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1 Mobility of antimony in contrasting surface environments of a mine site –  
2 Influence of redox conditions and microbial communities

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15

16 **Abstract**

17 Microbial processes can influence the complex geochemical behaviour of the toxic  
18 metalloid antimony (Sb) in mining environments. The present study is aimed to evaluate the  
19 influence of microbial communities on the mobility of Sb from solid phases to water in different  
20 compartments and redox conditions of a mining site in southwest (SW) Spain. Samples of  
21 surface materials presenting high Sb concentrations, from two weathered mining waste dumps  
22 and an aquatic sediment were incubated in slurries comparing oxic and anoxic conditions. The  
23 initial microbial communities of the three materials strongly differed. Incubations induced an  
24 increase of microbial biomass and an evolution of the microbial communities' structures and  
25 compositions, which diverged in different redox conditions. The presence of active bacteria  
26 always influenced the mobility of Sb, except in the neutral pH waste incubated in oxic

27 conditions. The effect of active microbial activities in oxic conditions was dependent on the  
28 material: Sb oxic release was biologically amplified with the acidic waste, but attenuated with  
29 the sediment. Different bacterial genera involved in Sb, Fe, and S oxidation or reduction were  
30 present and/or grew during incubation of each material. The results highlighted the wide  
31 diversity of microbial communities and metabolisms at the small geographic scale of a mining  
32 site and their strong implication in Sb mobility.

33 **Key words:** antimony, microbial communities, redox conditions, incubations, mine waste, aquatic  
34 sediment

35

## 36 1. Introduction

37 Antimony (Sb) is a strategic element with a large range of applications. It is involved as  
38 a flame retardant in the composition of plastics, coatings and electronics, but also utilised in  
39 manufacturing batteries, ammunitions, glasses, semiconductors, diodes, infrared detectors,  
40 paints, rubbers, and Hall-effect devices (Krachler et al. 2001; Dupont et al. 2016). The current  
41 order of magnitude of annual Sb production is 150,000 tons (USGS 2021). Antimony is mainly  
42 extracted from stibnite ( $Sb_2S_3$ ) mines, but also from Sb oxides and, as a secondary product,  
43 from Pb, Au, Ag, Cu, Ni or Zn ores (Anderson 2012; Dupont et al. 2016; Herath et al. 2017).  
44 This element, although necessary for the elaboration of many manufactured products,  
45 presents health and environmental issues. Acute and chronic toxicity of Sb have been reported  
46 (Sundar and Chakravarty 2010). Results from recent studies indicate that Sb might be  
47 genotoxic (El Shanawany et al. 2017), and Sb trioxide has been recognised as a potential  
48 carcinogen (Schildroth et al. 2021).

49 Antimony concentration in water compartments of mining environments, i.e. porewater,  
50 groundwater and surface water, ranges from a few  $\mu\text{g L}^{-1}$  to more than  $10 \mu\text{g L}^{-1}$  (Ashley et al.  
51 2003; Casiot et al. 2007; Filella et al. 2009; Fawcett et al. 2015; Cidu et al. 2018; Radková et  
52 al. 2020). In most cases, Sb(V) is the main Sb chemical species detected in solution (Casiot

53 et al. 2007; Fawcett et al. 2015; Cidu et al. 2015), suggesting that the oxidation state is a major  
54 driver of Sb mobility. From a geochemical point of view, environmental behaviour of Sb  
55 depends on its speciation, redox conditions, pH, and the presence of major and trace elements,  
56 as Sb can be integrated in a large range of secondary minerals (Roper et al. 2012; Herath et  
57 al. 2017). Climatic conditions and mineralogy influence the biogeochemical Sb transfer and  
58 soil enzymatic parameters in mining environments (Gallego et al. 2021; Esbri et al. 2022).  
59 Meanwhile, the microbial communities naturally present in most environments may influence  
60 Sb mobility through several indirect or direct processes.

61         Aerobic dissolution of Sb sulphides by S- and Fe-oxidising bacteria classically involved  
62 in acid mine drainage generation has been shown to produce high Sb concentrations in acidic  
63 liquors (Torma and Gabra 1977; Hafeez et al. 2017). Conversely, in anoxic zones, where  
64 microbial sulphate reduction occurs, Sb might be chemically reduced by dissolved sulphide  
65 (Polack et al. 2009), precipitated as  $Sb_2S_3$  (Zhang et al. 2016), or form mobile thioantimonate  
66 complexes (Ye et al. 2019). Direct transformations of Sb species by microorganisms include  
67 Sb(III) oxidation, Sb(V) reduction and methylation. More than 60 bacterial strains, belonging to  
68 27 genera, presented the ability to enzymatically oxidise Sb(III) into Sb(V) (Deng et al. 2021).  
69 They may contribute to increasing the mobility of Sb from solid phases to water, in oxic and  
70 neutral pH conditions, as shown by Loni et al. (2020). Anaerobic bio-reduction of Sb(V) into  
71 Sb(III), involved in respiratory metabolism, has been evidenced with strains of *Sinorhizobium*  
72 (Nguyen and Lee 2014), *Desulfuribacillus* (Abin and Hollibaugh 2017), *Dechloromonas*,  
73 *Propionivibrio* (Yang et al. 2020) and *Geobacter* (Nguyen et al. 2018, Yamamura et al. 2021).  
74 Microbial methylation of Sb has also been shown to occur, particularly in reducing and organic  
75 matter-rich environments (Caplette et al. 2021). A few studies have reported microbiologically  
76 influenced Sb behaviour in environmental samples. Biotic versus abiotic incubations of  
77 sediments impacted by ancient mining activity indicated an effect of the microbial compartment  
78 on Sb release (Casiot et al. 2007) that increased in oxygenated conditions and decreased in  
79 anoxic conditions. This overview of the state of the art highlights the potential involvement of

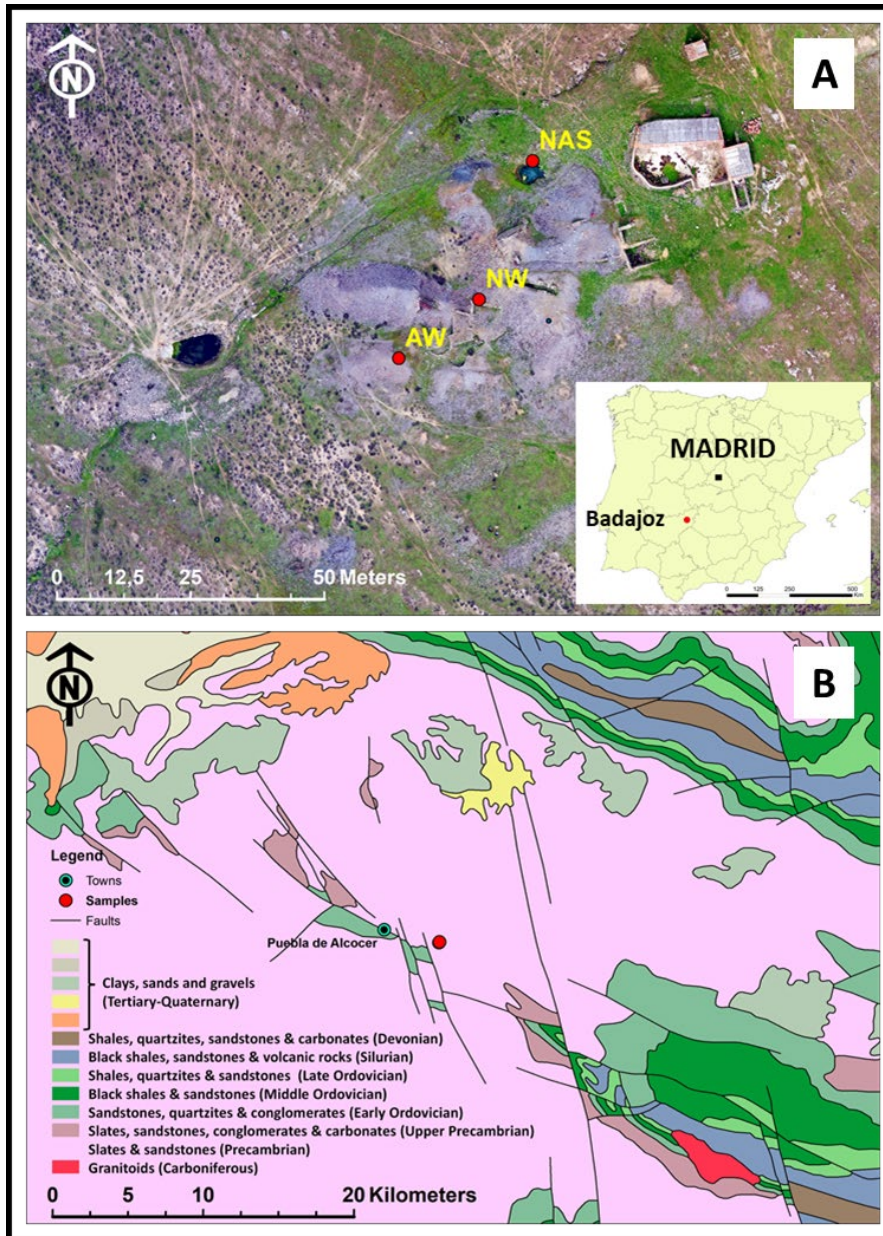
80 a large range of microbial processes in either release of Sb from solid phases to water, or  
81 conversely, immobilisation of this toxic element in mineral sinks. However, considering the  
82 complexity of Sb geochemical behaviour, significant gaps still need to be addressed to  
83 evaluate the global resulting impact of biogeochemical processes, compared with non-  
84 biological mechanisms, in different environmental compartments of mining sites. The present  
85 study is aimed to: (1) evaluate the influence of microbial communities on Sb mobility from solid  
86 phases to water, in different surface compartments of a mining site, comparing oxidising versus  
87 reducing conditions; and (2) examine the composition and evolution of microbial communities  
88 in these different systems.

89

## 90 **2. Material and Methods**

### 91 **2.1 Site description and sampling**

92 Samples were taken from the abandoned Susana mine, located near La Puebla de  
93 Alcocer, Badajoz province, SW Spain (Fig. 1). Gumiel (1983) described this Sb mineralisation  
94 as a small vein-type deposit, exploited through two shallow shafts and galleries, with N130°E  
95 direction, hosted by the Pre-Ordovician "*Complejo Esquisto Grauváquico*" (Lotze 1956), a  
96 monotonous succession of shales/schists and greywackes, where an important number of Sb  
97 mineralizations are present (Gumiel & Arribas 1987). The area lacks any remediation  
98 measures and presents abandoned mine waste dumps. These wastes are very heterogeneous  
99 in grain size, with frequent debris containing stibnite ( $Sb_2S_3$ ), the only ore mineral recognised  
100 in mineralisation. There is no evidence of in situ ore processing actions, consequently the  
101 wastes correspond exclusively to non-processed materials.



