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**Insight into the magnetic properties of Pb-doped iron oxide nanoparticles during
Fe(III) bio-reduction by *Shewanella oneidensis* MR-1**

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Abstract

Anthropogenic activities have led to a significant accumulation of Pb in the environment, posing a threat to ecosystems. Iron oxides that display magnetic properties are ubiquitous in the environment and Pb partitioning onto these minerals is considered one of the most critical geochemical processes controlling its environmental fate. In anoxic environments, iron oxides undergo redox cycling due to biotic and abiotic routes, resulting in their transformation/dissolution and evolution of their magnetic characteristics. However, there is still a lack of knowledge on the impact of Pb on the dynamic of iron oxides bioreduction. Furthermore, there is little information available regarding the nature of biogenic minerals and their magnetic signatures.

Here we incubated Pb-bearing ferrihydrites (Fh_Pb) with various Pb/(Fe + Pb) molar ratios (*i.e.*, 0, 2 and 5%) with the iron-reducing bacterium *Shewanella oneidensis* MR-1, for 21 days. XRD analyses of the initial Fh_Pb displayed characteristic features of Fh and a decrease of magnetization in the presence of Pb. During the bio-reduction process, Pb doping led to a drop in the rate and extent of reduction. At the end of the incubation period, the analysis of the aqueous solutions revealed a minor proportion of Pb in solution, indicating that a significant proportion of the Pb is sorbed onto the biogenic minerals. Magnetite (95%) and siderite (5%) formed during the bioreduction of Fh, while magnetite (~80%) and goethite (~20%) precipitated in the presence of Pb as revealed by transmission Mössbauer spectroscopy. Furthermore, the size of the magnetite particles decreased from about 11 nm in absence of Pb to 6 nm with 2% of Pb-bearing, while 5% of Pb led to particles too small to be quantified with our TEM measurements. The combined effect of the decrease in particle size of magnetite, substitution of Pb and the precipitation of goethite in the Pb-doped assay led to a significant decrease of magnetization at room temperature. Overall, this study highlights the effect of Pb on iron oxides

51 bio-reduction and transformation processes and the sensitivity of magnetism to serve as a
52 monitoring method.

53

54 **Keywords: Pb-bearing ferrihydrite, bioreduction, magnetite, goethite, transmission**
55 **Mössbauer spectroscopy, SQUID**

56

1. Introduction

Anthropogenic activities including mining and smelting have contributed to the release of important levels of lead (Pb) in the environment (Singh and Li, 2014). Lead possesses three oxidation states, Pb(0), Pb(II) and Pb(IV), with Pb(II) being the most frequently encountered form (Abadin et al., 2007). More than 200 mg kg⁻¹ of Pb are commonly measured in contaminated soils, while concentrations lower than 20 mg kg⁻¹ of Pb are generally detected in pristine soils (Gis Sol, 2011; Gutiérrez et al., 2016; Hernandez et al., 2003; Kabata-Pendias and Pendias, 2000; National Academies of Sciences, Engineering, and Medicine, 2017; Villen-Guzman et al., 2018). High levels of Pb poses a threat to human health and ecosystems in the form of contaminated soil and groundwater, and reduce food quality and land arability. In this context, the United States Environmental Protection Agency (US EPA) recommends Pb concentrations in drinking waters below 15 µg L⁻¹ (Martin and Griswold, 2009), while the World Health Organization (WHO) sets a maximum permissible Pb level of 100 mg kg⁻¹ in agricultural soils (Chiroma et al., 2014).

It was well established that Pb speciation in the environment is largely governed by physicochemical processes. It can precipitate as poorly soluble Pb compounds, can be complexed by organic matter and/or adsorbed on the mineral soil matrix (i.e. carbonate, clays, Mn-and Fe oxides) (Bradl, 2004; Fleming et al., 2013; Jensen et al., 2006; Liu et al., 2020). Fe oxides are of particular interest among the carrier phases of Pb. They are ubiquitous in the environment, accounting for up to 5% in weight of specific fractions (silts, clays) of surface soils and sediments (Journet et al., 2014). They display a high surface area and a reactive surface; hence, they exhibit significant sorption capacities for organic and inorganic species. Thus, Fe oxides exert significant control on the reactivity, speciation, and mobilization of

metals and metalloids such as Pb, Ni, Co, As, Sb (Erbs et al., 2010; Frierdich et al., 2011; Zachara et al., 2001; Zegeye et al., 2021). Pb, like other metals, interacts with the surface of Fe oxides and their (hydroxyl) functional groups (Cornell and Schwertmann, 2003). These interactions may lead to the surface adsorption (*via* complexation), or structural incorporation of the metals into the iron oxide crystal, and/or physical isolation within nanosized pores of iron oxides aggregates (Davranche and Bollinger, 2000; Hesterberg et al., 2011; Lu et al., 2011; Seda et al., 2016). Thus, Pb sequestration by iron (oxyhydr)oxides is one of the most critical geochemical processes controlling its environmental fate, transport, and bio-availability (Hassellöv and von der Kammer, 2008).

Numerous mineralogical and geochemical studies have proven the occurrence of a significant amount of iron oxides and various heavy metals such as Pb in a polluted environment (Jordanova et al., 2003). Given the magnetic properties of iron oxides, the interaction between metallic pollutants and Fe(III) minerals can be investigated through magnetic and structural measurements. Thus, magnetic approaches have been applied for soil heavy metal content monitoring and pollution assessment during the last decades (Canbay, 2010; Chaparro et al., 2006; Orosun et al., 2020; Schmidt et al., 2005; Wang et al., 2018). Several studies indicated significant positive correlations between soil magnetic parameters (*e.g.*, magnetic susceptibility, MS) and heavy metal contents. For instance, increasing arsenic concentration in the mineral structure led to an elevated magnetic signal (Ouyang et al., 2020). Therefore, magnetic signatures have the potential to detect the level of heavy metal sorbed on iron oxides due to the carrier phase (*i.e.*, iron oxide) magnetic properties.

