INTERACTIONS OF MICROORGANISMS WITH GOLD IN REGOLITH MATERIALS FROM A GOLD MINE NEAR MOGO IN SOUTH EASTERN NEW SOUTH WALES

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Prokaryotes, i.e. eubacteria and archaea, have been shaping their environment and thus the earth's lithosphere, hydrosphere and pedosphere for billions of years. Despite their simple morphologies prokaryotes display an enormous range of metabolic capabilities. Prokaryotes are present in a wide array of geologic environments and have been isolated from extreme surroundings such as deep-marine sediments, deep-sea hydrothermal vents, deep rock fractures, deserts, polar regions, and acidic, heavy metal polluted mine wastes (Brock *et al.* 1994). Microorganisms have been found to control most element cycles on the earth's surface, e.g. the carbon-, nitrogen-, sulfur-, phosphorus-, iron-, manganese-, mercury- or vanadium cycles (Brock *et al.* 1994)

In their 1985 article Mossman & Dyer claim microbial processes to be responsible for the solubilisation, migration and deposition of gold during Witwatersrand times and thus for the formation of the Witwatersrand gold deposit. Further evidence for microbial interactions with gold have since then accumulated. Morphological evidence of gold encrusted bacterial and fungal microfossils have been reported from gold flakes panned in Alaska, Venezuela and Australia (Watterson 1992, Bischoff *et al.* 1992, Bischoff 1994). In pure culture studies *Thiobacillus ferrooxidans* has been found to promote the liberation of gold from ore through sulfide dissolution (Mossmann *et al.* 1999). Microbially derived low-molecular-weight-organic-acids (LMWOAs) and amino acids have been found to form complexes with gold and thus may enhance its transport through soils (Mossmann *et al.* 1999). Enzyme catalyzed microbial redox reactions have been shown to be involved in gold deposition (Mossmann *et al.* 1999). Gold colloids have been immobilized by *Bacillus subtilis* in pure culture studies (Southam *et al.* 1994).

Despite the studies undertaken to date little is known about the mobility of gold and its interactions with microorganisms in a complex natural environment like the regolith. The aims of this project are: (i) to determine, if a microbially driven biogeochemical gold cycle like in the hypothetical model shown in Figure 1 exists in the Australian regolith; (ii) to evaluate the contributions of bacteria and fungi to this cycle; (iii) to assess the use of microorganisms as biological indicators in gold exploration.

