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Effect of resident microbiota on the solubilization of gold in soil from the Tomakin Park Gold Mine, New South Wales, Australia

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Abstract

The processes influencing the solubilization and observed mobility of Au in soil were studied using a combination of geochemical and microbiological techniques. In this study, we demonstrate for the first time that biotic processes mediated by the resident microbiota are likely to control the mobilization of Au in auriferous soils and other regolith materials. Microcosms with auriferous soils from the Tomakin Park Gold Mine in temperate south eastern New South Wales, Australia, were incubated under biologically active versus inactive (sterilized) conditions. The soils were incubated oxic and anoxic, unamended and Au pellet- or cycloheximide amended for 70 days in a 1:4 (w:v) aqueous slurry at 25 °C in the dark. In biologically active unamended Ah- and B-horizon microcosms up to 80 wt.% of total Au was detected in solution after 45 days of incubation. In biologically active Au pellet amended microcosms Au was liberated from the soil and also from added Au pellets. Scanning electron microscopy and nucleic acid staining combined with confocal stereo laser microscopy revealed the presence of bacterial biofilms on Au pellets incubated in the biologically active microcosms. The biologically inactive microcosms displayed no or significantly reduced Au solubilization. After 40–50 days of incubation Au was generally re-adsorbed to the solid soil fractions. The results of sequential extractions conducted with dried slurry samples collected from the biologically active Ah-horizon microcosms after 0, 10, 20, 30, 40, and 68 days of incubation indicated a continuous microscale solubilization and re-adsorption of Au. In samples taken after 40 days of incubation more than 80 wt.% of the Au was extracted from the operationally defined organic fraction, which appears to act as a final re-adsorption site for Au in the soil. In samples taken after 10 days of incubation from microcosms amended with 100 $\mu g\,g^{-1}$ (d.w. soil) of Au as $AuCl_4^-$ 95 wt.% of the Au was associated with the organic fraction. To establish a mechanistic link between Au dissolution and re-adsorption with the activity of the heterotrophic bacterial community, analysis of the community structure based on carbon utilization patterns using was conducted. The bacterial community structure changed from a carbohydrate- and polymer-utilizing to a carboxylic- and amino acid utilizing community concurrently with the change from Au solubilization to re-adsorption. The bacterial community in the early stages of incubation (0–30 days) apparently produced an excess of amino acids, which are known to form stable amino acid Au complexes. The bacterial community in the later stages of incubation (after 40–50 days) metabolized these Au complexing ligands and Au, which apparently became unstable in the solution, was re-adsorbed to the solid soil fractions.
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1. Introduction

The formation of lateritic Au deposits and anomalies in the regolith depends on the mobilization, transport, and precipitation of Au in the weathering environment (e.g., Wilson, 1984), and recent studies using sequential extractions have shown that Au is mobile in soils and deeper regolith materials in and around mineralized zones (e.g., Mann, 1984; Grimm and Friedrich, 1988; Gray and Lintern, 1998; Reith et al., 2005). For instance, at the Tomakin Park Gold Mine approximately 50 wt% of the total Au in the Ah-horizon overlying the mineralized zone was associated with the following operationally defined fractions:

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water-, ammonium acetate-, sodium pyrophosphate-, and hydroxylamine hydrochloride-soluble fractions. In contrast, in the unweathered quartz-vein material more than 95 wt.% of the Au was only extractable with concentrated aqua regia and appeared to be strongly bound in pyrite and arsenopyrite (Reith et al., 2005).

The processes involved in the mobilization of Au in the regolith are believed to be abiotic. For instance, Gray (1998) postulated the following mechanisms in an attempt to explain the observed solubilization of Au: (i) oxidation and dissolution of Au due to higher concentration of dissolved O_2 in the supergene environment; (ii) release of Au during abiotic mineral weathering; (iii) mechanical abrasion of native Au due to swelling of minerals during alteration; and; (iv) increased concentration of ligand molecules such as chloride, sulfide, or thiosulfate. However, Au is widely present in the biosphere indicating that biogeochemical cycling and processes are important (e.g., Korobushkina et al., 1983).

There is growing evidence that suggests that biota are important in affecting the cycling of Au. Plants, algae, actinomycetes, and fungi have been found to accumulate Au (e.g., Korobushkina et al., 1983; Hosea et al., 1986; Mossman et al., 1999; Nakajima, 2003). Studies have also shown that common bacteria such as *Bacillus subtilis* or *Escherichia coli* are capable of precipitating Au from solution and accumulating it extra- and intracellularly (Beveridge and Murray, 1976; Karamushka et al., 1987a,b; Ulberg et al., 1992; Southam and Beveridge, 1996; Savvaidis et al., 1998; Karthikeyan and Beveridge, 2002; Nakajima, 2003). Morphological evidence for microbially mediated authigenic formation of secondary Au nuggets in regolith- and placer deposits has also been reported (Bischoff, 1994, 1997; Bischoff et al., 1992; Keeling, 1993; Mann, 1992). In addition, Au has been shown to play a functional role in the membrane-bound enzyme NADH-oxidase used by *Micrococcus luteus* during the oxidation of methane (Levchenko et al., 2002).

Microbiologists have conducted in vitro experiments with a variety of common soil bacteria that have shown their capacity to mobilize Au. Pares and Martinet (1964) found that Au from laterites and various other Au-bearing materials can be solubilized by unidentified autotrophic N_2-fixing bacteria and various heterotrophic bacteria such as *Serratia marcescens* and *Pseudomonas fluorescens*. Lyalikova and Mockeicheva (1969) found that a number of heterotrophic bacteria are able to dissolve Au. A strain similar to *Bacillus alvei* isolated from a Au deposit dissolved up to 600 $\mu g\ L^{-1}$ of Au in a microbial growth medium within 3 weeks (Boyle, 1979). Strains of *Bacillus megaterium*, *Bacillus mesentericus*, *Pseudomonas liquefaciens*, and *Bacterium nitrificans* were found to dissolve up to 35 $mg\ L^{-1}$ of Au during 30 days of incubation (Korobushkina et al., 1974). Iron- and sulfur-oxidizing bacteria such as strains of *Acidithiobacillus sp.* and *Leptospirillum sp.* have been observed to mediate the release Au by breaking down the sulfides in sulfidic Au ore (e.g., Iglesias and Carranza, 1995; Sandstroem and Peterson, 1997; Ubaldini et al., 2000), and some of these strains are used in industrial bioleaching processes to extract metals from ore (e.g., Bosecker, 1997).

Understanding the role of microorganisms in the regolith is complicated because regolith materials consist of complex inorganic and organic matrices with distinct abiotic and biotic phases. We need to know if the microbiota present in the regolith can mobilize Au, and thus facilitate its transport, and also if they play a role in precipitation and adsorption processes that counteract the mobilization of Au. Gold complexes in solution are readily adsorbed by solid inorganic and organic phases. For instance, Au(I/III)-complexes have been shown to readily adsorb onto clays, carbonates, Fe-oxides and -oxyhydroxides such as hematite and goethite and organics such as humic and fulvic acids as well as microbiota (e.g., Linter and Butt, 1993; Greffie et al., 1996; Ran et al., 2002; Nakajima, 2003).

Microcosm experiments under well-controlled biogeochemical conditions provide an opportunity to demonstrate that microbiota resident in auriferous soils and deeper regolith materials play a role in the solubilization of Au, but to the authors' knowledge no microcosm studies have been conducted. Therefore, the aims of this study are to: (i) evaluate if the resident microbiota of auriferous soil can mobilize Au; (ii) assess the microbially mediated solubilization and re-adsorption of Au in natural samples by developing suitable microcosm experiments; (iii) explore the association of Au with the different solid soil fractions known to adsorb trace elements, and; (iv) link the functional structure of bacterial community with the process of Au solubilization.

These aims were accomplished by using microcosm experiments, in which the changes in solution composition, microbial activity and populations, and the association of Au with the solid phases of the soil were monitored. Soil samples from the Tomakin Park Gold Mine in south eastern Australia were used for this study, because the regolith profile on top of the underground mine is undisturbed, the regolith is well characterized, and a previous study has indicated that Au is mobilized during weathering (Reith et al., 2005).

2. Study area description

The Tomakin Park Gold Mine is located 2 km west of the coastal village of Tomakin in south eastern New South Wales, Australia, at S 35°48′51.9″ and E 150°10′26.4″ (Fig. 1). A detailed description of the geology, the regolith-, and soil properties and the mineralogy is given in Reith et al. (2005), so we only provide a brief summary here. The mine is located in the Molong—South Coast Anticlinorial Zone, which is a structural subdivision of the Lachlan Fold Belt. The regional geology consists of an anticlinorial zone, which displays a large, north–south elongated, cratonised block of Ordovician flysch sediments overlain by Middle to Late Devonian rift volcanics and

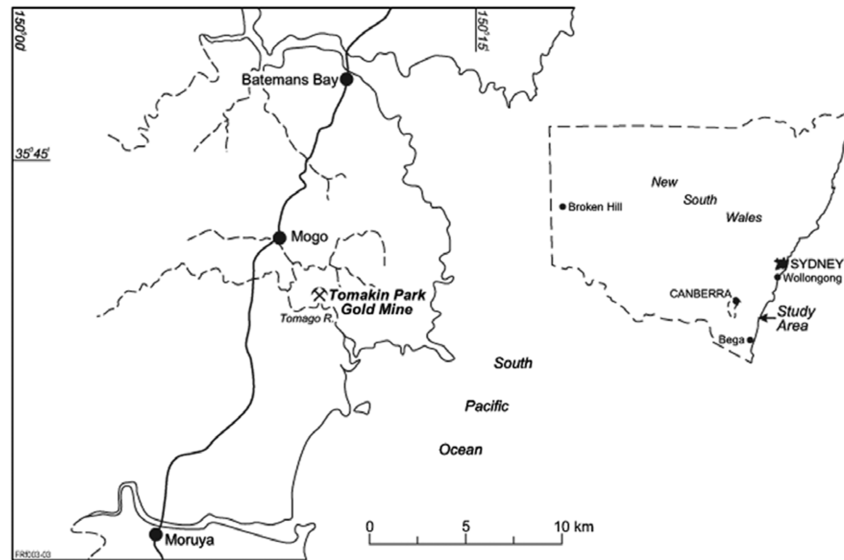


Fig. 1. Location map of the Tomakin Park Gold Mine in south eastern New South Wales, Australia.

