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Impact of microbial communities from tropical soils on the mobilization of trace metals during dissolution of cinnabar ore

Clarisse Balland-Bolou-Bi, Benjamin Turc, Vanessa Alphonse, Noureddine Bousserrhine*

Université Paris-Est Créteil Val de Marne, Institute of Ecology and Environmental Sciences of Paris (UMR 7618), 61 avenue du Général De Gaulle, 94010 Créteil cedex, France

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Biodissolution experiments on cinnabar ore (mercury sulphide and other sulphide minerals, such as pyrite) were performed with microorganisms extracted directly from soil. These experiments were carried out in closed systems under aerobic and anaerobic conditions with 2 different soils sampled in French Guyana. The two main objectives of this study were (1) to quantify the ability of microorganisms to mobilize metals (Fe, Al, Hg) during the dissolution of cinnabar ore, and (2) to identify the links between the type and chemical properties of soils, environmental parameters such as season and the strategies developed by indigenous microorganisms extracted from tropical natural soils to mobilize metals. Results indicate that microbial communities extracted directly from various soils are able to (1) survive in the presence of cinnabar ore, as indicated by consumption of carbon sources and, (2) leach Hg from cinnabar in oxic and anoxic dissolution experiments via the acidification of the medium and the production of low molecular mass organic acids (LMMOAs). The dissolution rate of cinnabar in aerobic conditions with microbial communities ranged from 4.8×10^{-4} to 2.6×10^{-3} μmol/m^2/day and was independent of the metabolites released by the microorganisms. In addition, these results suggest an indirect action by the microorganisms in the cinnabar dissolution. Additionally, because iron is a key element in the dynamics of Hg, microbes were stimulated by the presence of this metal, and microbes released LMMOAs that leached iron from iron-bearing minerals, such as pyrite and oxy-hydroxide of iron, in the mixed cinnabar ore.

Introduction

Tropical soils and, in particular, ferralsols (World Reference Base, 2006), are soils where the mineral weathering is very advanced and independent of the organic matter (Duchaufour, 2001). In French Guiana, soils are developed on parental materials that contain naturally high levels of trace metals, including mercury (Hg), copper (Cu), and zinc (Zn), etc. (Roulet and Lucotte, 1995; Guedron et al., 2006, 2009). As with any other metal in soil, Hg can occur as a dissolved form (free ion), a non-specifically adsorbed form (weak electrostatic bond), a specifically adsorbed form (covalent bond), especially on iron and aluminium oxy-hydroxides that were in large amount in tropical soils, a chelated form (bound to organics), or precipitated in mineral form (*i.e.*, carbonate, hydroxide, sulphide) (Schuster, 1991). Mercury in French Guyana comes from two main sources: natural as mercury sulphide minerals (cinnabar) naturally present in the continental crust and in the soil (Pujos et al., 1990) but also anthropogenically through gold mining, particularly illegal gold mining. The concentration of

* *Corresponding author*. E-mail: Clarisse.bolou-bi@u-pec.fr (Clarisse Balland-Bolou-Bi).

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Hg in Guianese ferralitic soils are 3 times more elevated compared to published data for background levels of Amazonian soils, which vary from 30 to 100 ng/g d.w. (Porcella et al., 1995). Illegal gold mining used mercury (under cinnabar form) to amalgamate gold. So, great quantities of mercury are present in certain part of French Guyana and can reach 20 ppm (Guedron et al., 2006).

In contrast to Cu or Zn, which are key micronutrients for living organisms and become toxic only at high concentrations (Baize, 1997), Hg has no recognized role in living organisms and is toxic even at low concentrations. The study of the speciation and mobility of these metals (Hg, Fe, Al) when released in soil solution is of primary importance for constraining their bioavailability and their dynamics in the environment, especially in ferralsol due to the natural presence of iron and aluminium oxy-hydroxides.

