started to decrease after 7 or 14 days till the end of the leaching experiments.

Apparently, pH change depends on the type of bacterial species used during bioleaching assay. Though generally *Acidithiobacillus* decreases pH during growth on reduced sulfur compounds due to sulfur oxidation and production of sulfuric acid, which results in mobilization of metals according to Eqs. (1) and (2) (Chen and Lin 2001), pH increased, after an initial drop, in the following weeks of incubation (in particular at 10% pulp density; Fig. 5).

$$2S + 3O_2 + 2H_2O \xrightarrow{\text{S-oxidizing bacteria}} 2SO_4^{2-} + 4H^+ \quad (1)$$

$$H_2SO_4 + \text{Sediment} - M \rightarrow \text{Sediment} - 2H + MSO_4 \quad (2)$$
$$M: \text{ metal}$$

Rise in pH of the SSP-6c systems can be explained as a result of bacterial ferrous ion (Fe^{2+}) oxidation (Eq. (3)), which is an acid-consuming reaction at low pH (Kinnunen et al. 2006; Marhual et al. 2008; Singer and Stumm 1970). Generally, ferric ion (Fe^{3+}) oxidizes sulfide minerals, thereby converting the insoluble metal sulfides to soluble metal sulfates and ferric iron to more ferrous ion (Fe^{2+}) (Eqs. 4 and 5) (Sand et al. 2001; Kinnunen et al. 2006). Thus, at low pH, this cyclic oxidation/reduction reaction continues and causes more and more solubilization of metals (Marhual et al. 2008). Hence, the solubilized metal concentrations

were continuously raising with time. Due to the low amount of sulfur in slag, the reaction may be limited, as well as the production of H^+ according to Eq. 4 (cf. Tewelde 2004).

$$2Fe^{2+} + 0.5O_2 + 2H^+ \xrightarrow{\text{Fe-oxidizing bacteria}} 2Fe^{3+} + H_2O \quad (3)$$

$$FeS_2 + 8H_2O + 14Fe^{3+} \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (4)$$

$$CuFeS_2 + 4Fe^{3+} \rightarrow Cu^{2+} + 5Fe^{2+} + 2S^0 \quad (5)$$

In cultures inoculated with SSP-4b (*Leptospirillum* sp. YQP-1), after a week of bioleaching, pH values reached 2.63 and 2.85 for 5% and 10% substrate pulp density, respectively. Again, the oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) by iron-oxidizing bacteria according to Eq. (3) results in pH increase. It is known from other studies that pH between 2.3 and 3.5 is suitable for precipitation of ferric ion (Fe^{3+}) as $Fe(OH)_3$ (Eq. (6)) and also favorable for the formation of jarosite (Eq. (7)) (Xiang et al. 2000). Both reactions are acid producing and may lower pH after the 7th day of bioleaching till the end.

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \quad (6)$$

$$3Fe^{3+} + K^+ + 2HSO_4^- + 6H_2O \rightarrow KFe_3(SO_4)_2(OH)_6 + 8H^+ \quad (7)$$

Low pH and heavy metals in solution will finally inhibit activity even of acidophilic and heavy-metal-tolerant bacteria (Chen and Lin 2001). In addition, elemental sulfur (S^0), produced according to reactions Eqs. (4 and 5) forms a passivation layer on the surface of metal sulfides, causing a decrease in metal solubilization rates with time (Hirato et al. 1987; Saxena and Mandre 1992).

Also in abiotic controls, the pH decreased slightly after 7 or 14 days of leaching experiments (Fig. 5), due to abiotic oxidation of ferrous ion to ferric ion (Fe^{3+}) (Ilbert and Bonnefoy 2013). However, continuous pH decrease of the controls can also be explained in terms of oxidation of pyrite or other sulfide minerals in the presence of molecular oxygen as in Eqs. (8 and 9) (Bonnissel-Gissinger et al. 1998).

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \quad (8)$$

$$2Fe^{2+} + 1/2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (9)$$

Removal of heavy metals from Sangerhausen slag pile (SSP) substrate

Due to low pH and microbial activity, transition metals are leaching out of the copper slag. Leaching was observed over a time period of 35 days. Maximum concentrations of soluble ions in the supernatants were observed with 10% (w/v) pulp density of Sangerhausen slag pile (SSP) substrate as depicted in Table 1 and Fig. 6. Among all metal ions, Cu and Zn concentrations were highest, followed by Mn, Pb, and Ni.

