Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

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

ARTICLE INFO

Article history:
Received 6 January 2016
Revised 19 September 2016
Accepted 8 October 2016
Available online 19 October 2016

Keywords:
Low molecular mass organic acids
Cinnabar ore
Microbial communities
Tropical soils

ABSTRACT

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).

http://dx.doi.org/10.1016/j.jes.2016.10.003
1001-0742/

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 and Poszwa, 2012). These compounds can lead to the dissolution of minerals (silicate phosphate, oxide, sulphide or carbonate) (Berthelin and Bourrelier, 1988; Balland et al., 2010). In addition, respiration processes acidify the surrounding environment by the production of CO_2 and subsequently increase the mineral weathering rate (Welch and Ullman, 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 preculture 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

water drainage. Halfway down the slope, ferralsols steadily evolve to acrisols, a massive alteritic horizon with a high content of fine silts at a shallow depth (<1 m). Downslope, the soils become hydromorphic, with dominant sands. A permanent aquifer is present and slowly drained by the river, thus imposing reducing conditions. We have chosen to collect only the upper part of the studied watershed (ferralsols and acrisols) because these 2 soils have contrasted physico-chemical characteristics given in Table 1 that could interfere on the structure of bacterial communities and thus on their activities. These soils are considered to be pristine based on the soil structure and texture (Guedron et al., 2009). The sampling took place in November 2012 with a rainfall of ~300 mm and a temperature of 27°C, corresponding to the wet season, and in June 2013 with a rainfall of ~40 mm and a temperature of 28°C, corresponding to the end of the wet season. The choice of these two campaigns, according to season, has been motivated by the different condition of oxygenation in soil, with waterlogged condition in November that can favour some anaerobic condition and well-drained condition in June.

1.2. Soil sampling and microorganism extraction

The two soil profiles were sampled systematically every 10 or 20 cm, down to a depth of 1–2 m, using an auger, and were collected in sterile polyethylene bags. For the extraction of microorganisms, only the first 10 cm were used, consisting of a composite of 5 subsamples (sampled at a distance of five meters). After sampling, the soils were frozen until extraction.

Before extracting bacterial communities, each soil sample was physically dispersed using a solution composed of 50 mL of Milli-Q water, mixing for 2 hr with previously defrosted soil with a weight equivalent to 5 g of dry soil. This method improved the desorption of bacteria from the soil particles. The mixture was then allowed to settle for 1 hr. The supernatants (to avoid soil particles and eventually cinnabar particles) were then used to inoculate the batch experiments.

1.3. Experimental set up for cinnabar ore biodissolution

Cinnabar ore biodissolution experiments with microbial communities were run in flasks at 24°C. All laboratory material was pre-cleaned (HNO_3 weakly concentrated) and autoclaved for 30 min at 110°C with a semi-automatic laboratory sterilizer. The flasks were agitated briefly each day. Each flask contained 140 mL of pre-sterilized melin norkran modified Melin Norkran medium (MMN) and 100 mg of cinnabar ore.

Experiments were conducted in aerobic conditions and anaerobic conditions. For aerobic conditions, flasks were closed by carded cotton that avoid contaminations but permit the circulation of air. For anaerobic conditions, flasks were closed with a septum, and the air initially present in the flask was flushed by N_2 gas. All the solutions were then sampled under a vertical laminar flow hood (ADS laminaire) with a needle through the septum to decrease the risk of contamination.

Microorganisms extracted from the soil were then grown in the presence of cinnabar ore. The cinnabar ore come from the Almaden district in Spain. The ore crystal was crushed and sieved (5 to 100 μm), then rinsed and sonicated with distilled deionized water to remove fine particles. The sample was then X-rayed to determine its mineralogical composition (data not show). This analysis was realized by the Alysés service at IRD Bondy (France) on an X-ray diffractometer (DRX, X'PERT POWDER, Panalytical). It is composed of cinnabar, quartz, pyrite, and Fe/Al oxy-hydroxide, such as goethite.

The cinnabar ore was also acid-digested to quantify the proportion of Hg, Fe and Al in the sample. This method has consisted to dissolve 50 mg of cinnabar powder in a mixture of concentrated acids (HNO_3-HCl, 3–3 mL) for 48 hr at 80°C. The mixture was evaporated to dryness. All obtained residues were dissolved in 10 mL 5% HNO_3 before analyses by inductively coupled plasma optical emission spectrometer (SPECTROBLUE ICP-OES, SPECTRO Analytical, France).

The studied cinnabar ore contains 55% of Hg, 10.3% of iron (Fe) and 19.5% of aluminium (Al). A specific surface area of 0.23 (±0.3) m^2/g was determined by an N_2 adsorption Brunauer–Emmet–Teller method. The powder was dried and sterilized at 80°C for two days.

The crushed cinnabar was added to the culture media as Hg and metal source. The culture medium used is a Melin Norkans medium containing per 1 L: 0.1 g KH_2PO_4 (Carl Roth), 0.05 g $(NH_4)_2HPO_4$ (Rectapur), 0.01 g $CaCl_2$ (Sigma), 0.005 g NaCl (Rectapur), 0.03 g $MgSO_4$ (Prolabo), 0.001 g thiamine (Sigma), 1.25 g glucose (VWR) and 5 g malt extract (Prolabo). Mineral media were sterilized by autoclaving (30 min at 110°C). In addition, 10 mL of extracted microbial community suspension were added to the media. All the experiments were repeated 3 times and took 17 days. On days 3, 5, 7, 11, 14, and 17, solution samples were collected. These supernatants were then centrifuged, filtered at 0.2 μm (filter PTFE, VWR) and stored at 4°C prior to chemical and biological analyses.