Late Devonian transitional and cratonic sediments. The primary ore consists of massive and disseminated arsenopyrite and pyrite that contain Au in solid solution or as small inclusions (Bowman, 1979).

The climate in the region is temperate and controlled by its proximity to the Pacific Ocean (Fig. 1). Average annual temperatures and rainfall approximate are 15 °C and 1000 mm, respectively. The study area lies between 10 and 60 m above sea level and the landscape is characterized by narrow ridgelines with gentle to very steep side slopes. The regolith cover is moderately to intensely weathered to depths of up to several meters. The vegetation covering the area is predominantly *Sclerophyll* forest, as described in Reith et al. (2005).

Field analyses of soils, after McDonald et al. (1998), show that the soils are colluvial cambisols. A typical soil profile (thickness) consists of 1–3 cm O-horizon, 3–12 cm Ah-horizon, 15–20 cm B_1-horizon, and 50–100 cm B_2-horizon, which overlie a moderately weathered saprock C-horizon. The soil textures are predominantly sandy or silty clay loams with gravel contents of up to 30 vol%. A few, i.e., <2%, orange to pale brown mottles were detected in the B-horizon. The mineralogy, grain size total C and N and pH of the soils and deeper regolith materials used in our experiments are given in Table 1.

Table 1
Mineralogy and properties of the auriferous soil and vein-quartz samples used in the microcosms

	Ah-horizon	B-horizon	Quartz-vein material
Mineralogy[a] (%)			
Quartz	80.1	47.3	96.4
Albite	1.3	1.2	1.4
Muscovite	12.2	42.5	0.7
Kaolin	1.0	5.1	0.1
Illite	0.2	0.2	0.2
Goethite	0.4	1.3	0.6
Microcline	2.1	n.d.	n.d.
Halloysite	0.2	n.d.	n.d.
Pyrophillite	n.d.[b]	n.d.	0.4
Biotite	n.d.	1.4	n.d.
Rutile	n.d.	1.0	n.d.
Size fraction analysis (%)			
Coarse sand	23	24	n.a.[c]
Fine sand	30	20	n.a.
Silt	25	25	n.a.
Clay	11	32	n.a.
Total C [wt.%]	9.6	0.7	n.d.
Total N [wt.%]	0.33	0.09	n.d.
pH (1:5 extract in water)	5.5	6.0	5.2

[a] Mineralogy was summarized from Reith et al. (2005) and was measured using XRD combined with the SIROQUANT software.
[b] n.d., not detected.
[c] n.a., not applicable.

3. Materials and methods

3.1. Sampling procedures and locations

Soil and vein-quartz materials were collected in June 2002, July 2003, and October 2003 for microcosms, sequential extractions, and community structure analyses using a flame-sterilized hammer, hand shovel, and sieve. Soil samples from the Ah- and B-horizons were collected from 3 to 5 cm and 15 to 20 cm depths, respectively, from soil directly overlying the primary Au deposit (notated in tables and figures as A and B) and from a site located 100 m away from the outcropping vein (notated as A100, B100). Soil samples from 100 m distance represent background Au concentrations in the soil, based on our previous study (Reith et al., 2005). Soil samples were sieved on site to <2 mm size, stored in sterile plastic bags and transported over ice to the laboratory. Material was collected from the quartz-vein within the underground workings and hammered to rock chips on site using the method described

by (Hirsch et al., 1995). In the laboratory, the quartz chips were ground in a flame-sterilized tungsten-carbide ring mill and sieved to <200 μm size under sterile conditions.

3.2. Microcosm experiments

Batch-type microcosms with soils and vein-quartz materials were incubated as aqueous slurries on a rotary shaker at 25 °C for up to 70 days. Seventy-five grams (dry weight) of solid material (biologically active or inactive—see below) plus 300 ml double deionized sterile water (1:4 (w:v); chosen to allow up to ten 5 ml water samples per experiment) were placed under sterile conditions into 500 ml infusion bottles (Ochs, Bovenden-Lenglern, Germany). Experiments were conducted under oxic or anoxic conditions without amendment, or amended with Au pellets, 100 $\mu g\,g^{-1}$ (d.w. soil) of dissolved Au ($AuCl_4^-$) or cycloheximide. Specific conditions for all experiments are listed in Table 2. Microcosms were named according to a code based on the experimental conditions, for instance, AJ2-a + Au stands for a microcosm with Ah-horizon material collected in June 2002 that was incubated biologically active and amended with 2 Au pellets. Oxic microcosms were plugged with sterile cotton wool to allow O_2 exchange with the atmosphere and incubated on a shaking incubator at 25 °C and 100 rpm (rounds per minute) in the dark. Dissolved oxygen (DO) levels in the water were checked in microcosms AJ2-a and BJ2-a using a 1469-00 OX-2P dissolved-O_2 Kit (Hach, Belgium) at days 10 and 68 and ranged between 4.7 and 6.3 $mg\,L^{-1}$, compared with DO = 7.9 $mg\,L^{-1}$ in water saturated with atmospheric O_2 at 25 °C. Other experiments under oxic conditions were assumed to contain similar concentrations of oxygen. Anoxic conditions in microcosms AJ2-a + Au-an and BJ2-a + Au-an were established by flushing the infusion bottles with sterile Ar for 20 min after the solid samples were introduced, closing the bottles with rubber stoppers and screw-cap seals and then adding anoxic water from sterile syringes through the rubber stoppers. Gas chromatography was used to check remaining $O_{2(g)}$ levels in the bottles and none was detected. Anoxic microcosms were incubated static at 25 °C in the dark. Some microcosms were amended with sterilized 99.999% pure Au pellets (0.1–0.2 mm in diameter; Johnson Matthey, Sydney, Australia) to assess if more Au than contained in the soils could be solubilized. In some experiments cycloheximide (1000 $\mu g\,ml^{-1}$ aqueous solution) was added to inhibit the growth of eukaryotes such as fungi, yeast, or algae, because they have been shown to actively adsorb complexed or colloidal Au complexes from solution (e.g., Korobushkina et al., 1983; Hosea et al., 1986; Savvaidis et al, 1998; Mukherjee et al., 2002; Nakajima, 2003). Five milliliters aqueous samples were aseptically collected from all experiments using sterile one-way plastic syringes (Terumo, Tokyo, Japan) and centrifuged at 15,000 rpm for 20 min in a microfuge (Eppendorf, Germany). The supernatant was decanted, filtered through a Whatman No. 4 paper filter, and analyzed using ICP-MS. Note that this method does not distinguish between dissolved and colloidal Au. This was chosen to measure how much of both forms of Au can be released from solid soil fractions, and thus to more closely represent what might happen in the field. In addition, the measured Au concentrations were too low to measure the speciation of Au reliably. Biologically active experiments contained field-fresh materials and were commenced within 24 h of arrival in the laboratory.

For the biologically inactive microcosms, i.e., sterilized control experiments, the solid materials were sterilized by autoclaving at 125 °C and 1.1 atm for 1 h before sterile double deionized water was added; vein-quartz material had to be autoclaved three times to sterilize (Trevors, 1996). Sterility was tested optically using phase contrast microscopy and by plating of 1:10 (v:v) soil dilutions on nutrient agar plates (Oxoid, Basingstoke, UK) after the incubation; only those experiments that had remained sterile were used for further evaluation. It is possible that autoclaving affected the regolith materials, in particular how the Au is associated with the different fractions, so we checked for possible effects by analyzing field-fresh and autoclaved samples using the first three steps of a sequential leaching procedure (see below). No discernable changes to the fractionation of Au were detected in the autoclaved compared to the field-fresh materials. Repeatability/reproducibility was established by running many experiments in duplicate or triplicate, as shown in Table 2. Biologically inactive microcosms were conducted in duplicate to reproducibly establish that no or significantly less Au was mobilized when no active microbiota were present. Microcosms with samples from July 2003 (triplicate) and October 2003 were conducted biologically active (Table 2) to determine the repeatability and variability of Au mobilization in samples collected at different sampling times.

3.3. Total and sequential extractions

Solid materials were analyzed for total Au concentrations and the fractionation of Au in the different solid phases. Total Au concentrations in all regolith materials were determined by digesting 1 g of sample for 24 h in 10 ml concentrated aqua regia at 25 °C. The supernatant was filtered through a No. 4 Whatman paper filter and diluted with double deionized water to final HNO_3 and HCl concentrations of ≈2 and 0.7 vol%, respectively. Sequential extractions were used to assess changes in the association of Au with different operationally defined solid soil fractions during incubation for two Ah-horizon microcosms incubated biologically active in triplicate, i.e., AJ3-a and AJ3-a + $AuCl_4^-$ amended with 100 $\mu g\,g^{-1}$ (d.w. soil) of Au as $AuCl_4^-$. Solid samples were aseptically removed from AJ3-a after 0, 10, 20, 30, 40, and 68 days of incubation, and after 10 days from AJ3-a + $AuCl_4^-$. The latter experiment was to test for the capacity of the soil to adsorb Au and to determine the fractionation of the adsorbed Au to the solid soil fractions. The sequential leaching procedure used in this study was developed by Reith et al. (2005) based on methods used in previous studies (e.g.,

Table 2
Sampling times, incubation conditions, and analyses in microcosm experiments conducted with soil- and quartz-vein materials from the Tomakin Park Gold Mine

