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*Technical Article*

# Studies on Microbial Heavy Metal Retention from Uranium Mine Drainage Water with Special Emphasis on Rare Earth Elements

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**Abstract.** Microbial heavy metal retention was studied using seepage water sampled from a former uranium mining site in Eastern Thuringia, Germany. The seepage water has a low pH and contains high concentrations of metals, including uranium, rare earth elements (REE), and other heavy metals. Microbial influence on sorption and/or active uptake of heavy metals was studied using REE patterns. Incubation of seepage water with the bacterium *Escherichia coli* caused sorption of heavy metals to biomass. Incubation with the fungus *Schizophyllum commune*, however, had a much more pronounced effect, including significant fractionation of REE, pointing to the possibility of a specific active uptake mechanism. Extraction factors and fractionation coefficients are given to show the capacity of the presented bioextraction for future applications.

**Key words:** Abandoned mine; bioremediation; Eastern Thuringia; Germany; heavy metals; microbial incubation; radionuclides; rare earth elements; uranium

## Introduction

More than half of the East German uranium production (about 220 kt of $U_3O_8$) was mined in the Ronneburg mining district using underground and open pit mining techniques (Lange 1995; Jakubick et al. 2002). On the whole, 16 dumps, with a volume of 200 x $10^6$ $m^3$, were deposited on an area of 466 ha (Geletneky et al. 2002). Mining was terminated at the former S(D)AG WISMUT (1946-1954: Sowjetische Aktiengesellschaft Wismut; 1954-1990: Sowjetisch Deutsche Aktiengesellschaft Wismut) mine site in Eastern Thuringia and Saxony (Germany) in 1990. Afterwards, the newly founded Wismut GmbH began a major effort to restore and remediate the area.

In order to minimize metal leaching and ecological contamination, the mines are being flooded and the spoil material is either being moved into the former Lichtenberg mine pit (Eastern Thuringia, Ronneburg district) or remediated on-site (Geletneky et al. 2002). Low pH seepage waters enriched in heavy metals emerged prior to and during remediation at the base of (former) dumps such as the Nordhalde waste rock dump and the former Gessenhalde leaching dump. The efflux waters still show high levels of radioactive heavy metals, such as uranium, and non-radioactive heavy metals, including rare earth elements (REE).

REE show smooth but continuous variations in chemical behaviour as a function of their atomic number. After normalization to PAAS (Post-Archean Australian Shale, Taylor and McLennan 1985) REE patterns can be used as tracers to monitor water flow paths (Johannesson et al. 1997; Johannesson and Lyons 2000), to indicate processes such as sorption on biological materials (Texier et al. 1999), uptake into (FengFu et al. 2000; Ozaki and Enomoto 2001; Tyler and Olsson 2001; Weltje et al. 2002) and sequestration in plants (Wang et al. 1999; Zhang et al. 2001). REE patterns have also been used to study water-rock interactions in acid mine drainage (AMD) areas (Worall and Pearson 2001). Fractionation of REE points to processes different from dilution, such as dissolution of minerals (Hannigan and Sholkovitz 2001), sorption (Aagaard 1974; Texier et al. 1999; Astrom 2001), (co)precipitation (Byrne and Kim 1993; Liu and Byrne 1997) or complexation (Wood 1990; Lee and Byrne 1993; Johannesson et al. 1996; Schijf and Byrne 2001). For this reason, REE patterns can be used to investigate reactive transport in AMD and AMD remediation.

Microbes play important roles in the remediation of heavy metals and radionuclides from surface waters (Ouyang 2002). For example, fungi can concentrate radioactive heavy metals, as was observed after the Tchernobyl fall-out (Gaso et al. 1998; Kirchner and Daillant 1998; Baeza et al. 2000). Great variance in concentration of radioactive heavy metals between fruitbodies collected from different sites, between different fungal species, and between different substrates were reported, making it difficult to determine general enrichment factors for fungi. Therefore, the characterization of well known strains under standardized, *in vitro* conditions is necessary. The processes of heavy metal accumulation by fungi must be identified in order to be able to use defined strains with known properties in remediation approaches. The retention of heavy metals may be

due to adsorption processes, such as by the chitin cell wall of fungi, as well as other polyglucans excreted by fungi (Gutnick and Bach 2000; Kamnev and Lelie 2000; Gabriel et al. 2001) or to active processes of uptake into the living cell.

Soil bacteria can also accumulate heavy metals (Nies 1992; Cooksey 1994; Valls et al. 1998; Cervantes et al. 2001). Processes on the molecular level are better understood for bacteria than for fungi, since strains from communal waste water treatment units have been characterized in detail over the past decades. Soil bacteria are less well characterized, but comparable mechanisms are assumed to be present. Well characterized laboratory strains can be used to determine general factors for adsorption, e.g., on the bacterial cell wall and lipopolysaccharide coat. In addition, it might be possible to construct strains for waste water treatment in future applications.

Passive adsorption contrasts with active heavy metal uptake and storage mechanisms observed in both eukaryotic (fungi) and prokaryotic (bacteria) organisms. Passive processes generally are slower, but can lead to higher enrichment, which would be useful for bioextraction. In the future, it may be attractive to construct strains that combine different strategies. Therefore, the characterization of strains that can be easily manipulated and have been used in biotechnological production processes is essential. These easily manipulated strains can be used to express resistance factors found in other, more adapted strains and then used to remediate heavy metal contamination on-site.

In order to evaluate the potential of microbes to decrease the heavy metal contamination of mine waters, both a fungal and a bacterial strain were incubated with AMD. Heavy metal extraction from the water column was investigated using two well characterized organisms, the basidiomycete *Schizophyllum commune* (S. *commune,* ATCC strain 4-40) and the proteobacterium *Escherichia coli* (*E. Coli*, strain K12 DH5α). The potential of microbes to decrease heavy metal concentration was evaluated by analysing for a variety of elements including U, REE, and other heavy metals. REE patterns, after normalization of REE-concentration to PAAS, were used to tentatively differentiate between the processes of sorption and uptake of heavy metals by biological materials.