Table 1 Maximum concentrations of metals (mg/l) solubilized from Sangerhausen slag pile (SSP) substrate over a time period of 35 days (cf. Fig. 6)

Organism	*Acidithiobacillus ferrivorans* SCUT-1		*Leptospirillum* strain YQP-1	
Substrate pulp density	5%	10%	5%	10%
Metals (mg/l)				
Cu	789	1725	480	652
Zn	663	715	227	242
Mn	73	85	173	207
Pb	49	80	85	86
Ni	36	62	66	75

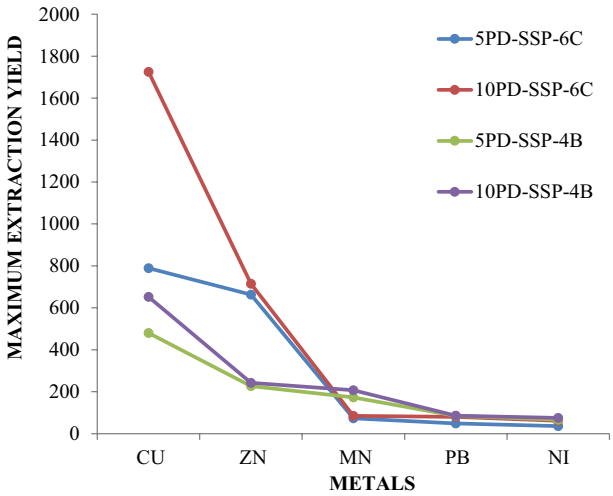

Fig. 6 Maximum removal of heavy metals (Cu, Zn, Mn, Pb, and Ni) from Sangerhausen slag pile (SSP) substrate at 5% (5pd) and 10% (10pd) pulp density (cf. Table 1). SSP-6c: cultures inoculated with enrichments of iron- and sulfur-oxidizing *Acidithiobacillus ferrivorans* SCUT-1. SSP-4b: cultures inoculated with iron-oxidizing bacteria *Leptospirillum* sp. YQP-1. (Other conditions: 30 °C, 125 rpm for *A. ferrivorans* SCUT-1 inoculated systems and 20 °C, no agitation for *Leptospirillum* sp. YQP-1 inoculated systems; total incubation time 35 days)

The high leaching rate of Cu and Zn by iron- and sulfur-oxidizing bacteria (*A. ferrivorans* SCUT-1) is due to their sulfur-oxidizing ability that is presumably lacking in iron-oxidizing *Leptospirillum* strain YQP-1. Generally, oxidation of sulfides in copper sulfide minerals drives mobilization of copper and other transition metals as shown in Eq. (10–14) (Hutchins et al. 1986; Escobar et al. 2010).

$$CuFeS_2(\text{chalcopyrite}) + 2Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5FeSO_4 + 2S^0 \quad (10)$$

$$CuS(\text{covellite}) + Fe_2(SO_4)_3 \rightarrow CuSO_4 + 2FeSO_4 + S^0 \quad (11)$$

$$Cu_2S(\text{chalcocite}) + 2Fe_2(SO_4)_3 \rightarrow 2CuSO_4 + 4FeSO_4 + S^0 \quad (12)$$

$$Cu_5FeS_4(\text{bornite}) + 6Fe_2(SO_4)_3 \rightarrow 5CuSO_4 + 13FeSO_4 + 4S^0 \quad (13)$$

$$FeS_2 + Fe_2(SO_4)_3 \rightarrow 3FeSO_4 + 2S^0 \quad (14)$$

Unlike Cu and Zn, Mn, Pb, and Ni were more efficiently mobilized by *Leptospirillum* strain YQP-1 inoculated systems.

Conclusion

There is a high demand for preservation measures for the enormously increasing industrial heritage. Waste of mining activities and from smelting processes in particular, is an important resource for industry archaeology (e.g., Leblanc et al. 2000; Mateus et al. 2011). Important key information about the process is preserved in "time capsules" of the glassy slag, whereas in most cases, all other edifices, such as smelting plants or furnaces are already degraded or overprinted by more recent buildings. Processes even from the middle ages are mainly known from detailed descriptions (cf. Agricola 1950) and analysis of the metals and slag incurring during processes (Asmus 2014).

Today, much is known about bioleaching technology for the cost-effective removal of heavy metals from contaminating sites. However, this process has, until now, not been evaluated in view of the—sometimes conflicting—contexts of protection of historic monuments vs. environmental protection.

In our experiments, we could show that natural enrichments of bacteria are capable of leaching considerable amounts of metals from waste material. These organisms are common in acid drainages of abandoned mines, where cost-intensive mine water management is neglected. The mine drainages represent natural resources for microbes used in our leaching approach. Copper slag, as used in our experiments, is stable under circumneutral pH conditions. Remarkably, at low pH, leaching efficiency strongly depends on the enriched bacterial community, which develops according to the environmental conditions (here either oxic or dysoxic conditions, favoring either *Acidithiobacillus* or *Leptospirillum*).

With respect to tailings as cultural heritage, there are several implications: Mine waste is subject to change due to microbial activity. Waste, including slag, which is particular important for the oldest, pre-historic slag piles (Radivojevic et al. 2010) is subjected to microbial "stress" and degradation under specific conditions and the "geochemical" inventory may get lost under certain circumstances. Certain microbial communities may promote specific mobilization of minerals and metal ions from waste material, which will contaminate the environment during long time periods after dumping. This depends on pH, which decreases, e.g., when available reduced sulfur compounds are (microbially) oxidized and enhances mineral mobilization. At circumneutral pH, however, glassy slag in particular, appears to be relatively stable for longer time periods.

Acknowledgements The support of Gerhard Rosenkranz and the "Marsberger Heimatbundes e. V." (Marsberg) is gratefully acknowledged.

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