Iron oxides are also the most abundant terminal electron acceptors in several anaerobic subsurface environments for dissimilatory iron-reducing bacteria (DIRB), such as *Geobacter* sp. and *Shewanella* sp (Bleam, 2012). These phylogenetically diverse bacteria can couple the oxidation of an organic (*e.g.*, formate, pyruvate, lactate) or inorganic (*e.g.*, H₂) electron source

to the reduction of Fe(III). The Fe(II) generated by the bio-reduction process is either liberated in the solution or further reacts with mineral surfaces and can lead to the precipitation of biogenic minerals such as magnetite (Fe₃O₄), goethite (α -FeOOH) and siderite (FeCO₃) (Hansel et al., 2018; Hansel et al., 2005, 2003; Pallud et al., 2010; Zachara et al., 2002). Concomitantly, adsorbed or co-precipitated metals, such as Pb, could either be released in the solution or partitioned on the solid phases (*i.e.*, initial iron oxide and/or the biogenic mineral). The mineralogic transformation due to bacterial activity also affects the magnetic signature of the system (Byrne et al., 2015; Porsch et al., 2010).

Relatively little is known regarding the effect of Pb on the microbiologically mediated Fe redox reactions, the precipitation of biogenic minerals, and the subsequent redistribution of Pb. It is also of interest to monitor the magnetic signal variation of iron oxides during their incubation with iron-reducing bacteria in order to depict the bio-reduction processes. To broaden our understanding of Fe and Pb biogeochemical cycles and the relevance of magnetic signature measurements, our study aims to investigate how various Pb concentrations impact the bio-reduction of iron oxides, the precipitation of biogenic minerals and their magnetic signals. To this end, we incubated Pb-bearing ferrihydrites with variable molar ratios in the presence of *Shewanella oneidensis* MR-1. With this set-up we aimed to study the changes in: i) the rate and extent of ferrihydrite bioreduction, ii) the redistribution of Pb among the liquid and solid phases, and iii) the nature of the minerals formed upon the bio-reduction and their magnetic signature.

2. Material and methods

All reagents and acids were of analytical grade and solutions were prepared using 18.2 MΩ cm⁻¹ deionized water. Anaerobic experiments and sample collection were conducted under strictly anaerobic conditions in a re-circulating anoxic chamber (Coy Laboratory Products Inc.) where trace amounts of oxygen were scavenged by palladium catalysts. A 98% N₂ + 2% H₂ atmosphere was maintained by automatized injection of the gas mixture.

2.1. Pb(II)-bearing ferrihydrite synthesis

Ferrihydrite was prepared by neutralization of 0.4 M FeCl₃ with 4 M NaOH. Pb-bearing ferrihydrites were synthesized similarly to pristine ferrihydrite except for the addition of PbCl₂ (8 to 20 mM PbCl₂ solutions) to the ferric salt before hydrolysis at Pb/(Fe+Pb) molar ratios of 0.02 and 0.05 (Schwertmann and Cornell, 2000). These samples will be named Fh, Fh_Pb 0.02, and Fh_Pb 0.05 (or the Fh_Pb series) hereafter. All precipitates were washed free of electrolytes by dialysis (1 nm pore size Spectra/Por® 7 dialysis membranes) until a steady conductivity < 20 μS cm⁻¹ was obtained.

2.2. Bioreduction of Pb(II)-bearing ferrihydrites

Shewanella oneidensis MR-1 cells were streaked under aerobic conditions on tryptic soy agar from -80 °C glycerol stock. They were sub-cultured once, and the colonies were used to prepare a suspension to inoculate 400 mL of trypticase soy broth (TSB). Cells were grown aerobically to the exponential growth phase in TSB under agitation at 180 rpm and at 28 °C.

They were harvested by centrifugation, washed twice with sterile 0.9% NaCl, and concentrated in the same medium (70 mL).

The bio-reduction assays were performed in a mineral medium (Table S1) that was heat sterilized, purged with filter-sterilized N₂ and dispensed into sterile 100 mL serum bottles, in the presence of Fh and Fh_Pb series (6.25 g L⁻¹ initial solids) and crimp sealed with butyl rubber stoppers. Sodium formate, as the sole electron source, and anthraquinone-2,6-disulfonate (AQDS), as an electron shuttle (Watanabe et al., 2009), were sterilized by filtration (filter pore size 0.2 µm), purged with filter-sterilized N₂ and were introduced in the culture medium under aseptic conditions in order to achieve 35 mM and 100 µM respectively in each 80 mL assay. *S. oneidensis* MR-1 cell suspensions were added to obtain final concentrations of $(1.85 \pm 0.15) \times 10^9$ cell mL⁻¹. The initial pH after mixing all the components was adjusted to ca. 7. The assays were incubated in the dark for 21 days in triplicates at 28°C. The control experiments were cell-free and otherwise identical to the biotic assays.

2.3 Chemical analyses

Total Fe and Pb concentrations in the initial solids were quantified using atomic absorption spectroscopy (AAS) after dissolution in 4 M HCl.

