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

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1   Short title: **Iron oxide and mercury biogeochemistry in tropical soils**

2

3   **General research paper**

4

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6   **French Guyana**

7

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1   **Abstract**

2

3   Iron oxy(hydr)oxides (oxides) are important mercury sinks in tropical oxisols and the

4   geochemistry of these two elements are thus closely entwined. We hypothesized that bacterial

5   Fe-oxide reduction in anoxic conditions could be a significant mechanism for mobilizing

6   associated Hg. Iron oxide and mercury solubilisation in presence of two chemical reducers

7   (ascorbate and dithionite, dissolving amorphous and amorphous plus well crystallized Fe-

8   oxides, respectively) was compared to their solubilisation in presence of autochthonous ferri-

9   reducing bacteria. This work was carried out on two soil profiles from a small catchment

10   basin in French Guyana, an oxisol (O) from a well drained slope and a water-saturated

11   hydromorphic soil (H).

12   The chemical reductions showed that in the oxisol 20 and 48 % of total Hg (Hg_T) was

13   associated to amorphous and well crystallized iron oxides, respectively. However, in the

14   hydromorphic soil, no Hg seemed to be associated to amorphous iron oxides while the well

15   crystallized fraction contained less than 9 % of Hg_T. Chemical Fe-oxide reduction showed

16   that Hg solubility was correlated to Fe reduction in the oxisol, demonstrating a relationship

17   between the geochemistry of these two metals.

18   During bacterial growth, while bacterial iron reduction solubilised up to 3.2 mg Fe g^{-1}

19   soil in the oxisol sample, Hg_T remained unchanged. No mercury was detected in the culture

20   medium either. However, chemical analysis showed a decrease of the amounts of Hg

21   associated to amorphous and well crystallized Fe-oxides after 14 days of incubation,

22   underlining the potential for iron-reducing bacteria to modify mercury distribution in soil.

23

24

25

1 Introduction

2

3 Amazonian soils have been accumulating atmospheric mercury (Hg) for several millions of

4 years and register natural background levels of Hg up to ten times those found in temperate

5 soils (Carmouze *et al*., 2002). On top of that, human activities, and in particular gold mining,

6 have contributed to raising the levels of Hg in these regions. Indeed, it has been estimated that

7 around 5 000 tons of Hg have been dumped in the whole Amazonian area since the beginning

8 of the gold rush towards the end of the 19th century (Nriagu, 1994).

9   In pristine soils of the Amazonian basin, high mercury concentrations have been

10 measured in Fe-oxide rich oxisols that contain little organic matter. Conversely, in

11 hydromorphic soils that contain little Fe, lower Hg concentrations have been measured.

12 Several studies have identified relationships between Hg and Fe contents in soils (Roulet and

13 Lucotte, 1995; Roulet *et al*., 1998; Roulet and Grimaldi, 2001) and it is now generally

14 accepted that in organic-poor soils, such as oxisols, Fe-oxides play an important role in

15 adsorbing Hg. Thus, the geochemistry of these two elements may be strongly linked. This

16 explains the lower amounts of Hg measured in hydromorphic soils in which most of the iron

17 has been reduced and exported, along with the mercury, via the aquifer.

18   The Hg adsorbed to Fe-oxides has little risk of being exported from the well drained

19 oxisols, except via natural surface erosion and soil transformation. However, modern gold

20 mining and deforestation expose soils to sunlight and rain and the excavation of the gold-

21 containing soils causes previously immobile mercury to encounter conditions that (i) favour

22 its erosion and transport in watersheds (Oliveira (de) *et al*., 2001; Lacerda *et al*., 2004;

23 Béliveau *et al*., 2009) and (ii) create disorganized hydromorphic areas where all the

24 geochemical conditions necessary to favour methylation are brought together (Guedron,

25 2008). Indeed, Hg methylation is generally considered to occur in anoxic soils and sediments

1   (Morel *et al.*, 1998), mainly by microbial methylation of inorganic Hg (Gadd, 1993; King *et*

2   *al.*, 2002). Once methylated, methylmercury (MeHg) can be transported via the aquifer or by

3   soil water runoff or erosion, to streams and rivers (Morel *et al.*, 1998; Wasserman *et al.*,

4   2003). Once in the water system, MeHg is readily accumulated along the food chain and can

5   become an environmental and sanitary hazard as it can be consumed by local fish-eating

6   populations (Veiga *et al.*, 1999).