102  
103 **Figure 1.** Presentation of the site, location (A); geological context (B)

104  
105 From a climatic point of view, the area corresponds to the BSk Köppen climate (Cold  
106 semi-arid), which implies a dry area with low precipitation and extreme temperature conditions,  
107 particularly during winter. These conditions also imply a low degree of pedogenesis, and very  
108 poor vegetal cover, constituted by poor pastures and scattered presence of *Retama*  
109 *sphaerocarpa* bushes and the very occasional presence of acorn trees (*Quercus rotundifolia*).

110 Different constituents of the local landscape around the mine were sampled. Two samples  
111 were taken from a mine waste dump, including acidic pH waste (AW) and neutral pH waste  
112 (NW). The third sample corresponded to a sediment (NAS for neutral pH aquatic sediment)  
113 taken from a small, flooded area, some 1x1x1 meter in size, in which reducing conditions were  
114 suggested by a black substrate highly enriched in organic matter, and supporting an active  
115 population of tadpoles when the sampling was performed.

116 All samples were taken using plastic spoons, after homogenisation of the profile (0-15  
117 cm deep) with a clean geologist's hammer and placed in plastic bags for transportation to the  
118 laboratory. Samples were air-dried at ambient temperature for 14 days, sieved to remove the  
119 <2 mm fraction, and split to obtain three subsamples: one to be stored, one for physicochemical  
120 determinations, and a last subsample to be milled for analyses. Non-dried samples were kept  
121 at 5°C in closed bags for incubation experiments.

122

## 123 **2.2 Characterisation of solids**

124 Preliminary analysis of the elemental composition of the samples was carried out by  
125 means of energy dispersive X-Ray fluorescence spectrometry (EDXRF), on milled samples,  
126 using an Epsilon 1 (from Malvern Panalytical). Mineralogical characterisation was performed  
127 using X-Ray diffractometry using a Bruker D8 Advance A25 in a 15-minute work program, on  
128 ground and pressed samples. Interpretation was carried out with Match! Software for phase  
129 analysis using powder diffraction data. Interpretation of diffractograms focused on the  
130 identification of the presence of quartz, Fe oxides and mineral phases containing Sb.

131 For incubation experiments, sub-samples of each material were subjected to  
132 autoclaving under N<sub>2</sub> atmosphere, three successive times at 120°C for 20 min, at 24 h intervals,  
133 in order to strongly attenuate microbial activity. This treatment did not kill 100% of the  
134 microorganisms; however, by convention the corresponding conditions are thereafter labelled  
135 as "Sterilised - St". In order to determine different mineral phases bearing Sb in soil samples

136 and to search for possible differences between autoclaved and non-autoclaved materials, 6  
137 samples (3 autoclaved and 3 non-autoclaved) were analysed using SEM-EDS. The 6 soil  
138 samples were compressed using a hydraulic press (10 tons), each sample containing between  
139 70 g and 110 g of material. The resulting samples were put into resin and thinly polished in  
140 order to be analysed using a ZEISS Merlin Compact Scanning Electron Microscope (SEM).  
141 The SEM was operated at 15KV, and it was provided with an EDXRF spectrum analyser (EDS,  
142 Bruker QUANTAX-XFlash6-30mm2-resolution 129eV), and a Bruker Micro XRF (XTRACE-  
143 Tube Rh-Filters Al, Ti, Ni). The results were analysed using ESPRIT Software.

144 Multielemental characterisation including total sulphur was carried out by EDXRF,  
145 using a Bruker S2 Ranger with a Pd detector with 10 g of ground and pressed samples.

146 Total organic carbon (TOC), humic and fulvic acids were analyzed according to the  
147 protocol described by Schnitzer (1983). For the determination of the TOC, 10 g of soil were  
148 added to 50 mL of extractant solution (44.6 g of 0.1 M pyrophosphate and 4 g of NaOH in a  
149 total volume of 1 L). Samples were shaken for 2 h and centrifuged for 15 min at 800 g. After  
150 that, 0.098 g of  $K_2Cr_2O_7$  were added to 1 mL of the supernatant of the samples; then, 4 mL of  
151 concentrated  $H_2SO_4$  were added and heated using a STUART SBH 200D/3 digester at 150°C  
152 for 30 min. After 12 h, 5 mL of ultrapure  $H_2O$  were added, finally obtaining the result using a  
153 Spectronic UNICAM Helios Epsilon spectrophotometer at a wavelength of 590 nm. For the  
154 determination of humic and fulvic acids, 10 mL of the extract were taken and acidified with 0.2  
155 mL of  $H_2SO_4$ . The samples were centrifuged for 15 min at 1,000 g. The resulting supernatant  
156 represents fulvic acids. Ten mL of NaOH 0.5 M were added to the resulting precipitate (humic  
157 acids). After stirring, 0.098 g of  $K_2Cr_2O_7$  was added to 1 mL of the supernatant of the samples,  
158 and 4 mL of concentrated  $H_2SO_4$  were added and heated using a STUART SBH 200D/3  
159 digester at 150°C for 30 min. After 12 h, 5 mL of ultrapure  $H_2O$  were added, and the solution  
160 was analysed at 590 nm using a Spectronic UNICAM Helios Epsilon spectrophotometer.

161 Evaluation of geochemical Sb mobility was performed through the following steps. The  
162 leachable fraction was evaluated using the Modified Geological Survey Field Leach Test



163 (Hageman 2007): 5 g of soil were mixed with 100 mL of deionised water shaken at 50 rpm for  
164 2 h in a water bath. The supernatant was filtered through 0.45  $\mu\text{m}$  PTFE filters. The available  
165 fraction was studied by means of  $\text{AcNH}_4$  selective extraction: 5 g of soil were mixed with 100  
166 mL of 1 M  $\text{AcNH}_4$  solution at pH 7, shaken at 50 rpm for 2 h in a shaking water bath. The  
167 supernatant was filtered through a 0.45  $\mu\text{m}$  PTFE filter. Modified BCR sequential extraction  
168 was carried out according to Quevauviller et al. (1997) in order to obtain data about  
169 fractionation of Sb and related elements. This sequential extraction procedure provides  
170 information about four fractions: fraction 1 (F1), extracted by a solution of 0.11 M acetic acid,  
171 releases the species that are soluble in water, easily leached by weak acids and associated  
172 with soluble carbonates; fraction 2 (F2), extracted by 0.5 M hydroxylamine hydrochloride,  
173 consists of easily reducible species associated with Fe and Mn oxy-hydroxides; fraction 3 (F3),  
174 extracted by 8.8 M hydrogen peroxide and 1.0 M ammonium acetate solutions, consist in easily  
175 oxidisable compounds, mainly associated with organic matter and sulphide minerals; and the  
176 residual fraction (RF) was determined by calculating the difference between pseudo-total and  
177 mobile fractions. A suitable certified reference material (BCR 701) was used to evaluate Pb  
178 recovery (between 95-98%). Extractions were filtered using Whatman filters (8  $\mu\text{m}$ ). The  
179 analysis of Sb, Pb, As, Fe, Mn and Cu in all solutions was performed by High-Resolution  
180 Atomic Absorption Spectrometry (HR-AAS), using an Analytik Jena ContraA-800D.

181

## 182 **2.3 Microcosms**

183 Four types of microcosms were used: biotic and sterilised (St) in aerobic and anaerobic  
184 conditions. To establish the biotic microcosms, fresh samples (equivalent to 20 g dry weight)  
185 were mixed in sterile 500 mL glass bottles with 200 mL of sterilised Mont Roucoux mineral  
186 water (composition close to rainwater, pH = 6.38, EC = 160  $\mu\text{S}\cdot\text{cm}^{-1}$ ,  $\text{Cl}^-$  = 56  $\mu\text{M}$ ,  $\text{NO}_3^-$  = 24  
187  $\mu\text{M}$ ,  $\text{SO}_4^{2-}$  = 16  $\mu\text{M}$ ,  $\text{Na}^+$  = 109  $\mu\text{M}$ ,  $\text{K}^+$  = 7.7  $\mu\text{M}$ ,  $\text{Ca}^{2+}$  = 25  $\mu\text{M}$ ,  $\text{Mg}^{2+}$  = 21  $\mu\text{M}$ ) and covered  
188 with cotton plugs (Ox: oxic conditions) or rubber stoppers (AOx: anoxic conditions). To create  
189 conditions with strongly attenuated microbial activities, bottles were prepared according to the

190 same protocol with St materials. At the beginning of the experiment and every week, 20 µl of  
191 formaldehyde was added to each St flask to prevent growth of the microorganisms that were  
192 not killed by autoclaving. All conditions were tested in triplicates, except the NAS St conditions,  
193 that were prepared in duplicates, due to the limitation of the quantity of material. In anoxic  
194 conditions, oxygen was purged from the microcosms with sterile N<sub>2</sub> for 20 minutes and  
195 pressurised with sterile H<sub>2</sub> (1 bar over atmospheric pressure). All microcosms were incubated  
196 for 41 days at 20°C with rotational shaking (100 rpm) in the dark to prevent photo-induced  
197 oxidation of metals. Every week, samples were taken from the bottles and the water that  
198 evaporated in oxic conditions was compensated by adding sterile deionised water. For anoxic  
199 microcosms, sampling was conducted under strictly anaerobic conditions in an oxygen-free  
200 glove box and H<sub>2</sub> pressure was renewed after each sampling event.