## Material and Methods

Water was collected at two seeps located between the communities of Kauern and Ronneburg in Eastern Thuringia, Germany (Figure 1). Seepage water Q4 was taken directly from a drainage site at the NW boarder of the Nordhalde waste rock pile; the second seep (G16) drains the site of the former Gessenhalde spoil heap. The drainage waters used in this study were sampled from Q4 on 18-07-01 and G16 on 07-11-01 (at the end of a rainy period) (see Figure 1 for sampling locations). The water samples (3 L) were filtered in the field using glass fibre prefilters (Millipore, Eschborn, Germany) and cellulose acetate filters (Sartorius, Göttingen, Germany) with a pore size of 0.45 µm. Additionally, pH, electrical conductivity, redox potential, and temperature of the unfiltered samples were measured directly in the field (WTW, Weilheim, Germany). The samples were transferred to the laboratory the same day and stored at 6°C until analysis. The water samples were partly filtered in the lab using a 0.2 µm cellulose acetate filter (Sartorius, Göttingen, Germany) and autoclaved using standard microbiology techniques (20 min at 121°C and 1 bar) before inoculation.

*S. commune* and *E. coli* were grown in minimal medium (Schwalb and Miles 1967) and Standard I (Merck, Darmstadt, Germany), respectively, to late logarithmic growth phase, separated from the nutrient solution, washed 3 times in sterile, distilled water and inoculated 1:10 in mine drainage water. The minimal medium consisted of aspartic acid (2 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $KH_2PO_4$ (0.46 g/L), $K_2HPO_4$ (1 g/L) and thiamin (120 µg/L) with a pH adjusted to

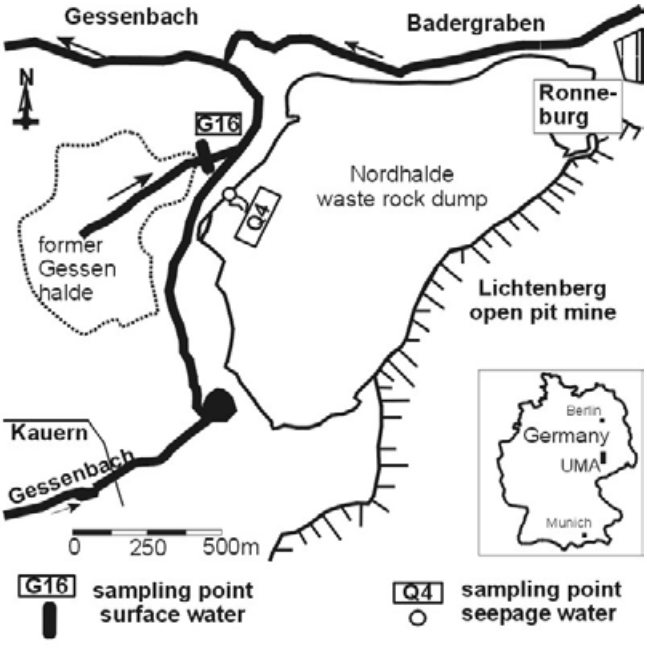


**Figure 1.** Former uranium mining area (UMA) of Ronneburg (Eastern Thuringia, Germany) with sampling locations for seepage water used for incubation experiments (modified from Geletneky et al. 2002); in 2003, the Nordhalde dump was removed and the material was moved into the Lichtenberg open pit

6.3 by the addition of NaOH. Standard I consisted of NaCl (6 g/L), pepton (15 g/L), yeast extract (3 g/L), and glucose (1 g/L) at a pH of 7.5.

The seepage waters were incubated with *E. coli* and *S. commune* for 1 and 4 weeks, respectively. For the 4 week incubation with *S. commune*, a control with pH set to 5.3 by addition of NaOH was used to check the influence of pH on growth of *S. commune* and sorption/uptake of heavy metals. Each incubation experiment was performed twice, in 500 ml Erlenmayer flasks, using 150 ml of seepage water each time, under identical experimental conditions to check the reproducibility of the method. Control samples were analyzed under unsterile conditions.

After incubation, the samples were centrifuged at 6000 rpm and 4°C (Beckman, Palo Alto, USA) and filtered twice, including a 0.2 µm sterile filter (Sartorius, Göttingen, Germany). The clear supernatant was analyzed hydrochemically using atomic absorption spectrometry (AAS, AAS 5EA and AAS 5FL, Carl Zeiss, Jena, Germany), inductively coupled plasma mass spectrometry (ICP-MS, PQ3-S, Thermo Elemental, Winsford, UK), titration (Titrino 716 DMS, Metrohm, Filderstadt, Germany) and spectrophotometry (DR/4000U, Hach, Loveland, USA). The analyses included U, Y, REE, Al, a series of transition elements (Cd, Co, Cu, Fe, Mn, Ni), alkaline (K, Na) and alkaline earth elements (Ca, Mg, Sr), as well as $Cl^-$, $SO_4^{2-}$, $PO_4^{3-}$ and nitrogen compounds ($NH_4^+$, $NO_3^-$).

## Results and Discussion

### Sampling details

The seepage waters had a low pH, and high redox potential and electrical conductivity (Table 1). The pH of Q4 seepage water (2.9) was significantly lower than that of G16 (4.9). The total dissolved solids (TDS) content can be estimated from the measured electrical conductivity (Freeze and Cherry 1979). Due to the lower pH, TDS is nearly a factor of two higher in seepage water Q4 (≈9 g/L) than in G16 (≈5.2 g/L).