At each time point, the assays were shaken to ensure homogeneity of the slurries before sampling and extraction with 4 M HCl. Total Fe(II) (Fe(II)_{tot}) was determined by the ferrozine method (Viollier et al., 2000). The same procedure was used to measure total Fe (Fe_{tot}) but after reduction of the samples with hydroxylamine hydrochloride. The apparent reduction rate was computed from the first derivative of a nonlinear curve fit (Eq. 1).

$$\%(Fe(II)_{tot}/Fe_{tot})_t = \%(Fe(II)_{tot}/Fe_{tot})_{max} \times [1 - e^{-k_{obs} \times t}] \quad (1)$$

Where $\%(\text{Fe(II)}_{\text{tot}}/\text{Fe}_{\text{tot}})_t$ is the $(\text{Fe(II)}_{\text{tot}}/\text{Fe}_{\text{tot}})$ (%) produced at time t , $\%(\text{Fe(II)}_{\text{tot}}/\text{Fe}_{\text{tot}})_{\text{max}}$ is the maximum $\%(\text{Fe(II)}_{\text{tot}}/\text{Fe}_{\text{tot}})$ observed at the end of the reduction period (*i.e.*, 21 days), and k_{obs} is the pseudo first-order constant.

At the end of the incubation period, 1 mL was withdrawn from the assays, filtered through 0.22 μm pore filters and acidified in 2 M HCl to measure Pb in the aqueous phase by AAS.

2.4 Characterization of the solid phase

Powder X-ray diffraction (XRD) was performed using D8 Bruker diffractometer equipped with a monochromator, a position-sensitive detector and a Co anode ($K\alpha_1 = 0.178897$ nm radiation). The diffractograms were recorded in the $3\text{--}80^\circ$ 2θ range and a 0.03° 2θ step size with a collecting time of 3 s per point, using sample holders equipped with glass domes and filled under a glovebox to maintain the samples under a N_2 atmosphere. Phase identification was performed using the software DIFFRAC.EVA.

TEM observations and selected area electron diffraction (SAED) were carried out using a Philips CM200 TEM (200 kV) at the end of the incubation period (*i.e.*, 21 days). One drop of the suspension was laid on an amorphous carbon-coated grid and loaded into the analysis holder of the microscope under 10^{-8} Torr vacuum.

SQUID Magnetic measurements were performed using Quantum Design Superconducting Quantum Interference Device (SQUID). Magnetization versus magnetic field hysteresis loops were measured at 300 K and 2 K with field sweep range up to 7 T. Zero field cooled (ZFC) and field cooled (FC) measurements were also performed. The ZFC procedure was as follows: The sample were demagnetized at 300 K using an oscillatory decaying field from 1 T to zero field and then cooled to 2 K with a rate of 1 K min^{-1} . Afterwards ZFC magnetic moments were measured while the temperature was ramped back up from 2 K to 300 K at a

rate of 1 K min⁻¹ under 7.5 mT constant field. The FC procedure consists in cooling down the sample from 300K under 7.5 mT and then measuring moment while heating it up from 2 K back to 300 K.

⁵⁷Fe Mössbauer spectroscopy Transmission Mössbauer spectroscopy was performed between 298 K and 11 K with a constant acceleration Mössbauer spectrometer, 512 multichannel analyzer (Halder Electronic GmbH), and a 50 mCi source of ⁵⁷Co in Rh maintained at room temperature (RT). Data were obtained from appropriate amounts (10 mg of Fe per cm²) of solid samples to get optimal experimental conditions. Analyzes were carried out between ambient and 11 K on the ARS cryostat (Advanced Research Systems, Macungie USA) equipped with vibration isolation stand developed in the LCPME laboratory. Mössbauer spectra were collected in transmission mode. The 50 mCi source of ⁵⁷Co in Rh matrix was maintained at room temperature and mounted at end of a Mössbauer velocity transducer. The spectrometers were calibrated with a 25 µm foil of α-Fe at RT. Spectra were computer-fitted (recoil Software, Ottawa University) with the Voigt-based fitting model for paramagnetic sites corresponding to doublet which have a distributions of quadrupole splittings (QSDs) and magnetic sites corresponding to sextet which have a distribution of magnetic hyperfine fields (HFDs), as implemented in the Recoil software.

3 Results and discussion

3.1 Fh_Pb series characterization

The XRD pattern for the pristine Fh and Pb-bearing Fh (Fh_Pb(0.02) and Fh_Pb(0.05)) display only two diffraction peaks, at about 0.149 and 0.258 nm (Figure 1) (Schwertmann and Cornell, 2000). However, in the presence of Pb, we observe a shift of the most intense diffraction lines (*i.e.*, 0.258) to higher d values relative to the peak of the pristine Fh. In addition, the patterns of the Pb-bearing Fh indicate a line broadening which may imply either a decrease of the crystallite size or the incorporation of Pb in the Fh structure, lowering its crystallinity. No other peak indicative of additional phases was observed in the diffraction data.

The Fe and Pb contents of the co-precipitates were measured by AAS, and the obtained concentrations were approximately equal to the initial molar ratio of Pb/(Pb+Fe) (*i.e.*, 0.02, and 0.05) used to precipitate the solids.

To gain more insight into the influence of Pb-doping on ferrihydrite magnetic properties, we performed both isothermal magnetization versus field loops at different temperatures and ZFC/FC magnetization versus temperature measurements, on Fh, Fh_Pb(0.02) and Fh_Pb(0.05). At 250 K, Fh shows a linear increase of magnetization with field intensity with a slope of $1.5 \text{ A m}^2 \text{ kg}^{-1} \text{ T}^{-1}$ (Figure 2a). The same linear behavior is observed for Fh with 2% and 5% Pb but with a smaller slope, down to $1.1 \text{ A m}^2 \text{ kg}^{-1} \text{ T}^{-1}$ for 5% Pb. These results suggest that the moment of Fh decreases when adding Pb. In recent experiments on Sb-doping of Fh, whose chemical and structural characterization is reported in Zegeye et al. (2021), we also observed a similar trend (Figure 3a). As the concentration in Sb increases from 0 to 10%, the slope of the magnetic susceptibility at 250 K decreases from 1.45 to $0.65 \text{ A m}^2 \text{ kg}^{-1} \text{ T}^{-1}$.