201

## 202 **2.4 Characterisation of water samples**

203 At the beginning of the experiment and every week, one aliquot of the supernatant was  
204 sampled and pH, oxidation-reduction potential (ORP), electric conductivity (EC), Fe(II)/Fe(III)  
205 and Sb content were analysed. Additionally, Pb and As content were determined at the  
206 beginning, mid-time and end of the experiment. The pH, EC and ORP parameters were  
207 measured immediately in the suspensions using a Consort C3040 apparatus equipped with  
208 pH, ORP and EC electrodes. Supernatants were centrifuged at 10,000 g and filtered at  
209 0.45 µm. For dissolved Fe speciation and quantification, a 1.5 mL aliquot was immediately  
210 acidified with concentrated HCl. Dissolved Fe(II) was measured using the ortho-phenantroline  
211 colorimetric method (Murti et al. 1966; Mamindy-Pajany et al. 2013). For this, 0.5 mL of acetate  
212 buffer pH 4.5, 0.1 mL of orthophenantroline 0.5 % and 0.9 mL of the filtered sample were  
213 added to a spectrophotometer cuvette. The mixture was left at room temperature for 15 min in  
214 the dark and the absorbance was recorded at 510 nm using a UV-Vis spectrophotometer. The  
215 total dissolved Fe concentration was determined using the same protocol but with the addition  
216 of 0.1 mL of hydroquinone 1 % prepared in acetate buffer pH 4.5. Samples for HR-AAS were

217 immediately acidified with 1 % of concentrated HNO<sub>3</sub>. Concentrations of As, Sb and Pb were  
218 quantified by HR-AAS using a ContrAA-800 equipment (Analytik Jena, Germany) equipped  
219 with a xenon short-arc lamp and using the flame technique. All calibration standard solutions  
220 were prepared using 1 g L<sup>-1</sup> primary certified standard solutions (Inorganic Ventures).

221 Speciation of Sb was determined at the end of incubation with the method described by  
222 Nguyen and Lee (2015) according to Yu et al. (2002). Samples were diluted in ultrapure water  
223 in order to reach a concentration lower than 250 µg L<sup>-1</sup>. In glass tubes, 1 mL of sample was  
224 mixed with 3.5 mL of ultrapure water and 1 mL of 1 % pyrrolidine dithiocarbamate solution.  
225 After 10 min of reaction, the mixture was transferred to an Agilent SampliQ C18ODS (6 mL)  
226 solid-phase extraction cartridge, previously conditioned with 1 mL analytical grade methanol  
227 then 1 mL ultrapure water and placed above a collection tube. The cartridge was rapidly rinsed  
228 with 5 mL of ultrapure water, and Sb(III) was retained in the cartridge. Sb was quantified by  
229 oven Atomic absorption spectrometry (VARIAN) in the collection tube that received Sb(V) from  
230 the initial sample. The difference between total Sb and Sb(V) was considered to represent  
231 Sb(III).

232

## 233 **2.5 Microbial growth and viability**

234 The viability of microorganisms in each flask was determined at the beginning, mid-  
235 time and end of the incubation process, using a LIVE/DEAD® BacLight Bacterial Viability Kit  
236 (Molecular Probes 2004) to stain the bacteria fixed by filtration of 100 µL of incubated slurries  
237 diluted in 1 mL of sterile physiological water on GE Whatman™ Nuclepore polycarbonate  
238 membrane. Filters were observed under UV-light using a ZEISS Imager Z1 optical microscope  
239 (DSRED and FITC filters). Twelve images were taken in randomly chosen areas of each filter.  
240 Determination of live and dead cells was conducted by counting the number of green (live) and  
241 red (dead) points. Points were counted using a recorded macro in ImageJ (National Institute  
242 of Health, USA).

## 243 **2.6 Characterisation of microbial communities**

### 244 **2.6.1 Extraction and quantification of soil DNA**

245 DNA extractions were performed on initial soils and pellets obtained at the middle and  
246 end of the experiment after centrifuging the samples at 10,000 g. Pellets of triplicate  
247 microcosms were pooled before DNA extraction. Exclusively for the biotic AW in AOx  
248 conditions, DNA extraction and 16S fragment sequencing was also performed on individual  
249 replicates, because results in terms of Sb concentration strongly differed between these  
250 replicates. We used the FastPrep DNA Spin Kit for soil (MP Biomedicals) following the  
251 manufacturer's instructions. Total extracted DNA was quantified using a QUANTUS  
252 Fluorometer (Promega) and stored at -20 °C until the next analysis.

253

### 254 **2.6.2 16S rRNA amplification and next-generation sequencing**

255 Bacterial and archaeal diversity was determined by 16S rRNA gene metabarcoding,  
256 generated by INRAE Transfert (Narbonne, France). The V4-V5 hypervariable region of the  
257 16S rRNA gene was amplified using the barcoded, universal primer set 515WF/918WR (Wang  
258 et al. 2009). PCR reactions were performed using AccuStart II PCR ToughMix kit and cleaned  
259 (HighPrep PCR beads, Mokascience). Forward and reverse primers contained the adapter  
260 sequences (5'GTGYCAGCMGCCGCGGTA) and (3'ACTYAAAKGAATTGRCGGGG),  
261 respectively, used in Illumina sequencing technology. The amplicons were sequenced using  
262 an Illumina MiSeq at the GeT-PlaGe platform (Auzeville, France).

263

### 264 **2.6.3 Processing of sequencing**

265 The datasets from MiSeq sequencing were processing using the FROGS (Find,  
266 Rapidly, OTUs with Galaxy Solution) bioinformatics pipeline (Escudié et al. 2017). Fastq paired  
267 reads were merged with Vsearch software, and clustered into OTU (Operational Taxonomic

268 Units) with Swarm and an aggregation distance clustering of 1. Chimera and OTU with a  
269 proportion less than 0.0005% of all sequences were removed. Taxonomic affiliation was  
270 performed using BLAST and the Silva 138.1 database for 16S rRNA gene sequences. The  
271 raw datasets can be found on the European Nucleotide Archive system under project  
272 accession number **[in progress]**. InvSimpson diversity indexes were calculated based on the  
273 Illumina sequence data.

274

## 275 **2.7 Statistics**

276 Data about the evolution of soil microcosms were tested for homogeneity of variance  
277 and normal distribution. In this way, analysis of variance (ANOVA) was carried out using SPSS  
278 version 21.0 followed by a Tukey test between means of treatments to determine the significant  
279 difference ( $p \leq 0.05$ ).

280 Redundancy analysis (RDA), using the vegan R package, was performed to identify the  
281 effects of environmental variables on bacterial phylum structure, based on the relative  
282 abundance of the detected OTU in pellets at t0, t21 and t41 days. This analysis was calculated  
283 based on a Hellinger-transformed phylum abundance basis of 16S rRNA data (Borcard et al.  
284 2011). Environmental explanatory tested variables were standardised and the function ordistep  
285 in the vegan package was used to identify the significant explanatory environmental variables.  
286 The significance of the RDA model was tested by ANOVA based on the Monte Carlo test with  
287 999 permutations. This analysis helps to determine the most influential factors and the extent  
288 to which different environmental parameters affected the bacterial phylum.

289

290

291

292

## 293 **3. Results**

### 294 **3.1 Characterisation of solids**

#### 295 **3.1.1. Mineralogy**

296 The major Sb phases identified by XRD in rock samples from the site included stibnite  
297 ( $\text{SbS}_3$ ) as the primary phase and stibiconite ( $\text{Sb}^{3+}(\text{Sb}^{5+})_2\text{O}_6(\text{OH})$ ) as the oxidised phase.  
298 However, in the soil and sediment samples used in this study, XRD analyses revealed only an  
299 oxidised phase of Sb and As, stenhuggarite  $\text{CaFeSb}(\text{AsO}_3)_2\text{O}$  (5.3% in NAS sample), in  
300 addition to quartz as the majority phase (95-99%) and Fe and Mn oxides (5.6% in AW sample).

301 According to the SEM-EDS observations (Supplementary Table 1 and Supplementary  
302 Fig. 1), the most frequently observed Sb-bearing mineral phases are stibnite (in all samples),  
303 and Sb-oxides, including cervantite ( $\text{Sb}^{3+}\text{Sb}^{5+}\text{O}_4$ ), bindheimite ( $\text{Pb}_2\text{Sb}_2\text{O}_6(\text{O},\text{OH})$ ), and other  
304 Sb-oxides that would need further studies to be more precisely determined. Jamesonite  
305 ( $\text{Pb}_4\text{FeSb}_6\text{S}$ ) is also present in lesser quantities. Besides these Sb-containing minerals, other  
306 important minerals present in both St and non-St samples—include quartz, rutile, Fe oxides  
307 (hematite), arsenopyrite, and monazite. Regarding Sb-bearing minerals, no significant  
308 difference was found between St and non-St samples through these qualitative analyses.  
309 Nevertheless, a detailed quantitative study of the Sb-oxides might lead to some important  
310 differentiation.

311

#### 312 **3.1.2 Chemical composition**

313 All samples are siliceous, with variable contents of Fe (4.6-16.5 %), Sb (1.02-4.41 %)  
314 and As (878-9,800  $\text{mg kg}^{-1}$ ), as shown in Table 1. The NAS sample clearly differs from the  
315 other samples, especially in terms of higher concentrations of Fe, As, TOC (Table 1), P, Mn,  
316 Cr and Ca (Supplementary Table 2).

317

318 **Table 1.** Characteristics of initial materials. All data are expressed in %. (\*) % of TOC

	<b>AW</b>	<b>NW</b>	<b>NAS</b>
Fe	4.61	4.67	16.49
Sb	4.41	1.02	1.08
S	0.53	0.13	1.11
As	0.22	0.09	0.98
TOC	2.35	2.43	9.59
Humic Acid	0.89 (37.8%)*	1.29 (53.1%)*	2.65 (27.6%)*
Fulvic Acid	1.46 (62.1%)*	1.14 (46.9%)*	6.94 (72.3%)*

319

### 320 **3.1.3 Geochemical mobility of Sb**

321 A clear differentiation was found in the fractionation between different samples and  
 322 different considered elements. The first noteworthy finding is the overwhelming proportion of  
 323 Sb in the residual phase in all samples, although the proportion of Sb in more available phases  
 324 is very slightly higher in the NAS sample (Supplementary Figure 2). This is confirmed by the  
 325 negligible proportion of Sb extracted in a leaching test, or even extracted with AcNH<sub>4</sub>  
 326 (Supplementary Figure 2A). The fraction extracted with AcNH<sub>4</sub> tends to be higher in the AW  
 327 sample than in the NAS sample. With the BCR protocol, Fe is extracted in higher proportions  
 328 (more than 40%) in the soluble/available fractions (Supplementary Figure 2B) only in the NAS  
 329 sample, with the highest values corresponding to the F2 fraction linked to Fe and Mn oxides.  
 330 However, the low proportion of extracted Sb in F2 suggests that most of the Sb is not  
 331 associated with oxides in the NAS sample. Iron is only present in very small proportions as  
 332 oxides in the NW and AW samples.