Microcosm	Sampling material			Sampling date			Biological activity		Amendments and conditions				Analyses
	Ah-horizon (A)	B-horizon (B)	Quartz (Q)	June 2002 (J2)	July 2003 (J3)	October 2003 (O3)	Active (a)	Inactive (i)	None	Au pellets (+Au)	Cyclo-heximide (+C)	Other	
AJ2-a	X			X			X		X				pH, O_2, cell numbers
AJ2-i	X			X				X	X			Duplicate	
AJ2-a + C	X			X			X				X		pH
AJ2-i + C	X			X				X			X	Duplicate	pH
AJ2-a + Au	X			X			X			X			pH
AJ2-i + Au	X			X				X		X		Duplicate	pH
AJ2-a + Au-an	X			X			X					Anoxic (an)	
BJ2-a		X		X			X		X				pH, O_2,cell numbers
BJ2-i		X		X				X	X			Duplicate	pH
BJ2-a + Au		X		X			X			X			pH
BJ2-i + Au		X		X				X		X		Duplicate	pH
BJ2-a + Au-an	X			X			X			X		Anoxic (an)	
QJ2-a			X	X									pH, cell numbers
QJ2-i			X	X								Duplicate	pH
AJ3-a	X				X		X		X			Triplicate	AAS, CLPP, Sel. Leach.
AJ3-a + Au	X				X		X			X			CLPP
AJ3-a + $AuCl_4^-$	X				X		X					Triplicate, +$AuCl_4^-$	Sel. Leach.
A100J3-a	A100				X		X		X				CLPP
A100J3-a + Au	A100				X		X			X		Triplicate	Biofilm
BJ3-a		X			X		X		X				CLPP
B100J3-a		B100			X		X			X			CLPP
AO3-a + Au	X					X	X			X		30 °C	

Chao, 1984; Hall et al., 1995, 1998; Xueqiu, 1998; Gray et al., 1999). The reagent and operationally defined fraction for each extraction was: double deionized water for the water-soluble fraction, 1 M ammonium acetate (NH_4OAc) for the exchangeable, clay- and carbonate-bound fraction, 1% sodium pyrophosphate ($Na_4P_2O_7$) for the organic fraction, 0.25 M hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) in 0.25 M hydrochloric acid (HCl) for the amorphous Fe- and Mn-oxides fraction, and 4 M HCl for the crystalline Fe-oxide fraction. The procedure for each extraction was: (i) 1 g of sample was added to 15 ml Falcon tubes; (ii) 10 ml of leaching solution was added; (iii) the tubes were shaken for 6 h at 25 °C; (iv) the tubes were centrifuged for 30 min at 4000*g* after each extraction step; (v) the supernatant was decanted and filtered through Whatman No. 4 paper; (vi) the residue dried in air overnight at 60–70 °C; (vii) the next leaching solution was added; and (viii) steps 3–7 were repeated for each leaching step. All extract solutions were stored in the dark at −20 °C until analysis. Note that the extractions are operationally defined and may not necessarily reflect the fractions that are intended in any of these methods (Chao, 1984; Hall et al., 1995, 1998; Xueqiu, 1998; Gray et al., 1999); however, because our experiments were conducted in triplicate, we were able to discern statistical differences between the results of the different extractions. To evaluate if the observed differences between the analogous extractions on days 0, 10, 20, 30, 40, and 68 were significant, two-tailed *t* tests were performed at significance levels of $p = 0.05$ using the Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS, 2001).

3.4. Chemical analysis

Gold in the liquid samples was measured using an Agilent 7500S ICP-MS, equipped with a concentric nebulizer and an automatic sampler. The detection limit for Au was 2.8 ng L^{-1} and analytical precision lay within 5%. Total carbon, C_{tot}, and total nitrogen, N_{tot}, contents of Ah- and B-horizon samples were measured using a Leco CNS 2000 element analyzer. Minerals were identified and their abundances determined at Geoscience Australia using XRD on powdered samples using the SIROQUANT software following the procedures outlined in Taylor (1991). Size fraction analysis of the <2 mm Ah- and B-horizon samples was conducted using the pipette method (USDA Method 3A1, 1996). The concentration of free amino acids in soil solutions was measured in selected experiments by a commercial laboratory (Australian Proteome Facility, Sydney, Australia) using high-pressure liquid chromatography (HPLC). The pH values of the liquid samples were measured with an Activon pH electrode and meter.

3.5. Microbiological analysis

3.5.1. Bacterial numbers determined by direct cell counts

During the incubation of three unamended microcosms, i.e., AJ2-a, BJ2-a, and QJ2-a slurry samples were aseptically collected for direct optical cell counts. Cells were counted with using a haemocytometer with a Thoma counting chamber (Assistent, Germany) and a phase contrast microscope without previous dyeing. Samples were diluted 10^3–10^4 times with sterile 0.9% NaCl solution so that the number of organisms counted in a single square ≈2–12 cells. One hundred of the 400 fields were counted for each sample, and then the average bacterial number and standard error for each sample was calculated.

3.5.2. Analysis of biofilms on Au pellets

To assess the formation of biofilms on the added Au pellets, pellets from the biologically active experiments, i.e., AJ2-a + Au, AJ3-a + Au, and A100J3-a + Au, were aseptically collected after 70 days of incubation and thoroughly rinsed with sterile deionized water. One pellet from each experiment or replicate was then mounted on stubs using carbon tape and examined uncoated with a Cambridge S360 scanning electron microscope (SEM) operated at 20 kV and ≈20 mm working distance, and equipped with a Tracor Northern EDXA detector. To study the composition of the coatings discovered on the Au pellets, samples were analyzed with a JEOL JSM 6400 scanning electron microscope equipped with an Oxford Instruments light element EDXA detector and Link ISIS software. The second Au pellet from each experiment or replicate was stained using the nucleic-acid-specific fluorochrome 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI; Sigma, St. Louis, MO; Larsson et al., 1996). The samples were stained on ice with 1 ml of 10 μl ml^{-1} DAPI solution for 20 min and then washed with deionized water and air dried. The stained biofilms were examined with a Leica TCS-SP2-UV confocal laser microscope with Leica imaging software (Leica, Wetzlar, Germany).

3.5.3. Community structure analysis

The community-level physiological profiling method (CLPP) using BIOLOG™ Ecoplates (BIOLOG, Hayward, CA, USA) was used in this study to evaluate changes in the culturable, heterotrophic, and aerobic bacterial community in the soil samples during incubation of the microcosms. CLPP fingerprints were obtained according to a method developed by Garland and Mills (1991), which has successfully been used to differentiate between microbial activity in soil samples subject to different environmental influences such as heavy metal contamination, and to subsequently infer microbial community population changes due to different substrate utilization profiles (e.g., Garland and Mills, 1991; Mueller et al., 2001). BIOLOG™ Ecoplates contain 31 carbon sources (three replicates for each) with tetrazolium redox dye and one control well containing the dye but no carbon source. Samples for CLPP fingerprinting were taken from five microcosms, i.e., AJ3-a, AJ3-a + Au A100J3-a, BJ3-a, and B100J3-a (Table 3), on day 0, 10, 20, 30, 40, and 68 of the incubation. One milliliter

Table 3
Concentrations of free amino acids in solutions from microcosm AJ3-a

Amino acid	Concentration (μM)		
	Day 0	Day 20	Day 50
Aspartic acid	1.3	3.6	n.d.
Serine	1.4	4.2	2.2
Glutamic acid	n.d.[a]	6.0	n.d.
Glycine	3.9	9.3	3.7
Glutamine	n.d.	0.9	n.d.
Arginine	n.d.	2.1	n.d.
Threonine	n.d.	2.1	n.d.
Alanine	1.4	18.3	1.6
Tyrosine	n.d.	1.9	n.d.
Valine	0.5	6.9	0.5
Isoleucine	n.d.	3.1	n.d.
Leucine	n.d.	2.8	n.d.
Phenylalanine	n.d.	3.0	n.d.
Total	8.5	64.2	8.0

[a] n.d., not detected.

of soil slurry was aseptically removed from the microcosms and diluted in 25 ml of 0.9 wt% NaCl solution, to make a final dilution of 1:100. BIOLOG™ Ecoplates were inoculated with 125 μl of sample per well and incubated at 25 °C in the dark for 120 h (BIOLOG, Hayward, USA). The absorbance of the plates were read with a Biolog microplate reader (590 nm) at 6 h intervals for 60 h and then at 12 h intervals for a further 60 h.

The community-level response was separated into two major components: (i) the overall rate of color development; and (ii) the emerging pattern of substrate utilization. The overall rate can be estimated by the rate of AWCD, and is a function of the inoculum density (Garland, 1996). In this study, the AWCD was measured over time and used as a measure of total activity. To eliminate weak false positive responses a threshold of 0.1 absorbance units was used (Garland, 1996).

To analyze for similarities and differences in the patterns of the microbial responses, a variety of multivariate statistical approaches have been used (e.g., Hitzl et al., 1997; Preston-Mafham et al., 2002; Yan et al., 2000). In this study, we calculated the area under the absorbance curve versus time curve for every well using the trapezoidal approximation to the area under the curve according to Guckert et al. (1996). Only area values greater than 1 were used for further statistical analysis. Using this method differences in lag times, rates of increase and maximum optical densities can be accurately assessed, making it a robust method to study differences in patterns of the microbial responses (Guckert et al., 1996). A principal component analysis with Kaiser normalization was used to evaluate further differences in patterns. Several methods of matrix rotations, i.e., varimax, quartimax, equamax, and promax, were tried, but varimax rotation with Kaiser normalization was found to be the most effective in separating the data. All calculations were made using SPSS version 11.0.

4. Results and discussion

This section consists of following subsections: (i) solubilization of Au in biologically active and inactive (sterilized) microcosms; (ii) analysis of the biofilms growing on Au pellets incubated in biologically active microcosms; (iii) changes occurring in the structure of the heterotrophic, aerobic bacterial communities in selected auriferous and non-auriferous soil microcosms during the incubation; and (iv) a discussion on the implications of microbial processes on behavior of and exploration for Au in the regolith.

4.1. Solubilization of Au in microcosm experiments

In general, Au was solubilized in all biologically active microcosms with auriferous soils (Figs. 2–4). Gold was detectable in solution within 4–30 days of the start of the incubation, generally reached maximum concentrations between days 30 and 45, decreased thereafter and in many cases no Au was detected in solution by the end of the experiments (Figs. 2A and B, 3, and 4A and B). In the biologically inactive microcosm, Au concentrations in solution were typically below detection or much lower than in the equivalent biologically active microcosms (Figs. 2A and B, 4A and B).

