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Mercury mobilization by chemical and microbial iron oxide reduction in soils of French Guyana

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Abstract Iron oxy(hydr)oxides (oxides) are important mercury sinks in tropical oxisols and the geochemistry of these two elements are thus closely entwined. We hypothesized that bacterial Fe-oxide reduction in anoxic conditions could be a significant mechanism for mobilizing associated Hg. Iron oxide and mercury solubilisation in presence of two chemical reducers (ascorbate and dithionite, dissolving amorphous and amorphous plus well crystallized Fe-oxides, respectively) was compared to their solubilisation in presence of autochthonous ferri-reducing bacteria. This work was carried out on two soil profiles from a small catchment basin in French Guyana, an oxisol (O) from a well drained slope and a water-saturated hydromorphic soil (H). The chemical reductions showed that in the oxisol 20 and 48% of total Hg (Hg_T) was associated to amorphous and well crystallized iron oxides, respectively. However, in the hydromorphic soil, no Hg seemed to be associated to amorphous iron oxides while the well crystallized fraction contained less than 9% of Hg_T. Chemical Fe-oxide reduction showed that Hg solubility was correlated to Fe reduction in the oxisol, demonstrating a relationship between the geochemistry of these two metals. During bacterial growth, while bacterial iron reduction solubilised up to 3.2 mg Fe g^{-1} soil in the oxisol sample, Hg_T remained unchanged. No mercury was detected in the culture medium either. However, chemical analysis showed a decrease of the amounts of Hg associated to amorphous and well crystallized Fe-oxides after 14 days of incubation, underlining the potential for iron-reducing bacteria to modify mercury distribution in soil.

Keywords Mercury · Iron oxides · Chemical reduction · Ferri-reducing bacteria · French Guyana

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Introduction

Amazonian soils have been accumulating atmospheric mercury (Hg) for several millions of years and register natural background levels of Hg up to ten

times those found in temperate soils (Carmouze et al. 2002). On top of that, human activities, and in particular gold mining, have contributed to raising the levels of Hg in these regions. Indeed, it has been estimated that around 5 000 tons of Hg have been dumped in the whole Amazonian area since the beginning of the gold rush towards the end of the 19th century (Nriagu 1994).

In pristine soils of the Amazonian basin, high mercury concentrations have been measured in Fe-oxide rich oxisols that contain little organic matter. Conversely, in hydromorphic soils that contain little Fe, lower Hg concentrations have been measured. Several studies have identified relationships between Hg and Fe contents in soils (Roulet and Lucotte 1995; Roulet et al. 1998; Roulet and Grimaldi 2001) and it is now generally accepted that in organic-poor soils, such as oxisols, Fe-oxides play an important role in adsorbing Hg. Thus, the geochemistry of these two elements may be strongly linked. This explains the lower amounts of Hg measured in hydromorphic soils in which most of the iron has been reduced and exported, along with the mercury, via the aquifer.

The Hg adsorbed to Fe-oxides has little risk of being exported from the well drained oxisols, except via natural surface erosion and soil transformation. However, modern gold mining and deforestation expose soils to sunlight and rain and the excavation of the gold-containing soils causes previously immobile mercury to encounter conditions that (i) favour its erosion and transport in watersheds (Oliveira (de) et al. 2001; Lacerda et al. 2004; Béliveau et al. 2009) and (ii) create disorganized hydromorphic areas where all the geochemical conditions necessary to favour methylation are brought together (Guedron 2008). Indeed, Hg methylation is generally considered to occur in anoxic soils and sediments (Morel et al. 1998), mainly by microbial methylation of inorganic Hg (Gadd 1993; King et al. 2002). Once methylated, methylmercury (MeHg) can be transported via the aquifer or by soil water runoff or erosion, to streams and rivers (Morel et al. 1998; Wasserman et al. 2003). Once in the water system, MeHg is readily accumulated along the food chain and can become an environmental and sanitary hazard as it can be consumed by local fish-eating populations (Veiga et al. 1999).

Although geochemical conditions and erosion are a well recognized means of remobilizing Hg, little attention has been given to soil microbial communities, although their activity could play an important role in Hg distribution and speciation. Indeed, in natural environments, and in particular in the tropics, microbial Fe-reduction is a major factor in the mobilization of Fe (Stemmler and Berthelin 2003). Ferri-reducing bacteria reduce Fe in anoxic conditions by using it either as a major final electron accepter in anaerobic respiration or as a minor electron accepter during fermentation metabolism. During this process, it has been demonstrated that heavy metals adsorbed to iron oxides can be mobilized. (Addy et al. 1976; Schwertmann and Latham 1986; Francis and Dodge 1989; Lovley 1993; Trolard et al. 1995; Bousserrhine et al. 1999; Quantin et al. 2001, 2002; Cornell and Schwertmann 2003; Burnol et al. 2007; Bradl 2004; Neaman et al. 2004; Noubactep et al. 2005; Cooper et al. 2006). However, although bacterial Fe reduction is frequently mentioned as being an important factor of heavy metal mobilization, to our knowledge, there is no available data on the impact of this reductive dissolution on Hg mobility.

The aim of this study was to evaluate and compare the impact of chemical and microbial Fe-oxide reduction on the mobilization and distribution of associated Hg in tropical ferralitic soils. The chemical reducers used were ascorbate and dithionite that extract amorphous (Fe_{Asc}) and amorphous plus well crystallized (Fe_{CBD}) Fe-oxides, respectively. Microbial reduction was carried out with autochthonous soil bacteria. The soils studied were an oxisol (O) and a hydromorphic soil (H) from a small catchment basin in French Guyana of which the downstream flats were gold-mined towards the end of the 19th century and again in the middle of the 20th century. Thus, high Hg concentrations could be found locally.