1.4. Chemical analyses

During the cinnabar dissolution experiment, the pH of the solution was followed and measured at each sampling date. In addition, Fe and Al leached in solution was determined using an ICP-OES. The detection limits for these elements were 0.005 mg/L.

The quantification of Hg leached in solution was performed using an advanced mercury analyser (Altec AMA-254, Symalab, France) without sample pre-treatment or pre-

Table 1 – Physico-chemical characteristics of soils.

	pH	Carbon content	Nitrogen content	Exchange capacity (meq/100 g)	Hg content (mg/kg)	Sand content	Silt content	Clay content
Ferralsol	4.10	7.44%	0.52%	29.85	0.507	22.71%	8.21%	69.08%
Acrisol	3.87	4.49%	0.36%	16.10	0.247	33.81%	28.09%	38.12%

concentration. A sample is burned in an oxygen-rich atmosphere (99.5%) and the evolved gasses are then transported using an oxygen carrier gas through specific catalytic compounds (to remove interfering impurities, *e.g.*, ash, moisture, halogens, and minerals) to an Au-plated ceramic (an amalgamator), which collects the mercury in the vapour. The amalgamator is then heated to 700°C to release mercury into the detection system, which contains the Hg-specific lamp, emitting light at a wavelength of 253.7 nm. A silicon UV diode detector is used for mercury quantification. The working range is 0.05–500 ng.

1.5. Biological analyses

Biological activities were evaluated through measuring the consumption of carbon sources and the production of LMMOAs in solution.

The assay of total sugar was performed by spectrophotometry using the protocol of Dubois (1956) to quantify the consumption of carbon sources. 0.2 mL of sample was mixed with 0.2 mL of 5% phenol, followed by addition of 1 mL of concentrated sulphuric acid (98%). After stirring and 30 min of cooling, the optical density is measured at 490 nm using a spectrophotometer (Genesys 10 UV scanning device®). A calibration curve was made with glucose, and this helped to calculate the amount of carbon in solution from the degradation of sugars initially present in mineral media (glucose and malt extract).

The quantification of LMMOAs was performed by High Pressure Liquid Chromatography (HPLC) following the method of Van Hees et al. (1999). Samples were run on a C18 stationary phase (AQUASIL C18, 5 μm, 250 × 4.6 mm) using a mobile phase of 1% ACN/99% 0.05 mol/L KH_2PO_4, pH 2.8 at a flow rate of 1.25 mL/min. The column was operated at 20°C to detect oxalic, malic, maleic, malonic, succinic, fumaric and citric acid. The acids were detected at 210 nm by an UV detector.

2. Results and discussion

2.1. Effect of cinnabar ore on biological activities

Microbial activities during the kinetics of cinnabar ore biodissolution are monitored through the pH, consumption of carbon sources over time and measurement of the production of LMMOAs at the end of the experiments.

2.1.1. pH

Whatever the modalities (with or without oxygen), cinnabar biodissolution experiments have the same tendency. Microbial communities quickly acidified the media after 1 day (Fig. 1). In aerobic conditions, this decrease is higher with microbial communities extracted in June (from 6.2 to 3.5 for both acrisol and ferralsol) compared to November ones (from 6.2 to 4.0 and 4.2 for acrisol and ferralsol, respectively), according to the analysis of variance realized on repeated measures and the Tukey test ($p = 0.05$) (Fig. 1a). From the 1st day to the 9th day, the pH stabilized at approximately 3.5. After the 9th day, the pH increased slightly for microbial communities extracted in June to 3.75 and 3.85 for acrisol and ferralsol, respectively compared to November ones (4.0 and 4.25 for acrisol and ferralsol, respectively). The same pattern of acidification was observed for anaerobic experiments (Fig. 3b). The pH is an important parameter to consider, in particular during dissolution of minerals (Brantley, 2003). Moreover, Soares et al. (2015) have shown that soils with pH below 5.0 (even with high organic matter content) retained smaller amounts of mercury than eutrophic soils with near-neutral pH and with larger organic matter contents.

2.1.2. Consumption of carbon sources

Whatever the modalities (with or without oxygen), cinnabar biodissolution experiments have the same tendency. Microbial