4.1.1. Gold solubilization in microcosms of the Ah-horizon

To determine the effect of resident microbiota on the solubilization of Au, the concentrations of Au in solution in unamended biologically active and inactive microcosms, i.e., AJ2-a and AJ2-i, respectively, were measured. In AJ2-a 65 $ng\,g^{-1}$ (d.w. soil) of Au was detected in solution at day 4 and then none until day 44 and day 45 where 1134 and 534 $ng\,g^{-1}$ (d.w. soil) were detected, respectively (Fig. 2A). No Au was detected in solution between 45 days and the end of the experiment at day 70. Based on the aqua regia digests conducted with soils from AJ2-a, that yielded total Au concentrations of 1453 $ng\,g^{-1}$ (d.w. soil), this amounts to a maximum of ≈80 wt.% of Au dissolved at day 44. Gold was not detected in the solution during the incubation in either of the duplicates of AJ2-i.

The addition of cycloheximide, designed to inhibit the growth of eukaryotes, e.g., fungi, yeasts, and algae, resulted in an early increase in solubilized Au relative to the unamended equivalent microcosms, but the pattern of solubilization was similar. Gold was detected in solution in AJ2-a + C during the first 40 days in concentration of 34–260 $ng\,g^{-1}$ (d.w. soil); the maximum Au concentration was 664 $ng\,g^{-1}$ (d.w. soil) at day 45 (Fig. 2A). Gold was not detected in the solution in either of the duplicates of the AJ2-i + C microcosm (Fig. 2A). The early release of Au suggests that Au may have been constantly solubilized during the first 40–50 days of the incubation in all microcosms, but that active eukaryotes re-adsorb Au rapidly.

Gold solubilization patterns in experiments amended with Au pellets were similar those detected in unamended

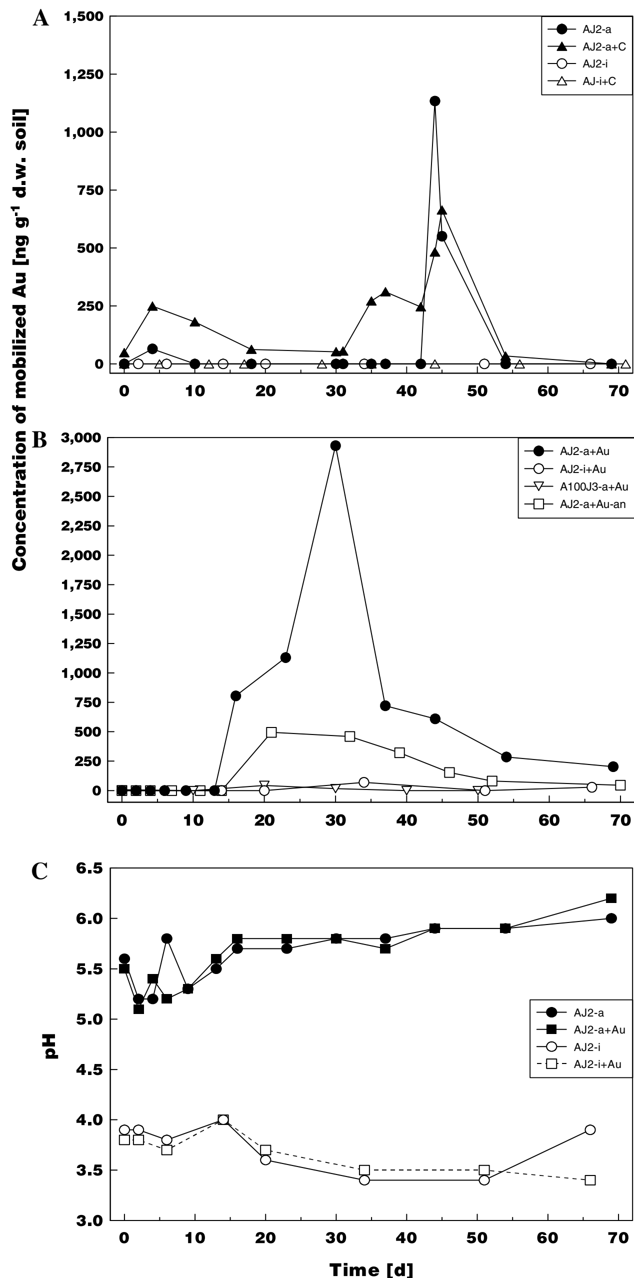


Fig. 2. Concentration of solubilized Au (A and B) and pH (C) in solutions from microcosms incubated biologically active or inactive (sterilized) with samples from the Ah-horizon collected in June 2002.

microcosms, although Au was solubilized earlier and in higher concentrations (Fig. 2B). In AJ2-a + Au, Au was detected in solution after 14 days of incubation and reached a maximum concentration of 2930 $\mathrm{ng\,g^{-1}}$ (d.w. soil) of Au at day 30 (Fig. 2B). The maximum was approximately three times higher than in the unamended microcosm and was approximately twice the concentration of Au contained in the soil samples indicating that the microbiota were able to solubilize more Au than was present in the soils by mobilizing it from the added Au pellets.

Anoxic microzones are generally present in oxic soils (e.g., van der Lee et al., 1999) and anaerobic bacteria constitute ≈10% of the total culturable bacterial community in oxic soils (e.g., Küsel et al., 1999; Skinner, 1975). Therefore, an experiment (AJ2-a + Au-an) was conducted under anoxic conditions to compare its results to those of the equivalent oxic experiment; amendment with Au pellets was chosen to potentially maximize the effect. The results of an anoxic microcosm indicate that the anaerobic microbiota are capable of solubilizing the Au that is disseminated in the solid soil fractions, but that in contrast to the oxic microcosm the organisms were not able to solubilized Au from the added Au pellets. In the anoxic experiment, the pattern of Au solubilization was similar than in the equivalent oxic experiment; however, the maximum concentrations of solubilized Au were lower, i.e., 481 $\mathrm{ng\,g^{-1}}$ (d.w. soil) compared to 2930 $\mathrm{ng\,g^{-1}}$ (d.w. soil), respectively (Fig. 2B).

The microcosms using samples with background Au concentrations (A100) displayed no Au solubilization, even when they were amended with Au pellets. The unamended A100 sample contained little Au that could be mobilized, i.e., 2–3 ng of Au $\mathrm{g^{-1}}$ (d.w soil), and in A100J3-a + Au less than 70 $\mathrm{ng\,g^{-1}}$ (d.w. soil) of Au was solubilized, which lay within the range of the sterile control experiment (Fig. 2B). The result indicates that the appropriate microorganisms were either not present or not active in the experiment.

The variation of the solution pH occurring between biologically active and inactive microcosms may influence the observed solubilization patterns of Au. To account for this, the pH values were measured in several biologically active and inactive microcosms during the incubation (Fig. 2C). At the start of the experiment, the pH lay between 3.5 and 4.0 in the autoclaved controls and was throughout the incubation 1.0–1.5 U lower compared to the biologically active microcosms (Fig. 2C). A lowering of pH in autoclaved soil was observed in other studies (e.g., Shaw et al., 1999), but did in our experiments apparently not enhance the solubilization of Au.

Differences in Au solubilization in microcosms with samples from different sampling times were detected and

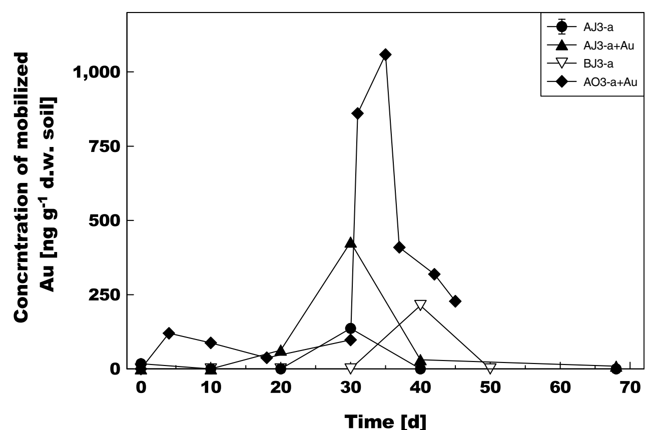


Fig. 3. Concentration of solubilized Au in solutions from microcosms incubated biologically active with samples from the Ah- and B-horizon collected in July and October 2003.

may be the result of differences in the community structures and/or activity of the resident microbiota. Biologically active microcosms were conducted with Ah-horizon samples collected in July and October 2003, i.e., AJ3-a, AJ3-a + Au, AO3-a + Au (Fig. 3). The solubilization of Au followed a similar pattern than in the equivalent biologically active microcosms from June 2002, however, the timing of Au release and its concentration generally differed (Fig. 3). In AJ3-a a Au concentration of 136 ± 2 ng g^{-1} (d.w. soil) was detected in solution after 30 days, Au in solution was not detected at the other sampling times (Figs. 3 and 5). AJ3-a + Au yielded much lower maximum concentrations of Au in solution, i.e., 427 ng g^{-1} (d.w. soil), compared to AJ2-a + Au. AO3-a + Au was incubated at 30 °C and Au was detected in all solution samples taken after day 4 day, reaching a maximum of 1058 ng g^{-1} (d.w. soil) after 35 days, and then decreasing to ≈200 ng g^{-1} (d.w. soil) at the end of the incubation at day 45.