Materials and methods

Sample collection

Soil samples were collected in December 2005 from the catchment basin of the Combat creek (1 km^2), situated at approximately 10 km from the village of Cacao in French Guyana (52°23′ W, 4°35′ N). Soil profiles up to 1 m deep were sampled with a metal auger, and each 10 cm layer was immediately sealed

in sterile hermetic polyethylene bags. Two soil profiles were considered in this study: an oxisol (O) collected from a well drained slope and a hydromorphic soil (H) from the toeslope. These soils did not appear to have been directly affected by gold-mining as a logical continuity in vertical variations was observed; for example organic matter getting scarce with depth.

Soil preparation and characterization

The collected samples were air dried at 25°C, sieved at 2 mm and hermetically sealed at 4°C until use. Initial analyses were carried out on all samples by the National Institute of Agronomic Research (INRA, Arras, France). They consisted in the determination of 5 granulometric fractions without decarbonation (NF X 31-107), organic carbon (C) and total nitrogen (N) (NF ISO 10694 and NF ISO 13878), and total Fe (NF EN ISO 11885). Finally, pH was measured in water with a 1:2.5 ratio soil: solution (NF ISO 10390).

Chemical iron reduction

Chemical iron reduction was followed over time on homogenised samples of the 70–80 cm deep horizon of O and H using ascorbate and dithionite to reduce amorphous (Fe_{Asc}) and well crystallized Fe-oxides (Fe_{CBD}), respectively. These samples were chosen firstly because of their depth which induced low organic matter contents, thus increasing the possibilities of interactions between mercury and mineral elements, and secondly for their total iron and mercury contents which made them representative of their soil type (i.e. oxisol or hydromorphic soil).

Amorphous Fe-oxides were extracted using a modified application of the ascorbate method as described by Ferdelman, (1988). Briefly, 10 g of sodium citrate ($Na_3C_6H_5O_7$) and 10 g of sodium bicarbonate ($NaHCO_3$) were added to 200 ml of ultra pure water that was deaerated by bubbling with filtered nitrogen, before adding 4 g of ascorbic acid, for a final pH of 8. One gram of soil (dry weight, dw) was placed in a 30 ml polypropylene centrifugation tube (Nalgene, France) with 10 ml of reagent and agitated at room temperature. This temperature was chosen in order to avoid Hg volatilization.

Crystallized Fe-oxides were extracted using the Carbonate, Bicarbonate, Dithionite (CBD) method of Mehra and Jackson (1960), modified by Jeanroy et al. (1991). Briefly, 0.2 g soil (dw) was placed in a centrifuge tube with 20 ml of reagent (78.4 g l^{-1} sodium citrate and 9.8 g l^{-1} sodium bicarbonate in ultra pure water) for a 1/100 soil weight/reagent ratio. Sodium dithionite was added at a ½ ration soil weight/dithionite (0.4 g $tube^{-1}$), the tubes were agitated and incubated under agitation at room temperature.

During the ascorbate extraction, triplicate samples were harvested after 6, 12, 24, 48, 72 and 120 h of extraction, and during the dithionite extraction, triplicate samples were harvested after 2, 4, 8, 12, 24, 48 and 96 h of extraction. Samples were immediately centrifuged at 5000×*g* for 10 min at 4°C. The pellets were resuspended in 2 ml of ultra pure water, centrifuged a second time and both supernatants were pooled. The supernatant was then filtered (0.45 µm acid rinsed filtered, Minisart SRP 25, Sartorius) and acidified (pH 1) with HCl (37%) to prevent metal precipitation and preserved at 4°C until analysis. The remaining soil pellet was air dried and crushed to <100 µm and also conserved at 4°C until further analyses.

Chemical extracts were analyzed for Hg and Fe, as described below. Moreover, Fe_{Asc} and Fe_{CBD} were also extracted from the 50–60 and 90–100 cm deep horizons of soils O and H, with an extraction time of 72 h. This extraction time was chosen after the extraction kinetics demonstrated that the maximum amount of iron was extracted after 72 h.

Microbial iron reduction

As chemical extractions showed (see results), significant quantities of Hg and Fe were leached only in the Fe-rich oxisol (O), thus, only this soil was investigated in bacterial iron reduction experiments. The three depths used correspond to different soil colours, described according to the Munsell Soil Colour Charts (2000). At 50–60 cm, the soil was dark yellowish brown (10YR4/6), at 70–80 cm it started evolving towards a heterogenic dark brown and red colour, and at 90–100 cm, it was clearly double phased with brownish-yellow and red volumes (5YR5/6 and 10R4/8).

Microbial iron reduction was followed in soil microcosms set up in sterile hermetic 250 ml glass plasma bottles (BioBlock, France). Microcosms consisted in 5 g of each soil sample and 100 ml of modified Bromfield medium (Bousserrhine 1995), of the following composition for 1 l of MilliQ® water; $MgSO_4.7H_2O$ (0.5 g); K_2HPO_4 (0.5 g); $(NH_4)_2SO_4$ (1 g), yeast extract (0.15 g) and glucose (1 g). Each soil sample was prepared in triplicates. Anoxic conditions were initiated by flushing microcosms with N_2 for 10 min. Incubations were carried out in the dark at 28°C for 14 days without shaking except prior to sampling.

During the incubation, several parameters were measured in the culture medium: carbon mineralization, glucose consumption, Fe(II) production and Hg solubilisation. At the end of the incubation, the microcosm medium was recovered and analyzed for Hg and Fe, and total Hg was measured in the soil. Moreover, the soil was subjected to Fe_{Asc} and Fe_{CBD} selective extractions as described previously.