7

8   Although geochemical conditions and erosion are a well recognized means of

9   remobilizing Hg, little attention has been given to soil microbial communities, although their

10   activity could play an important role in Hg distribution and speciation. Indeed, in natural

11   environments, and in particular in the tropics, microbial Fe-reduction is a major factor in the

12   mobilization of Fe (Stemmler and Berthelin, 2003). Ferri-reducing bacteria reduce Fe in

13   anoxic conditions by using it either as a major final electron accepter in anaerobic respiration

14   or as a minor electron accepter during fermentation metabolism. During this process, it has

15   been demonstrated that heavy metals adsorbed to iron oxides can be mobilized. (Addy *et al.*,

16   1976; Schwertmann and Latham, 1986; Francis and Dodge, 1989; Lovley, 1993; Trolard *et*

17   *al.*, 1995; Bousserrhine *et al.*, 1999; Quantin *et al.*, 2001; Quantin *et al.*, 2002; Cornell et

18   Schwertmann, 2003; Garrido *et al.*, 2003; Bradl, 2004; Neaman *et al.*, 2004; Noubactep *et al.*,

19   2005; Cooper *et al.*, 2006). However, although bacterial Fe reduction is frequently mentioned

20   as being an important factor of heavy metal mobilization, to our knowledge, there is no

21   available data on the impact of this reductive dissolution on Hg mobility.

22

23   The aim of this study was to evaluate and compare the impact of chemical and

24   microbial Fe-oxide reduction on the mobilization and distribution of associated Hg in tropical

25   ferralitic soils. The chemical reducers used were ascorbate and dithionite that extract

1 amorphous (Fe$_{Asc}$) and amorphous plus well crystallized (Fe$_{CBD}$) Fe-oxides, respectively.

2 Microbial reduction was carried out with autochthonous soil bacteria. The soils studied were

3 an oxisol (O) and a hydromorphic soil (H) from a small catchment basin in French Guyana of

4 which the downstream flats were gold-mined towards the end of the 19th century and again in

5 the middle of the 20th century. Thus, high Hg concentrations could be found locally.

6

7 **Material and methods**

8

9 *Sample collection*

10

11 Soil samples were collected in December 2005 from the catchment basin of the Combat creek

12 (1 km^2), situated at approximately 10 km from the village of Cacao in French Guyana

13 (52°23'W, 4°35'N). Soil profiles up to 1 m deep were sampled with a metal auger, and each

14 10 cm layer was immediately sealed in sterile hermetic polyethylene bags. Two soil profiles

15 were considered in this study: an oxisol (O) collected from a well drained slope and a

16 hydromorphic soil (H) from the toeslope. These soils did not appear to have been directly

17 affected by gold-mining as a logical continuity in vertical variations was observed; for

18 example organic matter getting scarce with depth.

19

20 *Soil preparation and characterization*

21

22 The collected samples were air dried at 25°C, sieved at 2 mm and hermetically sealed at 4°C

23 until use. Initial analyses were carried out on all samples by the National Institute of

24 Agronomic Research (INRA, Arras, France). They consisted in the determination of 5

25 granulometric fractions without decarbonation (NF X 31-107), organic carbon (C) and total

1   nitrogen (N) (NF ISO 10694 and NF ISO 13878), and total Fe (NF EN ISO 11885). Finally,

2   pH was measured in water with a 1:2.5 ratio soil: solution (NF ISO 10390).

3

4   *Chemical iron reduction*

5

6   Chemical iron reduction was followed over time on homogenised samples of the 70-80 cm

7   deep horizon of O and H using ascorbate and dithionite to reduce amorphous (Fe_{Asc}) and well

8   crystallized Fe-oxides (Fe_{CBD}), respectively. These samples were chosen firstly because of

9   their depth which induced low organic matter contents, thus increasing the possibilities of

10 interactions between mercury and mineral elements, and secondly for their total iron and

11 mercury contents which made them representative of their soil type (i.e. oxisol or

12 hydromorphic soil).

13

14   Amorphous Fe-oxides were extracted using a modified application of the ascorbate

15 method as described by Ferdelman, (1988). Briefly, 10 g of sodium citrate ($Na_3C_6H_5O_7$) and

16 10 g of sodium bicarbonate ($NaHCO_3$) were added to 200 ml of ultra pure water that was

17 deionised by bubbling with filtered nitrogen, before adding 4 g of ascorbic acid, for a final pH

18 of 8. One gram of soil (dry weight, dw) was placed in a 30 ml polypropylene centrifugation

19 tube (Nalgene, France) with 10 ml of reagent and agitated at room temperature. This

20 temperature was chosen in order to avoid Hg volatilization.