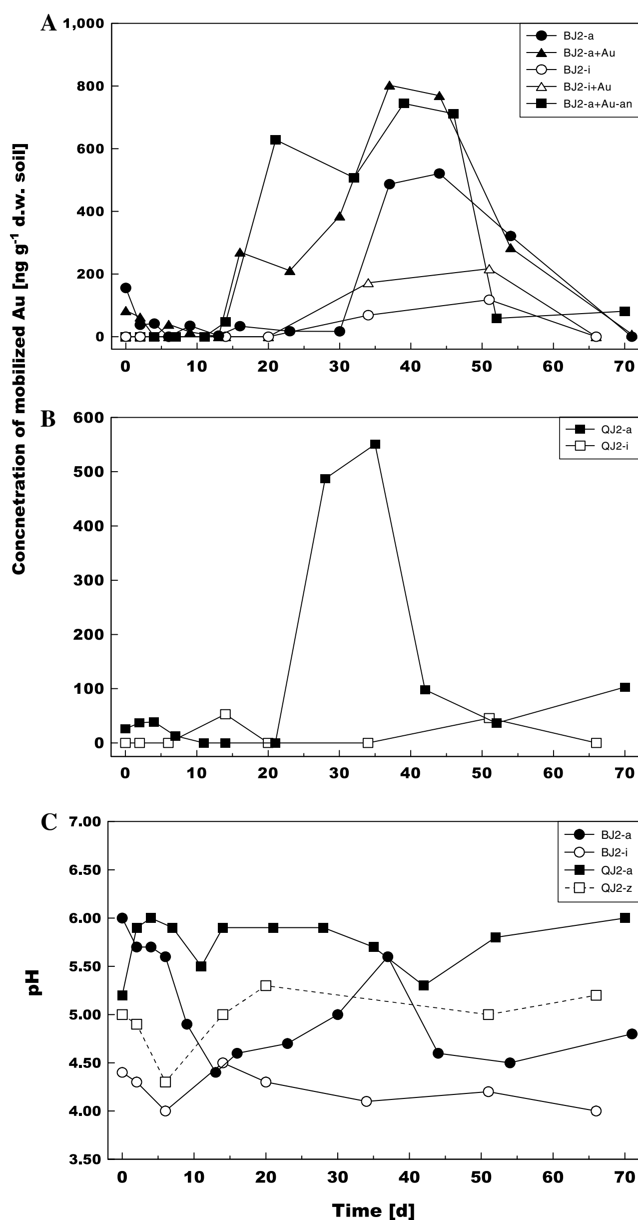


Fig. 4. Concentration of solubilized Au (A and B) and pH (C) in solutions from microcosms incubated biologically active or inactive (sterilized) with samples from the B-horizon (A) and the quartz-vein material (B) collected in June 2002.

4.1.2. Gold solubilization in microcosms of the B-horizon

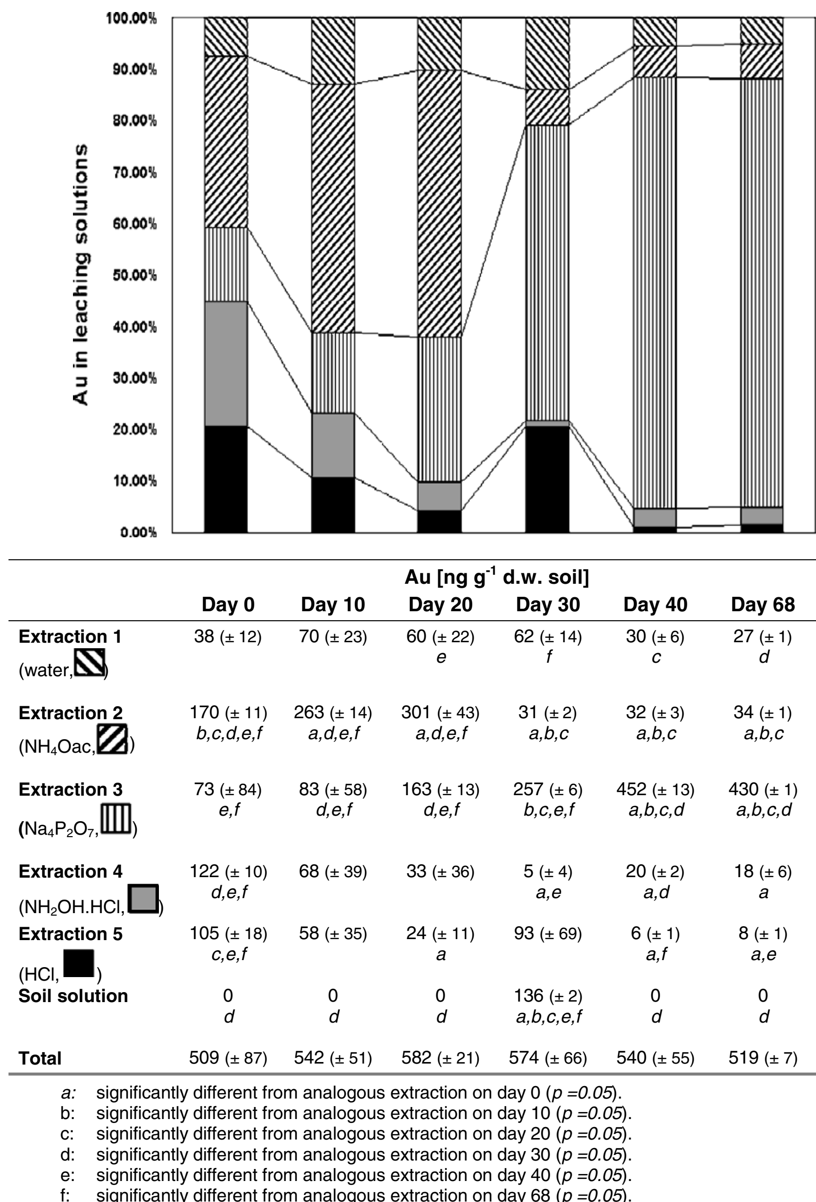
In the biologically active microcosms with B-horizon samples, similar solubilization- and re-precipitation patterns were detected compared to the corresponding Ah-horizon experiments (Fig. 4A). Gold concentrations in solution were much higher in biologically active, i.e., BJ2-a and BJ2-a + Au, compared to inactive microcosms, i.e., BJ2-i and BJ2-i, indicating an effect of the resident microbiota on the solubilization of Au. The highest concentrations of Au in solution in BJ2-a Au were detected at days 37 and 44, i.e., 486 and 521 ng g^{-1} (d.w. soil), respectively (Fig. 4A). The maximum concentration represents 80 wt.% of the total Au content, i.e., 652 ng g^{-1} (d.w. soil), in solution. BJ2-i displayed a maximum Au concentration in solution in one of the duplicates of 117 ng g^{-1} (d.w. soil) at day 51, but generally no or much lower Au concentration were detected (Fig. 4A).

In BJ2-a + Au we observed a similar pattern as in the unamended experiments, although Au in solution was detected earlier at day 15. The maximum Au concentration in solution in BJ2-a+Au was 802 ng g^{-1} (d.w. soil). One of the duplicates in sterile control experiment, BJ2-i + Au, had up to 217 ng g^{-1} (d.w. soil) Au in solution (day 51; Fig. 4A), but typically had much lower values, and in all cases less than in the equivalent biologically active experiment.

BJ2-a + Au-an was run under anoxic conditions and the results indicate that the anaerobic microbiota of the B-horizon similarly to those of the Ah-horizon appears capable of solubilizing Au. In B100J3-a (background sample) Au was detected in the solution after 14 days of incubation, from day 20 to day 50 the Au concentration in solution fluctuated between 500 and 730 ng g^{-1} (d.w. soil), after which it decreased sharply to ≈50 ng g^{-1} d.w. soil between day 50 and 52 (Fig. 4A). BJ3-a showed that the mobilization of Au in the B-horizon was repeatable with samples collected at different times; 213 ng g^{-1} (d.w. soil) of Au was mobilized at day 40 of the incubation (Fig. 3). The pH values, which were 1.5 U lower and changed little during the incubation time in the biologically inactive compared to active microcosms, did not lead to an enhanced Au solubilization (Fig. 4C).

4.1.3. Solubilization of Au in microcosms with vein-quartz material

The solubilization and precipitation pattern detected in the vein-quartz microcosms was generally similar to those detected in Ah- and B-horizon microcosms (Fig. 4B). Gold was detected in solution in the biologically active vein-quartz microcosm, i.e., QJ2-a, during the first days of



	Au [ng g^{-1} d.w. soil]					
	Day 0	**Day 10**	**Day 20**	**Day 30**	**Day 40**	**Day 68**
Extraction 1 (water)	38 (± 12)	70 (± 23)	60 (± 22) *e*	62 (± 14) *f*	30 (± 6) *c*	27 (± 1) *d*
Extraction 2 (NH_4Oac)	170 (± 11) *b,c,d,e,f*	263 (± 14) *a,d,e,f*	301 (± 43) *a,d,e,f*	31 (± 2) *a,b,c*	32 (± 3) *a,b,c*	34 (± 1) *a,b,c*
Extraction 3 ($Na_4P_2O_7$)	73 (± 84) *e,f*	83 (± 58) *d,e,f*	163 (± 13) *d,e,f*	257 (± 6) *b,c,e,f*	452 (± 13) *a,b,c,d*	430 (± 1) *a,b,c,d*
Extraction 4 ($NH_2OH.HCl$)	122 (± 10) *d,e,f*	68 (± 39)	33 (± 36)	5 (± 4) *a,e*	20 (± 2) *a,d*	18 (± 6) *a*
Extraction 5 (HCl)	105 (± 18) *c,e,f*	58 (± 35)	24 (± 11) *a*	93 (± 69)	6 (± 1) *a,f*	8 (± 1) *a,e*
Soil solution	0 *d*	0 *d*	0 *d*	136 (± 2) *a,b,c,e,f*	0 *d*	0 *d*
Total	509 (± 87)	542 (± 51)	582 (± 21)	574 (± 66)	540 (± 55)	519 (± 7)

a: significantly different from analogous extraction on day 0 ($p = 0.05$).
b: significantly different from analogous extraction on day 10 ($p = 0.05$).
c: significantly different from analogous extraction on day 20 ($p = 0.05$).
d: significantly different from analogous extraction on day 30 ($p = 0.05$).
e: significantly different from analogous extraction on day 40 ($p = 0.05$).
f: significantly different from analogous extraction on day 68 ($p = 0.05$).

Fig. 5. Association of Au with different solid soil fractions of the Ah-horizon and the aqueous solution in microcosm AJ3-a (Table 2; Fig. 2). The five fractions are operationally defined as: (i) water-soluble, (ii) clay- and carbonate-bound and exchangeable, (iii) organic, (iv) amorphous Fe and Mn oxides, and (v) crystalline Fe oxides (Reith et al., 2005). Given in the table are the means and standard deviations of the triplicate samples and the significant differences between analogous extractions determined by two-tailed t tests at significance level of $p = 0.05$.

incubation in concentrations below 40 ng g^{-1} (d.w. quartz-vein material), and reached a maximum of 550 ng g^{-1} (d.w. quartz-vein material) at day 35 of the incubation and decreased afterwards (Fig. 4B). In the biologically inactive control experiment, i.e., QJ2-i, no Au was detected in the solution, except for two samples from one of the duplicates at days 14 and 50 days which with 50 ng g^{-1} (d.w. quartz-vein material), and thus lay in the range of the water-soluble Au extracted from the quartz host rock during sequential leaching (Reith et al., 2005). The starting pH in biologically active microcosms was 5.2 and in sterilized microcosms approximately 5 (Fig. 4C). Generally, the pH increased in the biologically active microcosms to ≈6.0 and remained constant after day 25. In sterile microcosms the pH stabilized at ≈5.0.