Mercury analysis

Total Hg (Hg_T), in soils and in chemical extracts, was analyzed with an Automatic Mercury Analyzer (AMA 254, Courtage Analyses, France), detection limit 0.01 ng. Soil samples were crushed in an agate mortar to <100 μm. About 100 mg were then placed in the AMA 254 and analyzed. In brief, the sample is heated to 550°C and all products of decomposition, including mercury, are carried by a stream of oxygen through a catalytic tube where Hg_T is transformed into elementary mercury (Hg°) which then readily adsorbs to a gold-trap. The fixed mercury is then released by heating the gold-trap at 450°C and the mercury quantified by atomic adsorption spectrometry. Concentrations of standard reference materiel (MESS-3) did not exceed the range of concentrations announced (0.091 ± 0.008 μg g^{-1}). Extraction supernatants were analyzed using the same method by analyzing 400 μl of each sample. All liquid samples were previously filtered (<0.45 μm Teflon filters, Minisart SRP 25, Sartorius) and acidified to pH 1 (HCl, 37% Suprapur).

Total dissolved Hg (Hg_D) in bacterial microcosm medium was analyzed by Cold Vapor Atomic Fluorescence Spectrophotometer (CVAFS), detection limit 17 pg, as described by Cossa et al. (2002). All liquid samples were previously filtered (<0.45 μm Teflon filters, Minisart SRP 25, Sartorius) and acidified to pH 1 (HCl, 37% Suprapur). Briefly, the sample is mineralized with $BrCl_2$, and reduced to elemental mercury (Hg°) with stannous chloride ($SnCl_2$). A bubbling system then causes the elemental mercury to be caught on a gold trap before being measured using a 2500 CVAFS Mercury detector (TEKRAN, Canada). Concentrations of standard reference material (ORMS3) did not exceed the range of concentrations announced (12.6 ± 1.1 pg g^{-1}).

Iron analysis

Chemical extracts and microcosm medium were analyzed after filtration (0.45 μm acid-washed membrane filter, Minisart SRP 25, Sartorius) and acidification at pH 1 with HCl (37%) by ICP-AES (Vista MPX, Varian) for total Fe. Standards were prepared from an ICM 240 (Promochem, VWR) solution.

Reduced Fe (Fe^{II}), was also measured in the medium at regular intervals (after 1, 2, 3, 4, 9 and 14 days of incubation) using a colorimetrical method with orthophenanthroline-chlorydrate. Briefly 0.1–1 ml of filtered samples (0.45 μl acid rinsed filters, Minisart SRP 25, Sartorius) were put in a 25 ml graduated glass vial with 1 ml of orthophenanthroline (0.5%) and completed to 25 ml with MilliQ water. After 10 min the absorbance was read at 490 nm. Readings were compared to a standard curve established with dissolved anhydrous ferrous sulphate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$).

Bacterial metabolism

During the incubations, carbon mineralization was followed by measuring CO_2 evolution daily in the microcosm's atmosphere with an infrared spectrophotometer (Berly 100, Cosma).

Glucose concentration in microcosms was measured daily in samples of microcosm medium with a D-Glucose enzymatic test (Boehringer Mannheim, France). Samples were filtered (0.45 μm, Minisart, Sartorius) prior to analysis.

Sulphate reduction was followed by measuring sulphide (S^{2-}) formation in the culture medium. This was carried out using a kit (Spectroquant, Merck), analogue to NF ISO 10530. This kit measures

sulphide concentrations in a range of 0.02–1.5 mg $S^{2-}l^{-1}$. Samples were filtered (0.45 μm, Minisart, Sartorius) prior to analysis.

Finally, redox potential and pH were measured in microcosms at the beginning and the end of the incubation with Eh/pH electrodes (Metrohm, France).

Statistical analysis

Homogeneity of variances was checked using a Bartlett's test and normality using a Shapiro–Wilk's test. When variances were not homogeneous between replicates, real standard deviations (SD) were presented in the figures; otherwise, the overall SD was calculated and displayed as the interval associated to each sample mean. Our data (CO_2 and iron solubilisation) did not follow a normal distribution (*P*-value <0.05), thus, a Friedman non parametrical test for paired data was run for each soil depth to identify the effect of time on data distribution, and a Kruskal–Wallis non parametrical test and a multicomparaison test were run to identify differences between soil depths. The results for CO_2 and Fe solubilisation identified an effect of time (i.e. increase during the experiment) and no differences between soil depths ($P_{Fe} = 0.643$ and $P_{CO2} = 0.617$).

Regression analyses were carried out between Hg and Fe solubilised during the chemical Fe extractions and between CO_2 evolution and Fe solubilisation in the microcosms. The R^2 and *P*-value are given in the corresponding paragraphs and significant correlations are presented graphically.

Student *t* tests were run on the molar ratios calculated between Fe and Hg, in the iron oxide CBD and ascorbate extractions, at the beginning and the end of microcosm incubations (Fig. 6), to determine whether differences were significant.

Extraction and incubation quality assurance and control (QA/QC)

To avoid contamination, all materials used in this work were acid-washed twice with HNO_3 (5%), then rinsed several times with Milli-Q® water before use.

The extraction reagents (ascorbate and CBD) were prepared in Milli-Q® water and analysed for Hg contents as blanks. On average we measured 0.089 ng Hg $g^{-1} \pm 0.003$ in the ascorbate and 0.223 ng Hg $g^{-1} \pm 0.007$ in the dithionite. These values are very low compared to the amounts measured in the samples.

Blank microcosms containing sterile Bromfield medium were incubated in the same conditions as the soil containing ones and analysed for CO_2 evolution, Fe(II), Fe_T and Hg_T. Whereas these tests were negative for CO_2 and Fe, we measured 0.87 ± 0.3 pg Hg l^{-1} medium.