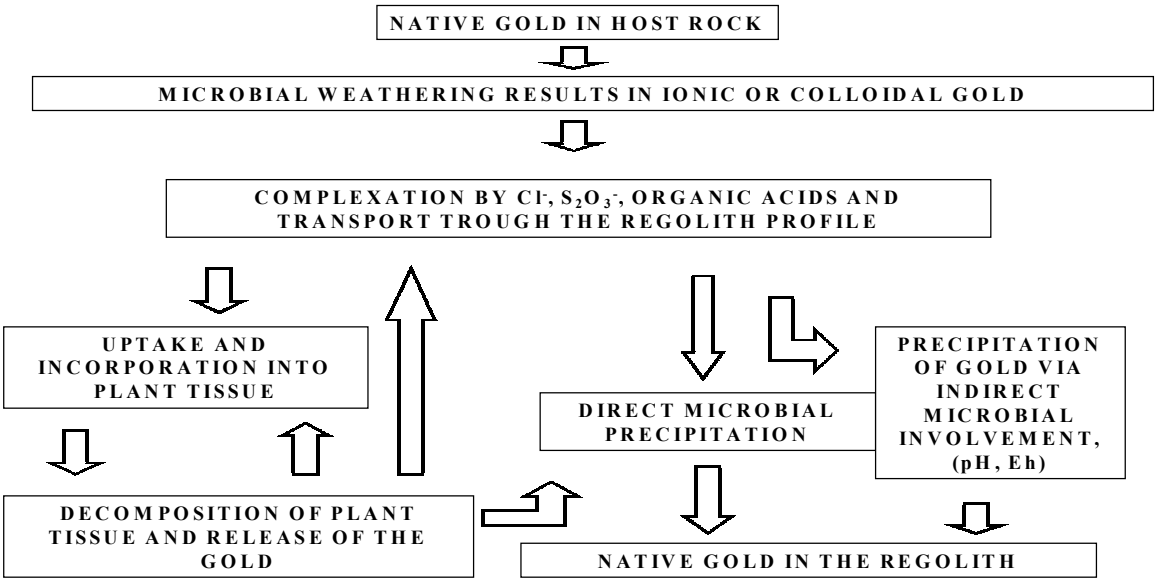

Figure 1: Hypothetical model for a gold cycle in the Australian regolith.

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To understand the interactions of gold and microbes different experiments are being conducted. These were grouped in the following categories: (i) morphological studies; (ii)sequential selective and total extractions of regolith materials; (iii) kinetic experiments; (iv) culture studies in microbial media.

(i) MORPHOLOGICAL STUDIES

Gold flakes were panned from soil surrounding an old underground gold mine near the coastal town of Mogo in south eastern New South Wales. Here the Moruya Batholith intrudes Middle and Late Ordovician metasediments and is associated with relatively abundant vein gold mineralization. The quartz vein itself is between 20 and 30 cm wide and gold appears to be present in pyrite, possibly as microscopic inclusions. The mine itself consists of one shaft of about 30 m length and 20 m depth, which follows the gold-quartz-vein. The material surrounding the vein appears to be a strongly weathered phyllite. The soil above the mine was a clay rich loam with approximately 10-15% organic carbon in the A_h horizon and a soil pH of around 5. The gold flakes panned from the soil around the mine were 0.1-1.0 mm in diameter. The samples were analyzed using scanning electron microscopy (SEM) with secondary and backscattered electron imaging and electron dispersive spectrometry (EDS). The morphological features discovered closely resemble those described by Bischoff (1994) as bacterial gold, (Figure 2A, 2B). EDS analysis of areas on the gold flakes covered with microfossils showed 10-20% higher carbon concentrations compared to areas without colonialisation, indicating organic origin of the structures.

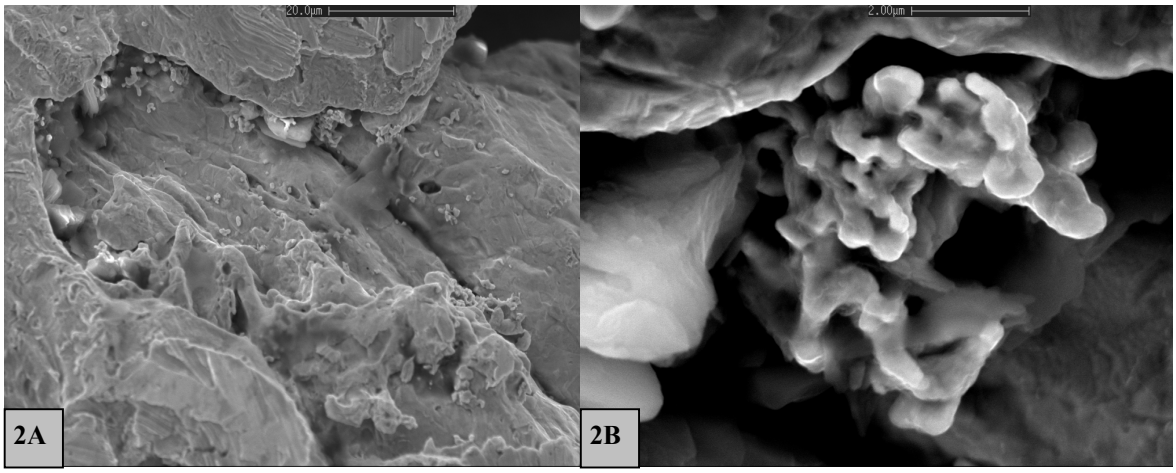

Figure 2A: Fossilized colonies of *Pedomicrobium sp. australiensis*? in depression of a gold flake panned from soil close to the quartz vein at Mogo gold mine.