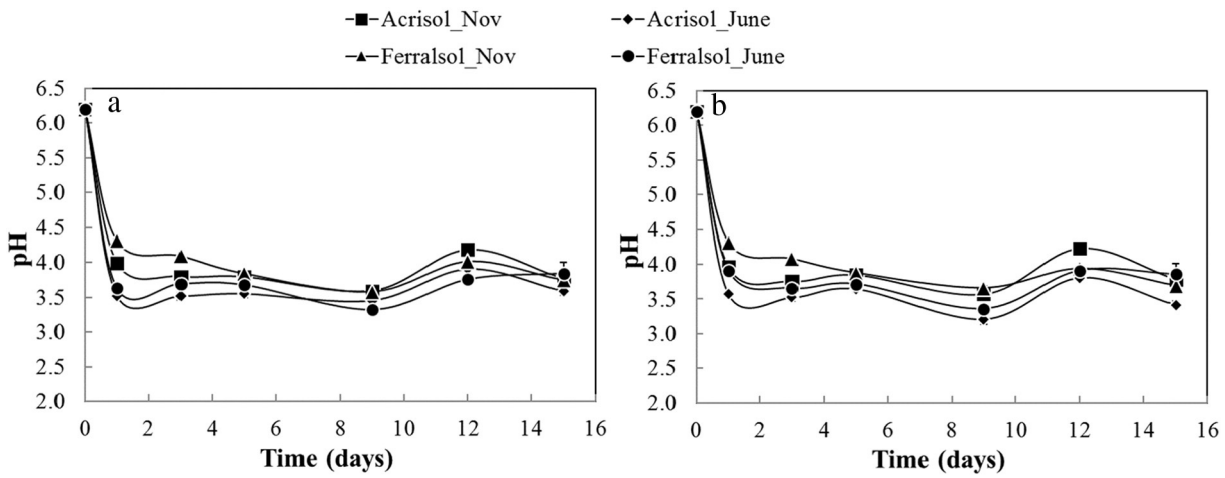

Fig. 1 – Evolution of pH during the cinnabar ore biodissolution in aerobic (a) and anaerobic (b) conditions. Each symbol represents the mean and standard deviation calculated from three replicates. According to analysis of variance repeated measures and the Tukey test ($p = 0.05$) on three replicates, whatever the conditions (aerobic or anaerobic) and whatever the soil (acrisol or ferralsol), the experiments performed with microbial communities isolated in June 2013 are significantly different that experiments performed with microbial communities isolated in November 2012.

communities quickly consume the carbon source after 1 day, as indicated by the decrease of carbon in the nutrient solution (Fig. 2). In aerobic conditions, this decrease is higher with microbial communities extracted from acrisol (1.4 g C/L to 0.38 g C/L and 0.42 g C/L for June and November, respectively) compared to the ferralsol ones (0.7 and 0.9 g C/L for June and November, respectively). After this strong decrease of the carbon in solution, the consumption of carbon decreases progressively without significant difference between the soil treatments for each soil. However, carbon consumption remains slightly higher for experiments with microbial communities isolated in June; they are significantly different from experiments performed with microbial communities isolated in November, according to the test ANOVA realized on repeated measures and the Tukey test ($p = 0.05$). At the end of the experiment, the initial carbon added to the solution decreased an average of 0.1 g/L with no difference between soil treatments. The same pattern of carbon consumption was observed for anaerobic experiments (Fig. 2b), but the decrease of carbon in solution is similar for both treatments (0.5 g C/L for June and November, respectively, for acrisol and 0.62 g C/L for June and November, respectively, for ferralsol). In addition, the remaining carbon in solution is higher for experiments with microbial communities isolated in June in comparison to aerobic condition experiments. At the end of the experiment, initial carbon added to the solution decreased to 1.4 g C/L to an average of 0.3 g C/L with no difference between soil treatments, higher but not significant compared to the aerobic condition experiments. These results suggest that anaerobic conditions seem to induce metabolism consuming more energy, for microbial communities extracted from the soils of June.

Thus, the biological activity of the microbial community that was related to partial oxidation of carbon sources into LMMOAs, led to CO_2 release during respiration or fermentation (Gottschalk, 1986) and also to SO_4^{2-} leached during experiments (Holley et al., 2007). As a consequence of the production of LMMOAs, the media became more acid.

2.1.3. Low molecular mass organic acids

LMMOAs were identified and quantified on day 10 before the carbon sources (glucose and malt extract) become a limiting factor (Table 2). Only oxalic, formic, acetic and citric acids were quantified. In aerobic conditions, microbial communities extracted from ferralsol exhibit the greatest release of LMMOAs with 41 mmol/L and 31 mmol/L of LMMOAs for June and November, respectively, in comparison to microbial communities extracted from acrisol with 23 mmol/L and 21 mmol/L of LMMOAs for June and November, respectively. Communities from ferralsol permit the release of great quantities of chelating organic acids, such as oxalic and citric acids. The presence of these poly-functional acids could increase the silicate dissolution rate by 3–10 times compared to the silicate dissolution rate in experiments performed with mono-functional acids, such as acetic acid (Robert and Berthelin, 1986; Welch and Ullman, 1999). In anaerobic conditions, microbial communities extracted from ferralsol permit the release of LMMOAs with 37 mmol/L and 23 mmol/L of LMMOAs for June and November, respectively. Microbial communities extracted from acrisol produced 18 mmol/L and 43 mmol/L of LMMOAs for June and November, respectively.

Experiments performed in anaerobic conditions exhibit the greatest release of acetic acid in comparison with experiments performed in aerobic conditions. Acetic acid is produced during fermentation (Gottschalk, 1986).

The nature and concentration of LMMOAs produced by microbial communities during the experiments are similar to LMMOAs measured in soil compartments with concentrations ranging from 0.1 to 1000 μmol/L (Van Hees et al., 2005; Jones, 1998; Balland-Bolou-Bi and Poszwa, 2012; Fujii et al., 2012). LMMOAs are transitory in soils, especially in tropical soil with