Results

Soil characteristics

Table 1 presents soil texture and organic carbon contents in three depths of each soil profile. The oxisol (O) was very rich in clay, up to 75% with quantities of fine silt and coarse sand ranging from 5 to 15%. The texture triangle defines this soil as clay. The hydromorphic soil (H) was not as rich in clay (<35%), its dominant component being silt (~45%), followed by sand (up to 40%). The texture triangle describes this soil as a fine clay-loam soil. Organic carbon contents were low and decreased with depth in both soils.

Fe_T and Hg_T soil contents were very different in O and H (Table 2). Quantities of Fe_T were up to 10 times higher in O compared to H and ranged from 198.1 mg Fe g^{-1} soil in the 50–60 cm horizon, to 290.6 mg Fe g^{-1} soil in the 90–100 cm horizon. In H, the maximum Fe_T was found at 70–80 cm: 20.7 mg g^{-1} soil, it was of 13.4 and 8.4 mg Fe g^{-1} soil in the 50–60 and the 90–100 cm horizons, respectively. Hg_T decreased slightly with depth in O, from 294.1 ng Hg g^{-1} soil in the 50-60 cm horizon, to 248.7 ng Hg g^{-1} soil in the 90–100 cm one. In H, Hg_T was on average 134 ng Hg g^{-1} soil in the first two horizons and then increased to 338.6 ng Hg g^{-1} soil in the 90–100 cm one.

The selective extractions carried out before kinetic reductions (Table 2) showed a good repeatability. In O, the percentage of Fe_{Asc}/Fe_T decreased with depth from 6.9 to 1.7%, as did the percentage Hg_{Asc}/Hg_T (19.8 to 8.5). Also in O, CBD extracted up to 77.3% of Fe_T, and 48% of Hg_T. Although significant quantities of Hg were leached with Fe_{Asc} and Fe_{CBD} extractions, no significant correlation was observed between the two elements.

Table 1 Soil texture and organic carbon contents distribution in the two studied profiles

Soil sample		Clay g/100 g (<2 μm)	Fine silt g/100 g (2–20 μm)	Coarse silt g/100 g (20–50 μm)	Fine sand g/100 g (50–0.2 μm)	Coarse sand g/100 g (0.2–2 mm)	Carbon g/100 g
O	50–60 cm	75.9	5.3	1.6	3.5	13.5	2.1
	70–80 cm	69.1	8.7	2.1	3.6	16.4	1.1
	90–100 cm	61.1	12.4	2.5	4.6	19.2	0.7
H	50–60 cm	32.2	28.1	19.0	9.8	10.8	0.32
	70–80 cm	27.9	21.5	12.9	7.8	29.6	0.31
	90–100 cm	31.9	33.1	13.4	6.4	15.0	0.2

Table 2 Total, ascorbate extracted and CBD-extracted Fe and Hg in soils O and H at 50–60, 70–80 and 90–100 cm depth. Extraction intervals represent ± mean standard error calculated for each determination

Soil samples	Depth (cm)	Fe_T (mg g $soil^{-1}$)	Hg_T (ng g $soil^{-1}$)	Fe_{asc}/Fe_T (%)	Hg_{asc}/Hg_T (%)	Fe_{Dit}/Fe_T (%)	Hg_{Dit}/Hg_T (%)
O	50–60	198.1	294.1	6.9 ± 0.3	19.8 ± 0.2	70.1 ± 5	40.0 ± 2.1
	70–80	263.1	265.9	2.7 ± 0.4	13.07 ± 0.97	77.3 ± 5.7	47.9 ± 5.6
	90–100	290.6	248.7	1.7 ± 0.5	8.52 ± 0.62	66.9 ± 4.5	38.8 ± 7.1
H	50–60	13.4	135.6	4.5 ± 0.2	–	80.6 ± 7.4	3.0 ± 0.13
	70–80	20.7	133.9	14.4 ± 0.7	–	46.7 ± 4.6	8.9 ± 1.7
	90–100	8.4	338.6	4.7 ± 0.6	–	122.6 ± 9.5	1.6 ± 0.1

In H, the ascorbate extraction yielded up to 14% of Fe_T, however, Hg_{Asc} was not detectable (nd) by AMA 254 in the extractant (Table 2). The CBD extracted 100% of Fe_T in the 90–100 cm horizon and up to 9% of Hg_T in the 70–80 cm horizon. No significant correlations were observed between Fe and Hg in H.

Kinetics of chemical iron oxide reduction

The kinetics of chemical Fe-oxide reduction by ascorbate and CBD are presented in Fig. 1. The citrate in CBD acts as a complexant and prevents Al, Fe or other metals from precipitating. In O, Hg and Fe were leached simultaneously over time with both ascorbate and CBD. During ascorbate extraction, the maximum of Hg_{Asc} and Fe_{Asc} were leached after 48 h, while during CBD extraction, Hg_{CBD} was leached faster (12 h) than Fe_{CBD} (48 h). In H, on the contrary, no Hg_{Asc} was detected along with Fe_{Asc}, but Hg_{CBD} appeared to be leached simultaneously to Fe_{CBD} in this soil, although values were a lot smaller than in O.

Regression analyses were run between Hg and Fe leached during Fe chemical extractions when possible. Results demonstrate a clear correlation ($R^2 = 0.84$, $P = 0.003$) between Fe and Hg extracted with dithionite and a less significant correlation ($R^2 = 0.51$, $P = 0.070$) for ascorbate (Fig. 2). In H on the other hand, the relation between Fe and Hg for dithionite gave a $R^2 = 0.21$ and could not be calculated for ascorbate as Hg was not detectable.