333

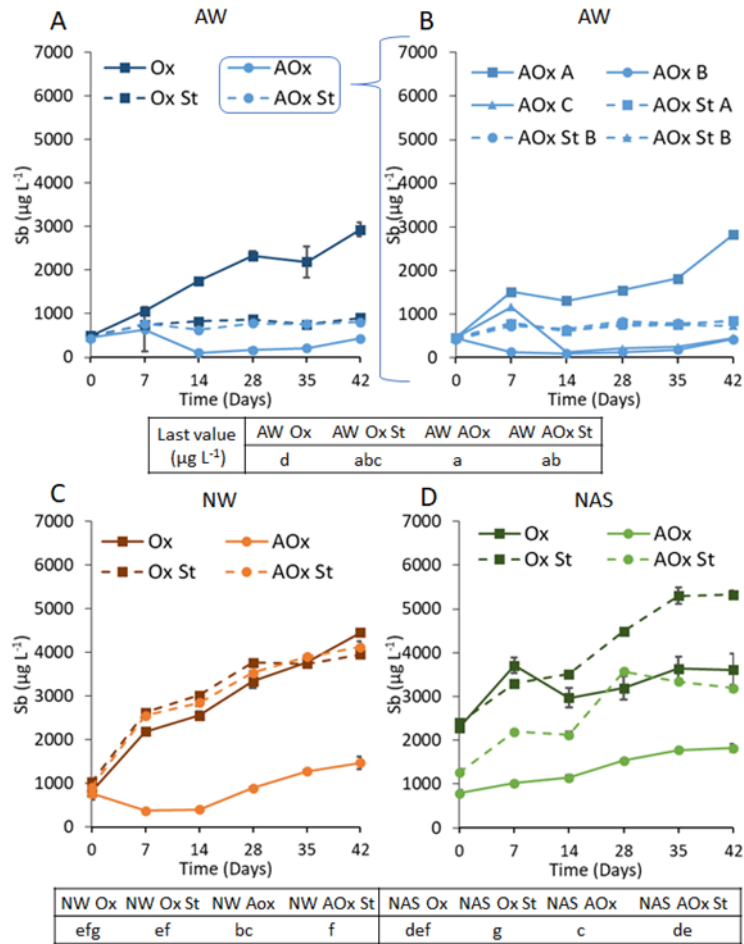
## 334 **3.2 Microcosms**

### 335 **3.2.1 Evolution of physico-chemical parameters**

336 In the AW microcosms, oxidising conditions with microbial growth produced a strong  
 337 release of Sb ( $\approx$  495 % of the initial concentration, Fig. 2A). However, under anoxic conditions,

338 the three replicates in biotic conditions behaved differently (Fig. 2B): Sb was released more in  
339 solution than in the St condition for replicate A, but immobilised between days 6 and 15 in  
340 replicate B and immobilised from the beginning of incubation in replicate C. In all replicates, a  
341 late release of Sb into water was observed during the last week of incubation. Few changes  
342 were observed in the St microcosms. On the other hand, in NW microcosms (Fig. 2C), a  
343 release of Sb was observed in all cases (452 % Ox, 283 % Ox St, 353 % AOx St), although it  
344 was much less pronounced in the AOx microcosm (92 %). Regarding NAS microcosms (Fig.  
345 2D), a release was observed in all cases; however, it was much stronger in the St microcosms  
346 than in the biotic microcosms for both Ox and AOx conditions (58 % Ox, 122 % Ox St, 131 %  
347 AOx, 152 % AOx St). More precisely, in oxic conditions Sb was released more in biotic than in  
348 St conditions during the first week, then the Sb concentration decreased sharply between day  
349 6 and day 13. Arsenic had a more homogeneous behaviour within the three studied materials  
350 (AW, NW and NAS, Table 1 and Supplementary Fig. 3). In all cases, the microcosm under  
351 anoxic conditions and with microorganisms produced a release of the metalloid, while in the  
352 rest of the microcosms the release was lower. No release of Fe(III) was observed in any of the  
353 microcosms (data not shown). However, under anoxic and biotic conditions, an increase in the  
354 content of soluble Fe(II) could be observed mainly with AW and a few with NW, while in the  
355 rest of the microcosms it remained almost unchanged (Fig. 3).





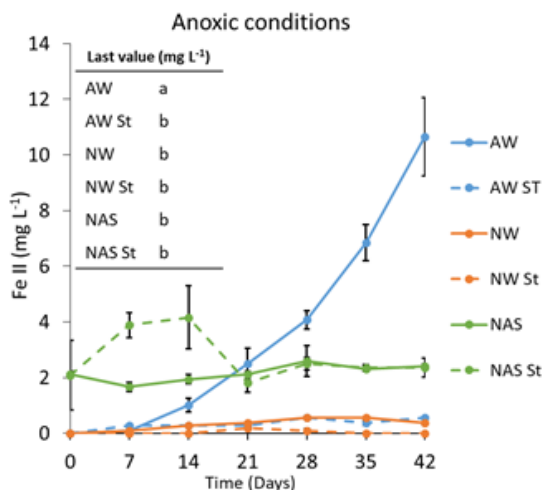
356

357 **Figure 2.** Evolution of Sb concentration in the liquid phase of acidic pH waste (AW, A & B),  
 358 neutral pH waste (NW, C) and neutral pH aquatic sediment (NAS, D) microcosms. Ox (oxic-  
 359 biotic conditions), Ox St (oxic-abiotic conditions), AOx (anoxic-biotic conditions) and AOx St  
 360 (anoxic-abiotic conditions). Values show the mean of three replicates  $\pm$  SE. Values with the  
 361 same small letters are not significantly different at  $P \leq 0.05$  (ANOVA, Tukey test).

362

363 AW microcosms showed lower pH as compared to the other two soil types (NW and  
 364 NAS, Supplementary Fig. 4). In the AW microcosm with microorganisms, the pH decreased in  
 365 Ox conditions and increased in AOx conditions, whereas no change of pH was observed in  
 366 abiotic conditions. With NW, pH increased in AOx conditions while no major pH change was  
 367 observed in the other conditions. Similar behaviour was observed in NAS, where an increase  
 368 in pH was observed only under AOx conditions with microorganisms. The ORP evolved

369 similarly for all materials (AW, NW and NAS, Supplementary Fig. 5): it reached values of  
 370 around 200-300 mV (Ref. Ag/AgCl) in microcosms under Ox conditions, while in AOx  
 371 conditions it decreased down to -300 mV, with no clear influence of living microbes in any  
 372 condition.



373  
 374 **Figure 3.** Evolution of Fe(II) concentration in reducing conditions of acidic pH waste (AW, A),  
 375 neutral pH waste (NW, B) and neutral pH aquatic sediment (NAS, C) microcosms. St indicates  
 376 abiotic conditions. The Fe(II) concentration did not vary under oxidising conditions. Values  
 377 show the mean of three replicates  $\pm$  SE. Values with the same small letters are not significantly  
 378 different at  $P \leq 0.05$  (ANOVA, Tukey test).

379  
 380 The monitoring of EC showed differences between the studied microcosms  
 381 (Supplementary Fig. 6). A very strong increase of EC was observed in AW and NAS incubated  
 382 in Ox conditions with microorganisms (400% and 162%, AW and NAS, respectively), whereas  
 383 with NAS, EC tended to decrease in biotic AOx microcosms. In the NW microcosm, there was  
 384 only a slight increase over the weeks in all conditions.

385 The final speciation of Sb in the water phase (Table 2) showed the dominance of Sb(V)  
 386 in all conditions, except in the biotic AOx incubations of NW, in which Sb(V) represented only  
 387 20 to 30% of total Sb.

389 **Table 2.** Speciation of Sb, total Sb and total As in the water phase at the end of the incubation  
 390 experiment (day 42).

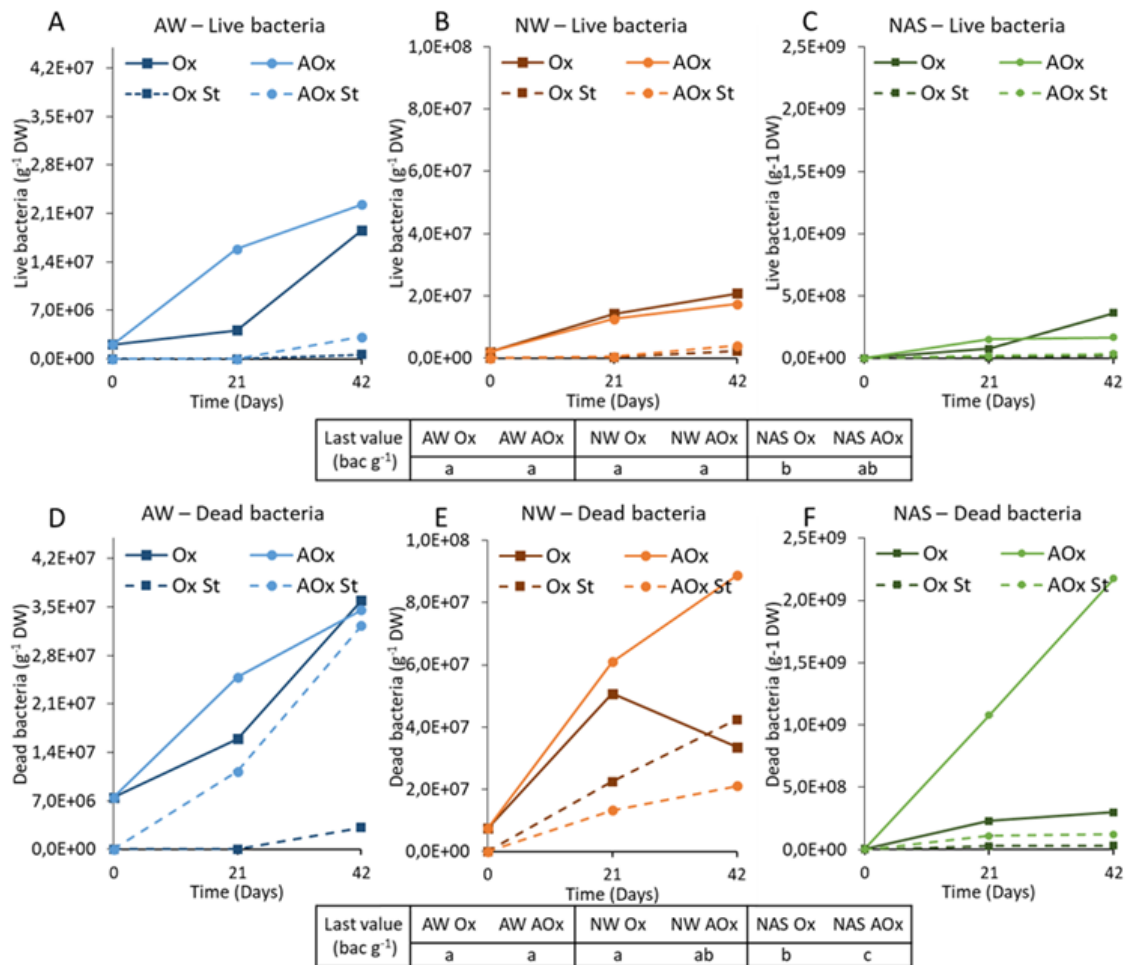
Condition	Replicates	Total Sb ( $\mu\text{g L}^{-1}$ )	Sb(V) ( $\mu\text{g L}^{-1}$ )	Sb(V) (%)	Total As ( $\mu\text{g L}^{-1}$ )
AW Ox	A	2231	2030	91	208
	B	2657	2475	93	217
	C	2693	2253	84	240
AW Ox St	A	1316	1205	92	256
	B	887	768	87	265
	C	1507	1368	91	304
AW AOx	A	3516	3318	94	1337
	B	664	559	84	2955
	C	576	353	61	2963
AW AOx St	A	1049	819	78	360
	B	992	816	82	322
	C	943	752	80	249
NW Ox	A	4596	3981	87	908
	B	4898	4175	85	850
	C	5320	4847	91	892
NW Ox St	A	4030	3691	92	650
	B	3950	3547	90	839
	C	3534	3216	91	854
NW AOx	A	2039	629	31	9764
	B	2199	649	30	10540
	C	2184	440	20	10676
NW AOx St	A	4250	3696	87	1146
	B	4552	3960	87	1109
	C	4370	3985	91	1069
NAS Ox	A	3200	3059	96	118
	B	3758	3564	95	116
	C	2652	2424	91	95
NAS Ox St	A	5516	5108	93	1250
	B	4474	4083	91	1764
NAS AOx	A	2021	1453	72	15045
	B	1808	1539	85	14986
	C	2155	1482	69	15515
NAS AOx St	A	3756	3356	89	2285
	B	2816	2561	91	2216