### Influence of sterilization by autoclaving

In the Q4 water, an opaque, orange precipitate was observed after autoclaving. The pH in the autoclaved samples of Q4 dropped from 3.4 to 2.4–2.7, probably due to precipitation of Fe(oxy)hydroxides. G16 water showed only minor amounts of white precipitate. For both G16 and Q4, the concentrations of the investigated elements in the water column were analyzed prior to and after autoclaving. Autoclaving increased TDS in Q4 by about 15% (data not shown), presumably due to evaporative water loss. The exceptions to this trend were Fe (elevated only 6%), $NH_4^+$ (increased by 32%) and $PO_4^{3-}$, which dropped by about 20%. Fe and $PO_4^{3-}$ can precipitate during autoclaving whereas dissolved N-species can be reduced to $NH_4^+$.

**Table 1.** Physico-chemical parameters of investigated seepage water samples

| Sample | Date | pH | Conductivity [µS/cm] | Redox potential [mV] | T [°C] |
|---|---|---|---|---|---|
| Q4 | 18-07-01 | 2.9 | 11300 | 606 | 14.7 |
| G16 | 07-11-01 | 4.9 | 6470 | 472 | 6.4 |

The TDS was about 5% higher in the G16 water after thermal treatment (Table 2). The exceptions to this general trend were again Fe, $NH_4^+$, and $PO_4^{3-}$. Fe and $NH_4^+$ were elevated by a factor of about 60 and 4, respectively. $PO_4^{3-}$ decreased by about 50%. The rather strong increase in dissolved Fe in the water column is probably due to partial dissolution of precipitated $Fe(OH)_x$. The general enhancement in concentration is most likely due to slight reductions in the volume of the liquid phase due to evaporation. This assumption is supported by the fact that the concentration of $Cl^-$, which is typically conserved, was also enhanced. For sample G16, reproducibility was tested during autoclaving (Table 2). For two sample aliquots autoclaved separately, the variations of the concentrations for analytes well above the detection limits was typically less than 2% of the relative standard deviation (see 1σ levels in Table 2). This is comparable to the variation in the two unsterilized samples.

It was important to examine the potential influence of sterilization on the REE patterns, since fractionation among the REE was being used to investigate the effects of microbial incubation of seepage water on the element contents. The REE patterns of sample G16 prior to and after autoclaving are displayed in Figure 2. No significant fractionation due to autoclaving is visible. In order to investigate fractionation more quantitatively, the quotients Lu/La (concentration normalized to PAAS of the heavy REE divided by the normalized concentration of the light REE), Sm/La (middle REE/light REE), and Lu/Sm (enrichment of heavy REE as compared to middle REE) were calculated both prior and subsequent to autoclaving. In the seepage water, heavy (HREE) and middle REE(MREE) were enriched 14 and about 8.5 times, respectively, over the light REE (LREE). HREE were only slightly enriched over MREE (≈ 1.65 times). As can be seen from the coefficients and the corresponding standard deviations (1σ) in Table 3, autoclaving does not lead to significant fractionation among the REE. Also, it can be seen that the REE

**Table 2.** Concentrations of various elements in water sample G16 from a former uranium mine site (Ronneburg, Germany) before and after autoclaving (sterile), and after incubation for 1 and 4 weeks with *E. coli* and *S. commune* and corresponding error levels (1σ for two replicates)

| Analyte | Not sterile | 1σ | Sterile | 1σ | *E. coli* 1 week | 1σ | *E. coli* 4 weeks | 1σ | *S. commune* 1 week | 1σ | *S. commune* 4 weeks | 1σ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cd (µg/L) | 44 | 1 | 47.16 | 0.02 | 42 | 1 | 43.3 | 0.3 | 35.0 | 0.4 | 33 | 6 |
| Sr (µg/L) | 732 | 7 | 763 | 3 | 706 | 13 | 723.1 | 0.1 | 595 | 6 | 599 | 64 |
| U (µg/L) | 634 | 6 | 681 | 5 | 88 | 18 | 120 | 1 | 236 | 3 | 66 | 34 |
| Y (µg/L) | 612 | 13 | 643 | 1 | 574 | 29 | 598 | 2 | 283 | 6 | 84 | 71 |
| Al (mg/L) | 47.4 | 0.8 | 51.0 | 0.2 | 44 | 1 | 45.8 | 0.2 | 32.08 | 0.04 | 8 | 6 |
| $NH_4^+$ (mg/L) | 0.03 | 0.01 | 0.12 | 0.01 | 1.4 | 0.5 | 2.0 | 0.1 | 3.6 | 0.1 | 90 | 37 |
| Ca (mg/L) | 721 | 18 | 754 | 16 | 671 | 10 | 683 | 13 | 563 | 7 | 574 | 1 |
| Co (mg/L) | 2.02 | 0.01 | 2.12 | 0.04 | 1.9 | 0.1 | 1.97 | 0.01 | 1.61 | 0.01 | 1.815 | 0.001 |
| Cu (mg/L) | 1.18 | 0.01 | 1.25 | 0.01 | 1.008 | 0.002 | 1.052 | 0.007 | 0.756 | 0.002 | 0.85 | 0.07 |
| Fe (mg/L) | 0.1 | 0.02 | 6.1 | 0.2 | 4.6 | 0.3 | 3 | 1 | 5.5 | 0.1 | 545 | 746 |
| K (mg/L) | 2.4 | 0.1 | 2.5 | 0.1 | 5.7 | 0.1 | 5.9 | 0.2 | 27.8 | 0.5 | 46.7 | 0.5 |
| Mg (mg/L) | 923 | 2 | 966 | 2 | 898 | 8 | 918 | 3 | 772 | 2 | 815 | 1 |
| Mn (mg/L) | 71.7 | 0.6 | 74.9 | 0.9 | 69 | 1 | 70.7 | 0.9 | 58.45 | 0.01 | 62 | 4 |
| Na (mg/L) | 33.2 | 0.5 | 34.9 | 0.1 | 32.7 | 0.2 | 34.8 | 0.4 | 28.5 | 0.2 | 28 | 1 |
| Ni (mg/L) | 11.4 | 0.1 | 11.9 | 0.2 | 10.9 | 0.2 | 11.30 | 0.04 | 9.5 | 0.1 | 10 | 1 |
| $Cl^-$ (mg/L) | 246 | 2 | 261 | 1 | 236 | 4 | 242 | 2 | 207.7 | 0.2 | 208 | 9 |
| $NO_3^-$ (mg/L) | 22 | 0.4 | 23 | 1 | 20.0 | 0.5 | 20.6 | 0.4 | 19.9 | 0.2 | 19 | 1 |
| $PO_4^{3-}$ (mg/L) | 0.10 | 0.01 | 0.05 | 0.01 | 0.43 | 0.04 | 0.28 | 0.03 | 14.0 | 0.3 | 4 | 4 |
| $SO_4^{2-}$ (mg/L) | 5250 | 100 | 5465 | 160 | 5035 | 163 | 5325 | 35 | 4125 | 35 | 4550 | 269 |
| Σ REE (µg/L) | 712 | 7 | 748 | 6 | 658 | 12 | 673.8 | 0.5 | 262 | 9 | 78 | 69 |