21   Crystallized Fe-oxides were extracted using the Carbonate, Bicarbonate, Dithionite

22 (CBD) method of Mehra and Jackson (1960), modified by Jeanroy *et al.* (1991). Briefly, 0.2 g

23 soil (dw) was placed in a centrifuge tube with 20 ml of reagent (78.4 g l^{-1} sodium citrate and

24 9.8 g l^{-1} sodium bicarbonate in ultra pure water) for a 1/100 soil weight/reagent ratio. Sodium

1   dithionite was added at a ½ ration soil weight/dithionite (0.4 g tube^{-1}), the tubes were agitated

2   and incubated under agitation at room temperature.

3   During the ascorbate extraction, triplicate samples were harvested after 6, 12, 24, 48,

4   72 and 120 hours of extraction, and during the dithionite extraction, triplicate samples were

5   harvested after 2, 4, 8, 12, 24, 48 and 96 hours of extraction. Samples were immediately

6   centrifuged at 5000 x g for ten minutes at 4°C. The pellets were resuspended in 2 ml of ultra

7   pure water, centrifuged a second time and both supernatants were pooled. The supernatant

8   was then filtered (0.45 µm acid rinsed filtered, Minisart SRP 25, Sartorius) and acidified (pH

9   1) with HCl (37 %) to prevent metal precipitation and preserved at 4°C until analysis. The

10   remaining soil pellet was air dried and crushed to < 100 µm and also conserved at 4°C until

11   further analyses.

12   Chemical extracts were analyzed for Hg and Fe, as described below. Moreover, Fe_{Asc}

13   and Fe_{CBD} were also extracted from the 50-60 and 90-100 cm deep horizons of soils O and H,

14   with an extraction time of 72 h. This extraction time was chosen after the extraction kinetics

15   demonstrated that the maximum amount of iron was extracted after 72 hours.

16

17   *Microbial iron reduction*

18

19   As chemical extractions showed (see results), significant quantities of Hg and Fe were

20   leached only in the Fe-rich oxisol (O), thus, only this soil was investigated in bacterial iron

21   reduction experiments. The three depths used correspond to different soil colours, described

22   according to the Munsell Soil Colour Charts (2000). At 50-60 cm, the soil was dark

23   yellowish brown (10YR4/6), at 70-80 cm it started evolving towards a heterogenic dark

24   brown and red colour, and at 90-100 cm, it was clearly double phased with brownish-yellow

25   and red volumes (5YR5/6 and 10R4/8).

1   Microbial iron reduction was followed in soil microcosms set up in sterile hermetic

2   250 ml glass plasma bottles (BioBlock, France). Microcosms consisted in 5 g of each soil

3   sample and 100 ml of modified Bromfield medium (Bousserrhine, 1995), of the following

4   composition for one litre of MilliQ® water; $MgSO_4.7H_2O$ (0.5 g); K_2HPO_4 (0.5 g);

5      $(NH_4)_2SO_4$ (1 g), yeast extract (0.15 g) and glucose (1 g). Each soil sample was prepared in

6   triplicates. Anoxic conditions were initiated by flushing microcosms with N_2 for 10 min.

7   Incubations were carried out in the dark at 28°C for 14 days without shaking except prior to

8   sampling.

9   During the incubation, several parameters were measured in the culture medium:

10   carbon mineralization, glucose consumption, Fe(II) production and Hg solubilisation. At the

11   end of the incubation, the microcosm medium was recovered and analyzed for Hg and Fe, and

12   total Hg was measured in the soil. Moreover, the soil was subjected to Fe_{Asc} and Fe_{CBD}

13   selective extractions as described previously.