391

### 392 3.2.2 Evolution of the microbial compartment

393 The cell density increased in all incubation conditions (Fig. 4), the highest cell  
 394 concentrations being found in the NAS. This result was in accordance with the DNA  
 395 quantification data (Supplementary Table 3). For AW microcosms, the AOx conditions gave  
 396 the highest abundance of live cells: however, in NW there was a similar abundance of living  
 397 cells in Ox and AOx, and in NAS more living cells were found in Ox at the end of incubation

398 (Fig. 4 A, B and C). Considering dead cells, their abundance in AW was similar between Ox  
 399 and AOx conditions; however, in NW and NAS materials, dead cells were more abundant  
 400 under AOx conditions (Fig. 4 D, E and F). Cells (live and dead) were observed in St conditions,  
 401 however, low DNA concentrations confirmed that growth in the presence of St materials was  
 402 limited.



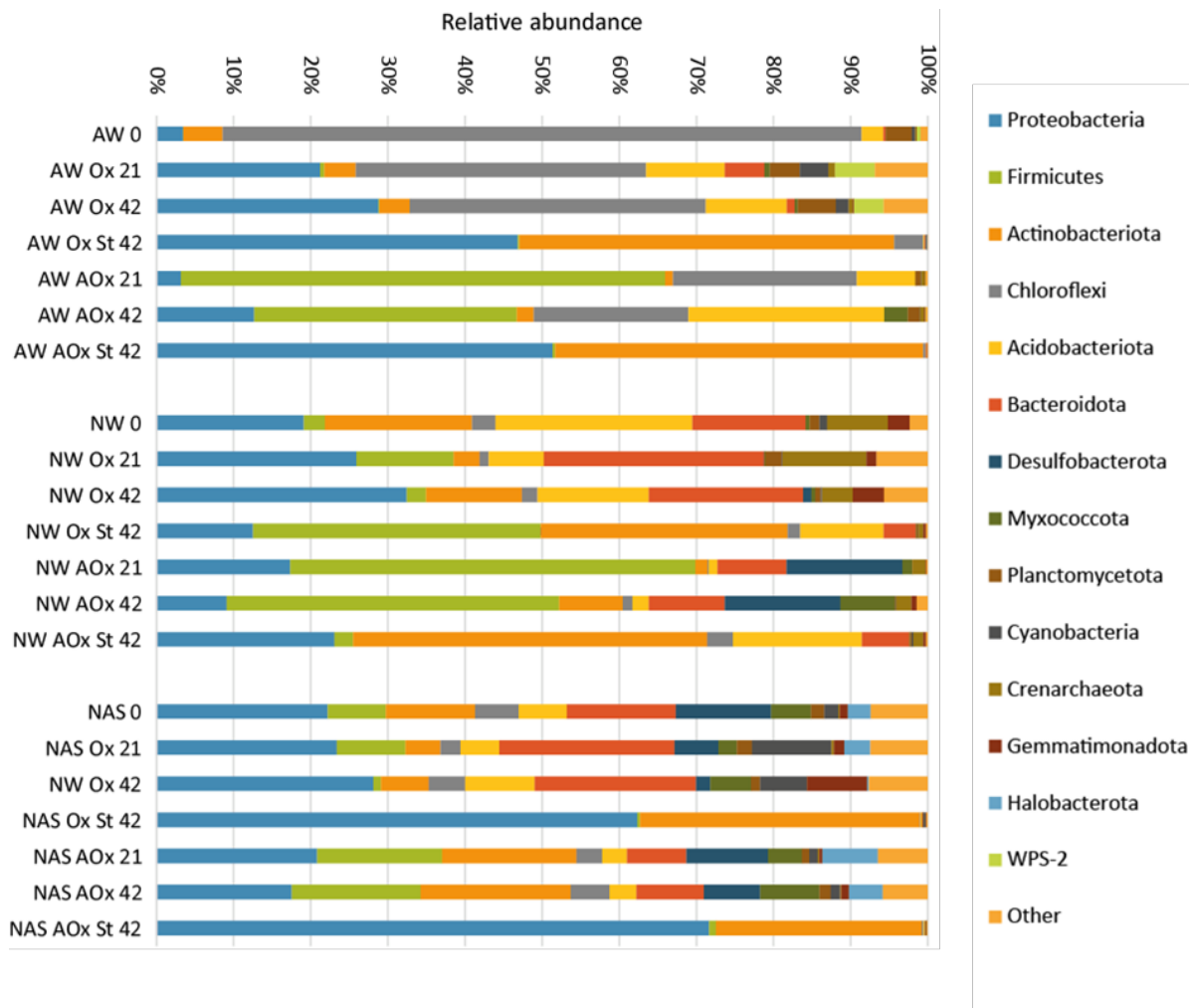
403

404 **Figure 4.** Evolution of live and dead cells abundance (cells g<sup>-1</sup> of solid dry weight) in acidic  
 405 waste (AW, A & D), neutral waste (NW, B & E) and neutral aquatic sediment (NAS, C & F)  
 406 microcosms. Ox (oxic-biotic conditions), Ox St (oxic-abiotic conditions), AOx (anoxic-biotic  
 407 conditions) and AOx St (anoxic-abiotic conditions). Values show the mean of three replicates  
 408 ± SE. Values with the same small letters are not significantly different at P ≤ 0.05 (ANOVA,  
 409 Tukey test).

410 A total of 382,567 sequences of 16S rRNA gene were obtained from the 21 samples.  
411 The highest richness was observed in the NAS (Supplementary Figure 7). It ranged from 657  
412 OTUs in the soil at the beginning of experiment (t0) to 570 in the Ox condition after 42 days.  
413 In NW, 514 OTUs were observed at t0. This sample shows the greatest difference between  
414 the Ox and AOx conditions: after 21 days, in AOx conditions, the richness decreased to 321  
415 before increasing to 508 at the end. The lowest richness was observed in AW with a number  
416 of OTUs between 300 and 400. OTUs were observed in St conditions: with AW and NAS, few  
417 OTUs were present at the end of the St experiment however in NW about 400 OTUs were  
418 observed in both St conditions. The diversity based on the InvSimpson index decreased in all  
419 conditions between 0 and 21 days, except for NW Ox and AW Ox conditions (Supplementary  
420 Figure 7). Between 21 and 42 days, the diversity increased in NW Ox and NAS AOx, and  
421 decreased in AW Ox. At the end of the experiment, diversity was higher in the Ox condition  
422 than in the AOx condition for AW and NAS, but higher in the AOx than in the Ox condition for  
423 NAS. Diversity in the St conditions was very low, except for the NAS condition, where it was  
424 close to the biotic condition.

425 The relative abundance of the major bacterial and archaeal phyla is shown in Fig. 5.  
426 AW was characterised by a predominance of Chloroflexi. Ox conditions favoured the  
427 development of Proteobacteria, Bacteroidota, Planctomycetota, Cyanobacteria, and WPS-2,  
428 while AOx conditions promoted the development of Firmicutes (which became the main  
429 phylum) and Acidobacteria. NW was dominated by five phyla: Proteobacteria,  
430 Actinobacteriota, Acidobacteriota, Bacteroidota, and Crenarchaeota. Ox conditions enhanced  
431 the development of Proteobacteriota and Bacteroidota, whereas Firmicutes, Desulfobacterota  
432 and Myxococcota emerged in AOx conditions. Quite similar compositions at the phylum level  
433 were observed in NW and NAS; however, three phyla were important in this sediment:  
434 Desulfobacterota, Myxococcota, and Halobacterota. As observed in NW, Proteobacteria and  
435 Bacteroidota abundance increased in NAS microcosms with Ox conditions, but a decrease in  
436 abundance of Firmicutes, Desulfobacterota, and Halobacterota was also observed. In AOx

437 conditions, the NAS phylum composition was very similar during the experiment with no major  
 438 variations. The main phyla observed in AW and NAS St conditions were Proteobacteria and  
 439 Actinobacteria, meanwhile Firmicutes, Acidobacteriota, and Bacteroidota were also observed  
 440 in proportions greater than 5% in NW.