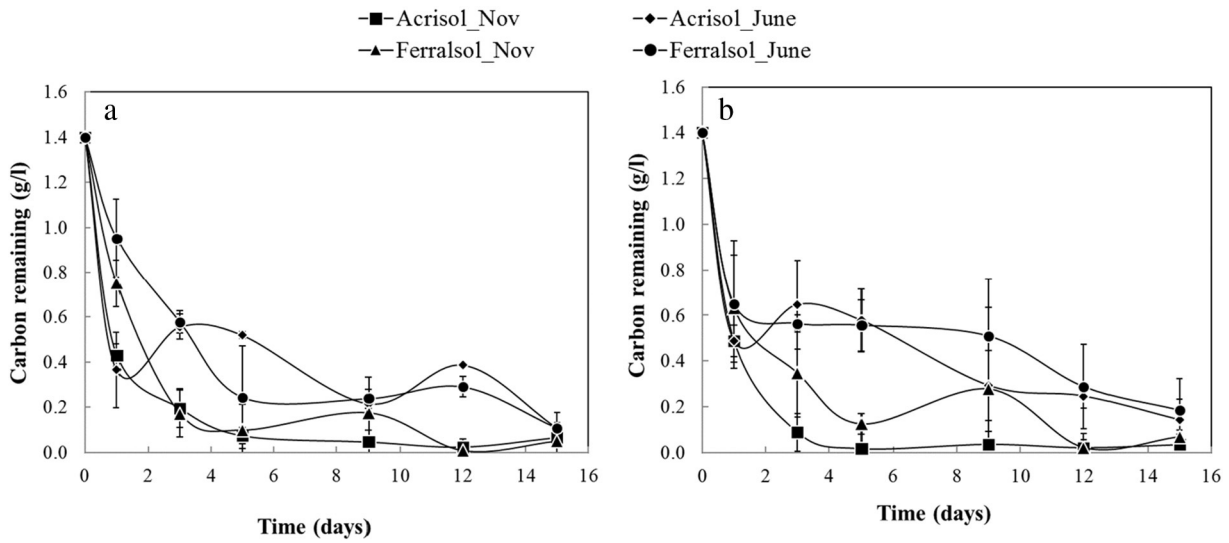

Fig. 2 – Evolution of carbon remaining during the cinnabar ore biodissolution in aerobic (a) and anaerobic (b) conditions. Each symbol represents the mean and standard deviation calculated from three replicates for each measurement. According to analysis of variance repeated measures and the Tukey test ($p = 0.05$) based on three replicates. Whatever the conditions (aerobic or anaerobic) and whatever the soil (acrisol or ferralsol), the experiments performed with microbial communities isolated in June 2013 are significantly different that experiments performed with microbial communities isolated in November 2012.

Table 2 – Low molecular mass organic acids (LMMOAs) concentrations released in solution by microbial communities during cinnabar ore leaching (unit: mmol/L).

	Oxalic acid		Acetic acid		Formic acid		Citric acid		∑ LMMOAs	
	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
Acrisol_Nov_O_2	16.09	1.87	1.25	2.17	0.01	0.01	3.63	6.29	20.99 b	8.60
Acrisol_June_O_2	11.02	1.44	1.65	1.69	0.00	0.01	10.19	0.38	23.55 b	0.66
Ferralsol_Nov_O_2	21.66	3.20	0.74	0.16	0.01	0.02	8.65	2.41	31.05 ab	3.65
Ferralsol_June_O_2	28.93	4.27	0.98	0.22	0.02	0.03	11.55	3.22	41.48 a	4.88
Acrisol_Nov	28.90	2.33	3.47	0.91	0.07	0.03	10.41	0.39	42.84 a	1.26
Acrisol_June	11.51	2.07	2.50	1.56	0.01	0.01	4.19	7.25	18.20 b	8.01
Ferralsol_Nov	15.61	1.81	3.77	0.71	0.01	0.01	3.52	6.10	22.91 b	6.03
Ferralsol_June	23.17	3.42	4.43	1.86	0.01	0.02	9.25	2.58	36.87 ab	5.67

The symbols a, b and ab correspond to the different groups of significance obtained by analysis of variance and by the Tukey test (P = 0.05) on three replicates.

a fast turnover of organic matter. Thus, their pool sizes are maintained through continuous production and consumption (Van Hees et al., 2005).

2.2. Biological control of cinnabar ore dissolution

2.2.1. Mercury and metals (Fe, Al) leaching during solution

Microbial communities extracted directly from Guyanese soils are able to (1) survive in the presence of cinnabar ore (production of LMMOAs, consumption of carbon sources) and (2) leach mercury from cinnabar dissolution in oxic and anoxic dissolution by acidification of growth media and production of LMMOAs.

In general, microbial communities have permitted the leaching of cinnabar ore (Fig. 3). The leaching of iron and aluminium in the experiments seems to have the same profile. In more precise detail, the behaviour of microbial communities in leaching cinnabar ore was the same for iron and aluminium. In aerobic conditions, the percentage of the leached element reached a value range of approximately 2% and 0.25% for Fe and Al, respectively, except for microbial communities extracted from ferralsol in June that permit the release of more elements (4% for Fe and 0.7% for Al). In anaerobic conditions, the percentage of leached iron reached a value range of approximately 3% except for microbial communities extracted from acrisol in November. The percentage of leached aluminium reached a value range of approximately 0.35% except for microbial communities extracted from ferralsol in June.

For Hg, whatever the conditions of oxygenation of the experiments, microbial communities permitted the release of 0.02% of this element in solution, except for microbial communities extracted from ferralsol in June 2013 and in November 2012 that leached, respectively, 0.06% and 0.11% of Hg in aerobic conditions, according to the test ANOVA repeated measures and the Tukey test ($p = 0.05$) on three replicates.

As a consequence of the production of LMMOAs, the media became more acidic and induced the release of Fe (2% to 5%), Al (0.1% to 0.8%) and Hg (0.02%) into the media. Iron is preferentially leached from iron-bearing-minerals such as pyrite, poorly crystallized iron oxy-hydroxides (*e.g.*, goethite) and Al from Al-oxy-hydroxide poorly crystallized from cinnabar ore. Indeed, it seems that the percentage of leached elements depends largely on the nutrient needs of microbes (Fe > Al ⋙ Hg) (Pelmont, 1993). Mercury has no vital role for microbes, and the leaching of cinnabar is linked to an indirect action of leaching of the other minerals contained in cinnabar ore. As shown in Fig. 4., the preferential leaching element is indicated through the positive correlation between the percentage of metabolized carbon (defined as the amount of carbon produced contained in the LMMOAs normalized to consumed carbon sources) and the percentage of elements measured (Fe, Al, Hg) in solution. As more metabolites are produced, greater amounts of Fe and Al are released into the solution, which is important. However, there is no correlation between the metabolites and the amount of mercury released into the solution, although salts of LMMOAs (*e.g.*, citrate and tartrate) have been used to facilitate Hg mobilization in soils rich in organic matter (Wasay et al., 2001) Several works lead to similar conclusions, highlighting that mineral composition strongly influences the metabolic activities and the colonization pattern of minerals and rocks by microbes (Rogers and Bennett, 2004; Hutchens et al., 2008a, 2008b; Balland et al., 2010).