patterns were very reproducible (Figure 2). Thus, sterilized samples could be used for inoculation, incubation and subsequent measurement of dissolved elements in the water column.

Direct incubation of seepage water: Q4

Incubating Q4 with *E. coli* significantly decreased the concentration of most elements in the water column after 1 week of incubation (Table 4) by about 10% mass/volume (m/V). The decrease was higher for Al (15%), Ca (20%), and especially Fe (30%). $NO_3^-$ and K were introduced with the biomass and so significantly increased in concentration. For K, the concentrations after inoculation with *E. coli* were about 30% higher for 1 week of incubation. $NO_3^-$ concentrations were elevated by about 25% after the first week.

After 4 weeks of incubation, the element concentrations in the sterilized and inoculated samples were relatively similar to the uninoculated samples, though they were significantly elevated relative to what had been observed after 1 week of incubation with *E. coli*. The exceptions were U, K, and $PO_4^{3-}$. U concentrations decreased by 10% (m/V) after 4 weeks of incubation, compared to uninoculated samples.

The concentrations of $PO_4^{3-}$ and K in the water column were about 30% higher after 4 weeks of incubation with *E. coli*, compared to the samples not

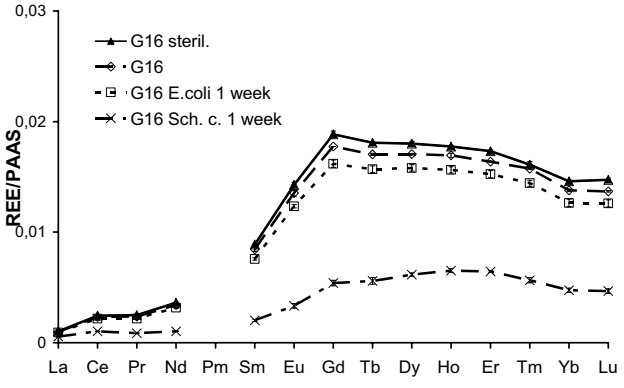


**Figure 2.** REE patterns of sample G16, G16 after autoclaving (steril.) and for G16 incubated for one week with *E. coli* and *S. commune* (Sch. c.)

**Table 3.** Enrichment factors of heavy (Lu/La) and middle REE (Sm/La) over the light REE, and enrichment of heavy REE compared to the middle REE (Lu/Sm) for water sample G16, sterilized seepage water sample and after 1 week of incubation with *E. coli* and *S. commune* and corresponding error levels (1σ for two replicates)

| Sample | Lu/La | 1σ | Sm/La | 1σ | Lu/Sm | 1σ |
|---|---|---|---|---|---|---|
| Not sterile | 14.0 | 0.2 | 8.6 | 0.1 | 1.63 | 0.01 |
| Sterile | 14.4 | 0.1 | 8.6 | 0.1 | 1.67 | 0.01 |
| *E. coli* | 13.7 | 0.1 | 8.2 | 0.1 | 1.68 | 0.01 |
| *S. commune* | 8.5 | 0.1 | 3.7 | 0.1 | 2.31 | 0.01 |

**Table 4.** Influence of direct incubation of seepage water Q4 after autoclaving (sterile), with *E. coli* and *S. commune* on concentrations of various elements in the water column and corresponding error levels (1σ for two replicates)