The linear $M(H)$ curve shown in Figure 2a and Figure 3a at 250 K is typical of a paramagnetic material. Although a Néel temperature for 2-line ferrihydrite of about 350 K was once extracted from neutron reflectivity experiments (Seehra et al., 2000), for nanometer-size ferrihydrite particles, several studies demonstrated an additional paramagnetic-like signal for temperature much lower than 350 K. It is attributed to the presence of uncompensated surficial spins (Guyodo et al., 2006; Punnoose et al., 2004) either from reduced coordination and broken exchange bonds (Knyazev et al., 2021) or the depletion of Fe and enrichment of OH groups (Michel et al., 2010; Hiemstra, 2018) on the ferrihydrite nanoparticles surface. The magnetic susceptibility measured here around $1.5 \text{ A m}^2 \text{ kg}^{-1} \text{ T}^{-1}$ at 250 K for Fh matches values reported in Punnoose et al. (2004). In the same article, the authors found a decrease of the moment per particle of 35% for 5% Ir or Mo doping. For Pb and Sb, from the slopes of the paramagnetic $M(H)$ loop in Figure 2a and Figure 3a, we obtain a decrease of 27% and 35%, respectively. This is consistent with a general scenario where the surface Fe(III) of ferrihydrite nanoparticles is preferably substituted by the dopant, as already reported for Co doping (Stolyar et al., 2017). So, unlike magnetic dopants that can improve exchange (Stolyar et al., 2017), we conclude that non-magnetic dopants such as Pb and Sb could reduce the exchange coupling between ferrihydrite uncompensated spins and therefore lead to a decrease in the moment per particle.

Next, we measured $M(H)$ loops at 2 K. For all three concentrations of Pb (as well as for samples in the Sb-bearing ferrihydrite series), the loop was a S-type hysteresis loop without any magnetic saturation even under 7 T field. A typical example is shown for 2% Pb-doping in inset of Figure 2a. Magnetization reversal steps also occurred at low magnetic field. Both features are the signature of spin glass systems (Ghosh et al., 2017; Mydosh, 2014) where the magnetic moments are driven by strong dipolar interactions (Berquó et al., 2009; Seehra et al., 2000) and/or surface electronic exchange coupling. This is typical of dense assemblies of

agglomerated nanoparticles (Fiorani and Peddis, 2014; Fraerman and Sapozhnikov, 2002; Mydosh, 2014) and was already experimentally revealed in agglomerated ferrihydrite nanoparticles (Duarte et al., 2006; Punnoose et al., 2004). The superspin-glass behaviour of our ferrihydrite nanoparticles assemblies is confirmed by the ZFC/FC curves presented in Figure 2b and inset of Figure 3b (De Toro et al., 2013; Duarte et al., 2006; Fiorani and Peddis, 2014). The difference between ZFC and FC characterizes the difference of randomness in the collective random freezing of ferrihydrite particle moments depending on the field-induced symmetry breaking below a critical temperature, so-called spin-glass transition temperature (T_g). It is to be noted that the curve for pure Fh in the Pb-doping series does not show a parabolic shape below T_g (Figure 2b), unlike typical spin-glass system and unlike the pure Fh sample in the Sb-doping series (Zegeye et al., 2021). We have no clear explanation for this discrepancy since all the other curves match very well the superspin-glass scenario. As depicted in Figure 3b, T_g decreases when the dopant concentration increases for the ferrihydrite Pb-series and for the ferrihydrite Sb-series. Moreover, the height of the dip below T_g decreases as the dopant concentration increases. It can be seen in the normalized plot of $M/M(T_g)$ value as a function of temperature (inset of Figure 3b). The ferrihydrite nanoparticle diameter does not vary much with a few percent of doping. Furthermore, the interparticle interaction energy is much larger than the individual particle anisotropy energy in a strongly interacting superspin-glass system. We conclude that the dip height and T_g change must originate mainly from the gradual reduction of the ferrihydrite inter-particles interaction because of the decrease of each particle moment when adding Pb or Sb dopant.

3.2 Bioreduction of the Fh_Pb series and Pb partitioning

We monitored the bioreduction of the Pb-bearing Fh series for 21 days (Figure 4). No measurable Fe(III) reduction took place in the cell-free assays. The presence of Pb decreased the rate and extent of reduction (Figure 4a). The apparent reduction rate was 0.07 ± 0.01 (Fe(II)_{tot}/Fe_{tot}) day⁻¹ for assays run with Fh, while it decreased to 0.014 ± 0.001 (Fe(II)_{tot}/Fe_{tot}) day⁻¹ for those run with Fh_Pb(0.05). Thus, the reduction rate declined by 15% for every molar ratio increase of 0.01 in Pb-bearing Fh (Figure 4b). Although the linear decrease tends to indicate a complete inhibition of the reduction for a higher Pb/(PB + Fe) molar ratio relative to our study, further assays will be necessary to conclude on the impact of lead loading, higher than 5%, on the bioreduction process. The precipitation of Pb with Fh alters the physicochemical properties of Fh, as shown for other elements (Ekstrom et al., 2010; Erbs et al., 2010). The decrease of the reduction rate observed in this study may be related to a significant decrease of reactive sites in the Fh surface due to Pb loading. This trend is consistent with bioreduction data presented by Ekstrom et al., 2010 and Fredrickson et al., 2001 that showed a decrease of the reduction rate with increasing Al or Ni content for substituted Fh. Fh is a metastable mineral and transforms to a thermodynamically stable phase such as goethite and magnetite in the presence of Fe(II) (Benali et al., 2001; Boland et al., 2014; Hansel et al., 2005; Pedersen et al., 2005; Williams and Scherer, 2004). The extent of reduction reached 35% for Fh (Figure 4), indicating that 35% of Fe(II) was enough to induce its mineralogical transformation. On the other hand, the extent of reduction levelled around 25% irrespective of the Pb/(Fe+Pb) molar ratio. Lower Fe(II) concentrations are thus required to induce Fh transformation via Fe(II) catalyzed dissolution-reprecipitation in the presence of Pb, indicating higher lability of Fh_Pb in the concentration range tested in this study. These results indicate that the presence of Pb decreased the Fe(II)/Fe(III) ratio required to induce Fh transformation compared to that of Fh.

