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Bacillus cereus, gold and associated elements in soil and other regolith samples from Tomakin Park Gold Mine in southeastern New South Wales, Australia

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Abstract

Efficient exploration for new Au deposits is increasingly important as existing deposits become depleted. This is particularly relevant in Australia, where exploration can be difficult because of a thick regolith cover. New and effective methods of exploration need to be developed, and possibilities lie in geomicrobiological methods. For instance, *Bacillus cereus*, a common soil bacterium, has been shown to act as a biogeochemical indicator for concealed mineralisations, including vein-type Au deposits. We report the results of the first Australian case study of the association of *B. cereus* and Au at the Tomakin Park Gold Mine in southeastern New South Wales. Soil samples from the Ah horizon were analysed for *B. cereus* spores and 56 major and trace elements. The results show enrichment of Au, As, *B. cereus* spores and, to a lesser extent, Sb, Bi and Pb over the top of the Au deposit. Gold concentrations over the mineralisation range from 100 ppb to 1.1 ppm compared to a background of 2 ppb and As concentrations are enriched to 100 ppm from a background of 5 ppm. *B. cereus* spore counts were up to 10 times higher in soils with elevated concentrations of Au. Factor analysis indicates four main associations: TiO_2+lanthanides+actinides; CaO+MgO+Cs+Be+Ba(+Ga+Pb+Rb); *B. cereus*+Au+As+Sb+Bi(+Pb); Fe_2O_3+MnO+Co+Ni+Cu+Mo. Selective sequential leaching was used to study the fractionation of Au and As in soils, other regolith materials and Au-bearing vein quartz to infer their mobility and bioavailability. In unweathered quartz vein material, the majority of the Au was extracted only in the strongest, final step, with aqua regia. However, in soils from the Ah horizon, 50% of the Au was present in the water-, ammonium acetate- and sodium pyrophosphate- and hydroxylamine hydrochloride-extractable fractions. In contrast, As displays little change in fractionation with an increasing degree of weathering, and is predominantly associated with the operationally defined Mn- and Fe-oxides and oxyhydroxides. These results indicate that: (i) Au is mobilised during the weathering of the host rock; (ii) Au is bioavailable in these soils; and (iii) the increase in *B. cereus* spores is likely to be linked to elevated concentrations of bioavailable Au in these soils. The results indicate also that an effective biogeochemical

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exploration technique may be developed, where *B. cereus* spore counts are measured in the field and used as a pre-screening method to target areas useful for further sampling and complete geochemical analysis.

Keywords: Bacillus cereus; Gold; Arsenic; Regolith; Soil; Selective sequential leaching; Australia

1. Introduction

Mining is a major industry in Australia, and to replace Au and other ore deposits as they are mined out, it is vital for the mining industry to locate new deposits (Smith, 1996). However, in Australia this is becoming increasingly difficult as deeply weathered residual regolith as well as transported cover in excess of 100 m depth may conceal the mineralisation. One aim of geochemical exploration in such terrains is to find easily accessible sampling materials and appropriate analytical procedures, which provide indicators for the presence of buried deposits. Sampling and analysis of regolith materials, such as gossans, calcrete or laterite and soil, or vegetation are used to locate buried ore deposits (Lintern and Butt, 1993; Lintern et al., 1997; Butt and Lintern, 2000). Samples are commonly analysed for "pathfinder elements" associated with Au such as Sb, As, Bi, Cu, Pb, Se, Ag, Te and Zn besides the Au itself (Boyle and Jonasson, 1973; Boyle, 1979).

Previous studies have shown that some microorganisms may also indicate the presence of buried deposits (e.g., Parduhn et al., 1985; Parduhn, 1991), because the composition of the microflora in natural and polluted environments is strongly influenced by the concentration of the heavy metals and their bioavailability (Hassen et al., 1998). However, no geomicrobiological studies have been published on indicator organisms in Australia. The use of the soil bacterium *Bacillus cereus* as an indicator organism for Au and other metals has been explored in studies of different terrains in Belgium, China, Argentina and Mexico (Neybergh et al., 1991; Melchior et al., 1994, 1996; Wang et al., 1999). *B. cereus* is an aerobic spore-forming soil bacterium that displays a high metal and penicillin tolerance. Its spores have been isolated from most soils and sediments in numbers of several hundred colony-forming units (CFUs) per gram dry weight of soil (Watterson, 1985). However, the spore counts in polymetallic soils, especially those with high Au concentrations, were up to several orders of magnitude higher, indicating an unambiguous association of bacterial population with polymetallic soils (Watterson, 1985; Neybergh et al., 1991; Melchior et al., 1994, 1996; Wang et al., 1999). Due to their high oxidation potentials, most Au(I) and Au(III) complexes can act as strong bactericides even at low concentrations (Karthikeyan and Beveridge, 2002). Hence, mobile Au complexes could be directly responsible for the increase of *B. cereus* spores in Au-bearing polymetallic soils by suppression of competing microbial species.

Selective sequential extraction techniques have been used to study the association of Au with principle soil and other regolith phases to understand how Au is mobilised and trapped in the regolith in order to develop effective exploration strategies for Au deposits (e.g., Boyle, 1979; Grimm and Friedrich, 1988; Gray and Lintern, 1998; Lintern, 1989; Xueqiu, 1998). These leaching techniques can also be used to understand the bioavailability of heavy metals, such as Au, in soils, other regolith materials and sediments (Wallmann Fig. 1B). The objective of selective sequential extraction is to determine the amount of an element that is associated with specific fractions of a soil or weathered material by using a sequence of reagents to extract elements from successively stronger binding fractions (Hall, 1998; Hall et al., 1998). The main phases that trap migrating elements in the supergene environment comprise clays and carbonate minerals, humic substances, and Fe- and Mn-oxides and oxyhydroxides, where the elements are either sorbed to the solid surfaces or precipitated with secondary minerals (Hall et al., 1998). Bioavailable forms of metals and metalloids in regolith and soil can be present as: (i) ions, complexes or colloids soluble in water; (ii) exchangeable complexes, or absorbed to clays or carbonates; (iii) bound to soil organics, such as fulvic and humic acids or humins; and (iv) bound to amorphous or poorly crystalline Mn- and Fe-oxides and oxyhydroxides (Salomons, 1993; Hall, 1998).