| Analyte | sterile | 1σ | *E. coli* 1 week | 1σ | *E. coli* 4 weeks | 1σ | *S. commune* 1 week | 1σ | *S. commune* 4 weeks | 1σ | *S. commune* 4 weeks pH 5.3 | 1σ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cd (µg/L) | 342 | 10 | 314 | 1 | 336 | 6 | 266 | 10 | 278 | 2 | 280 | 13 |
| Sr (µg/L) | 280.6 | 0.2 | 247 | 1 | 268 | 1 | 215 | 8 | 224 | 3 | 234 | 9 |
| U (µg/L) | 1155 | 27 | 1004 | 6 | 1029 | 7 | 856 | 33 | 867 | 4 | 709 | 34 |
| Al (mg/L) | 375 | 6 | 339 | 5 | 365 | 2 | 296 | 12 | 316 | 6 | 168 | 9 |
| $NH_4^+$ (mg/L) | 5.0 | 0.1 | 4.4 | 0.2 | 6.4 | 0.4 | 5.0 | 0.1 | 4.7 | 0.4 | 46.8 | 0.1 |
| Ca (mg/L) | 1257 | 5 | 1008 | 11 | 1201 | 10 | 837 | 198 | 969 | 22 | 788 | 11 |
| Co (mg/L) | 11.4 | 0.4 | 10.7 | 0.2 | 11.2 | 0.1 | 8.78 | 0.04 | 9.6 | 0.1 | 10.0 | 0.5 |
| Cu (mg/L) | 4.9 | 0.2 | 4.38 | 0.01 | 4.74 | 0.01 | 3.68 | 0.02 | 4.04 | 0.03 | 3.0 | 0.2 |
| Fe (mg/L) | 2595 | 93 | 1867 | 25 | 2446 | 16 | 1493 | 3 | 2051 | 11 | 1260 | 1 |
| K (mg/L) | 6.9 | 0.2 | 9.6 | 0.1 | 10.2 | 0.1 | 49.3 | 0.1 | 51 | 1 | 55.0 | 0.3 |
| Mg (mg/L) | 1282 | 32 | 1204 | 3 | 1257 | 6 | 1056 | 17 | 1098 | 4 | 1103 | 9 |
| Mn (mg/L) | 142 | 4 | 133 | 2 | 141 | 2 | 115 | 3 | 122 | 1 | 127 | 6 |
| Na (mg/L) | 31 | 1 | 29.7 | 0.2 | 32.0 | 0.2 | 25.4 | 0.3 | 26.3 | 0.1 | 1024* | 40* |
| Ni (mg/L) | 28 | 1 | 25.6 | 0.1 | 26.8 | 0.1 | 21 | 1 | 23.0 | 0.1 | 21 | 1 |
| Y (mg/L) | 4.1 | 0.1 | 3.7 | 0.1 | 3.95 | 0.05 | 3.17 | 0.02 | 3.4 | 0.1 | 3.29 | 0.01 |
| $Cl^-$ (mg/L) | 21 | 1 | 19.91 | 0.05 | 21 | 1 | 16 | 1 | 17 | 1 | 17 | 1 |
| $NO_3^-$ (mg/L) | 6.6 | 0.4 | 9 | 1 | 6.3 | 0.4 | 11.6 | 0.1 | 10.1 | 0.3 | 8.4 | 0.1 |
| $PO_4^{3-}$ (mg/L) | 1.0 | 0.1 | 1.4 | 0.4 | 1.3 | 0.4 | 23 | 1 | 17.2 | 0.3 | 0.5 | 0.1 |
| $SO_4^{2-}$ (mg/L) | 14100 | 212 | 12950 | 354 | 14035 | 474 | 11700 | 106 | 11763 | 265 | 11150 | 424 |
| Σ REE (µg/L) | 4017 | 134 | 3651 | 24 | 3878 | 35 | 3068 | 127 | 3261 | 27 | 3044 | 140 |

* NaOH added for adjustment of pH

incubated. The most likely explanation is that $PO_4^{3-}$ and K were released from the biomass, whereas U was either bound to the biomass or precipitated.

Two alternative explanations can be given for the observation that 4 weeks of incubation led to higher concentrations in the water column than 1 week of incubation. The first one is a simple loss in volume due to the prolonged experiment. However, this assumption is not supported by the concentration of $Cl^-$, which presumably behaved conservatively. The concentration of $Cl^-$ does not change significantly between 1 and 4 weeks of incubation. Therefore, it seems more probable that incubation with *E. coli* led to sorption or uptake of heavy metals from the water phase onto/into the actively growing cells. After reaching stationary conditions, the process stopped and metals were released as the bacteria died and the cells lysed. This was supported by an incubation experiment with a seepage water resembling Q4 with a pH of 3.15 (data not shown). After 4 weeks, no living cells were detectable, supporting the view that the cells could not survive under these harsh conditions.

After 4 weeks of incubation of sample Q4 with the bacteria, the REE-patterns were determined (Figure 3). As with seepage water G16, shale-normalized REE patterns of seepage water Q4 showed enrichment of HREE and MREE relative to the LREE. However, for Q4, the REE patterns show an even more pronounced enrichment of HREE (Lu/La: ≈26, Table 5) and MREE (Sm/La: ≈12, Table 5) compared to the LREE. In this case, incubation with *E. coli* had no pronounced effect. REE patterns in the water column before and after 4 weeks of incubation with *E. coli* were about the same (REE patterns of sterile Q4 compared to REE pattern of Q4 *E. coli* 4 weeks). The calculated coefficients indicate no significant fractionation (Table 5) after incubation with *E. coli*. Again, reproducibility of the REE patterns for the inoculated samples was excellent.

Incubation of seepage water Q4 with the fungus *S. commune* led to a much more pronounced decrease of element concentrations in the water phase compared to incubation with *E. coli* (Table 4). Typically, the decrease in most element concentrations was about 20% (m/V), being slightly higher after 1 week of incubation than after 4 weeks of incubation. However, Fe and Ca decreased by 55% and 65%, respectively, compared to the uninoculated samples. In contrast, a significant increase in $PO_4^{3-}$, $NO_3^-$, and K was observed after incubation with *S. commune* for 1 and 4 weeks, respectively, which was attributed to the addition of the pre-grown biomass.