4.1.4. Microbial mechanisms of Au solubilization

Gold concentrations in the experimental solutions were significantly higher in biologically active microcosms compared to the biologically inactive controls, where in most experiments Au was below detection. This indicates that resident microbiota play a major role in the solubilization of Au. Different physiological mechanisms of Au solubilization involving a variety of groups of bacteria have been investigated under in vitro conditions. The solubilization of Au in the QJ2-a might have been mediated by bacterial

oxidation of the pyrite and arsenopyrite, which include the Au at the Tomakin Park Gold Mine. Iron- and sulfur-oxidizing bacteria have been shown to liberate Au from sulfide minerals such as pyrite and arsenopyrite (e.g., Ubaldini et al., 2000; Rawlings, 2002). The organisms use ferrous iron and sulfide as electron donors in their metabolisms and oxidize them to ferric iron, thiosulfate, and sulfate, respectively (e.g., Brock et al., 1996). In this process, the mineral is dissolved and the Au, which is often bound in lattices or incorporated into the crystal structure of these minerals, is liberated (King, 2002; Rawlings, 2002).

The solubilization of Au in the top soils is more likely to be linked to the activity of the heterotrophic bacteria, which have been shown to dominate bacterial communities in oxic soils with high organic matter contents, i.e., 9.6 wt.% and 0.7 wt.% in the Ah- and B-horizon at the Tomakin Park Gold Mine (e.g., Paul and Clark, 1996). Heterotrophic bacteria excrete organic substances such as proteins and peptides, amino acids, carboxylic acids and nucleic acids (Korobushkina et al., 1983; Sand, 1997), some of which have been shown to be able to form stable complexes with Au (Vlassopoulos et al., 1990). Korobushkina et al. (1983) have shown that several mg L^{-1} (growth medium) Au can be solubilized in in vitro studies with heterotrophic bacteria in media containing metallic Au, and using electrophoretic methods they separated Au organic complexes and found that Au amino acid complexes dominated. To assess if the solubilization of Au in our experiments was linked to the changes in amino acid concentration in solution, the concentrations of free amino acids in AJ3-a at days 0, 20, and 50 was measured. Since there was no live biomass in the biologically inactive controls, as reported earlier, the production of amino acids would not occur, thus, the concentration of amino acids in the soil solution was only measured in biologically active Ah-horizon microcosms. The total concentration of amino acids was 8.5 μM at day 0, increased to 64.2 μM at day 20 and then decreased to 8.0 μM at day 50 (Table 3). This indicates an increase in amino acid production and excretion was followed by a increase in amino acid consumption in the microcosms during the incubation. Thirteen different amino acid were detected in solution at day 20 compared to 5 and 4 at days 0 and 50, respectively (Table 3). In particular, aspartic acid was detected at days 0 and 20 and threonine was detected at day 20 (Table 3); both these amino acids have been shown to dissolve Au (Korobushkina et al., 1983).

4.2. Association of Au with solid soil fractions in the Ah-horizon

In all microcosm experiments, the concentrations of Au in solution decreased after 40–60 days of incubation, and Au was apparently precipitated and/or adsorbed to the surfaces of solids such as minerals, organic matter or biota. This was investigated using sequential leaching of solid slurry samples collected at days 0, 10, 20, 30, 40, and 68 of incubator from AJ3-a. The results presented in Fig. 5 indicate that a continuous mobilization and re-precipitation of Au was occurring during the incubation of the microcosms and highlight the function of the organic matter as reductant and/or sorbent for Au in soils.

In biologically inactive Ah-horizon microcosms generally no Au was detected in solution, so it was assumed that since there was no active biomass no change in Au adsorption patterns would occur. The total Au concentration in the initial soil samples from the Ah-horizon over mineralization measured in aqua regia digests was ≈700 ng g^{-1} (d.w. soil) and lay within the 150–1200 ng g^{-1} (d.w. soil) range reported by Reith et al. (2005). The total concentration of Au added up from the five extractions was 509 ± 87 ng g^{-1} (d.w. soil; Fig. 5) compared to ≈700 ng g^{-1} (d.w. soil) in aqua regia digests indicating the presence of Au in the residual phase. The concentration of Au in the water-soluble fraction was approximately constant during the experiment, but Au concentrations in the remaining fractions changed significantly during the course of the experiment indicating a microbially mediated micro scale mobilization and re-precipitation of Au (Fig. 5). Apparently, Au was liberated from the operationally defined Mn- and Fe-oxides during the first 20 days of incubation and re-adsorbed to the clay-bound and exchangeable fraction. The apparent solubilization of Au from the Mn- and Fe-oxides continued until day 20, where more Au apparently re-adsorbed to the operationally defined organic matter. After day 20 Au appeared mostly associated with the organic matter indicating the important role of the organic matter for Au precipitation in the environment. These results are consistent with field studies that have found a marked Au enrichment in the organic horizons of soil (e.g., Sokoloff, 1950; Boyle, 1979), and where organic matter has been shown to readily bind to Au (e.g., Ong and Swanson, 1969; Gray, 1998).

The results from another microcosm, i.e., AJ3-a + $AuCl_4^-$, where 100 μg g^{-1} (d.w. soil) of Au as $AuCl_4^-$ was added to an Ah-horizon microcosm, confirmed the importance of organic matter as a reductant and/or sorbent for Au in soils (Fig. 6). Solid samples were taken after 10 days that were subjected to the five step leaching protocol yielded a total concentration of Au in the five phases of 99.38 ± 8.0 μg g^{-1} (d.w soil), and most of the Au, i.e., 91 μg g^{-1} (d.w soil), was associated with the operationally defined organic matter.

4.3. Characterization of biofilms on incubated Au pellets

Gold pellets that had been incubated for 70 days in biologically active microcosms (AJ2-a + Au, AJ3-a + Au) were partly covered with a macroscopically visible gray patches. Scanning electron micrographs revealed the presence of cellular structures resembling microbial biofilms (Figs. 7A and 8A). The cellular structures, cocci of approximately 1 μm in diameter, were uniform in size and morphology (Figs. 8A and B). Approximately one cell per

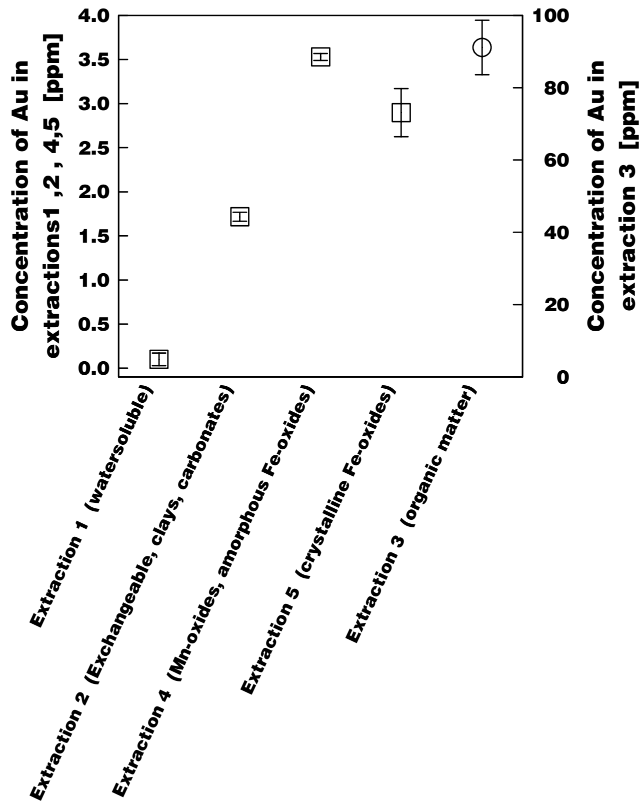


Fig. 6. Distribution of Au in operationally defined soil phases extracted by sequential leaching collected 10 days after amendment with 100 μg g^{-1} (d.w. soil) of Au as $AuCl_4^-$. AJ3-a + $AuCl_4^-$ was conducted in triplicate and error bars depict the standard deviations of the three replicates.

μm^2 was visible in the scanning electron micrographs. A cracked layer of extracellular polymeric substance (EPS) was apparent between the cells (Figs. 7A, and 8A). EPS layers have been shown to assist in the formation of microcolonies and microbial films on mineral and metal surfaces (Allison and Sutherland, 1987) and are formed by microorganisms immediately after their initial attachment to a surface (Little et al., 1997). The cracking of the EPS layer in our samples probably derived from the air drying and the high vacuum in used the scanning electron microscope.

Backscattered electron imagery (BSE) shows that these cellular structures consist of light elements, because they appeared dark in the secondary image suggesting that they are made up of carbonaceous materials (Fig. 7B). SEM-EDXA confirmed the presence of O, C, and possibly N, indicating that the observed structures consist of organic molecules (Fig. 7C).

Au pellets were stained using the unspecific nucleic acid stain DAPI. Areas with structures similar to those described above stained positive with DAPI, demonstrating the presence of nucleic acids in the apparent biofilms on Au pellets (Figs. 8C and D). High resolution imagery revealed an uneven distribution of the stain in the biofilm indicating the successful staining of nucleic acids in individual cells (Fig. 8D). Areas where no cellular structures were observed did not stain positive with DAPI, nor did Au pellets not incubated in the soil.