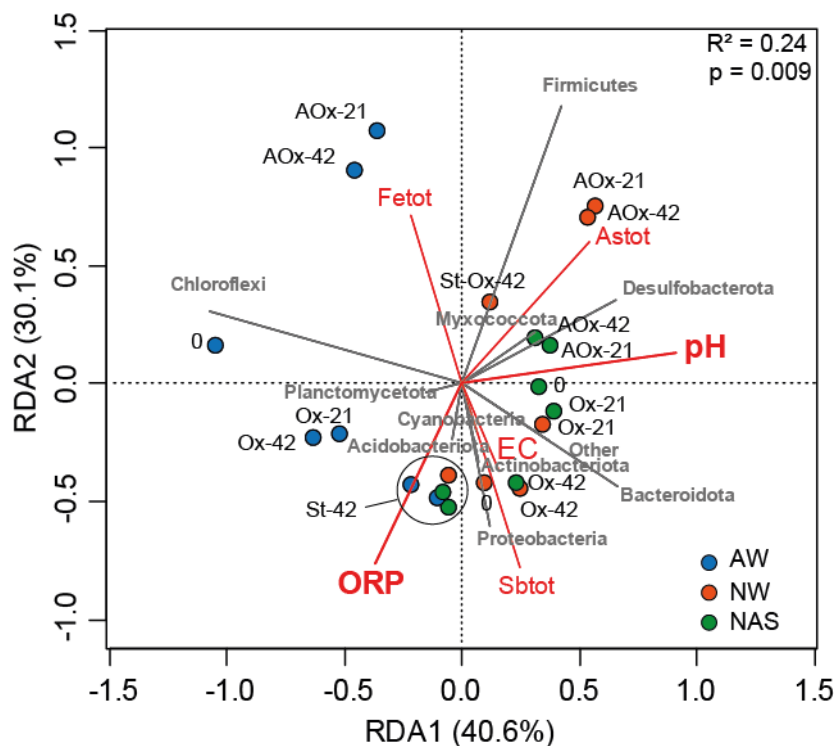


441  
 442 **Figure 5.** Relative abundance of the major bacterial and archaeal phyla in soils and samples  
 443 from acidic pH waste (AW), neutral pH waste (NW) and neutral pH aquatic sediment (NAS)  
 444 microcosms. Ox (oxic-biotic conditions), Ox St (autoclaved oxic-abiotic conditions), AOx  
 445 (anoxic-biotic conditions) and AOx St (autoclaved anoxic-abiotic conditions).

446 With the aim of identifying the influence on the microbial community structure of the  
 447 physico-chemical characteristics of the sampled water, an RDA was built (Fig. 6). The results  
 448 indicate that pH and ORP were explanatory variables that significantly influenced the

449 community structure at the phylum level, explaining 24 % ( $p = 0.009$ ) of the total variance. The  
 450 lowest pH favoured the presence of Chloroflexi, whereas high pH seemed to induce higher  
 451 proportions of Desulfobacterota and Bacteroidota. Low ORP, in AOx conditions, favoured the  
 452 development of Firmicutes. The St conditions had the same community structure, regardless  
 453 of their conditions, except for the NW Ox condition.

454



455

456 **Figure 6.** RDA of sequence abundances assigned at phylum level of bacterial and archaeal  
 457 communities in soils and microcosm samples in relation to environmental variables. Red lines  
 458 represent the environmental variables (significant variables in bold), grey lines represent the  
 459 Phyla, and circles represent the samples (AW in blue, NW in red and NAS in green). Ox (oxic-  
 460 biotic conditions), Ox St (autoclaved oxic-abiotic conditions), AOx (anoxic-biotic conditions)  
 461 and AOx St (autoclaved anoxic-abiotic conditions).

462 Some of the OTUs identified at the genus level correspond to genera that were shown  
 463 to be involved in the metabolisms of Sb, S or Fe (Table 3). Their proportion in the total number  
 464 of sequences for each sample, grouped by common metabolism, strongly varied between

465 conditions (Fig. 7). With the AW, these metabolic groups represented a very low proportion of  
466 the sequences in oxic conditions, whereas in anoxic conditions, Sb(III)-oxidising, sulphate-  
467 reducing bacteria (SRB) and Fe(III)-reducing groups emerged during incubation. At the end of  
468 the experiment (day 41), the two replicates AOx B and AOx C, in which Sb was immobilised,  
469 counted more Fe(III)-reducing bacteria and SRB, and fewer genera including Sb(III)-oxidising  
470 strains than the replicate AOx A, in which Sb mobility was higher. This result could be linked  
471 to the higher proportion of Sb(V) in AOx A than in AOx B and C (Table 2). With the NW, a high  
472 proportion of the Sb(III)-oxidising group was observed in oxic conditions, particularly in the St  
473 conditions, linked to the high proportion of sequences affiliated to the genera *Bacillus* and  
474 *Novosphingobium* in these samples (Supplementary Table 4). However, this might not lead to  
475 any biogeochemical interpretation, as biomass was very low in the St conditions  
476 (Supplementary Table 3). In anoxic conditions, NW was enriched in Sb(V)-reducing and Fe(III)-  
477 reducing metabolic groups, with a major contribution of *Geobacter* in these two groups  
478 (Supplementary Table 4). Sulphate-reducers also emerged in the NW biotic AOx incubation,  
479 to a lesser extent. With the NAS, the targeted metabolic groups did not appear in the St  
480 conditions, whereas, with the exception of the Sb(V)-red group, they were well represented in  
481 all the biotic conditions, even at t0. Incubation conditions seemed to favour the development  
482 of Sb(III)-oxidising and SOX groups in oxic conditions, and of Sb(III)-oxidising and more clearly  
483 Fe(III)-reducing groups in anoxic conditions, with *Anaeromyxobacter* and *Geothermobacter* as  
484 the main corresponding putative Fe(III)-reducers (Supplementary Table 4).

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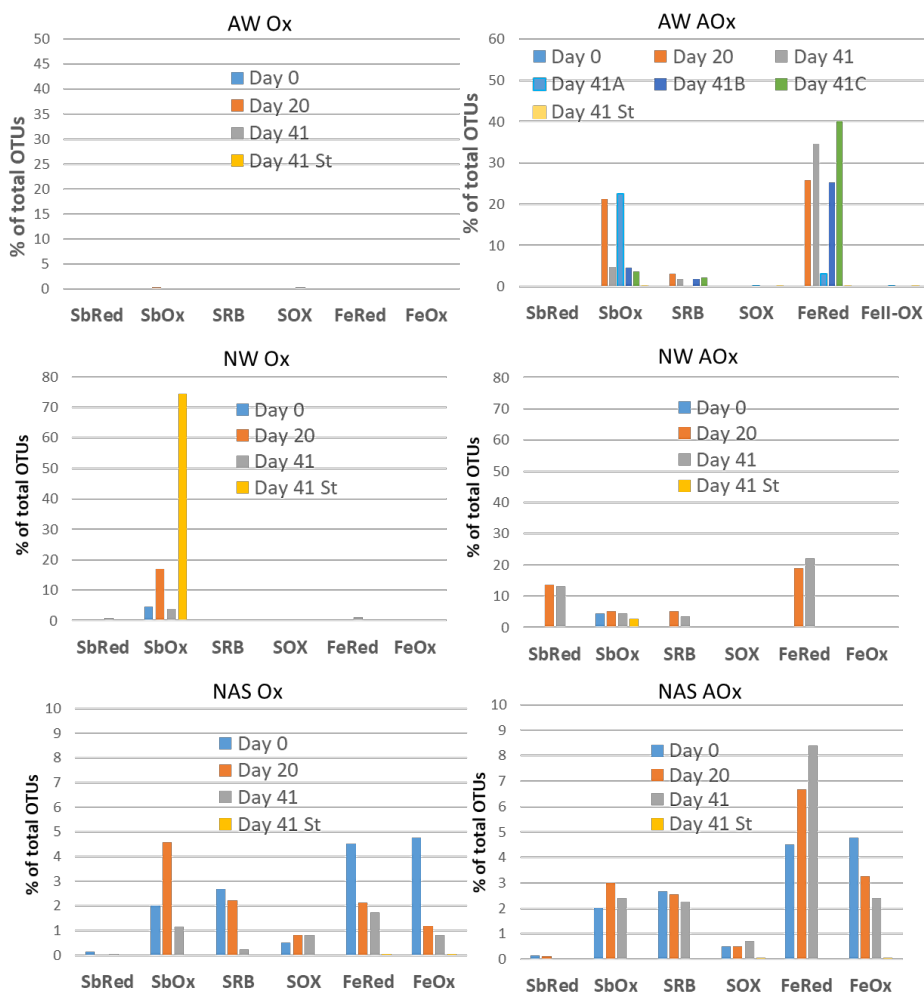
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491



492 **Table 3.** List of genera including strains with known metabolisms related to the biogeocycles  
 493 of Sb, S or Fe and detected in Susana sample incubations. Acidic pH waste (AW), neutral pH  
 494 waste (NW) and neutral pH aquatic sediment (NAS). Ox: oxic; AOx: anoxic; St: sterilised  
 495 conditions.