Microbial iron oxide reduction

Carbon mineralization (Fig. 3) increased considerably during the first 4 days of incubation in all three horizons (Friedman test, $P < 0.001$), before slowing down. The amount of C mineralized at the end of the experiments was 5.1 ± 0.3 mg g^{-1} soil on average and no significant differences were given between soil depths (Kruskal–Wallis test, $P = 0.617$). Glucose was totally consumed after 4 days (Fig. 3). The amount of carbon that evolved in the form of CO_2 in the microcosm atmosphere represented 72% of the carbon provided by the glucose.

The redox potential (Eh), decreased rapidly (data not shown) and the Eh measured at the end of the incubations was −190 ± 47 mV on average in all

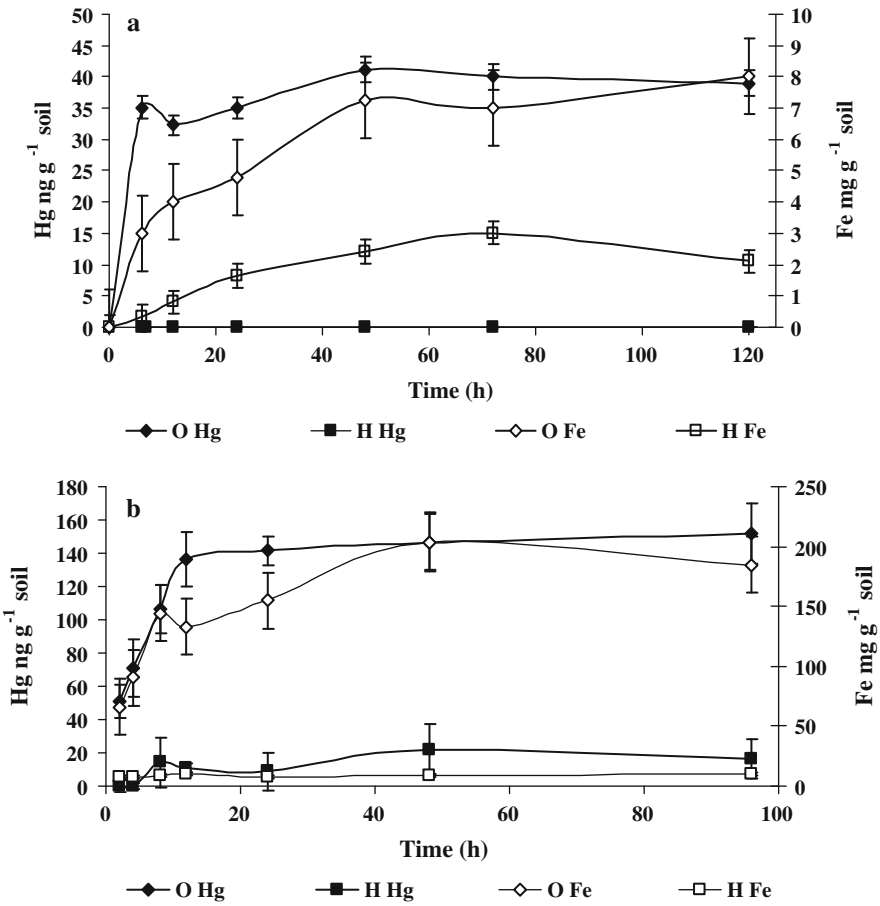

Fig. 1 Hg and Fe solubilised by chemical reduction over time in 70–80 cm horizon of the oxisol (O) and the hydromorphic soil (H). **a** Hg_{Asc} (ng g^{-1} soil) and Fe_{Asc} (mg g^{-1} soil); **b** Hg_{CBD} (ng g^{-1} soil) and Fe_{CBD} (mg g^{-1} soil). Intervals represent ±mean standard error calculated for each determination (3 replicates)

microcosms. The pH increased during incubations in all three soils from 4.7 ± 0.25 to 5.3 ± 0.25.

The formation of sulphides was detected from day 8 onwards, and at the end of the incubation we measured on average 80 µg S^{2-} g^{-1} soil in the microcosm medium (data not shown).

During the incubation, total dissolved Fe (Fe_D) increased in the microcosm medium (Fig. 4). Slightly more Fe was solubilised in the 90-100 cm horizon; 3.5 mg Fe g^{-1} soil, compared to the 50–60 and 70–80 cm horizons; 3 mg Fe g^{-1} soil at the end of the incubation but overall differences were not significant (Kruskal–Wallis test, $P = 0.643$). These solubilised quantities represent 2.1; 1.5; and 1.8% of Fe_{CBD} or 21, 42 and 70% of Fe_{Asc} in the 50–60, 70–80 and 90–100 cm horizons respectively. A colorimetric test with Orthophenantroline analysis indicated that all of this Fe was in the form of Fe^{II} (data not shown), confirming that Fe solubilisation was due to bacterial reduction. Moreover, a significant polynomial correlation was calculated between Fe solubilisation in the inoculation medium and CO_2 evolution (Fig. 5).

According to the chemical reduction of iron, the solubilisation of 2.1, 1.5 and 1.8% of Fe_{CBD} should have led to the solubilisation of at least 2.4, 1.6 and 1.3 ng Hg g^{-1} soil, and the solubilisation of 21, 42 and 70% of Fe_{Asc} to the solubilisation of 12.8, 14.6 and 15.1 ng Hg g^{-1} soil in the 50–60, 70–80 and 90–100 cm horizons, respectively. However, dissolved Hg (Hg_D) was very low and constant throughout the experiment, averaging 8.6 ± 2.3 ng g^{-1} soil, as was the total mercury in the solid phase.