The Hg dissolution rates found for microbial communities extracted from Guyanese soils ranged from 4.79×10^{-4} μmol/m^2/day to 2.62×10^{-3} μmol/m^2/day (Table 3). This was similar to the Hg dissolution rates obtained during dissolution of cinnabar in the presence of natural organic matter (Waples et al., 2005). However, our results were lower by a factor of from 10 to 100 compared with the results obtained during the dissolution of cinnabar in open systems with or without bacteria (Barnett et al., 2001; Waples et al., 2005; Holley et al., 2007). In our case, available carbon was the limiting factor leading to underestimation of the dissolution rates. Moreover, many parameters control the dissolution of cinnabar based not only on the hydrodynamics of the experimental systems and the adsorption of dissolved organic matter but also on the sorption of mercury on the surfaces of the oxy-hydroxides of iron and also on microorganisms (adsorption and absorption) (Barnett et al., 2001; Gabriel and Williamson, 2004). Some studies have shown that fungi are among other great accumulators of Hg (Bargagli and Baldi, 1984; Crane, 2011; Iram et al.,

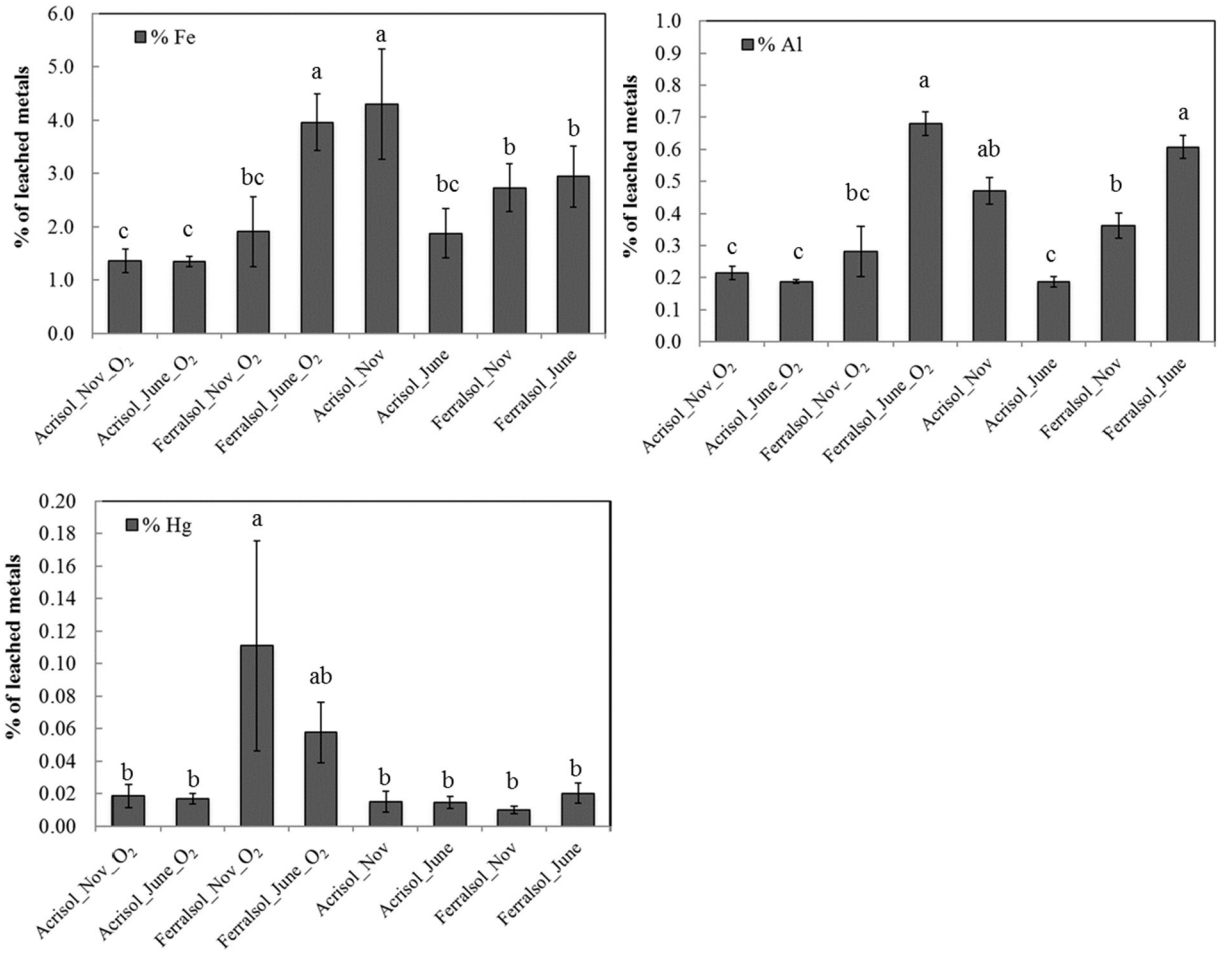

Fig. 3 – Percentage of leached metals (Fe, Al, Hg) at the end of cinnabar ore biodissolution experiments. Each bar represents the mean and standard deviation calculated from three replicates. The symbols a, b, c and d correspond to the different groups of significance obtained by analysis of variance and by the Tukey test (p = 0.05) on three replicates.