14

15   *Mercury analysis*

16

17   Total Hg (Hg_T), in soils and in chemical extracts, was analyzed with an Automatic Mercury

18   Analyzer (AMA 254, Courtage Analyses, France), detection limit 0.01 ng. Soil samples were

19   crushed in an agate mortar to < 100 µm. About 100 mg were then placed in the AMA 254 and

20   analyzed. In brief, the sample is heated to 550°C and all products of decomposition, including

21   mercury, are carried by a stream of oxygen through a catalytic tube where Hg_T is transformed

22   into elementary mercury (Hg°) which then readily adsorbs to a gold-trap. The fixed mercury

23   is then released by heating the gold-trap at 450°C and the mercury quantified by atomic

24   adsorption spectrometry. Concentrations of standard reference materiel (MESS-3) did not

25   exceed the range of concentrations announced (0.091 ± 0.008 µg g^{-1}). Extraction supernatants

1   were analyzed using the same method by analyzing 400 µl of each sample. All liquid samples

2   were previously filtered (< 0.45 µm Teflon filters, Minisart SRP 25, Sartorius) and acidified

3   to pH 1 (HCl, 37 % Suprapur).

4

5   Total dissolved Hg (Hg_D) in bacterial microcosm medium was analyzed by Cold

6   Vapor Atomic Fluorescence Spectrophotometer (CVAFS), detection limit 17 pg, as described

7   by Cossa *et al*. (2002). All liquid samples were previously filtered (< 0.45 µm Teflon filters,

8   Minisart SRP 25, Sartorius) and acidified to pH 1 (HCl, 37 % Suprapur). Briefly, the sample

9   is mineralized with $BrCl_2$, and reduced to elemental mercury (Hg°) with stannous chloride

10   ($SnCl_2$). A bubbling system then causes the elemental mercury to be caught on a gold trap

11   before being measured using a 2500 CVAFS Mercury detector (TEKRAN, Canada).

12   Concentrations of standard reference material (ORMS3) did not exceed the range of

13   concentrations announced (12.6 ± 1.1 pg g^{-1}).

14

15   *Iron analysis*

16

17   Chemical extracts and microcosm medium were analyzed after filtration (0.45 µm acid-

18   washed membrane filter, Minisart SRP 25, Sartorius) and acidification at pH 1 with HCl (37

19   %) by ICP-AES (Vista MPX, Varian) for total Fe. Standards were prepared from an ICM 240

20   (Promochem, VWR) solution.

21   Reduced Fe (Fe^{II}), was also measured in the medium at regular intervals (after 1, 2, 3,

22   4, 9 and 14 days of incubation) using a colorimetrical method with orthophenanthroline-

23   chlorydrate. Briefly 0.1 to 1 ml of filtered samples (0.45 µl acid rinsed filters, Minisart SRP

24   25, Sartorius) were put in a 25 ml graduated glass vial with 1 ml of orthophenanthroline (0.5

25   %) and completed to 25 ml with MilliQ water. After 10 min the absorbance was read at 490

1 nm. Readings were compared to a standard curve established with dissolved anhydrous

2 ferrous sulphate ($Fe(NH_4)_2(SO_4)_2.6H_2O$).

3

4 *Bacterial metabolism*

5

6 During the incubations, carbon mineralization was followed by measuring CO_2 evolution

7 daily in the microcosm's atmosphere with an infrared spectrophotometer (Berly 100, Cosma).

8   Glucose concentration in microcosms was measured daily in samples of microcosm

9 medium with a D-Glucose enzymatic test (Boehringer Mannheim, France). Samples were

10 filtered (0.45 μm, Minisart, Sartorius) prior to analysis.

11   Sulphate reduction was followed by measuring sulphide (S^{2-}) formation in the culture

12 medium. This was carried out using a kit (Spectroquant, Merck), analogue to NF ISO 10530.

13 This kit measures sulphide concentrations in a range of 0.02 to 1.5 mg $S^{2-}l^{-1}$. Samples were

14 filtered (0.45 μm, Minisart, Sartorius) prior to analysis.

15   Finally, redox potential and pH were measured in microcosms at the beginning and the

16 end of the incubation with Eh/pH electrodes (Metrohm, France).

17

18 *Statistical analysis*

19

20 Homogeneity of variances was checked using a Bartlett's test and normality using a Shapiro-

21 Wilk's test. When variances were not homogeneous between replicates, real standard

22 deviations (SD) were presented in the figures; otherwise, the overall SD was calculated and

23 displayed as the interval associated to each sample mean. Our data (CO_2 and iron

24 solubilisation) did not follow a normal distribution (P-value < 0.05), thus, a Friedman non

25 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} ± 0.003 in the ascorbate and 0.223 ng Hg g^{-1} ± 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.