Figure 2B: Fossilized colony of budding cells, possibly *Pedomicrobium sp. australiensis*, on a gold flake panned from soil close to the quartz vein at Mogo gold mine.

To show a possible colonialisation of gold flakes by microorganisms 99.99% pure gold pellets were obtained. These were buried in the A_h horizon above the quartz-gold-vein. Gold covered slit-pelosols were introduced to the soil and will be examined with SEM and SEM-EDS in the following months. Soil from the A_h horizons was supplemented with gold pellets and incubated at 25°C in the dark for 2 months under microaerophillic conditions. After 2 months approximately 50 % of the pellet surfaces have been covered by structures, which appear to be Siderocapsaceae cells, capsules and ferrihydrite particles precipitated by the organisms (Figure 3A). Similar structures have also been detected on gold flakes panned from Mogo mine soil (Figure 3B).

(ii) SELECTIVE AND TOTAL EXTRACTIONS

In soils gold can be associated with different phases, e.g. carbonates, organic mater, manganese oxides and amorphous or crystalline iron oxides. A six step selective leaching protocol was conducted with eight regolith samples from around the mine to assess the phases with which the gold is associated. Total extractions using conc. aqua regia were performed with soil samples from up to 200 m in either direction from the vein and results will be tested for correlation with *Bacillus cereus* spore counts.

(iii) KINETIC STUDIES

Gold can be released, transported and precipitated in the regolith. However, to this author's knowledge no experiments, to separate the biotic from the abiotic influences on these processes, have been undertaken to date. To test these influences microcosm experiments are currently being conducted. Soil from the A_h and B horizons and quartz from the gold-quartz vein are incubated field fresh and sterilized in a 1:4 w/w water

dilution under oxic and anoxic conditions. In similar experiments these regolith materials were supplemented with native Au, Au^{3+} and cycloheximide. To simulate field conditions drip column experiments with the same unsterilized and sterilized materials will be conducted. Water samples will be taken over a period of 2 months and the samples will be measured for gold and 24 other metals and metalloids using inductively-coupled plasma atomic emission spectrometry (ICP-AES) and inductively-coupled plasma mass spectrometry (IPC-MS).

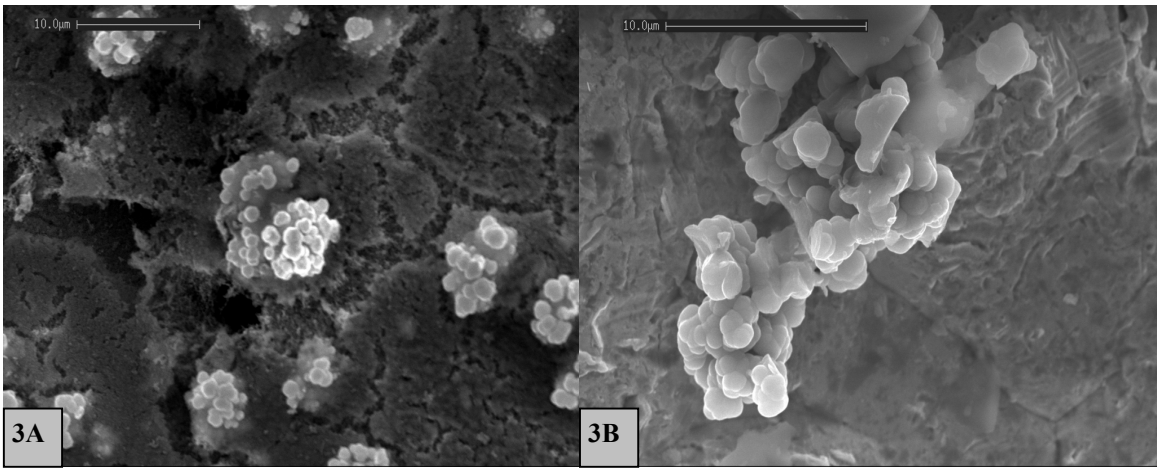

Figure 3A: Bacterial slime capsules and precipitated ferrihydrite particles on a gold pellet incubated in soil from Mogo gold mine at 25°C in the dark for 2 months.