2012; Martínez-Juárez et al., 2012). It is therefore possible that in closed systems such as those in our experiments, the steady state is reached quickly and a part of the Hg is trapped on

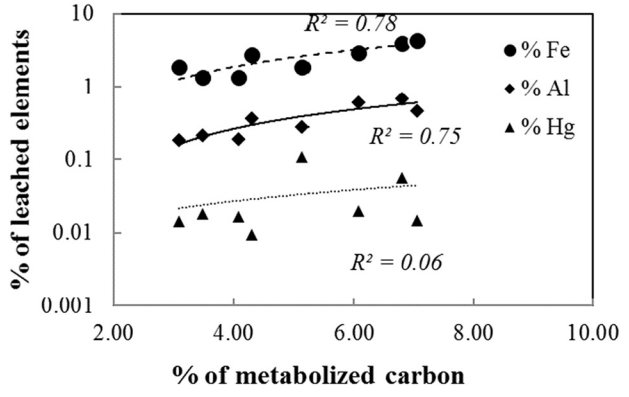

Fig. 4 – Percentage of leached metals (Fe, Al, Hg) versus percentage of metabolized carbon (amount of organic acids produced normalized at consumed carbon sources) on day 10.

Table 3 – Cinnabar release rates of our experiments and literature data.

	Cinnabar release rate ($\mu mol/m^2/day$)
Acrisol_Nov_O_2	4.37×10^{-4}
Acrisol_June_O_2	3.96×10^{-4}
Ferralsol_Nov_O_2	2.62×10^{-4}
Ferralsol_June_O_2	1.36×10^{-4}
Acrisol_Nov	3.56×10^{-4}
Acrisol_June	3.44×10^{-4}
Ferralsol_Nov	2.31×10^{-4}
Ferralsol_June	4.79×10^{-4}

The data from previous publication are as following: 3.15×10^{-2} to 5.87×10^{-2} $\mu mol/m^2/day$ (oxidative dissolution (abiotic), Barnett et al., 2001); 2×10^{-4} to 61.9×10^{-4} $\mu mol/m^2/day$ (performed with dissolved organic matter, Waples et al., 2005); 25.8 to 32.3 $\mu mol/m^2/day$ (performed in column with *A. ferrooxidans* (opened system), Wang et al., 2013); 6.45 $\mu mol/m^2/day$ (performed in column with HCl pH 1.7 (opened system), Wang et al., 2013); 2.64 to 6.16 $\mu mol/m^2/day$ (calculated from SO_4^{2-}, low flow fluvial system (opened system), Holley et al., 2007).

different surfaces, such as the oxy-hydroxide of Fe and also on the surface of HgS (Barnett et al., 2001), and in certain conditions (anaerobic), Hg could be transformed into methyl-mercury by iron-reducing bacteria (Harris-Hellal et al., 2009; Toubassy et al., 2014; Si et al., 2015).

We have shown that microbes amplified the leaching of cinnabar ore, especially in June. This implied that Hg that was in a stable form (trapped in the cinnabar), is now in soluble form and so bioavailable. Hg can be bioaccumulated in organisms (plants, macro, meso and microfauna), and through the trophic web, can impact human health. Microbial communities extracted from ferralsol in June (under aerobic conditions) and from acrisol in November (under anaerobic conditions) exhibit a more efficient leaching of cinnabar ore. These two experiments are close to field conditions; *i.e.*, the beginning of the dry season in late June and the rainy season in November. Ferralsols are very well-drained and oxygenated soils, so microorganisms are still under aerobic conditions and are richer in Hg and iron oxide than acrisol (Da Silva, 2013). However, acrisols during the rainy season are waterlogged and therefore under anaerobic conditions. This suggests that when experimental soils most closely approach the field conditions, microorganisms display greater growth, acidification and the metabolite production and therefore are more effective in releasing the elements from cinnabar ore. Recent papers suggested that soil-related changes in elements leaching from mineral and community structures of the cultivable mineral-weathering bacteria are a consequence of the possible roles of the microorganisms in element mobilization and speciation along the soil profile. (Balland-Bolou-Bi and Poszwa, 2012; Balland-Bolou-Bi et al., 2014; Wang et al., 2014; Yarwood et al., 2014).

Contribution of Hg from natural dissolution of cinnabar should be considered when establishing background Hg levels in contaminated catchments. The dissolution rate of cinnabar in aerobic conditions with microbial communities ranged from 4.79×10^{-4} to 2.62×10^{-3} μmol/m^2/day and was independent of the metabolites released by the microorganisms. These results were in agreement with the published literature and seem to indicate an indirect action of microorganisms on cinnabar dissolution. As a consequence, Fe is a key element in the dynamics of Hg. Iron is one of the main components of these soils. Iron bearing minerals (especially iron oxides) are susceptible to adsorb significant quantities of Hg originating from surface anthropogenic inputs but also from cinnabar. Recently, the works of Hellal et al. (2015) have displayed that in anaerobic, iron rich conditions, Hg complexation and mobility is suggestively dominated by dissolved organic molecules resulting from organic matter degradation by bacteria and by inorganic complexes such as $HgCl_2$. Moreover, microbes were stimulated by the presence of this metal and released LMMOAs that leached iron from iron-bearing minerals (pyrite and oxy-hydroxide of iron). It would be interesting to investigate the adsorption of Hg on the surface of minerals and also its incorporation in microorganisms.

3. Conclusion

Microbial communities extracted directly from various soils are able (1) to survive in the presence of cinnabar ore as indicated by consumption of carbon sources and (2), to leach Hg from cinnabar in oxic and anoxic dissolution experiments via the acidification of the medium and the production of LMMOAs. The dissolution rate of cinnabar in aerobic conditions with microbial communities ranged from 4.79×10^{-4} to 2.62×10^{-3} μmol/m^2/day and was independent of the metabolites released by microorganisms (no correlation found). In addition, these results suggest an indirect action of the microorganisms on the cinnabar dissolution.