After the 21d-bioreduction, the concentration of Pb in solution was significantly lower than that initially associated with Fh ($Pb_{\text{solution}}/Pb_{\text{initial}} < 1\%$) (Table S2). These results suggest that a substantial amount of Pb is associated with secondary minerals. We hypothesize that adsorption and coprecipitation of Pb occur during bioreduction through three processes: i) the adsorption of Pb ions on the surface, structural defects and nano-pores of biogenic minerals (Vu et al., 2013, 2010), ii) the incorporation within the structure of biogenic minerals due to olation and oxolation processes occurring upon their formation (Dai et al., 2017; Hu et al., 2012; Scheck et al., 2016), and iii) the occlusion of Pb ions in the porosity of the aggregates formed by newly precipitated minerals. Similarly, Vu et al. (2010) observed that Pb partitioned mainly into the solid phase during the transformation Pb-bearing Fh into hematite.

3.3 Biogenic minerals formed by the bioreduction of the Fh_Pb series

The secondary minerals formed after 21 days of bioreduction were investigated by XRD (Figure 5), TEM (Figure 6) and TMS (Figure 7, Table 1). XRD results revealed the precipitation of magnetite in the Pb-free assay while magnetite and goethite were detected in the assays with Pb. Neither ferrihydrite signature nor Pb precipitates crystalized were identified in the systems after the incubation period. The XRD characterizations are in line with TEM observations. We performed TMS analyses to identify and quantify the biogenic minerals. Transmission Mössbauer spectroscopy has been proved to be a very suitable tool to identify the reduction-oxidation states, structural and magnetic properties of iron-bearing materials, especially iron oxides (Murad and Cashion, 2004; Vandenberghe et al., 2000). Iron oxides that were formed in the ambient environment, often have certain characteristics that may exert a major influence on their magnetic properties (size effects), and consequently also on their Mössbauer spectra (Rakotomalala Robinson et al., 2019). However, because nanostructured materials and in

343 particular iron oxides substituted in foreign element can adopt an organization into a
 344 nanostructure, they may have parameters that deviate noticeably from those obtained in bulk
 345 materials. Such deviations include higher quadrupole splitting and line widths, in the
 346 paramagnetic state; and lower magnetic ordering temperatures and hyperfine fields, in the
 347 magnetically ordered state. However, because temperature reduction also has an effect on these
 348 deviations, the Mössbauer spectra of the biogenic minerals formed in the bio-reduced Fh,
 349 Fh_Pb(0.02) and Fh_Pb(0.05) systems were recorded at 128 K (Figure 7a). The TMS measured
 350 at 128 K of the bio-reduced Fh (without Pb) exhibited a quadrupole doublet of siderite in low
 351 abundance, characterized by center shift (CS) and D values of 1.32 and 2.32 mm.s⁻¹ respectively
 352 (Benali et al., 2001) and magnetically ordered components attributable to magnetite, also
 353 identified at 77 K (Figure S1). Fh_Pb(0.02) systems exhibited mainly a magnetically ordered
 354 components with three distributions of hyperfine fields that consists of a broad sextet
 355 corresponding respectively to magnetite and goethite, as are indicated by their associated
 356 hyperfine parameters in Table 1. The spectrum of the bio-reduced Fh_Pb(0.05) system showed
 357 a complex magnetic structure which superimposed on a broad absorption envelope. For this last
 358 bio-reduced sample, spectral fits were obtained assuming the overlapping of spectral signatures
 359 of superparamagnetic magnetite (quadrupolar doublet and singlet), magnetic magnetite (two
 360 distributions of hyperfine fields, corresponding to Fe(III) ions in tetrahedral sites and to Fe(II)
 361 and Fe(III) in octahedral sites) and goethite. Mössbauer temperature profiles (Figure 7b) for the
 362 bio-reduction Fh_Pb(0.05) system were used to consolidate the presence and attribution of the
 363 previously suggested phases. The room temperature Mössbauer spectrum (Figure 7a) was fitted
 364 with one quadrupole D1 doublets, one broad singlet and two sextets with broadened lines width
 365 (Table 1). When the temperature decreases between 128 and 11 K, we observe a gradual
 366 evolution from broad single line and complex magnetic structure with asymmetrically
 367 broadened components, modelled by distribution of magnetic hyperfine fields; to magnetic