Figure 3B: Ferrihydrite particles possible precipitated on a bacterial slime capsule on gold flakes panned from soil around Mogo gold mine.

(iv) MICROBIAL CULTURE STUDIES

Organisms, which might play an important role in the gold cycle, are isolated using a nutrient and humic acid agar and liquid media supplemented with colloidal gold in concentrations of up to 2 g/L. Dilutions from soil and rock were plated on these media and incubated at 30°C in the dark. After 1 to 5 days, growth was reported on most of the agar plates: The colonies formed were bacterial as well as fungal. Several of fungi precipitated all the colloidal gold present in liquid media within 2 days along their the hyphae (Figure 4A, 4B).

Generally, gold in its soluble form is a toxin for most microorganisms. Some organisms like *B. cereus* form spores if environmental conditions become too harsh. In auriferous soils these organisms have a better chance of survival. *B. cereus* has been found closely associated with auriferous soils in the US. The analysis for *B. cereus* spores in soils might have implications for geochemical exploration because this analysis is simpler and cheaper to perform than chemical analysis. However, to date no *B. cereus* study has been undertaken in Australia. Using a protocol from Watterson (1985), *B. cereus* spores were counted in soil samples taken from above the gold-quartz vein and from up to 200 m in both directions from the vein. The results indicate 3 to 10

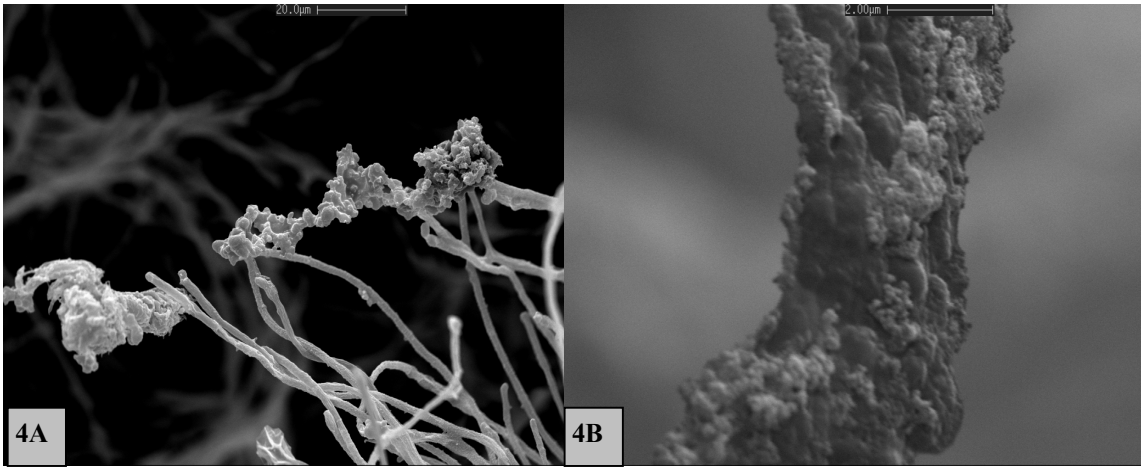

Figure 4A: Fungal hyphae with precipitated colloidal gold.

Figure 4B: Close up picture of gold colloids precipitated along a fungal hyphae isolated from Mogo mine soil

times higher spore counts with in 20 m of the vein compared to further away.

CONCLUSIONS

(i) The results of the morphological and media studies indicate the presence of microorganisms in Mogo mine soil, which might be actively mediating a gold cycle.
(ii) Bacteria and fungi are capable to precipitate gold from colloidal gold solution.
(iii) *B. cereus* spore counts show potential as cheap exploration tool for the detection of underground gold deposits.
(iv) The results of both the extraction, the microcosm and drip column experiments are necessary to evaluate a microbially induced solubilisation and migration of gold in regolith.

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