1 **Results**

2

3 *Soil characteristics*

4

5 Table 1 presents soil texture and organic carbon contents in three depths of each soil profile.

6 The oxisol (O) was very rich in clay, up to 75 % with quantities of fine silt and coarse sand

7 ranging from 5 to 15 %. The texture triangle defines this soil as clay. The hydromorphic soil

8 (H) was not as rich in clay (< 35 %), its dominant component being silt (~ 45 %), followed by

9 sand (up to 40 %). The texture triangle describes this soil as a fine clay-loam soil. Organic

10 carbon contents were low and decreased with depth in both soils.

11

12 Fe_T and Hg_T soil contents were very different in O and H (Table 2). Quantities of Fe_T

13 were up to 10 times higher in O compared to H and ranged from 198.1 mg Fe g^{-1} soil in the

14 50-60 cm horizon, to 290.6 mg Fe g^{-1} soil in the 90-100 cm horizon. In H, the maximum Fe_T

15 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

16 the 90-100 cm horizons, respectively. Hg_T decreased slightly with depth in O, from 294.1 ng

17 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

18 was on average 134 ng Hg g^{-1} soil in the first two horizons and then increased to 338.6 ng Hg

19 g^{-1} soil in the 90-100 cm one.

20

21 **Insert Table 1**

22

23 The selective extractions carried out before kinetic reductions (Table 2) showed a

24 good repeatability. In O, the percentage of Fe_{Asc}/Fe_T decreased with depth from 6.9 to 1.7 %,

25 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,

1 and 48 % of Hg_T. Although significant quantities of Hg were leached with Fe_{Asc} and Fe_{CBD}

2 extractions, no significant correlation was observed between the two elements.

3   In H, the ascorbate extraction yielded up to 14 % of Fe_T, however, Hg_{Asc} was not

4 detectable (nd) by AMA 254 in the extractant (Table 2). The CBD extracted 100 % of Fe_T in

5 the 90-100 cm horizon and up to 9 % of Hg_T in the 70-80 cm horizon. No significant

6 correlations were observed between Fe and Hg in H.

7

8   **Insert Table 2**

9

10 *Kinetics of chemical iron oxide reduction*

11

12   The kinetics of chemical Fe-oxide reduction by ascorbate and CBD are presented in

13 Fig. 1. The citrate in CBD acts as a complexant and prevents Al, Fe or other metals from

14 precipitating. In O, Hg and Fe were leached simultaneously over time with both ascorbate and

15 CBD. During ascorbate extraction, the maximum of Hg_{Asc} and Fe_{Asc} were leached after 48

16 hours, while during CBD extraction, Hg_{CBD} was leached faster (12 h) than Fe_{CBD} (48 h). In H,

17 on the contrary, no Hg_{Asc} was detected along with Fe_{Asc}, but Hg_{CBD} appeared to be leached

18 simultaneously to Fe_{CBD} in this soil, although values were a lot smaller than in O.

19

20   **Insert Fig. 1**

21

22   Regression analyses were run between Hg and Fe leached during Fe chemical

23 extractions when possible. Results demonstrate a clear correlation (R^2 = 0.84, P = 0.003)

24 between Fe and Hg extracted with dithionite and a less significant correlation (R^2 = 0.51, P =

1 0.070) for ascorbate (Fig. 2). In H on the other hand, the relation between Fe and Hg for

2 dithionite gave a R² = 0.21 and could not be calculated for ascorbate as Hg was not detectable.

3

4   **Insert Fig. 2**

5

6 *Microbial iron oxide reduction*

7

8 Carbon mineralization (Fig. 3) increased considerably during the first 4 days of incubation in

9 all three horizons (Friedman test, P < 0.001), before slowing down. The amount of C

10 mineralized at the end of the experiments was 5.1 ± 0.3 mg g^{-1} soil on average and no

11 significant differences were given between soil depths (Kruskal Wallis test, P = 0.617).

12 Glucose was totally consumed after four days (Fig. 3). The amount of carbon that evolved in

13 the form of CO_2 in the microcosm atmosphere represented 72 % of the carbon provided by the

14 glucose.

15

16   **Insert Fig. 3**

17

18   The redox potential (Eh), decreased rapidly (data not shown) and the Eh measured at

19 the end of the incubations was -190 ± 47 mV on average in all microcosms. The pH increased

20 during incubations in all three soils from 4.7 ± 0.25 to 5.3 ± 0.25.

21   The formation of sulphides was detected from day 8 onwards, and at the end of the

22 incubation we measured on average 80 µg S^{2-} g^{-1} soil in the microcosm medium (data not

23 shown).