The better extraction capacity of the fungus can be attributed to the greater stability of fungi under acidic conditions. Generally, fungi prefer a slightly acidic pH, such as 6.3, which was used for growth of the biomass prior to inoculation (see Materials and Methods section). However, in our experiments, the pH was much lower, and after 4 weeks of incubation in the Q4-like water, no living cells were detectable. This suggests that the higher capacity of *S. commune*

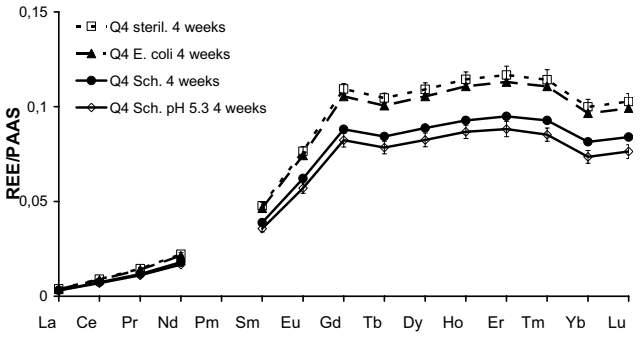


**Figure 3.** REE patterns for 4 week incubation of seepage sample Q4 with *E. coli* and *S. commune*

for extraction of heavy metals is linked to the longer survival of the fungus at highly acidic pH. After 4 weeks, however, the fungal cells also have started to lyse, giving rise to the elevated concentrations.

Since pH plays an essential role in survival of fungal cells, a sample was adjusted to a pH of 5.3 by addition of NaOH prior to incubation. For the samples set to a pH of 5.3, $NH_4^+$ and K were enriched 10 and 8 times, respectively, compared to the uninoculated samples. Both species were most probably introduced with the inoculated biomass. The enrichment in Na was due to the addition of NaOH for adjustment of pH. The concentration of elements forming insoluble hydroxides (Al, Ca, Cu, and Fe) or known to co-precipitate (U), however, were drastically reduced. The concentration of the respective elements in the water phase decreased to 45% (Al, Fe) or 60% (Ca, Cu, U) compared to samples prior to inoculation. $PO_4^{3-}$ concentrations in this case were also lowered, probably due to co-precipitation. The decrease in REE concentrations was even more pronounced in the water samples incubated with *S. commune* and set to a pH of 5.3. Fractionation among the REE was observed for incubation of the Q4 water with *S. commune* only after the pH was changed. After changing the pH, a small but significant fractionation of the REE patterns was observed, as indicated by the coefficient Lu/La changing from 26.6±0.2 to 25.1±0.1 (Table 5, Figure 3). This bioextraction might be attributed to active uptake or to adsorption on polymers in the fungal cell wall. The latter has been shown for plant cell walls. However, dead fungal cell walls were also present after 4 weeks of incubation at the lower pH, making processes depending on living cells and the metabolism of the fungus a more straightforward explanation.

Direct incubation of seepage water: G16

The concentrations of the investigated elements in the water column with and without inoculation and the corresponding standard deviations (1σ) of the two replicate measurements are listed in Table 2. Again,

**Table 5.** Enrichment factors of heavy (Lu/La) and middle REE (Sm/La) compared to the light REE, and enrichment of heavy REE compared to the middle REE (Lu/Sm) for Q4 after 4 weeks of incubation with *E. coli* and *S. commune* and corresponding error levels (1σ for two replicates)

| Sample | Lu/La | 1σ | Sm/La | 1σ | Lu/Sm | 1σ |
|---|---|---|---|---|---|---|
| sterile | 26.4 | 0.3 | 12.3 | 0.1 | 2.15 | 0.01 |
| *E. coli* | 26.6 | 0.2 | 12.4 | 0.1 | 2.13 | 0.01 |
| *S. commune* | 26.8 | 0.2 | 12.4 | 0.1 | 2.17 | 0.01 |
| *S. commune* (pH 5.3) | 25.1 | 0.1 | 11.8 | 0.1 | 2.14 | 0.01 |

reproducibility of sterilization and incubation experiments was very good, with one exception. In one culture, production of slime was observed after 4 weeks of incubation with *S. commune*, due to production of excessive amounts of extracellular polysaccharides. That sample, therefore, showed biosorption/uptake combined with additional bioextraction processes. The mixed effects led to results that deviate from the other samples. Reproducibility was worse for highly charged cations like Al, Fe, REE, Y, and U, while elements like Mg or $Cl^-$ with less sorptive tendency show results more comparable to samples lacking the additional polyglucans (Table 2).

In general, the concentration of the investigated elements in the water phase decreased by 5% to 10% (m/V) when incubating seepage water G16 with *E. coli* (Table 2). U decreased to about 15% of the concentration of the uninoculated sterilized G16 sample. K, $NH_4^+$, and $PO_4^{3-}$ were again introduced with the inoculation. Incubation with bacteria did not result in higher depletion of heavy metals from the water column upon 4 weeks of incubation. Again, as with Q4, concentrations of most elements in G16 were slightly higher after 4 weeks of incubation. This general trend was also observed for the REE (Table 2). Incubation with *E. coli* led to a small but significant decrease in concentration and also to fractionation among REE (Tables 2 and 3). The fractionation among the REE can be seen from the Lu/La (14.4±0.1 for the sterilized sample and 13.7±0.1 for the sample inoculated with *E. coli*) and Sm/La coefficients (8.6±0.1 and 8.2±0.1, respectively) in Table 3.

In contrast to incubation with the bacteria, incubation of seepage water G16 with *S. commune* led to a decrease in metal loading of the water column of 20 to 25% (m/V) (Table 2). Extraction efficiencies were even greater after 4 weeks for Al, Cu, Y, REE, and U. Al and Cu were depleted to about 65%, compared to uninoculated samples. Y and U were depleted to about 45% and 35%, respectively, of the

concentrations in the uninoculated samples of G16 after 1 week of incubation with *S. commune*. The concentrations of REE decreased to about 25% of the concentration prior to incubation. Concentrations of $NH_4^+$, $PO_4^{3-}$, and K were again highly elevated in the water phase after inoculation with *S. commune* for both the 1 and 4 week incubation experiments.