368 structure fitted with six components, using the Lorentzian-shape lines model (Rancourt, 1998).
 369 The sextet characterized by magnetic field of 484 kOe and negative quadrupole shift ε (-0.14
 370 mm s⁻¹) is attributed to goethite (Benali et al., 2001; Murad and Johnston, 1987) while the five
 371 other sextets can be assigned to magnetite as established for this mineral at very low temperature
 372 in the literature (Vandenberghe et al., 2000).
 373 The precipitation of various secondary minerals implies that different mechanisms are involved
 374 in the transformation of Fh. In the bio-reduced Fh system 95% of magnetite formed along with
 375 only 5% of siderite. Siderite precipitated neither during the bio-reduction of Fh_Pb(0.02) nor
 376 Fh_Pb(0.05), which contained 78% of magnetite plus 22% of goethite, and 81% of magnetite
 377 plus 19% of goethite respectively. Magnetite precipitates either by the crystallization of
 378 dissolved Fe(II) and Fe(III) ions after the dissolution of Fh or by a solid-state conversion of Fh
 379 (Hansel et al., 2003; Jolivet et al., 2004; Tronc et al., 1992). The solid-state conversion results
 380 from Fe(II) adsorption onto Fh surface and forming a short-range ordered material with mixed-
 381 valence and exhibiting fast electron mobility. This electron mobility induces rearrangements of
 382 the local structure and spinel ordering (Gorski and Scherer, 2009; Jolivet et al., 2004; Tamaura
 383 et al., 1983; Tronc et al., 1992; Williams and Scherer, 2004). The presence of magnetite in the
 384 bio-reduced Fh_Pb(0.05) system was not detected by XRD analysis, while equivalent amounts
 385 of magnetite formed in the bio-reduced Pb-bearing systems as revealed by TMS (Table 1). This
 386 discrepancy may be related to the size of the magnetite particles. The shape and size of the
 387 magnetite obtained are detailed in Table S3. The particle size distribution for magnetite was
 388 centered around 11.04 ± 2.31 nm ($n = 150$) and 6.03 ± 1.50 nm ($n = 155$) in the bio-reduced Fh
 389 and Fh_Pb(0.02) systems, respectively. These particles size are in line with previous biogenic
 390 magnetite formed during the reduction of ferrihydrite by *Shewanella* species (Kukkadapu et al.,
 391 2005; Zachara et al., 2002; Zegeye et al., 2021). However, in the bio-reduced Fh_Pb(0.05)
 392 system, magnetite particles exhibited a superparamagnetic signature suggesting that the size of

these nano particles are smaller than that observed in the Fh_Pb(0.02) assays, making their detection by XRD difficult. Additionally, the presence of goethite hindered the accurate determination of the size of magnetite particles by TEM. Indeed, the size of magnetite particles can vary according to the pH, ionic strength, and presence of magnetite foreign ions in solution. Indeed, the presence of other ions than Fe(II) slows down the electron mobility within Fe(II)-adsorbed Fh, and in turn perturbs the formation of magnetite by solid-state conversion (Jolivet et al., 2004; Tronc et al., 1992). Thus, the formation of specific species (corner- and/or edge-sharing complexes) on the surface of magnetite influences its nucleation and growth, as revealed by Wang et al. (2008), investigating the formation of magnetite in the presence of As(III). The adsorption of foreign ions on magnetite nuclei may decrease interfacial tension, which is the particle growth driving force (Vayssières et al., 1998) limiting the diffusion of soluble species toward magnetite surface and thus inhibiting the growth of magnetite particles (Wang et al., 2008). We assume Pb induced such growth inhibition of the magnetite particles in the bioreduced systems containing Pb, hampering the detection of the magnetite formed in the bioreduced Fh_Pb(0.05) systems with XRD analysis.

The precipitation of goethite may occur after the dissolution of ferrihydrite and the crystallization of the dissolved Fe(III) ions with a planar tetramer $[\text{M}_4(\text{OH})_{12}(\text{OH}_2)_4]^0$ as intermediary product, prior to its condensation by ololation and oxolation reactions (Jolivet et al., 2004). The bioreduction kinetic of the Pb-bearing Fhs were slower compared to the pristine ferrihydrite bioreduction kinetic. This is due to the blocking of Fh surface reactive sites by Pb. In addition, this induces a lower production of Fe(II) ions, leading to lower Fe(II)/Fe(III) ratio ($< 0.1 \text{ mM Fe(II)/g Fh}$) and enabling the formation of goethite in the Pb-bearing systems.

The precipitation of 5% of siderite in the bioreduced Fh system may be explained by a local high concentration of Fe(II) and HCO_3^- in micro-aggregate where the mass transfer is slowed down (Pallud et al., 2010).

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419 The magnetic properties of the dried product of bioreduction of Fh series namely
420 biored_Fh, biored_Fh_Pb(0.02) and biored_Fh_Pb(0.05) were investigated by SQUID.
421 Moment per mass versus field are plotted for 300 K and 5 K in Figure 8. At 300 K, all the
422 samples show curves which can be fitted with Langevin functions characteristic of
423 superparamagnetic behaviour of nanoparticles; this is consistent with bio-formed 5 to 20 nm-
424 sized magnetite particles (Knobel et al., 2007; Mikhaylova et al., 2004) The magnetic
425 susceptibility decreases with increase in Pb concentration, which is consistent with the decrease
426 of particle size and therefore magneto-crystalline anisotropy energy. Moreover, the
427 magnetization at 4 T decreases monotonically with the amount of initial Pb dopant up to 5%.
428 When cooled down to 5 K, the hysteresis loop opens up for the bioreduced Fh, Fh_Pb(0.02),
429 and Fh_Pb(0.05) systems, with coercivity of the order of 30 mT. The low squareness of the
430 hysteresis loops, characterized by a remanent magnetization smaller than half of the saturation
431 magnetization, indicates a significant dipolar or ferromagnetic interaction between the
432 nanoparticles. This is not a surprise since the sample is dried and the nanoparticles aggregated.
433 More interestingly, the magnetization at 4 T (near saturation) at 5 K decreases from 82 A.m².kg⁻¹
434 down to 32 A.m².kg⁻¹ with 5%. The value for the bioreduced Fh system agrees with the
435 magnetization of pure magnetite nanoparticles at 5 K reported in the literature (Byrne et al.,
436 2013; Deepak et al., 2015). To explain the decrease of moment with Pb-content in Fh, we must
437 study how the magnetic moment is perturbed by the formation about 20% goethite particles, as
438 characterized by Mössbauer measurements. Goethite particles (Figure 6c) are anti-
439 ferromagnetic with a Neel temperature of 400 K (Forsyth et al., 1968) and carry no net
440 magnetization, unless their diameter is lower than few nanometer (Brok et al., 2014) which is
441 not the case here. Therefore, with Pb doped in Fh and the occurrence of 20% goethite, the
442 overall moment per mass must decrease compared to almost 100% of magnetite for the undoped