24

14

1   **Insert Fig. 4**

2

3   During the incubation, total dissolved Fe (Fe_D) increased in the microcosm medium

4   (Fig. 4). Slightly more Fe was solubilised in the 90-100 cm horizon; 3.5 mg Fe g^{-1} soil,

5   compared to the 50-60 and 70-80 cm horizons; 3 mg Fe g^{-1} soil at the end of the incubation

6   but overall differences were not significant (Kruskal-Wallis test, P = 0.643). These solubilised

7   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-

8   80 and 90-100 cm horizons respectively. A colorimetric test with Orthophenantroline analysis

9   indicated that all of this Fe was in the form of Fe^{II} (data not shown), confirming that Fe

10   solubilisation was due to bacterial reduction. Moreover, a significant polynomial correlation

11   was calculated between Fe solubilisation in the inoculation medium and CO_2 evolution (Fig.

12   5).

13

14   **Insert Fig. 5**

15

16   According to the chemical reduction of iron, the solubilisation of 2.1, 1.5 and 1.8 %

17   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

18   the solubilisation of 21, 42 and 70 % of Fe_{Asc} to the solubilisation of 12.8, 14.6 and 15.1 ng

19   Hg g^{-1} soil in the 50-60, 70-80 and 90-100 cm horizons, respectively. However, dissolved Hg

20   (Hg_D) was very low and constant throughout the experiment, averaging 8.6 ± 2.3 ng g^{-1} soil,

21   as was the total mercury in the solid phase.

22

23   The amount of Hg (nmol) extracted per µmol Fe in the ascorbate and CBD fractions

24   before and after the incubation is presented in Fig. 6. Results showed that Hg was much more

25   concentrated in the amorphous fraction (up to 14 nmol Hg $µmol^{-1}$ Fe) than in the CBD

1 fraction (< 0.25 nmol Hg µmol^{-1} Fe). After the incubation, these ratios had significantly

2 decreased (Student t tests) in the 50-60 and 70-80 cm horizons for the ascorbate extractions (P

3 < 0.05) but not in the 90-100 cm horizon (P = 0.077). They had also significantly decreased in

4 all the horizons in the CBD extraction (P < 0.05).

5

6 **Insert Fig 6**

7

8 **Discussion**

9

10 The aim of this study was to compare chemical and microbial reduction of iron oxides in

11 tropical ferrallitic soils of French Guyana, and to evaluate its impact on mercury release. It is

12 a well accepted fact that Fe-oxides are sinks for various major and trace metals including Al,

13 Mn, Cr, Ni, Cd, Zn, Co, Pb and U, and that bacterial Fe-reduction contributes to re-mobilizing

14 these metals (Schwertmann, 1991; Bousserrhine *et al*., 1998; Bousserrhine *et al*., 1999;

15 Quantin *et al*., 2001; Dominik *et al*., 2002; Quantin *et al*., 2002; Stemmler and Berthelin,

16 2003; Cooper *et al*., 2005; Noubactep *et al*., 2005; Cooper *et al*., 2006). It is also more and

17 more accepted that Fe-oxides play an important role in the geochemistry of Hg, especially in

18 iron-rich tropical soils (Roulet and Lucotte, 1995; Roulet *et al*., 1998; Grimaldi *et al*., 2001).

19 However, although re-mobilization of Hg by Fe-oxide reduction has often been suggested, to

20 our knowledge, there is no data that demonstrates it.

21 A kinetic approach of the reduction of Fe-oxides by chemical and microbial processes

22 was used to determine the potential solubilisation of Fe and Hg in tropical ferrallitic soils of

23 French Guyana. The results enabled us to estimate the amounts of Hg associated to

24 amorphous and well crystallized Fe-oxides, which can potentially be mobilized in anaerobic

25 conditions by ferri-reducing bacteria.

1

2 *Soil characteristics and Fe and Hg distribution*

3

4 Both soils under study, O and H, were very different from a textural point of view and

5 concerning Fe_T and Hg_T contents. O is an oxisol, containing a lot of clay and iron oxides, as

6 reported for these types of soils (Boulet *et al*., 1993), whereas the hydromorphic soil

7 contained little clay and Fe, but more sand and silt, due to soil transformations in anoxic

8 conditions and the export of fine particles (Boulet *et al*., 1993; Tessier *et al*., 2003). The Hg

9 concentrations measured in the oxisol were in the range of concentrations previously reported

10 in French Guyana; 240-320 ng g^{-1} soil (Roulet and Lucotte, 1995). In pristine hydromorphic

11 soils, these authors measured Hg contents in a range of 50-100 ng g^{-1} soil which is lower than

12 those measured in the present study (133-338 ng g^{-1} soil). This suggests that although soil

13 horizons were not apparently disturbed, past gold-mining had contaminated these soils.