It is now well-known that microorganisms have major impacts on the dynamics of cation and trace metal biogeochemical cycles, including plant nutrition via metabolites produced by microbes in boreal and temperate climates (*e.g.*, Bennett et al., 2001; Rogers and Bennett, 2004). Among these microorganisms, bacteria are indirectly involved in the solubilization processes of trace metals through the weathering of metal-bearing mineral phases by the production of strong acidic or complexing compounds in soils, both in aerobic and anaerobic conditions (Balland et al., 2010; Balland-Bolou-Bi et al., 2012). These compounds can lead to the dissolution of minerals (silicate phosphate, oxide, sulphide or carbonate) (Berthelin and Bourrelier, 1988; Balland, 2010). In addition, respiration processes acidify the surrounding environment by the production of CO_2 and subsequently increase the mineral weathering rate (Welch et al., 1999). In soil, the chemo-organotrophic bacteria (anaerobic bacteria (strict or optional)) are particularly active in the transformation of their mineral environment. These bacteria are able, in the absence of oxygen, to solubilize Fe(III) or Mn(IV) oxyhydroxides by a direct reduction process (Ehrlich, 1998; Bousserrhine et al., 1999; Bongoua-Devisme et al., 2013). This is the main process in the case of anaerobic respiration in hydromorphic environments. The oxyhydroxide dissolution process leads to the leaching of trace metals (Cu, Zn, Hg) associated with these minerals. In addition to these bacteria, chemo-lithotrophic bacteria are able to oxidize the sulphides (such as pyrite, FeS_2). Trace metals associated with sulphides are also leached into solution. However, this oxidation process may lead to the formation of metal-bearing minerals where trace metals can then be selected again by neoformed minerals. In strict anaerobic conditions, sulphate-reducing bacteria are responsible or jointly responsible for the precipitation of many elements as insoluble sulphides (Ehrlich, 1998). To summarize, the mobilization of trace elements in soils has been studied extensively in both aerobic and anaerobic conditions (Berthelin and Leyval, 1982; Lovley, 1991; Ehrlich, 1996; Rogers and Bennett, 2004; Uroz et al., 2011). However, these studies have focused on and used mostly pure bacterial strains under controlled conditions. The soil is a complex environment inhabited by microbial consortia with interactions between various actors (bacteria, fungi, plants). Moreover, only a few studies have investigated the dynamics of microorganisms in tropical soils contaminated by Hg (Harris-Hellal et al., 2009, 2011; Oliveira et al., 2010; Frey and Rieder, 2013), and there is little information regarding their contribution to the mobility and bioavailability of Hg in tropical soils. They have demonstrated that mercury has a significant effect on microbial activities and bacterial community structures depending on the quantity of mercury in soils. Other study conducted on tropical soils showed that ferri-reducing bacteria have solubilized iron oxides and their associated mercury (Harris-Hellal et al., 2011). Study Hg bioavailability, in closed systems, can give more information on the potential of microorganisms to mobilize and increase bioavailability of Hg from cinnabar in soils. This mobilized Hg can be transformed by ferri-reducing bacteria and sulfato-reducing bacteria on methyl mercury, a more toxic form of mercury which is a neurotoxic compound with high bioaccumulation rate in living organisms and a potential transfer to human through the trophic web.

Thus, the main objectives of this study were (1) to quantify the ability of microorganisms to mobilize trace metals (Fe, Al, Hg) during the dissolution of cinnabar ore (material model as a source of trace metals, and naturally or not present in these soils) and (2) to identify the strategies developed by indigenous microorganisms extracted from tropical natural soils. To achieve these objectives, biodissolution experiments on cinnabar ore (mercury sulphide and other sulphide minerals such as pyrite) were performed with microorganisms extracted directly from two different soils to avoid a pre-culture step that would have selected only cultivable microorganisms. These experiments were carried out in closed systems under aerobic and anaerobic conditions with two different soils of French Guyana. The first is a ferralsol, well-drained, and the second an acrisol (WRB, 2006), partially waterlogged and therefore with different physicochemical conditions. The ability of microorganisms to leach trace metals during cinnabar ore biodissolution was evaluated. In addition, some physiological parameters of microbial communities were followed over the course of time, such as the carbon source consumption (glucose and maltose), the acidification of the medium, and the production low molecular mass organic acids (LMMOAs).

1. Materials and methods

1.1. Studied site

The studied site is located in French Guiana on the Combat Creek watershed (52°23′ W, 4°35′ N), a small catchment of ~1 km^2 covered by tropical rain forest. The climate is tropical humid, with an annual average rainfall of ~4000 mm and an annual average temperature of 26°C.

The bedrock is a Proterozoic shield consisting primarily of dark schist and thin sandstones on which the soil is ferralitic (Guedron et al., 2009). Soil samples were sampled along a toposequence with two types of soils. The soil distribution within the Combat Creek watershed is related to soil position along the slopes (Guedron et al., 2006; Grimaldi et al., 2008). Ferralsols were predominant on the upslope, having typically high clay (<2 μm size fraction) content, a micro-aggregated structure and a depth of over 1 m, which allow good vertical

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