sample. In Figure 8d, we compare the measured moment per mass with the weighted average moment considering $82 \text{ A.m}^2.\text{kg}^{-1}$ for magnetite particle, zero moment for goethite particles and the goethite/magnetite ratio, namely 0/100, 22/78 and 19/81 for the bio-reduced Fh, Fh_Pb(0.02) and Fh_Pb(0.05) systems respectively. The experimental decrease is still larger than the calculated value. The additional loss of moment originates from the combination of two independent phenomena. From the one hand, as quantified by TEM, the magnetite nanoparticle diameter decreases with increasing the amount of Pb. A decrease of diameter of 11 nm to 6 nm as obtained with 2% Pb doping was already found to produce up to 10% decrease of magnetization at saturation at 5K (Caruntu et al., 2007; Kolhatkar et al., 2013). On the other hand, the Pb-doping of the magnetite itself can strongly affect the magnetization at 5 K. Deepak et al. (2015) reported that substitution of 5 to 10% Fe by Mn or Ni in magnetite leads to a decrease of 10 to 20% of the moment per mass (Deepak et al., 2015), while Padalia et al. (2016) reported a moment decrease of 20% for a Ce doping of up to 1.5% (Padalia et al., 2016). With our current data, we cannot quantify the relative impact of these two effects, but both must play a role.

The low squareness of the hysteresis loops at 5 K, characterized by a remanent magnetization smaller than half of the saturation magnetization, reflects the significant dipolar or ferromagnetic interaction between the nanoparticles due to nanoparticles aggregation. It is to note that the coercivity increases monotonically from 27 mT to 38 mT for the bio-reduced Fh system to the bio-reduced Fh_Pb(0.05) one. On the one hand, the decrease of moment, due to change in magnetite/goethite ratio lowers the dipolar and/or ferromagnetic interactions between particles. Consequently, the coercivity is indeed expected to increase. On the other hand, the decrease of magnetite moment produced by the Pb-doping of magnetite must also increase the coercivity due to a smaller Zeeman energy (Padalia et al., 2016).

467 To further study the change in magnetic features of the bioreduction product of Pb-polluted Fh
468 as a function of the level of Pb-doping, we performed ZFC/FC curves measurements, as shown
469 in Figure 9. For the ZFC measurement, each sample was demagnetized at 300 K and then cooled
470 to 2 K. Afterwards ZFC magnetic moments were measured while the temperature was ramped
471 back up from 2 K to 300 K under 7.5 mT constant field. The FC procedure consists in cooling
472 down the sample from 300K under 7.5 mT and then measuring moment while heating it up
473 from 2 K back to 300 K. The curves show clear difference as compared with the ferrihydrite
474 ones previous to bioreduction (see Figure 2b). Although the produced particles are still strongly
475 interacting, the FC curves show no sign of spin-glass behavior at low temperature. The
476 bioreduced Fh and Fh_Pb(0.02) systems do not exhibit signature superparamagnetic peaks
477 neither, at least not below 150 K (Figure 9). The magnetization is continuously increasing as
478 the temperature increases. Such behaviour is typical of strongly interacting nanoparticles.
479 Similar results have been obtained in Byrne et al. (2013) on interacting magnetite nanoparticles
480 having about 10 nm diameter. Strong inter-particle interactions lead to an effective volume
481 much larger than the actual nanoparticle volume, as measured with TEM; this leads to a mixed
482 behaviour between long- and short-range interacting spins and superparamagnetism. We now
483 turn attention to the ZFC/FC curve for the bioreduced Fh_Pb(0.05) system (Figure 9). ZFC and
484 FC separate around 25 K near a peak in ZFC. Such peak (humping) in ZFC, while FC is still
485 increasing, is signature of superparamagnetic behaviour in the particles for temperature higher
486 than the peak, so called blocking temperature (Bean and Livingston, 1959). In the simplest
487 model, blocking temperature (T_b) occurs when the thermal activation energy ($k_B T$) and the
488 uniaxial anisotropy energy KV (where K is the anisotropy constant and V the nanoparticle
489 volume) of non-interacting particles equalize. In Figure 9, we find T_b is about 25 K. Such value
490 would correspond to a magnetite nanoparticle of 7 nm diameter if we consider an uniaxial
491 anisotropy of $1.4 \times 10^4 \text{ J m}^{-3}$ (Byrne et al., 2013; Tournus and Tamion, 2011). This size is

consistent with the TEM results in Figure 6. Indeed, strong interaction between particles artificially increases the blocking temperature such that 7 nm is an upper limit for the real nanoparticle diameter.

3.4 Environmental significance

Given the ecological and environmental importance of bacterial Fe(III) reduction, the subsequent biotransformation of iron oxide has a broader implication than just the cycling of iron. The varying properties of secondary minerals such as crystallinity, solubility or surface area influence the mobility of pollutants such as Pb initially associated with iron oxides. While this study addresses the bioreduction of Pb-bearing Fh at the laboratory scale, our results may be of interest in improving our understanding of the fate of Pb in natural settings and the evolution of Fh's magnetic properties during its transformation. When extending the results of this study to the environment, it is critical to keep in mind the abundance of organic and inorganic species and geochemical conditions likely to affect the bioreduction process, the nature of the biogenic minerals, their properties, and the fate of Pb.

In natural setting, Fh particles are considered to precipitate first during hydrolysis of Fe(III) but are thermodynamically unstable with respect to more crystalline iron oxides. Fh particles are therefore estimated as the most important Fe(III) pool in sustaining the metabolism of Fe(III)-reducing bacteria. However, in our study, the presence of Pb decreased the rate and the extent of bioreduction. This implies that Pb-bearing Fh particles are likely less bioavailable for microbial respiration in contaminated environments.