The amount of Hg (nmol) extracted per µmol Fe in the ascorbate and CBD fractions before and after the incubation is presented in Fig. 6. Results showed that Hg was much more concentrated in the amorphous fraction (up to 14 nmol Hg μmol^{-1} Fe) than in the CBD fraction (<0.25 nmol Hg μmol^{-1} Fe). After the incubation, these ratios had significantly decreased

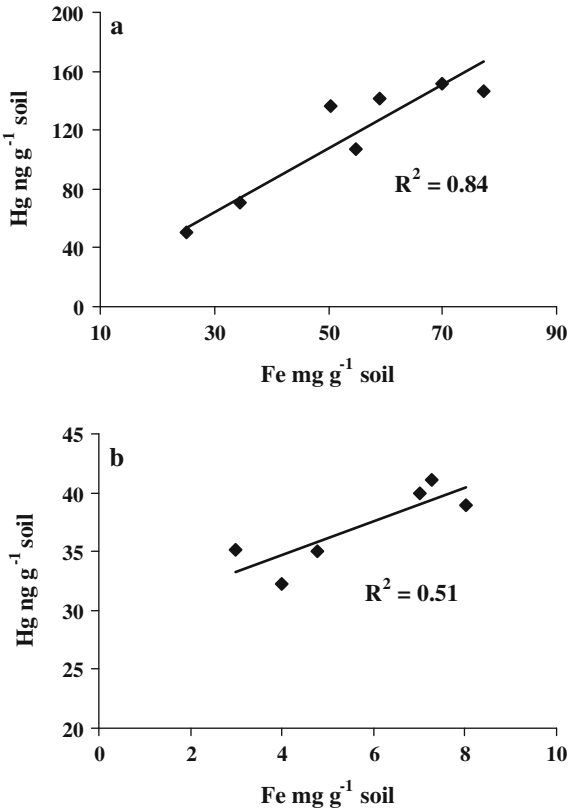

Fig. 2 Linear regressions between Fe and Hg leached during chemical extraction in the O soil, (**a**) by dithionite and (**b**) by ascorbate

(Student t tests) in the 50–60 and 70–80 cm horizons for the ascorbate extractions ($P < 0.05$) but not in the 90–100 cm horizon ($P = 0.077$). They had also significantly decreased in all the horizons in the CBD extraction ($P < 0.05$).

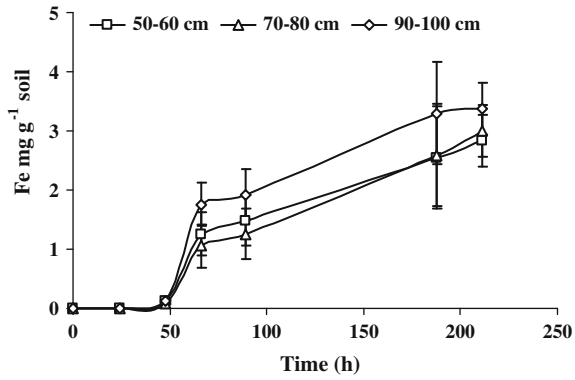

Fig. 4 Iron solubilisation over time in the three studied depths of the oxisol (O) in mg g^{-1} soil. Intervals represent ± mean standard error calculated for each determination (3 replicates)

Discussion

The aim of this study was to compare chemical and microbial reduction of iron oxides in tropical ferrallitic soils of French Guyana, and to evaluate its impact on mercury release. It is a well accepted fact that Fe-oxides are sinks for various major and trace metals including Al, Mn, Cr, Ni, Cd, Zn, Co, Pb and U, and that bacterial Fe-reduction contributes to re-mobilizing these metals (Schwertmann 1991; Bousserrhine et al. 1998; Bousserrhine et al. 1999; Quantin et al. 2001; Dominik et al. 2002; Quantin

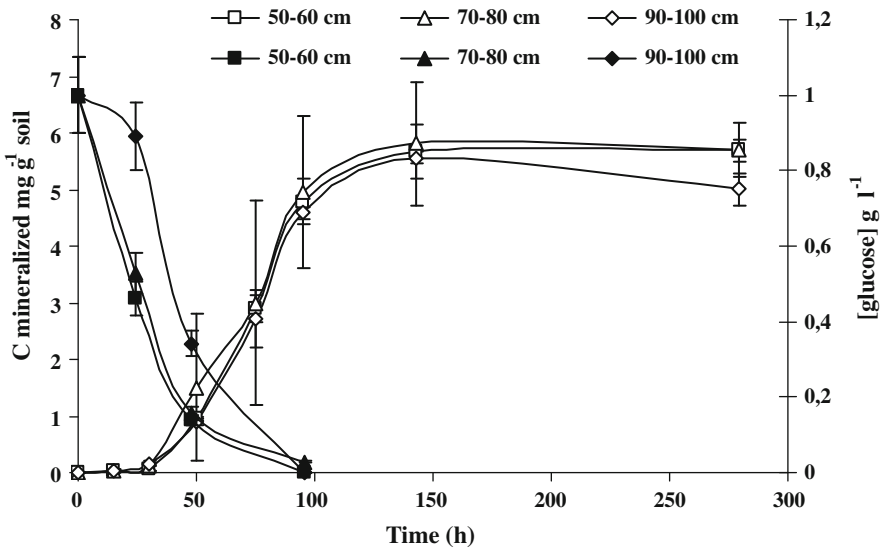

Fig. 3 CO_2 evolution and glucose consumption in the oxisol microcosms at the three studied depths (50–60, 70–80 and 90–100 cm) during the incubations, in mg C g^{-1} soil and g l^{-1}, respectively. Intervals represent ± mean standard error calculated for each determination (3 replicates)