In addition to the decrease in REE concentration in G16 after incubation with *S. commune*, strong REE fractionation was observed (Figure 2). As can be seen from Table 3, preferential extraction of heavy and especially middle REE from the water column was observed. The enrichment of HREE over LREE (Lu/La coefficient, Table 3) drops from 14.4±0.1 for the uninoculated sample to 8.5±0.1 for the sample inoculated with *S. commune*. This effect is even more pronounced for the MREE; for the sterilized sample, the Sm/La coefficient was 8.5±0.1, while after incubation with *S. commune*, it was only 3.7±0.1.

However, it was still unclear whether the decrease in concentration and fractionation among the REE were due to direct sorption to or uptake into biomass, or to changes in hydrogeochemistry of the water samples mediated by the biomass. Since during the original experiments, no redox potentials were measured and only a limited number of pH values were measured, the experiments were redone for *S. commune*. Since the seeps previously sampled no longer existed due to remediation, a seepage water with a pH (3.15), electrical conductivity/TDS (11600 µS/cm/ ≈ 9 g/L) and redox potential (650 mV) quite similar to sample Q4 was used. In this experiment, the pH in the autoclaved sample decreased to 2.7, showing the same trend as in the original experiments. After 1 week of incubation, a pH of 3.0-3.2 was measured for the two replicates; after 4 weeks of incubation, a pH of 3.5-3.8 was obtained. Redox potential decreased from 770 mV in the autoclaved uninoculated sample to about 630 mV after 1 week and to about 520 mV after 4 weeks of incubation. Thus, the data show a trend of increasing pH and decreasing redox potential with increasing incubation time.

In the original experiments, setting the pH to 5.3 for samples Q4 incubated with *S. commune* for 4 weeks led to significant, though rather weak fractionation among the REE (see Table 5). Thus, it seems improbable that the shifts in pH of 1 unit observed in the new experiments could have led to fractionation among the REE as observed for seepage water sample G16 inoculated with *S. commune* (see Table 3). However, the possible influence of co-precipitation with Fe(oxy)hydroxides had to be evaluated.

Co-precipitation of REE with Fe-oxyhydroxides is a well known method to pre-concentrate REE in water samples prior to analysis by ICP-MS (Welch et al. 1990; Klinkhammer et al. 1994; Lee et al. 2002). As can be seen from Figure 4, there is a rather good correlation between concentrations of total dissolved REE and dissolved Fe in seepage water Q4, pointing to inorganic precipitation rather than biologically driven precipitation. After incubation, concentrations for both dissolved Fe and total REE generally were lower. It seems very probable that precipitation of Fe-oxyhydroxides led to co-precipitation of REE and other trace metals. However, it is interesting to note that with seepage water Q4, no significant REE fractionation was observed (Table 5), although the concentration of precipitated Fe was very high (up to 1 g/L). The only exception is the sample adjusted to a pH of 5.3. It has been previously reported for AMD (Verplanck et al. 1999) that partitioning of REE into forming precipitates strongly increases with increasing pH.

In contrast to the Q4 water, there was no correlation between total dissolved REE and the concentration of dissolved Fe in the G16 samples (Figure 4). Thus,

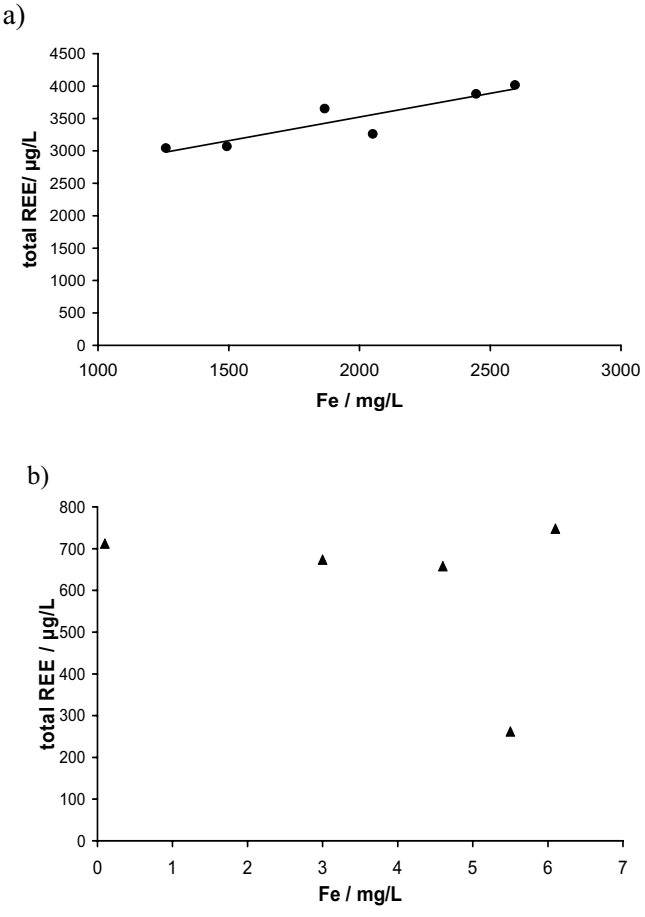


**Figure 4.** Total REE concentrations as a function of Fe-concentration in Q4 (a) and G16 (b); note, no value is given for 4 weeks of incubation with *S. commune* for G16, due to poor reproducibility

formation of Fe-containing precipitates had no direct influence on the concentration of REE in this sample and could not have led to fractionation among the REE. This is understandable since the concentration of dissolved Fe in seepage water sample G16 is below 10 mg/L and thus about two orders of magnitude lower than in samples Q4. Thus, although precipitation of Fe phases might have led to a decrease in concentration of REEs and other heavy metals, it was not responsible for fractionation among REE under the applied experimental conditions. Therefore, it would appear that the striking REE fractionation in G16 water can be attributed to the biomass, especially to the fungus. In addition, a specific uptake mechanism rather than unspecific sorption appears to drive the fractionation. Unspecific sorption should have been seen in Q4 samples irrespective of cell death and no REE fractionation was observed for Q4 unless the pH was set to 5.3.