In microcosm AJ2-a+Au a maximum of 2930 ng g^{-1} (d.w. soil) Au was mobilized compared to 1134 ng g^{-1} (d.w. soil) in the unamended microcosms conducted with the same samples. The total Au content of these samples ≈800–1500 ng g^{-1} (d.w. soil), indicating that approximately half of the Au of the 2930 ng g^{-1} (d.w. soil) was released from the Au pellets, possibly by the bacteria that form the biofilms. Biofilms have been shown to develop on a wide range of minerals such as iron oxides, carbonates, silicates, and sulfide minerals (Little et al., 1997). They also develop on materials such as steel and other metals, promoting the degradation of the metal surfaces (Beech, 2004). Interfacial processes in the EPS layers appear to play an important

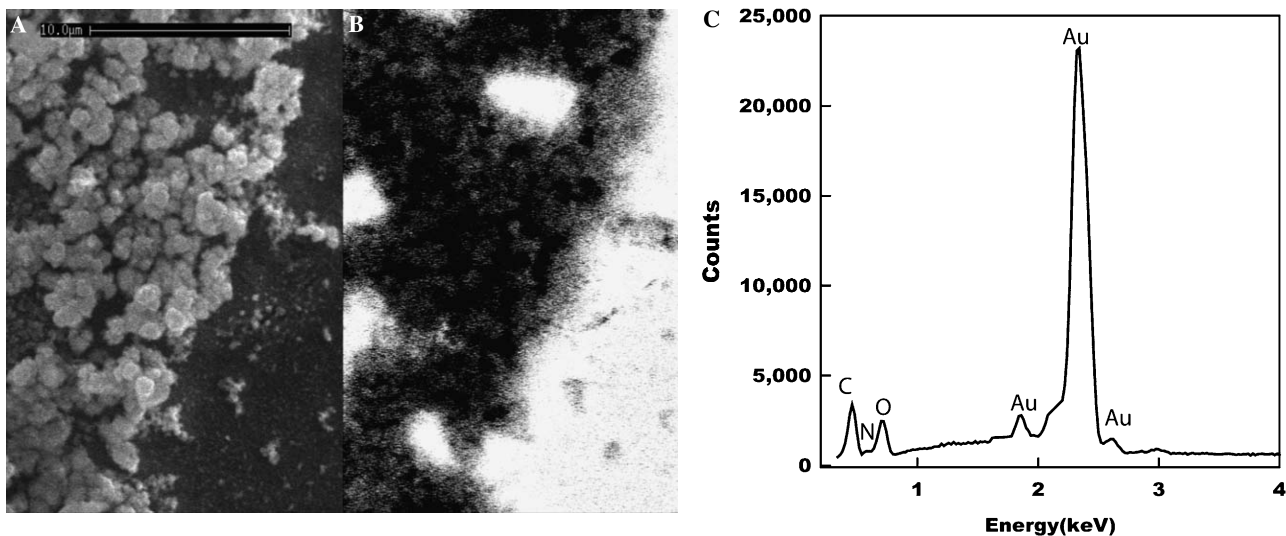


Fig. 7. SEM micrographs and EDX-analysis of a biofilm on a Au pellet incubated for 70 days in biologically active microcosm. (A) Secondary electron image (scale bar = 10 μm). (B) Backscatter electron image. (C) EDX-analysis of the area.

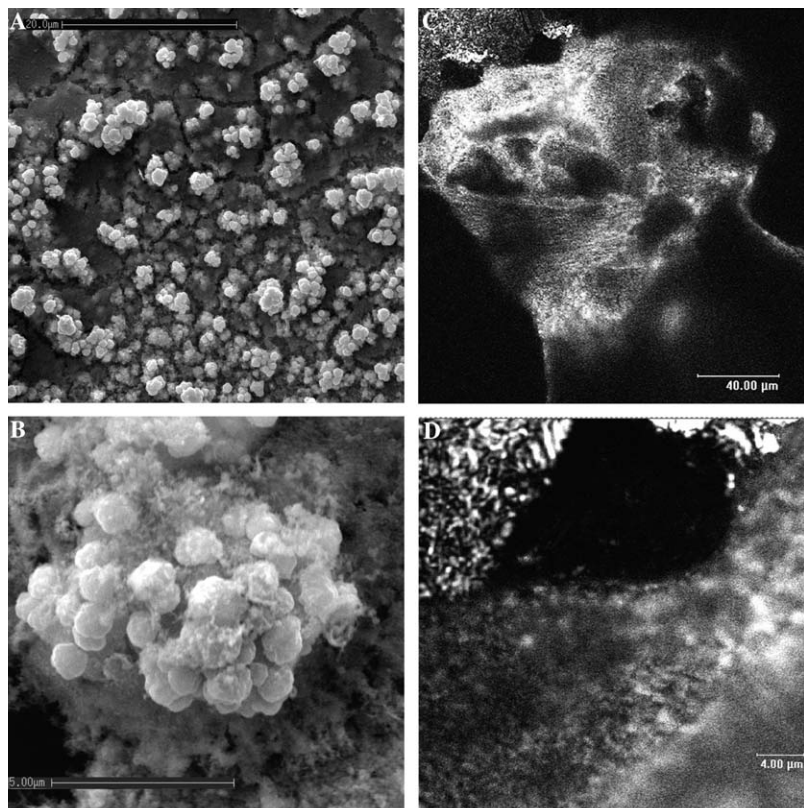


Fig. 8. SEM micrographs and confocal images of a biofilm growing on a Au pellet incubated for 70 days in biologically active microcosms AJ2-a + Au. (A) Secondary electron image showing bacterial cells and the cracked EPS layer (scale bar = 20 µm). (B) Secondary electron image showing a colony of cocci on the Au pellet (scale bar = 5 µm). (C) Confocal image of a biofilm on a Au pellet stained with DAPI (scale bar = 40 µm). Biofilms appear in light gray, unstained areas appear dark gray or black. (D) A DAPI stained area on a Au pellet biofilm showing cells or cell clusters (light gray), which are separated by unstained interstices (dark gray to black; scale bar = 4 µm).

role in this biocorrosion (Beech, 2004) and have also been shown to play a pivotal role in bioleaching of Au from sulfidic ores such as those containing pyrite (Banfield and Hamers, 2000; Little et al., 1997). Biofilms were not observed on any of the 6 Au pellets incubated in for 70 days in triplicate microcosm A100J2-a + Au, which might explain why Au was not detected in solution in these microcosms.

4.4. Community structure analysis

4.4.1. Total cell counts and average well color development of the heterotrophic microbial communities in the microcosm experiments

The results of the cell counts and the average well color development (AWCD) indicate that the microbiota were metabolically active for the length of the incubation in all biologically active microcosms (Fig. 9, Table 4). Total cell counts were conducted with samples from the unamended microcosms AJ2-a, BJ2-a, and QJ2-a. The initial cell counts in AJ2-a and BJ2-a were 6.74×10^{10} and 5.57×10^{10} cells g^{-1} (d.w. soil), respectively, as shown in Fig. 9. The number of cells in QJ2-a was one order of magnitude lower. During the first 4 days of incubation the cell number in AJ2-a increased, subsequently decreased to approximately half the initial number by day

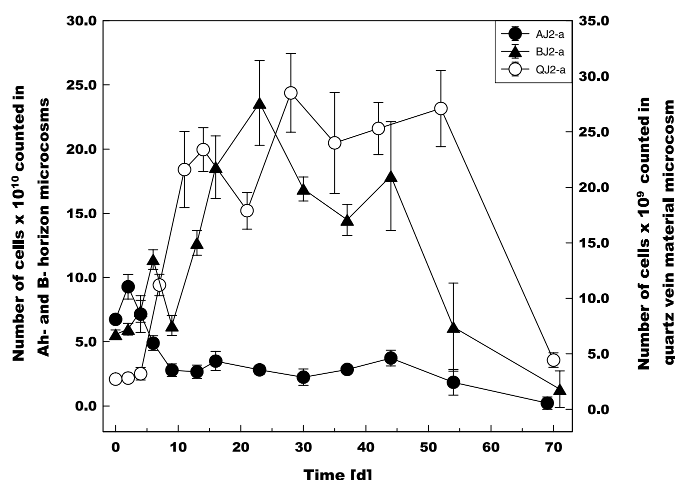


Fig. 9. Total cell counts of bacteria in the biologically active, unamended Ah- and B-horizons and the quartz-vein microcosms. The microcosms were incubated under oxic conditions in a 1:4 aqueous dilution (w:v) on a shaking incubator at 25 °C. Error bars depict the standard errors of the 100 of 400 fields counted on a haemocytometer.

10, is nearly constant up to day 50 and then decreased further after day 44 to 2.2×10^{9} cells g^{-1} (d.w. soil) at day 68. In BJ2-a, cell numbers increased ≈4 times the initial number after 23 days of incubation. Cell numbers

then remained approximately constant at three times the initial cell number and then decreased to less than the initial cell number after 70 days. Cell numbers in QJ2-a increased by a factor of 10 after 23 days, remained approximately constant until day 51 and then decreased to the initial number at day 68.

In our experiments, the dissolution of Au did not start immediately after the start of the incubation (Figs. 2–4). The majority of Au was released after 20–40 days of incubation. This might either indicate a lag-phase at the beginning of the incubation, which is often triggered by the change in the environmental conditions between field and laboratory (Brock et al., 1996). Gold was not detected in the experimental solutions after 50–70 days (Fig. 2–4), suggesting that the bacterial community in the microcosms changed, and that the new bacterial community did not promote the solubilization of Au.

To monitor the development and changes occurring in the heterotrophic aerobic bacterial community, BIOLOG™ Ecoplates were inoculated with slurry samples taken 0, 10, 20, 30, 40, and 68 days after the start of the incubation from microcosms AJ3-a, AJ3-a + Au, A100J3-a, BJ3-a, and B100J3-a (Table 4). Following an initial lag-phase of 24–30 h, the AWCD values increased markedly between 30 and 100 h after inoculation. For samples taken at day 0, maximum absorbance values of 1.44 and 1.51 were reached after 120 h after inoculation in the AJ3-a and AJ3-a + Au, respectively (Table 3). The AWCD of the BJ3-a and B100J3-a were generally lower compared to day 0. At day 20 the maximum AWCD was generally less by ≈10–20% in all experiments compared to the day 10 values (Table 4). Samples taken from the AJ3-a and A100J3-a at days 30, 40, and 68 displayed significantly lower AWCD values compared to the initial values, whereas in the BJ3-a and B100J3-a the maximum AWCD were in the range of the initial values (Table 4). AWCD values of AJ3-a + Au were between 1.24 and 1.33 for the first 40 days, and at day 68 the absorbance was lower. These results suggest that despite the higher cell number in the B-horizon microcosm, the activity of the organisms and their ability to rapidly use different carbon sources was higher in the Ah- than in the B-horizon.