Acknowledgment

This study was partially financially supported by ANR Interconnect. We are grateful to Sandrine Caquineau for the X-ray diffraction analysis of cinnabar ore, to E.B. BOLOU-BI for his helpful comments on this paper.

REFERENCES

Baize, D., 1997. Teneurs totales en éléments traces métalliques dans les sols: France.

Balland, C., Poszwa, A., Leyval, C., Mustin, C., 2010. Dissolution of phyllosilicate as a function of bacterial metabolic diversity. Geochim. Cosmochim. Acta 74, 5478–5493.

Balland-Bolou-Bi, C., Poszwa, A., 2012. Effect of Ca and Mg amendment on mineral weathering abilities of bacterial communities in acidic and silicate-rich soils. Soil Biol. Biochem. 50, 108–117.

Balland-Bolou-Bi, C., Poszwa, A., Mustin, C., 2014. Phlogopite weathering potential of bacteria isolated from different soil profiles. Am. Int. J. Biol. 2 (3 & 4), 35–63.

Bargagli, R., Baldi, F., 1984. Mercury and methylmercury in higher fungi and their relation with the substrata in a cinnabar mining area. Chemosphere 13, 1059–1071.

Barnett, M., Turner, R., Singer, P., 2001. Oxidadive dissolution of metacinnabar (–HgS) by dissolved oxygen. Appl. Geochem. 16, 1499–1512.

Bennett, P.C., Rogers, J.R., Choi, W.J., 2001. Silicates, silicate weathering, and microbial ecology. Geomicrobiol. J. 18, 3–19.

Berthelin, J., Bourrelier, P.H., 1988. Microbial weathering processes in natural environments. In: Lerman, A., M., M. (Eds.), Physical and Chemical Weathering in Geochemical Cycles, pp. 33–59.

Berthelin, J., Leyval, C., 1982. Ability of symbiotic and non-symbiotic rhizospheric microflora of maize (*Zea mays*) to weather micas and to promote plant growth and plant nutrition. Plant Soil 68, 369–377.

Bongoua-Devisme, A.J., Cébron, A., Kassin, K.E., Gballou, Y.R., Mustin, C., Berthelin, J., 2013. Microbial communities involved in Fe(III) reduction and mobility during soil organic matter (SOM) mineralization in two contrasted paddy soils. Geomicrobiol. J. 30, 347–361.

Bousserrhine, N., Gasser, U.G., Jeanroy, E., Berthelin, J., 1999. Bacterial and chemical reductive dissolution of Mn, Co, Cr, and Al-substituted goethites. Geomicrobiol. J. 16, 245–258.

Brantley, S., 2003. Reaction kinetics of primary rock-forming minerals under ambient conditions. In: Drever, J.I. (Ed.), Fresh Water Geochemistry, Weathering, and Soils. Pergamon Press, Oxford, pp. 73–118.

Crane, 2011. Mercury Effects on axenically Grown Fungal Isolates and on *Pinus rigida* and its Ectomycorrhizal Community (Dissertation) Graduate School-New Brunswik.