Extraction capabilities by microbial incubation

In order to evaluate the potential of microbial incubation for the remediation of heavy metal loaded waters, extraction factors (EF) for selected elements were calculated:

$$EF = \frac{c^{unincubated}}{c^{incubated}}$$

with $c^{unincubated}$ indicating the concentration of the element in the water column in autoclaved but uninoculated samples and $c^{incubated}$ for the concentration of the element in the water column after incubation. Typically, EF was higher after 1 week of incubation than after 4 weeks of incubation.

Among the REE, the highest extraction factor after 1 week of incubation of G16 with *S. commune* was about 4.3 for Sm; the lowest was about 1.8 for La (see Figure 5). The EF for seepage water G16 incubated with *S. commune* increased sharply from La to Sm, decreased from Sm to Er, and increased again from Er to Lu. Roughly the same trend was observed for inoculation of seepage water Q4 with *S. commune* when the pH was set to 5.3, though the results were much less pronounced. For water samples incubated with *E. coli*, the extraction factors were generally low (≈ 1.1) and constant for all REEs, indicating minor extraction by bacteria as compared to fungi and the absence of fractionation (Figure 5).

## Conclusions

REE were used to study adsorption and uptake of heavy metals by direct incubation of seepage water from a former uranium mine site with bacterial and

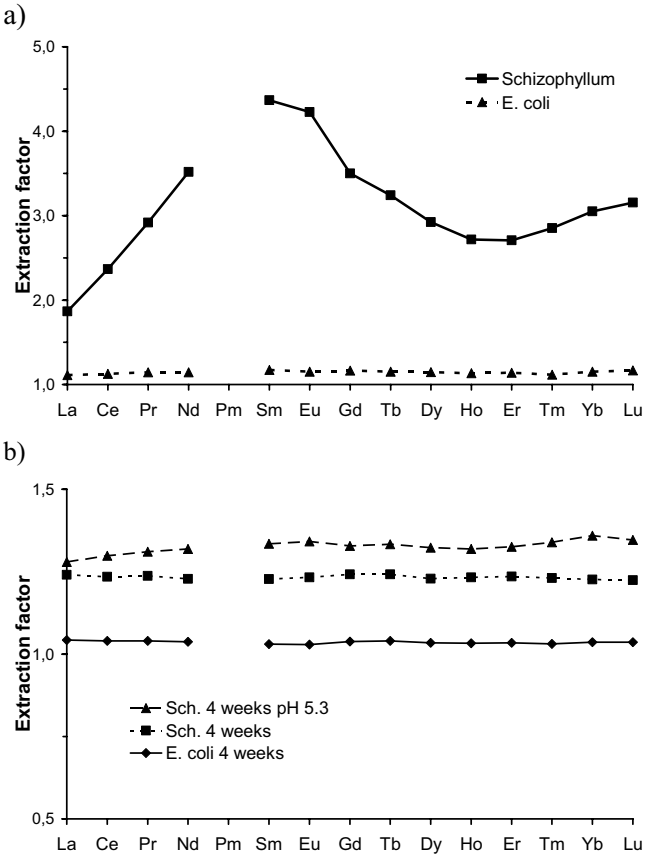


**Figure 5.** REE extraction factors for G16 seepage, 1 week incubation (a) and for Q4 seepage water, 4 week incubation (b) with both *E. coli* and *S. commune*

fungal biomass. Experiments performed twice under identical experimental conditions generally showed excellent reproducibility.

A significant decrease in concentrations of heavy metals and other elements in the water column was observed with direct incubation of the two investigated seepage waters with bacterial cells (*E. coli)*. Incubation with the fungus *S. commune* led to an even more pronounced decrease in heavy metal concentrations. This was either due to sorption of heavy metals to cell walls or to unspecific uptake mechanisms. For samples rich in Fe, precipitating Fe(oxy)hydroxides also exert a strong influence on the concentration of REE and other heavy metals. The decrease in heavy metal concentration in the water phase is highest during the first week of incubation when the cells are more active. The effect of re-elevated concentrations after 4 weeks of incubation is more pronounced for incubation of Q4 seepage water of rather low pH than for incubation of G16. This might be explained by desorption of heavy metals from the cell walls after accumulation of biomass has stabilized. This is also supported by the REE patterns obtained after incubation of water samples with *E. coli*. Only minor fractionation of REE patterns was

observed, pointing to the absence of active uptake processes. However, an active mechanism was postulated for heavy metal and REE uptake by *S. commune*, based on the REE pattern of the G16 samples, which showed strong fractionation among REE. No fractionation among the REE was observed due to precipitating Fe(oxy)hydroxides for the applied experimental conditions, even for samples extremely rich in Fe.

Our experiments indicate that bioextraction with fungal mycelia might be an alternative to plant growth and phytoextraction and might be preferable since no soil substrate is necessary for fungal growth. Incubation with fungal mycelia could be an option for bioremediation in heavy metal and radionuclide contaminated waters and possibly could replace some phytoremediation applications in wetlands. It will be interesting to investigate whether biological transport and intracellular storage mechanisms lead to fractionation rather than unspecific REE depletion. Future research must be directed towards genes for active transport, intra- or extra-cellular storage components and their application. Biotechnological use of such genes in, e.g., strains of *E. coli*, might yield highly useful bioremediation strains that could reduce the ecological effects of pollution from mining activities as well as from anthropogenic introduction of heavy metals during medical treatments.

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