A wide range of pollutants such as Pb may sorb on iron oxides in nature. Given that iron oxides are commonly the terminal electron acceptors to iron-reducing bacteria, the fate of adsorbed pollutants is often coupled to bacterial activity. There is a potential re-partitioning and release

of associated pollutants during Fe(II) induced transformation. Our study's trend indicates that Pb remains mainly associated with biogenic minerals, which suggests that iron-reducing activity may not mobilize Pb in the aqueous phase in natural settings. Furthermore, we measured a distinct magnetic signature between the biogenic minerals formed by Fh's bioreduction bearing different amounts of Pb. The macroscopic magnetometry measurement are in agreement with TMS data. Thus, these results highlight that magnetic measurement could be a cost-effective methodology to screen polluted environments as suggested elsewhere (Porsch et al., 2014, 2010).

4 Conclusion

Lead sequestration by iron (oxyhydr)oxides is recognized to be one of the most critical geochemical processes controlling its environmental fate, transport, and bio-availability. However, there is still a lack of knowledge on the impact of Pb on the bioreduction process of iron oxides and the subsequent precipitation of biogenic minerals.

Our study highlights that Pb doping decreased the rate and extent of microbially catalyzed Fh reduction. Indeed, the reduction rate declined by 15% for every Pb/(Pb + Fe) molar ratio increase of 0.01 in Pb-bearing Fh, and the extent of reduction declined of 10% irrespective of the Pb/(Pb + Fe) molar ratio compared to that of pure Fh. Lower Fe(II) concentrations are thus required to induce Fh transformation *via* Fe(II) catalyzed dissolution-reprecipitation in the presence of Pb, indicating that the presence of Pb decreased the Fe(II)/Fe(III) ratio required to induce Fh transformation compared to that of Fh.

The catalyzed secondary mineralization led to 95% magnetite and 5% siderite precipitation in the Fh system, while ~80% magnetite and ~20% goethite formed in the Fh_Pb systems. At the end of the bioreduction, less than 1% of Pb was measured in the aqueous phase indicating that a significant amount of Pb was associated with the biogenic minerals. The magnetite particles formed from the bioreduction of Fh_Pb(0.05) displayed a superparamagnetic property. These results reveal that Pb may influence the secondary mineralization pathway by decreasing the flux of Fe(II) and hampering the growth of magnetite particles.

Furthermore, the presence of Pb drastically affects the magnetic signal and features of Fh samples after bioreduction. Given the sensitivity of macroscopic magnetometry measurements, this method, in support of classical techniques, may be of interest to characterize the presence of metals such as Pb in redox active environments.

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Figure captions

Figure 1: X-ray diffraction pattern of pure Fh, Fh_Pb(0.02) and Fh_Pb(0.05).

Figure 2: Mass magnetization versus field (A) and temperature (B) obtained for pure (Fh) and substituted Fh (Fh_Pb(0.02) and Fh_Pb(0.05)). Insert in (A), presents the 2 K hysteresis-loop curve of Fh_Pb(0.02).

Figure 3: Mass magnetization versus field (A) obtained for Sb-bearing Fh (Zegeye et al., 2021) presented here for comparison purpose, and (B) the evolution of spin glass transition temperature (T_g) as a function of % of Pb or Sb with insert displaying mass magnetization versus temperature for the Fh_Sb series.

Figure 4: Bioreduction of Fh series over time. (A) Bioreduction of Fh (circle symbols), Fh_Pb(0.02) and Fh_Pb(0.05) square and triangle symbols, respectively. The initial rate of reduction was computed from the derivative of a nonlinear curve fit represented by the dotted lines. (B) Bacterial Fe(III) reduction rates versus % Pb/(Fe + Pb) molar ratio. Error bars represent the standard deviation of triplicate microbial reduction assays.

Figure 5: X-ray diffraction pattern of bioreduced Fh, Fh_Pb(0.02) and Fh_Pb(0.05).

Figure 6: TEM images of bioreduced Fh (A), Fh_Pb(0.02) (B) and (C) Fh_Pb(0.05), after 21 days of incubation displaying the formation of magnetite and goethite. Inserts represent the size distributions obtained for magnetite particles. The mean magnetite particles sizes were of 11.04 ± 2.31 nm ($n = 150$) and 6.03 ± 1.50 nm ($n = 155$) for the bioreduced Fh and bioreduced

867 Fh_Pb(0.02) respectively. The presence of goethite impeded to determine the size of magnetite
868 particles in the Fh_Pb(0.05) system (Table S3).

869

870 Figure 7: Mössbauer spectra of the secondary minerals formed after the 21 days of bioreduction.
871 (A) bioreduced Fh, Fh_Pb(0.02) and Fh_Pb(0.05) at 128 K , and (B) Fh_Pb(0.05) system at 11,
872 79, 128 and 298 K. Hyperfine parameters corresponding to these spectra are presented in Table
873 1.

874

875 Figure 8: Mass magnetization versus field of bioreduced (A) Fh, (B) Fh_Pb(0.02) and (C)
876 Fh_Pb(0.05). Inserts represents zooms of the mass magnetization versus field. (D) Evolution of
877 the experimental 5 K (black squares) and calculated (white circles) mass magnetization of
878 bioreduced Fh, Fh_Pb(0.02) and Fh_Pb(0.05) versus % of Pb. The dashed line shows the mass
879 magnetization of bulk magnetite.

880

881 Figure 9: Mass magnetization versus temperature of bioreduced Fh (blue), Fh_Pb(0.02) (red)
882 and Fh_Pb(0.05) (green).

883