14 Indeed, Guedron (2008) found droplets of metallic Hg in hydromorphic soils from the same

15 catchment basin as in this study that most probably came from past gold-mining.

16   In the oxisol, O, the maximum Fe_{CBD} yielded by selective extractions was 77 % of Fe_T.

17 The remaining iron is most probably resistant goethite or hard Fe nodules (Trolard *et al*.,

18 1995). Fe_{Asc} represented less than 7 % of Fe_T in all the studied depths of the O soil profiles,

19 and decreased with depth, indicating an increase in iron crystallinity (Fritsch *et al*., 2005).

20   In the hydromorphic soil, H, on the contrary, up to 100 % of Fe_T was extracted with

21 CBD, but not systematically. The presence of hard nodules in the soil profile indicates that

22 free iron has re-precipitated, probably during the dry seasons when soils are partially

23 oxygenated. Fe_{Asc} was slightly higher than in O (up to 14 %), and no relations were observed

24 with depth. In this blue-grey coloured soil, nearly all the iron oxides have been reduced and

25 exported, leaving only hard iron concretions.

1   Hg was only significantly leached during selective extractions of iron forms in O,

2   where Hg_{CBD} and Hg_{Asc} represented up to 48 and 19 % of Hg_T, respectively. However, there

3   wasn't a significant correlation between Fe_{Asc}, Fe_{CBD} and Hg_T. Although they didn't measure

4   Hg_{CBD}, Roulet and Lucotte (1995) did not observe any correlations between Fe_{CBD} and Hg_T in

5   soils of French Guyana, either. These authors concluded that although Fe-oxides play an

6   important role in Hg absorption, they do not solely control Hg retention in these soils.

7

8   *Hg was significantly associated to iron oxides in the oxisol*

9

10   Iron oxide dissolution is controlled by crystallinity and surface area (Trolard *et al*., 1995;

11   Cornell and Schwertmann, 2003). Moreover, several studies have demonstrated that high Al

12   substitution in Fe-oxides decreases their solubility (Rajot, 1992; Bousserrhine *et al*., 1998;

13   Bousserrhine *et al*., 1999). During the chemical Fe-oxide reduction, Fe was continuously

14   dissolved to a maximum as observed by other authors (Bousserrhine, 1995; Trolard *et al*.,

15   1995). Our results did not enable us to distinguish different iron oxides by variations in the

16   rates of dissolution as the dissolution curves were smooth; this has also been pointed out by

17   Trolard *et al*. (1995). The curves of Hg release, during chemical Fe-oxide reduction, were

18   similar to Fe release, especially in the CBD fraction of the oxisol. Although no significant

19   correlation was previously calculated between Hg_T and Fe_{CBD}, the regression analysis carried

20   out between Hg_{CBD} and Fe_{CBD} demonstrated a clear relationship between the two metal

21   fractions (R^2=0.84).

22   In the hydromorphic soil, H, little Fe or Hg was solubilised in the CBD or the

23   ascorbate fraction and no significant relationships could be established between the two

24   metals. However, total mercury concentrations in this soil went up to 338 ng g^{-1} soil in the

1   deepest sample (90-100 cm), which suggests that there could be other important mercury

2   adsorbing phases, other than iron oxides and organic matter.

3   In the oxisol, O, the percentages of Hg_T in the extraction fractions were higher for

4   CBD than for ascorbate, although the ratio nmol Hg / µmol Fe was much higher in the

5   amorphous Fe-oxide fraction, thus suggesting that it is only the small amount of amorphous

6   Fe-oxides that limits their Hg adsorption. Indeed amorphous Fe-oxides have the largest

7   surface areas and are reputed to be more efficient adsorbents for trace elements than well

8   crystallized oxides (Cornell and Schwertmann, 2003).