<b>Genus</b>	<b>Metabolisms</b>	<b>Detected in</b>	<b>Reference</b>
<i>Cupriavidus</i>	Sb(III)-oxidising	NW: AOx, AOx St	Nguyen and Lee 2015
<i>Pseudomonas</i>	Sb(III)-oxidising	AW: OxSt NW: Ox, OxSt, AOx, AOx St	Nguyen and Lee 2015
<i>Bacillus</i>	Sb(III)-oxidising	AW: Ox, OxSt, AOx, AOx St NW: Ox, OxSt, AOx, AOx St, NAS: Ox, AOx	Li et al. 2018
<i>Bosea</i>	Sb(III)-oxidising	NW: Ox, OxSt, AOx, AOx St	Xiang et al. 2022
<i>Acidithiobacillus</i>	Sb(III)-oxidising	AW: OxSt, AOx, AOx St	Nguyen and Lee 2015
<i>Flaviumibacter</i>	Sb(III)-oxidising	NAS: Ox, AOx	Han et al. 2016
<i>Hydrogenophaga</i>	Sb(III)-oxidising	NAS: Ox, AOx	Terry et al. 2015
<i>Novoshingobium</i>	Sb(III)-oxidising	NW: Ox, OxSt, AOx, AOx St NAS: Ox, AOx, AOx St	Deng et al. 2021
<i>Thiobacillus</i>	Sb(III)-oxidising S-oxidising	AW: OxSt, NW: Ox, NAS: Ox, AOx	Deng et al. 2021
<i>Roseomonas</i>	Sb(III)-oxidising	AW: OxSt NAS: Ox, AOx	Sun et al. 2020
<i>Geobacter</i>	Sb(V)-reducing Fe(III)-reducing	AW: OxSt NW: Ox, AOx NAS: Ox, AOx	Nguyen et al. 2018
<i>Thermithiobacillus</i>	S-oxidising	AW: Ox	Yang et al. 2015
<i>Acidithiobacillus</i>	S-oxidising Fe(II)-oxidising	AW: Ox St, AOx, AOx St NW: AOx St NAS: AOxSt	Quatrini and Johnson 2019
<i>Sideroxydans</i>	Fe(II)-oxidising	AW: Ox St NAS: Ox, Ox St, AOx	Weiss et al. 2007
<i>Gallionella</i>	Fe(II)-oxidising	NAS: Ox, AOx	De Vet et al. 2011
<i>Anaeromyxobacter</i>	Fe(III)-reducing	AW: AOx, Ox St NW: AOx NAS: Ox, AOx	Treude et al. 2003
<i>Geothermobacter</i>	Fe(III)-reducing	NAS: Ox, AOx, AOx St	Kashefi et al. 2003
<i>Desulfotobacterium</i>	Fe(III)-reducing, S-reducing	AW: AOx, ZOx St NW: Ox, AOx NAS: Ox, Ox St AOx	Finneran et al. 2002
<i>Desulfosporosinus</i>	S-reducing	AW: Ox, AOx NW: Ox, AOx NAS: Ox, AOx, Ox St	Alazard et al. 2010
<i>Desulfatirhabdium</i>	S-reducing	NAS: Ox, AOx	Balk et al. 2008
<i>Desulfotomaculum</i>	S-reducing	NW: AOx NAS: Ox	Kaksonen et al. 2004
<i>Desulfurivibrio</i>	S-reducing	NAS: AOx	Kaksonen et al. 2004
<i>Desulfobulbus</i>	S-reducing	NAS: Ox, AOx	Kaksonen et al. 2004
<i>Desulfovirga</i>	S-reducing	NAS: Ox, AOx	Deng et al. 2016
<i>Syntrophobacter</i>	S-reducing	NAS: Ox, AOx	Sánchez-Andrea et al. 2012



496

497 **Figure 7.** Proportion in each sample, according to the obtained 16S sequences, of the sum of  
 498 identified genera including Sb, S or Fe-transforming bacterial strains, grouped according to  
 499 metabolic processes. Incubations of acidic pH waste (AW), neutral pH waste (NW) and neutral  
 500 pH aquatic sediment (NAS). Ox: oxic; AOx: anoxic; St: sterilised conditions. SbRed: Sb(V)-  
 501 reducing; SbOx: Sb(III)-oxidising, SRB: sulphate-reducing bacteria; SOX: Sulphur-oxidising;  
 502 FeRed: Fe(III)-reducing; FeOx: Fe(II)-oxidising. Genera included in each group are detailed in  
 503 Table 2.

504

505

506

## 507 4. Discussion

### 508 4.1. Evolution of Sb concentrations

509 Dissolved Sb concentrations in the present experiments varied from 90  $\mu\text{g L}^{-1}$  (in the  
510 AW biotic anoxic conditions) to 5,400  $\mu\text{g L}^{-1}$  (in the NAS St oxic condition). These values are  
511 in the range previously found in mining environments, i.e. 2,200  $\mu\text{g L}^{-1}$  in the adit of Goesdorf  
512 mine, Luxembourg (Filella et al. 2009); 1,600  $\mu\text{g L}^{-1}$  in a pond receiving water from tailings of  
513 the Giant gold Mine in Yellowknife, Canada (Fawcett et al. 2015); from 2,900  $\mu\text{g L}^{-1}$  in surface  
514 water to 23,000  $\mu\text{g L}^{-1}$  in groundwater from Xikuangshan mine, China (Wen et al. 2016).

515 The mobility, bioavailability, and transport of Sb in soils are directly linked to its  
516 speciation as well as to the pH, redox conditions, type of minerals and microbial processes  
517 (Pitman et al. 1957; Filella et al. 2002; Wilson et al. 2010; Herath et al. 2017). Different  
518 behaviours of Sb were observed with our three studied materials presenting different pH  
519 values. Considering the results of the St microcosms, while in the AW (pH  $\approx$  4) the dissolved  
520 Sb concentration remained unchanged, in NW and NAS (pH  $\approx$  6.5) there was an increase in  
521 soluble Sb concentration. Whereas sequential extractions showed that most Sb is present in  
522 forms that are resistant to dissolution, the high total Sb concentration in all materials explains  
523 the dissolved Sb concentrations observed in the microcosms. Experiments carried out by Hu  
524 et al. (2016) showed different effects of pH on Sb release from mining soils, depending on  
525 mineralogy. Here, the three analysed materials contained stibnite and Sb oxides  
526 (Supplementary Table 1). Stibnite dissolves in oxygenated water to form the hydroxide  
527  $\text{Sb}(\text{OH})_3$  (Biver and Shotykh 2012). The corresponding dissolution rate is lower at near neutral  
528 pH than in acidic or alkaline conditions, thus this reaction could hardly explain our results in St  
529 condition. The resulting hydroxide  $\text{Sb}(\text{OH})_3$  presents a low solubility, as it can form a solid  
530 species  $\text{Sb}(\text{OH})_3(\text{s})$  (Brookins 1986; Shkol'nikov 2010). Our materials also contained Sb  
531 oxides, that might dissolve in aqueous solutions producing the Sb(V) species  $\text{Sb}(\text{OH})_6^-$ , which  
532 can exist over a wide range of pH (Pitman et al. 1957; Brookins 1986) including our  
533 experimental conditions, and the poorly soluble  $\text{Sb}(\text{OH})_3$ . As a matter of fact, in St conditions,

534 Sb was mainly present as Sb(V) at the end of incubation (Table 2), even in anaerobic  
535 conditions, suggesting a phenomenon linked to the abiotic dissolution of Sb(V)-containing  
536 minerals. Thermodynamic equilibrium data indicate that Sb(V) species should exist in aerobic  
537 conditions and Sb(III) species in anoxic and low-redox environments (Pitman et al. 1957; Filella  
538 et al. 2002), however the presence of oxidised Sb species in anaerobic systems has already  
539 been observed (Filella et al. 2002; Mitsunobu et al. 2006; Fawcett et al. 2015), suggesting the  
540 major role of kinetics in Sb speciation, that could be influenced by microbial catalysis  
541 phenomena. As a fact, we observed important differences in ORP (Supplementary Fig. 5) that  
542 did not induce differences in Sb concentrations (Fig. 2) in St conditions, whereas the redox  
543 conditions significantly influenced Sb behaviour in presence of microbes and the microbial  
544 communities (Fig. 6). Also, the adsorption and desorption of Sb by oxyhydroxides control its  
545 concentration in solution. Oxyhydroxides have strong affinity for Sb; however, adsorption  
546 efficiency depends on their type, pH and Sb species (Kong et al. 2016; Wang et al. 2021). The  
547 presence of Mn-, Fe- and Al oxyhydroxides affects the fate of Sb through sorption and oxidation  
548 and its affinity for Sb is affected by pH (He et al. 2019). Acidic pH conditions favour the sorption  
549 of both Sb(III) and Sb(V) onto Mn- (Wang et al. 2021), and Fe- (Leuz et al. 2006) oxides, with  
550 the rate of sorption decreasing subsequently with the increase in pH. These results appear  
551 consistent with those obtained in our study, where the most acidic material (AW St) released  
552 less Sb in solution than the other two studied materials (NW St and NAS St). Iron oxides were  
553 detected in most materials through mineralogical analysis (supplementary Table 1) and  
554 chemical extractions (Supplementary Fig. 2). The biogeochemical behaviour of Fe is strongly  
555 influenced by pH (Emerson and Weiss 2004), Fe(II) solubility being increased in low pH  
556 conditions (Frohne et al. 2011). This fact could explain the increase in soluble Fe concentration  
557 under reducing conditions in AW (Fig. 3), since it could be attributed to the bio-reduction of Fe  
558 hydroxides. However, Sb was rather immobilised than solubilised in the corresponding  
559 microcosm. These results suggest that adsorption/desorption mechanisms linked to Fe oxides  
560 were not the only processes controlling the fate of Sb in our microcosms.

561           **4.2. Significance of the comparison biotic vs. St conditions**

562           Through the present experiments, it was possible to evaluate the influence of microbial  
563 activities on the mobility of Sb in diverse surface compartments of a mining site, through  
564 comparison of complete microbial communities with systems in which microbial activities were  
565 greatly weakened. There is no efficient way to completely sterilise environmental samples  
566 (Otte et al. 2018). Here, the autoclaving of materials in anoxic conditions did not seem to  
567 strongly alter minerals, whereas it could have impacted the organic compartment, in particular  
568 in the NAS. Monitoring of cell densities by the LIVE/DEAD® BacLight Bacterial Viability Kit  
569 showed higher cell concentrations in anoxic conditions than in oxic conditions. Abundant  
570 growth in anoxic conditions could be linked to the availability of H<sub>2</sub> that could be used as an  
571 energy source. Dominance of “dead” cells in these samples might simply result from the  
572 experimental procedure as filtration and staining were performed in an oxygenated  
573 atmosphere. However, “live” cells were also observed in all incubations and their concentration  
574 increased, indicating active microbial metabolisms. Conversely, low cell counts, and low final  
575 DNA concentrations, indicated that autoclaving and formaldehyde injections greatly weakened  
576 microbial metabolism in the St conditions.

577

578           **4.3. Role of microbial communities**

579           **4.3.1. Comparison of environmental compartments**

580           Microbial communities presented very different compositions depending on the type of  
581 material, AW, NW or NAS, suggesting a diversity of biological processes depending on  
582 environmental compartments at the site. Interestingly, a few OTUs identified at the genus level  
583 that were retrieved in most of the samples, such as *Bacillus*, *Desulfosporosinus* and  
584 *Desulfitobacterium*, correspond to spore-forming microorganisms, able to resist adverse  
585 conditions. Not surprisingly, the most acidic environment presented the lowest diversity, and  
586 pH was one of the parameters influencing microbial communities' structure. Previous studies  
587 showed that Sb contamination could influence the composition of microbial communities on

588 mining sites (Deng et al. 2020; Duan et al. 2022). In sediments of a watershed contaminated  
589 by Sb mining, pH, temperature, ORP and dissolved Sb significantly structured the microbial  
590 communities (Sun et al. 2016). Here, we observed strong divergences between communities  
591 of surface environments at a small site scale that seemed to be more influenced by pH and  
592 redox conditions than by soluble Sb.