Da Silva, E., 2013. Interactions “sol - vers de terre” et dynamique du mercure en Guyane (phD Dissertation) Université Paris-Est, France.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Duchaufour, 2001. Introduction à La Science Du Sol. Edition Dunod, p. 331.
Ehrlich, H.L., 1996. How microbes influence mineral growth and dissolution? Chem. Geol. 132, 5–9.
Ehrlich, H.L., 1998. Geomicrobiology: its significance for geology. Earth Sci. Rev. 45, 45–60.
Frey, B., Rieder, S.R., 2013. Response of forest soil bacterial communities to mercury chloride application. Soil Biol. Biochem. 65, 329–337.
Fujii, K., Aoki, M., Kitayama, K., 2012. Biodegradation of low molecular weight organic acids in rhizosphere soils from a tropical montane rain forest. Soil Biol. Biochem. 47, 142–148.
Gabriel, M., Williamson, D., 2004. Principal biogeochemical factors affecting the speciation and transport of mercury through the terrestrial environment. Environ. Geochem. Health 26, 421–434.
Gottschalk, G., 1986. Bacterial metabolism. Springer Series in Microbiology. Springer-Verlag, New York.
Grimaldi, C., Grimaldi, M., Guedron, S., 2008. Mercury distribution in tropical soil profiles related to origin of mercury and soil processes. Sci. Total Environ. 401, 121–129.
Guedron, S., Grimaldi, C., Chauvel, C., Spadini, L., Grimaldi, M., 2006. Weathering versus atmospheric contributions to mercury concentrations in French Guiana soils. Appl. Geochem. 21, 2010–2022.
Guedron, S., Grangeon, S., Lanson, B., Grimaldi, M., 2009. Mercury speciation in a tropical soil association; consequence of gold mining on Hg distribution in French Guiana. Geoderma 153, 331–346.
Harris-Hellal, J., Vallaeys, T., Garnier-Zarli, E., Bousserrhine, N., 2009. Effects of mercury on soil microbial communities in tropical soils of French Guyana. Appl. Soil Ecol. 41, 59–68.
Harris-Hellal, J., Grimaldi, M., Garnier-Zarli, E., Bousserrhine, N., 2011. Mercury mobilization by chemical and microbial iron oxide reduction in soils of French Guyana. Biogeochemistry 103, 223–234.
Hellal, J., Guédron, S., Huguet, L., Schäfer, J., Laperche, V., Joulian, C., et al., 2015. Mercury mobilization and speciation linked to bacterial iron oxide and sulfate reduction: a column study to mimic reactive transfer in an anoxic aquifer. J. Contam. Hydrol. 180, 56–68.
Holley, E., McQuillan, A., Craw, D., Kim, P., Sander, S., 2007. Mercury mobilization by oxidative dissolution of cinnabar (–HgS) and metacinnabar (–HgS). Chem. Geol. 240, 313–325.
Hutchens, E., Clipson, N., McDermott, F.P., 2008a. Mineralogical influence on the structure and the diversity of bacterial communities associated with silicate minerals. Geophys. Res. Abstr. 10 (EGU2008-A-03930).
Hutchens, E., Mcdermott, F.P., Clipson, N., 2008b. Bacteria, fungi and archea on silicate minerals — a case for selective colonization. Geochim. Cosmochim. Acta 72, 404.
Iram, A., Zaman, A., Iqbal, Z., Shabbir, R., 2012. Heavy metal tolerance of fungus isolated from soil contaminated with sewage and industrial wastewater. Pol. J. Environ. Stud. 22, 691–697.
Jones, D.L., 1998. Organic acids in the rhizosphere — a critical review. Plant Soil 205, 25–44.
Lovley, D.R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. Microbiol. Rev. 55, 259–287.
Martínez-Juárez, V.M., Cárdenas-González, J.F., Torre-Bouscoulet, M.E., Acosta-Rodríguez, I., 2012. Biosorption of mercury(II) from aqueous solutions onto fungal biomass. Bioinorg. Chem. Appl. 1-5.
Oliveira, A., Pampulha, M.E., Neto, M.M., Almeida, A.C., 2010. Mercury tolerant diazotrophic bacteria in a long-term contaminated soil. Geoderma 154, 359–363.
Pelmont, J., 1993. Bactéries et environnement. Presse Universitaire de Grenoble, p. 899.
Porcella, D.B., Huckabee, J.W., Weathley, B., 1995. Mercury as a global pollutant. Water Air Soil Pollut. 80, 1336.
Pujos, M., Bouysse, P., Pons, J., 1990. Sources and distribution of heavy minerals in Late Quaternary sediments of the French Guyana continental shelf. Cont. Shelf Res. 10, 59–79.
Robert, M., Berthelin, J., 1986. Role of biological and biochemical factors in soil minerals weathering. In: Interactions of soil minerals with natural organic and microbes. Soil Sci. Soc. Amer. 17, 453–495.
Rogers, J.R., Bennett, P.C., 2004. Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates. Chem. Geol. 203, 91–108.
Roulet, M., Lucotte, M., 1995. Geochemistry of mercury in pristine and flooded ferralitic soils of a tropical rain forest in French Guyana, south America. Water Air Soil Pollut. 80, 1079–1088.
Schuster, E., 1991. The behavior of mercury in the soil with special emphasis on complexation and adsorption processes-a review of the literature. Water, Air, and Soil Pollut. 56, 667–680.
Si, Y., Zou, Y., Liu, X., Si, X., Mao, J., 2015. Mercury methylation coupled to iron reduction by dissimilatory iron-reducing bacteria. Chemosphere 122, 206–212.
Soares, L.S., Egreja Filho, F.B., Linhares, L.A., Windmoller, C.C., Yoshida, M.I., 2015. Accumulation and oxidation of elemental mercury in tropical soils. Chemosphere 134, 181–191.
Toubassy, M., Alphonse, V., Bousserhine, N., 2014. Impact of anaerobic bacterial activities on the dynamic and speciation of mercury in tropical soils in French Guiana. Mineral. Mag. 2507.
Uroz, S., Oger, P., Lepleux, C., Collignon, C., Frey-Klett, P., Turpault, M.P., 2011. Bacterial weathering and its contribution to nutrient cycling in temperate forest ecosystems. Res. Microbiol. 162, 820–831.
Van Hees, P.A.W., Dahlén, J., Lundström, J.S., Borén, H., Allard, B., 1999. Determination of low molecular weight organic acids in soil solution by HPLC. Talanta 48, 173–179.
Van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L., Lundström, U.S., 2005. The carbon we do not see: The impact of low molecular weight compounds on carbon dynamics and respiration in forest soils — a review. Soil Biol. Biochem. 37, 1–13.
Wang, Y.J., Li, H.Y., Hu, H.F., Li, D.P., Yang, Y.J., Liu, C., 2013. Using biochemical system to improve cinnabar dissolution. Bioresour. Technol. 132, 1–4.
Wang, Q., Wang, R.R., Lin, Y., Lu, J., Huang, Z., 2014. Changes in weathering effectiveness and community of culturable mineral-weathering bacteria along a soil profile. Biol. Fertil. Soils 50, 1025–1034.
Waples, J.S., Nagy, K.L., Aiken, G.R., Ryan, J.N., 2005. Dissolution of cinnabar (HgS) in the presence of natural organic matter. Geochim. Cosmochim. Acta 69, 1575–1588.
Wasay, S.A., Barrington, S., Tokunaga, S., 2001. Organic acids for the in situ remediation of soils polluted by heavy metals: soil flushing in columns. Water Air Soil Pollut. 127, 301–314.
Welch, S.A., Ullman, W.J., 1999. The effect of microbial glucose metabolism on bytownite feldspar dissolution rates between 5° and 35°C. Geochim. Cosmochim. Acta 63, 3247–3259.
World reference base for soil resources, 2006. World Soil Resources Reports No. 103. FAO, Rome.
Yarwood, S., Wick, A., Williams, M., Lee Daniels, W., 2014. Parent material and vegetation influence soil microbial community structure following 30-years of rock weathering. Microb. Ecol. 69, 383–394.