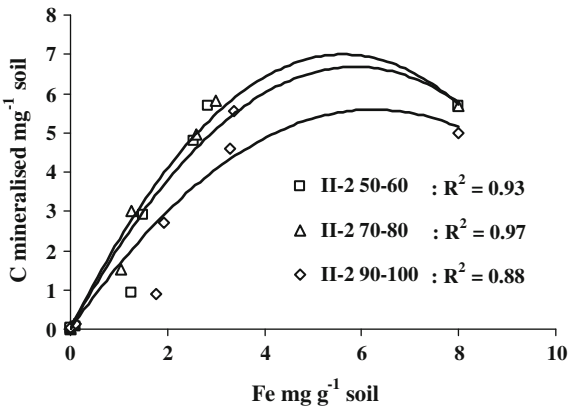

Fig. 5 Correlations between mineralised carbon and iron solubilisation in microbial incubations

et al. 2002; Stemmler and Berthelin 2003; Cooper et al. 2005, 2006; Noubactep et al. 2005). It is also more and more accepted that Fe-oxides play an important role in the geochemistry of Hg, especially in iron-rich tropical soils (Roulet and Lucotte 1995; Roulet et al. 1998; Grimaldi et al. 2001). However, although re-mobilization of Hg by Fe-oxide reduction has often been suggested, to our knowledge, there is no data that demonstrates it.

A kinetic approach of the reduction of Fe-oxides by chemical and microbial processes was used to determine the potential solubilisation of Fe and Hg in tropical ferrallitic soils of French Guyana. The results enabled us to estimate the amounts of Hg associated to amorphous and well crystallized Fe-oxides, which can potentially be mobilized in anaerobic conditions by ferri-reducing bacteria.

Soil characteristics and Fe and Hg distribution

Both soils under study, O and H, were very different from a textural point of view and concerning Fe_T and Hg_T contents. O is an oxisol, containing a lot of clay and iron oxides, as reported for these types of soils (Boulet et al. 1993), whereas the hydromorphic soil contained little clay and Fe, but more sand and silt, due to soil transformations in anoxic conditions and the export of fine particles (Boulet et al. 1993; Tessier et al. 2003). The Hg concentrations measured in the oxisol were in the range of concentrations previously reported in French Guyana; 240–320 ng g^{-1} soil (Roulet and Lucotte 1995). In pristine hydromorphic soils, these authors measured Hg contents in a range of 50–100 ng g^{-1} soil which is lower than those measured in the present study (133–338 ng g^{-1} soil). This suggests that although soil horizons were not apparently disturbed, past gold-mining had contaminated these soils. Indeed, Guedron (2008) found droplets of metallic Hg in hydromorphic soils from the same catchment basin as in this study that most probably came from past gold-mining.

In the oxisol, O, the maximum Fe_{CBD} yielded by selective extractions was 77% of Fe_T. The remaining iron is most probably resistant goethite or hard Fe nodules (Trolard et al. 1995). Fe_{Asc} represented less than 7% of Fe_T in all the studied depths of the O soil profiles, and decreased with depth, indicating an increase in iron crystallinity (Fritsch et al. 2005).

In the hydromorphic soil, H, on the contrary, up to 100% of Fe_T was extracted with CBD, but not systematically. The presence of hard nodules in the soil profile indicates that free iron has re-precipitated,

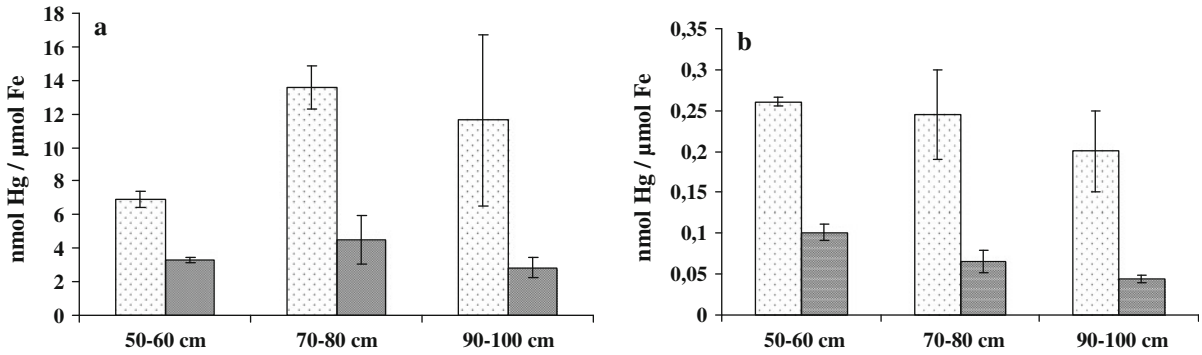

Fig. 6 Quantity of mercury (nmol) extracted per µmol of extracted Fe_{Asc} (**a**) and Fe_{CBD} (**b**), before incubation, T0, and after 14 days incubation; T14. T0:; T14:. Intervals represent ± mean standard error calculated for each determination (3 replicates)

probably during the dry seasons when soils are partially oxygenated. Fe_{Asc} was slightly higher than in O (up to 14%), and no relations were observed with depth. In this blue-grey coloured soil, nearly all the iron oxides have been reduced and exported, leaving only hard iron concretions.

Hg was only significantly leached during selective extractions of iron forms in O, where Hg_{CBD} and Hg_{Asc} represented up to 48 and 19% of Hg_T, respectively. However, there wasn't a significant correlation between Fe_{Asc}, Fe_{CBD} and Hg_T. Although they didn't measure Hg_{CBD}, Roulet and Lucotte (1995) did not observe any correlations between Fe_{CBD} and Hg_T in soils of French Guyana, either. These authors concluded that although Fe-oxides play an important role in Hg absorption, they do not solely control Hg retention in these soils.