593

#### 594 **4.3.2. Bio-processes in oxic conditions**

595 Previous studies have already shown that microbial activities could influence the  
596 mobility of Sb. Casiot et al. (2007) observed that Sb release from a sediment polluted by mining  
597 activities was enhanced by aerobic microbial activity and attenuated in anoxic biotic conditions.  
598 Loni et al. (2020) observed higher Sb solubilisation from mining rock samples, in oxic  
599 conditions, by an Sb(III)-oxidising micro-organism affiliated to the *Paracoccus* genus. Here, in  
600 the presence of oxygen, the overall effect of microbial processes differed between the types  
601 of materials: Sb release was enhanced by biological processes in the AW, not influenced in  
602 the NW, and attenuated in the NAS.

603 In AW, microbial activities in oxic conditions promoted Sb release and acidification;  
604 however these processes could not be clearly related to the analysis and evolution of the  
605 microbial community, that was largely dominated by OTUs belonging to Chloroflexi of the  
606 family of Ktedonobacteraceae, none identified at the genus level. This family includes  
607 filamentous, spore-forming, gram-positive heterotrophic bacteria from soil (Cavaletti et al.  
608 2006). However, although limited (0.6% of total OTUs on day 41), emergence of the known S-  
609 oxidising genus *Thermithiobacillus* was observed in these microcosms. The observed  
610 biologically-related release of Sb from AW might be related to the direct or indirect alteration  
611 of Sb-bearing phases, such as sulphides or organic matter. In NW, biological activities did not  
612 significantly influence Sb mobility in oxic conditions, and no known genus with metabolisms  
613 based on Sb, S or Fe transformations was identified in the microbial communities that seemed  
614 dominated by heterotrophic phyla. Conversely, the NAS incubated in oxic conditions showed

615 an overall immobilisation of Sb in biotic compared with St conditions, but only after a strong  
616 release of Sb that was observed in both conditions during the first week of incubation. These  
617 results could be explained by the early oxidation of amorphous Fe sulphide, often present in  
618 aquatic sediments (Andrade et al. 2010), that would release Fe(II), (bio)-oxidised into Fe(III),  
619 that would rapidly precipitate as fresh Fe oxides known to adsorb Sb (Ashley et al. 2003). The  
620 increase in the proportion in S-oxidising genus *Thiobacillus* between T0 and T20 days, and the  
621 presence of the Fe(II)-oxidising genera *Sideroxydans* and *Gallionella* in the sediment appear  
622 to be in agreement with this hypothesis. The observation of a colour change, from dark brown  
623 (St condition) to ochre-brown (biotic condition) also supported this scenario (Supplementary  
624 figure 8A). Moreover, the pH values that were lower in NAS than in NAS St, possibly linked to  
625 biologically-induced reactions (Fe hydroxydes precipitation, oxidation of sulphur compounds,  
626 oxidation of organic matter producing CO<sub>2</sub>), could favour Sb adsorption onto oxides (Leuz et  
627 al. 2006).

628

#### 629 **4.3.2 Bio-processes in anoxic conditions**

630 In the microcosms, Sb release was nearly always attenuated by living micro-organisms  
631 in anaerobic conditions. However, with the AW incubated in AOx conditions, the three  
632 replicates behaved differently in terms of Sb mobility and microbial communities. In two  
633 replicates, Sb was immobilised compared with the St condition, and the proportions of: (i) the  
634 Fe(III)-reducer *Desulfitobacterium* (25 to 40% of the total sequences) and (ii) the sulphate-  
635 reducer *Desulfosporosinus* increased. In the third replicate, Sb was more mobile than in the St  
636 condition, the Fe(III)-reducer proportion and identity were different (*Anaeromyxobacter*, “only”  
637 3%), and known sulphate-reducers were not detected. Conversely, this replicate exhibited a  
638 high proportion of sequences affiliated to the *Bacillus* genus, which includes known Sb(III)-  
639 oxidisers (Li et al., 2018) and strains able to grow either in aerobic or anaerobic conditions  
640 (Clements et al., 2002). The development of genera including Sb(III)-oxidisers in anoxic  
641 conditions seems paradoxical; however, some genera of this metabolic group are facultative

642 anaerobic (*Bacillus*, *Acidithiobacillus*...), thus they could grow in the absence of oxygen, and  
643 possibly oxidise Sb(III) using alternative electron acceptors such as nitrate (Terry et al. 2015).  
644 Other subtle differences between these replicates could be detected: ORP decreased less  
645 sharply, and Fe release was delayed in the microcosm with higher Sb mobility. These results  
646 show that Sb behaviour cannot be simply inferred from the redox context and could be strongly  
647 influenced by the growth sequence of different microorganisms belonging to different metabolic  
648 groups. Divergences of biogeochemical reactions linked with subtle differences in the  
649 composition of the initial microbial communities have already been observed in microcosms of  
650 sediments (Kwon et al. 2016; Battaglia-Brunet et al. 2022).

651 With the NW, anoxic incubation in the presence of active microbes induced both  
652 immobilisation of Sb and a clear increase of the Sb(III) proportion in dissolved Sb (**Table 2**).  
653 Immobilisation of Sb in reducing conditions may be linked to Sb(V) reduction, as Sb(III)  
654 hydroxide is less soluble than Sb(V) anions in neutral conditions (Brookins 1986; Shkol'nikov  
655 2010; Herath et al. 2017), or linked to the precipitation of Sb sulphide in the presence of  
656 biogenic H<sub>2</sub>S (Zhang et al. 2016). Here, NW AOx incubations induced the growth of bacteria  
657 belonging to the *Geobacter* genus, whose proportion of 16S sequences rose from non-  
658 detectable in the initial material to 13% on day 20. A *Geobacter* strain (Yamamura et al. 2021)  
659 was shown to be able to grow through dissimilatory Sb(V) reduction with acetate as an electron  
660 donor, producing Sb<sub>2</sub>O<sub>3</sub>, and this genus was detected as an active member of an Sb(V)-  
661 reducing community in incubated paddy soil (Sun et al. 2021). Hydrogen can be used as an  
662 energy source by members of the *Geobacter* genus (Caccavo et al. 1994), and this inorganic  
663 substrate was also shown to promote Sb(V) bio-reduction in soils (Nguyen et al. 2018). Thus,  
664 *Geobacter*-affiliated organisms could contribute to attenuating Sb mobility in NW incubations.  
665 In NAS microcosms incubated in anoxic conditions, Sb mobility was attenuated, and the Sb(V)  
666 proportion was slightly lower than in St conditions. In these microcosms, SRB were maintained  
667 as a major metabolic group identified at the genus level (2-3%), but the main enriched group  
668 corresponded to Fe(III)-reducers, whose proportion increased continuously during incubation,



669 from 4% (day 0) to 8% (day 41). The main Fe(III)-reducers in this group belonged to the genera  
670 *Anaeromyxobacter* and *Geothermobacter*. These microcosms contained diverse SRB, and  
671 among them, the proportion of *Desulfovibrio*-related sequences slightly increased during  
672 incubation. Thus, the biotic stabilisation of Sb in NAS AOx microcosms may have resulted from  
673 bio-sulphide precipitation, or bio-reduction of Sb(V) into Sb(III) by some micro-organisms  
674 whose Sb(V)-reducing activity has not been yet characterised. These mechanisms could be  
675 helped by the generation of Fe(II) by Fe(III)-reducing bacteria, as Fe(II) was shown to  
676 accelerate Sb sulphide precipitation by stimulating SRB activity (Xi et al. 2020). In the NAS  
677 incubations, dissolved the Fe(II) concentration remained around 2 mg L<sup>-1</sup>, probably because  
678 Fe(II) also precipitated with hydrogen sulphide as FeS. This hypothesis is supported by the  
679 darkening of the biotic slurries compared with the St NAS microcosms (Supplementary Fig.  
680 8B).

681 Globally, anoxic microbial activities decreased the mobility of Sb; however, they induced a  
682 significant release of As in water. The mobilisation of As from mine-contaminated materials in  
683 reducing conditions, linked to biological activities, is a well-known phenomenon (Drewniak and  
684 Sklodowska 2013; Rajpert et al. 2016), and the antagonist behaviour of Sb and As in mine  
685 sediments has previously been described (Casiot et al. 2007).

686

## 687 **5. Conclusions**

688 Microbial communities inhabiting different surface compartments of a small mining area  
689 presented very different compositions. The influence of microbial activities on Sb mobility and  
690 speciation was proven in nearly all conditions. Whereas Sb was mainly present in mineral  
691 phases resistant to chemical extractions, leaching of soils and sediments released several mg  
692 L<sup>-1</sup> of dissolved Sb. Several microbial activities could contribute to Sb immobilisation, including  
693 Fe(II)-oxidation, Sb(V)-reduction, and sulphate-reduction. In aquatic sediments, microbial  
694 activities attenuated Sb mobility in both aerobic and anaerobic conditions. Conversely, Sb(III)-

695 oxidising activities could enhance Sb solubilisation from solid phases of mine wastes. The  
696 overall results suggest that microbial processes influencing Sb behaviour on mining sites are  
697 very diverse. They should be carefully identified and quantified in each environmental  
698 compartment in order to avoid unreliable risk assessments. Moreover, the antagonist  
699 biogeochemical behaviour of As and Sb deserves specific awareness as these two toxic  
700 elements are very often associated in ore bodies. Specific management options, potentially  
701 exploiting microbial reactions, should be developed, aimed to prevent dissemination of these  
702 two metalloids in the environment.

703

704 **Abbreviations:** SW, south west; Sb, antimony; As, arsenic; AW, acidic pH waste; NW, neutral  
705 pH waste; NAS, neutral pH aquatic sediment; AOx, anoxic conditions; Ox, oxic conditions; St,  
706 sterilised; EC, electrical conductivity; ORP, oxidation reduction potential; TOC, total organic  
707 carbon.

708

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715

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717 The authors declare that they have no known competing financial interests or personal  
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719

720 **Author Contributions**

721 The contributions of each author to this study are as follows: Jesus Daniel Peco Palacios to  
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730

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