Table 4
Mean absorbance at 590 nm after 120 h of incubation of the Biolog plates for selected microcosms

Time	Microcosms				
[d]	AJ3-a	A100J3-a	BJ3-a	B100J3-a	AJ3-a + Au
AWCD[a] 590 nm					
0	1.44	1.51	0.84	0.94	n.d.[b]
10	1.50	1.28	0.74	1.05	1.27
20	1.33	0.97	0.62	0.84	1.24
30	0.94	1.06	0.76	0.77	1.32
40	0.90	1.34	0.89	0.77	1.33
68	0.67	1.28	1.04	0.88	1.11

[a] AWCD, average well colour development.
[b] n.d., not detected.

4.4.2. Changes in the substrate utilization patterns

Principal component analysis (PCA) performed on the carbon utilization patterns illustrates differences in the development of the bacterial community structures during the incubation of the microcosms that are likely to be linked to the solubilization and precipitation pattern of Au in the microcosms, as shown in Fig. 10. The first and second principle components, PC1 and PC2, accounted for 35.7% and 31.2% of the variance of the data. For the first 20 days of incubation the patterns for the AJ3-a, A100J3-a, and BJ3-a display similar component loadings of 0.8–0.9 on PC1 and 0.3–0.5 on PC2. The patterns evolving at and after day 30 for the A100J3-a, BJ3-a, and B100J3-a generally show loadings of 0.15–0.35 on PC1 and 0.7–0.9 on PC2. After 30 days of incubation, the AJ3-a and A100J3-a display factor loadings of 0.45–0.55 on PC1 and 0.35 on PC2. These results indicate a general change in the structure of the bacterial community of unamended microcosms between day 20 and 40.

To investigate which groups of carbon sources on the BIOLOG™ Ecoplates are reflected in PC1 and PC2, substrates were categorized into six substrate guilds, as previously described by Zak et al. (1994): carbohydrates (6); carboxylic acids (10); polymers (4); amino acids (5); amines (3); and miscellaneous (3), where numbers in parentheses represent the number of substrates in each group. These groups were analyzed by PCA. The results show that the utilization of polymers and carbohydrates were dominantly linked to PC1, whereas utilization of amino acids and carboxylic acids was mostly linked to PC2.

During the first 20–30 days of incubation the Biolog results show that the dominant organisms were those that utilize carbohydrates and amids. These groups of substances are found in soil as humic substances such as humic and

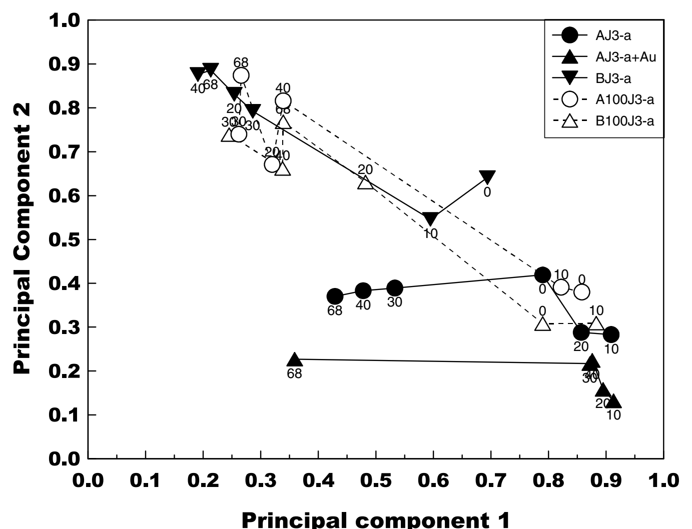


Fig. 10. Results of multivariate principal component analysis (PCA) on substrate class utilization patterns of bacterial communities during the incubation. Numbers next to symbols refer to the number of days for each experiment.

fulvic acids, and non-humic substances such as polysaccharides, lignins, and chitins, and form a major part of the non-reactive organic soil fraction (e.g., Baldock and Nelson, 2000). The initial bacterial communities of the soil in our experiments is well adapted to utilizing these substances as carbon- and energy sources. During the microbial decomposition of polymers and carbohydrates, organic acids such as amino acids were produced and released into the soil solution (this study; Paul and Clark, 1996). Thus, the rising concentrations of Au in the solution between day 20 and 45 might to be linked to the presence of Au complexing amino acids (as discussed in section 4.1.4), which are being produced and excreted more rapidly by the organisms initially dominating the bacterial community than they are consumed.

Gold precipitated or adsorbed to the solid soil fractions after 40–60 days of incubation and the organisms, which dominantly utilize amino acids and carboxylic acids and started to dominate the bacterial community in the microcosms after ≈40 days. This is also associated with much lower concentrations of amino acids at day 50, indicating an increasing consumption or a declining production and excretion of amino acids (Table 3). When the amino acids and carboxylic acids are been utilized more rapidly than they are produced, the Au-ions, if present in solution are left without complexing ligands, become unstable in solution, precipitate and/or re-adsorb to the solid soil phases, apparently dominantly to the organic fraction.

4.5. Implications the behavior of Au and exploration for Au in the regolith

Understanding the processes influencing the mobilization, transport, and precipitation of Au will lead to an improved understanding of the distribution of Au in the regolith, which will be vital for the development of successful Au exploration methods and -strategies. Many attempts have been made to link the primary lithology and mineralogy, the local climate, morphology, and topography or the abiotic chemical and physical characteristics of the weathering profile with the processes of Au mobilization, transport, and precipitation (e.g., Lakin et al., 1974; Mann, 1984; Webster and Mann, 1984; Wilson, 1984). However, no attempt had been made so far to assess the microbial influence on the dissolution of Au in the regolith in order to answer the question, whether or not we need to account for a microbial mediated Au dissolution in the environment. The work described in this paper clearly shows that we need to take the activity of Au-mobilizing microorganisms in natural systems into account. The results of previous studies conducted under in vitro condition have shown that Au was solubilized by amino acids, which had been produced and excreted by microorganisms (Korobushkina et al., 1983), the results of this study suggest that this process may be important for the dissolution of Au in natural systems. The amino acids tryptophan and lysine have been shown to from 4 to 8 nm sized water-dispersible Au nanoparticles (Selvakannan et al., 2004; Mandal et al., 2005). This indicates that amino acids might not only contribute to the dissolution of Au but also to its dispersion in the regolith.

The apparently rapid turnover of Au in soils, which are often used as sampling materials in geochemical exploration, makes it necessary to integrate microbially Au dissolution data into the interpretation of partial and sequential leaching exploration data. These leaching methods are designed to determine how an element is fractionated between different phases of regolith such as a soil or a calcrete to assess if the detected Au is residual or transported (e.g., Linter and Butt, 1993; McQueen et al., 1999). Many partial and sequential leaching procedures have been developed and tested in the recent years, and much effort was put into to the optimizing of the leaching conditions. However, this study has shown that within only 68 days of incubation of Ah-horizon samples with biologically active microbiota the patterns of Au association with host material shifted almost completely. This indicates that the results we obtain from partial- or sequential leaching may often only reflect momentary representations of the distribution of trace elements and may change rapidly over short periods of time. This study has also shown the significance of organic matter as a sorbent for Au in soil materials and it appears to be interesting to further assess the usage of soil organic matter as sampling medium for geochemical exploration.

5. Conclusions

The work presented in this paper represents the first successful trial of linking the ability of microorganisms to solubilize Au with actual Au mobility data derived from natural regolith samples. By using biologically active versus inactive microcosms an overall effect of the microbiota resident in auriferous soils on Au solubilization has been demonstrated. The results are a good indication of how microbial activity affects Au mobility in soils and regolith more generally, because contrary to studies conducted in microbial growth media with single strains of organisms, natural soil samples have been used. The microbiota resident in these auriferous soil are capable of dissolving finely disseminated Au bound within the soil fractions, up to 80 wt% of the Au in the soil. The microbiota of auriferous soils are also capable of dissolving Au from added Au pellets, given the concentrations were higher than in the soil sample itself and the presence of biofilms on the surfaces of the Au pellets. In contrast, the microbiota from soil 100 m from the mineralization, which displays only background Au values, did not mobilize Au nor form biofilms on added Au pellets indicating that the microbiota are different or act differently in auriferous soils. The changing adsorption patterns of Au within the solid soil fractions detected during the microcosm experiments indicate that Au is constantly mobilized and re-adsorbed. The solubilized Au shows high affinity for carbonates and clays as

transitional sorbants, but ultimately appears to be sorbed almost exclusively to the organic matter, this changes appears to take place through an intermediate stage where Au is in solution. These results show clearly that in terms of geochemical exploration the microbially mediated processes of Au solubilizations need to be taken into account, especially when interpreting selective or partial leaching data from near surface samples such as calcrete and soil.

Carbon utilization patterns to study the changes in the structure of the bacterial community combined with the analysis of amino acids in soil solution indicates that the process of excretion of amino acids was important in Au dissolution in organic rich natural environments, which is consistent with the results of several in vitro studies. In carbon limited system such as the quartz Au vein the resident microbiota also released Au, however, it appears likely that the Au release is appears to be linked to a different microbially mediated Au solubilization process, probably iron- or sulfide oxidation.

The results of this study clearly indicate that bacterial Au solubilization needs to be considered in understanding the mobility of Au in natural systems, but more research is necessary to elucidate the specific mechanisms of how the bacteria interact with the Au. Future microcosm studies should include measurement of all physical, chemical, and biological variables, subject to the need to minimize any disturbance to the microcosms. The species or groups of bacteria and other microorganisms that are important in affecting Au mobility need to be identified more specifically. In addition, the speciation of Au, e.g., dissolved complexes, colloids, needs to be identified. By making these detailed measurements we will be able to learn more about the mechanisms as well as the kinetics of these processes. Ultimately, we will be able to incorporate appropriate data into numerical geochemical models to predict Au transport, something useful in developing successful strategies for mineral exploration as well as innovative methods for processing Au ore.

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