Hg was significantly associated to iron oxides in the oxisol

Iron oxide dissolution is controlled by crystallinity and surface area (Trolard et al. 1995; Cornell and Schwertmann 2003). Moreover, several studies have demonstrated that high Al substitution in Fe-oxides decreases their solubility (Rajot 1992; Bousserrhine et al. 1998, 1999). During the chemical Fe-oxide reduction, Fe was continuously dissolved to a maximum as observed by other authors (Bousserrhine 1995; Trolard et al. 1995). Our results did not enable us to distinguish different iron oxides by variations in the rates of dissolution as the dissolution curves were smooth; this has also been pointed out by Trolard et al. (1995). The curves of Hg release, during chemical Fe-oxide reduction, were similar to Fe release, especially in the CBD fraction of the oxisol. Although no significant correlation was previously calculated between Hg_T and Fe_{CBD}, the regression analysis carried out between Hg_{CBD} and Fe_{CBD} demonstrated a clear relationship between the two metal fractions ($R^2 = 0.84$).

In the hydromorphic soil, H, little Fe or Hg was solubilised in the CBD or the ascorbate fraction and no significant relationships could be established between the two metals. However, total mercury concentrations in this soil went up to 338 ng g^{-1} soil in the deepest sample (90–100 cm), which suggests that there could be other important mercury adsorbing phases, other than iron oxides and organic matter.

In the oxisol, O, the percentages of Hg_T in the extraction fractions were higher for CBD than for ascorbate, although the ratio nmol Hg/μmol Fe was much higher in the amorphous Fe-oxide fraction, thus suggesting that it is only the small amount of amorphous Fe-oxides that limits their Hg adsorption. Indeed amorphous Fe-oxides have the largest surface areas and are reputed to be more efficient adsorbents for trace elements than well crystallized oxides (Cornell and Schwertmann 2003).

Bacterial iron reduction was related to carbon mineralisation

Due to the first results obtained with the chemical Fe-oxide reduction, bacterial Fe-reduction was only carried out in soils from the oxisol profile. The three horizons studied (50–60, 70–80 and 90–100 cm) were chosen because of the variations in colour, and thus theoretically their different contents in Fe-oxide types, e.g. goethite and hematite. These depths also differed in their carbon contents which could influence microbial density and activity. Thus, we tried to cover the vertical differentiation of our soil profile.

Although soil characteristics varied with depth, this did not interfere on carbon mineralisation, induced by the addition of glucose, or iron solubilisation as they were no significant differences between horizons ($P_{Fe} = 0.643$ and $P_{CO2} = 0.617$). Eh decreased rapidly in all microcosms and all of the dissolved Fe was ferrous Fe (Fe^{II}) indicating that Fe was dissolved by a reduction process. The correlation between Fe solubilisation and carbon mineralization indicated that reduction was biologically mediated (Lovley and Phillips 1986; Bousserrhine et al. 1999). All of the glucose was consumed after 3 days incubation and up to 72% of the provided carbon was transformed into CO_2. This also corresponded to a decrease in soil respiration and Fe^{II} production, thus suggesting that in the present study, available carbon was the limiting factor, in which case we have possibly underestimated the potential bacterial Fe-oxide reduction in these soils. Another limiting factor could be the accessibility of reducible iron as suggested by Stemmler and Berthelin (2003). Indeed, while in the case of chemical CBD extractions, a citrate buffer prevented metal precipitation in the solution, in natural conditions, however, soluble aluminium and iron can re-precipitate on the surfaces

of iron oxides and prevent their reduction by bacteria (Dominik et al. 2002).

Bacterial iron reduction influenced Hg distribution in soils

According to the chemical extractions, the bacterial reduction of 3 mg g^{-1} soil in the 50–60 and 70–80 cm horizons and of 3.5 mg g^{-1} in the 90–100 cm horizon could have led to the solubilisation of 2.4, 1.6 and 1.3 ng Hg g^{-1} soil if we consider the CBD fraction (amorphous plus well crystallised oxides) or to the solubilisation of 12.8, 14.6 and 15.1 ng Hg g^{-1} soil if we just consider the amorphous fraction. However, although we have observed the solubilisation of other trace metals such as Ni and Cr (unpublished data), in the present experiment we did not measure an increase of Hg_D in the microcosm medium, or a decrease of Hg_T in the solid phase.

Nevertheless, when selective extractions were performed on the soil samples after microbial incubations, the amounts of mercury associated to Fe_{Asc} and Fe_{CBD} had decreased significantly, even more than the theoretical amounts that should have been mobilized by iron reduction (in comparison to chemical reduction). This Hg could then have re-precipitated on metabolites or formed organo-mercurial complexes, or more likely, due to the presence of sulphates in Bromfield medium, and their reduction to sulphides towards the end of the incubation, Hg could have re-precipitated in the form of HgS. Indeed, for $[S^{2-}] > 1.8$ mg g^{-1} soil; all dissolved Hg forms HgS. However, in laboratory conditions, bacterial iron reduction carried out on pure goethite have shown that 100% of Fe_{CBD} can be reduced by ferri-reducing bacteria (Bousserrhine et al. 1999). If this were the case in the oxisol used in this study then in the right conditions and with a sufficient source of carbon, we can imagine that kilograms of mercury could rapidly be remobilized in the watersheds.

Conclusion

Our results demonstrate that a significant amount of Hg is adsorbed to Fe-oxides in the Fe-rich oxisol used in this study. During the chemical reduction of iron oxides, mercury was significantly leached from the soils, whereas mercury solubilisation was not detected during the microbially-mediated iron reduction. However, during the later, the distribution of Hg on amorphous and well crystallized iron oxides was modified. Further work would enable to (i) assess the dynamics of Hg associated to Fe oxides in soils, in relation to soil pedogenesis and water table fluctuations (ii) estimate the amounts of Fe and Hg that can be mobilized by bacteria in natural conditions, i.e. without glucose or Bromfield medium, and (iii) identify the micro-organisms present in the soils, and their potential to transform the available Hg.

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