9

10 *Bacterial iron reduction was related to carbon mineralisation*

11

12   Due to the first results obtained with the chemical Fe-oxide reduction, bacterial Fe-

13 reduction was only carried out in soils from the oxisol profile. The three horizons studied (50-

14 60, 70-80 and 90-100 cm) were chosen because of the variations in colour, and thus

15 theoretically their different contents in Fe-oxide types, e.g. goethite and hematite. These

16 depths also differed in their carbon contents which could influence microbial density and

17 activity. Thus, we tried to cover the vertical differentiation of our soil profile.

18   Although soil characteristics varied with depth, this did not interfere on carbon

19 mineralisation, induced by the addition of glucose, or iron solubilisation as they were no

20 significant differences between horizons (P_{Fe} = 0.643 and P_{CO2} = 0.617). Eh decreased rapidly

21 in all microcosms and all of the dissolved Fe was ferrous Fe (Fe^{II}) indicating that Fe was

22 dissolved by a reduction process. The correlation between Fe solubilisation and carbon

23 mineralization indicated that reduction was biologically mediated (Lovley and Phillips, 1986;

24 Bousserrhine *et al.*, 1999). All of the glucose was consumed after 3 days incubation and up to

25 72 % of the provided carbon was transformed into CO_2. This also corresponded to a decrease

1   in soil respiration and Fe^{II} production, thus suggesting that in the present study, available

2   carbon was the limiting factor, in which case we have possibly underestimated the potential

3   bacterial Fe-oxide reduction in these soils. Another limiting factor could be the accessibility

4   of reducible iron as suggested by Stemmler and Berthelin (2003). Indeed, while in the case of

5   chemical CBD extractions, a citrate buffer prevented metal precipitation in the solution, in

6   natural conditions, however, soluble aluminium and iron can re-precipitate on the surfaces of

7   iron oxides and prevent their reduction by bacteria (Dominik *et al.*, 2002).

8

9   *Bacterial iron reduction influenced Hg distribution in soils*

10

11   According to the chemical extractions, the bacterial reduction of 3 mg g^{-1} soil in the

12   50-60 and 70-80 cm horizons and of 3.5 mg g^{-1} in the 90-100 cm horizon could have led to

13   the solubilisation of 2.4, 1.6 and 1.3 ng Hg g^{-1} soil if we consider the CBD fraction

14   (amorphous plus well crystallised oxides) or to the solubilisation of 12.8, 14.6 and 15.1 ng Hg

15   g^{-1} soil if we just consider the amorphous fraction. However, although we have observed the

16   solubilisation of other trace metals such as Ni and Cr (unpublished data), in the present

17   experiment we did not measure an increase of Hg_D in the microcosm medium, or a decrease

18   of Hg_T in the solid phase.

19   Nevertheless, when selective extractions were performed on the soil samples after

20   microbial incubations, the amounts of mercury associated to Fe_{Asc} and Fe_{CBD} had decreased

21   significantly, even more than the theoretical amounts that should have been mobilized by iron

22   reduction (in comparison to chemical reduction). This Hg could then have re-precipitated on

23   metabolites or formed organo-mercurial complexes, or more likely, due to the presence of

24   sulphates in Bromfield medium, and their reduction to sulphides towards the end of the

25   incubation, Hg could have re-precipitated in the form of HgS. Indeed, for $[S^{2-}] > 1.8$ mg g^{-1}

1   soil; all dissolved Hg forms HgS. However, in laboratory conditions, bacterial iron reduction

2   carried out on pure goethite have shown that 100 % of Fe_{CBD} can be reduced by ferri-reducing

3   bacteria (Bousserrhine *et al.*, 1999). If this were the case in the oxisol used in this study then

4   in the right conditions and with a sufficient source of carbon, we can imagine that kilograms

5   of mercury could rapidly be remobilized in the watersheds.

6

7   **Conclusion**

8

9   Our results demonstrate that a significant amount of Hg is adsorbed to Fe-oxides in the Fe-

10   rich oxisol used in this study. During the chemical reduction of iron oxides, mercury was

11   significantly leached from the soils, whereas mercury solubilisation was not detected during

12   the microbially-mediated iron reduction. However, during the later, the distribution of Hg on

13   amorphous and well crystallized iron oxides was modified. Further work would enable to (i)

14   assess the dynamics of Hg associated to Fe oxides in soils, in relation to soil pedogenesis and

15   water table fluctuations (ii) estimate the amounts of Fe and Hg that can be mobilized by

16   bacteria in natural conditions, i.e. without glucose or Bromfield medium, and (iii) identify the

17   micro-organisms present in the soils, and their potential to transform the available Hg.

18

19

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