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Hugues Thouin, Fabienne Battaglia-Brunet, Marie-Paule Norini, Lydie Le Forestier, Mickael Charron, et al.. Influence of environmental changes on the biogeochemistry of arsenic in a soil polluted by the destruction of chemical weapons: A mesocosm study. *Science of the Total Environment*, 2018, 627, pp.216-226. 10.1016/j.scitotenv.2018.01.158 . insu-01700628

**HAL Id: insu-01700628**

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Submitted on 12 Feb 2018

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# Influence of environmental changes on the biogeochemistry of arsenic in a soil polluted by the destruction of chemical weapons: a mesocosm study

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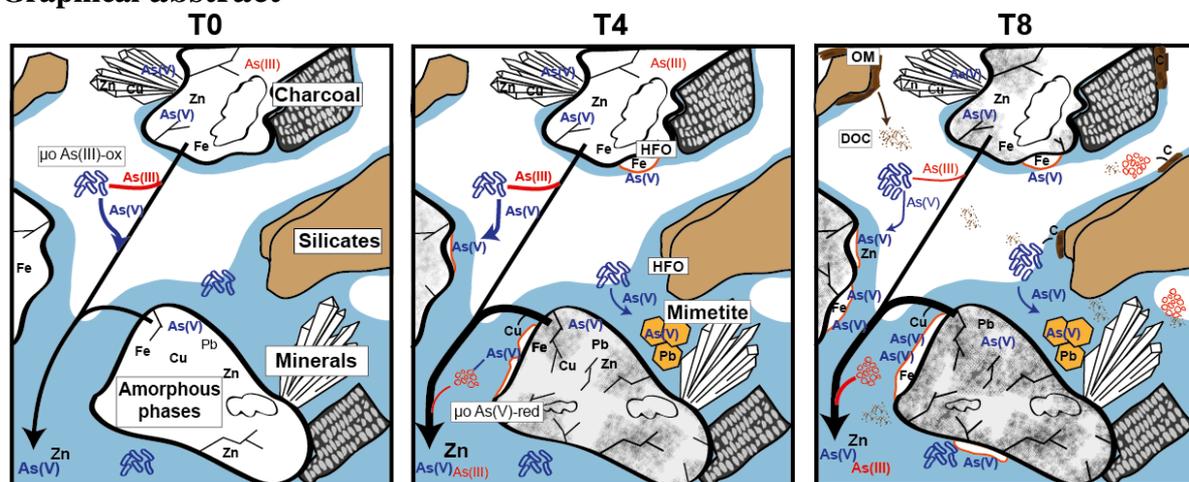
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## Highlights

- The surface of the main As, Cu and Zn carrier was altered in water saturated conditions
- Precipitation of As(V)-bearing mineral mimetite contributed to the immobilization of As and Pb
- The addition of OM contributed to the growth of As transformation microorganisms
- As(III)-oxidizing activity was decreased by OM but remained the major As transformation phenomenon in the system.

## Graphical abstract



## Abstract

Thermal destruction of chemical munitions from World War I led to the formation of a heavily contaminated residue that contains an unexpected mineral association in which a microbial As transformation has been observed.

A mesocosm study was conducted to assess the impact of water saturation episodes and input of bioavailable

organic matter (OM) on pollutant behavior in relation to biogeochemical parameters. Over a period of about eight (8) months, the contaminated soil was subjected to cycles of dry and wet periods corresponding to water table level variations. After the first four (4) months, fragmented litter from the nearby forest was placed on top of the soil. The mesocosm solid phase was sampled by three rounds of coring: at the beginning of the experiment, after four (4) months (before the addition of OM), and at the end of the experiment. Scanning electron microscopy coupled to energy dispersive X-ray spectroscopy observations showed that an amorphous phase, which was the primary carrier of As, Zn, and Cu, was unstable under water-saturated conditions and released a portion of the contaminants in solution. Precipitation of a lead arsenate chloride mineral, mimetite, in soils within the water saturated level caused the immobilization of As and Pb. Mimetite is a durable trap because of its large stability domain; however, this precipitation was limited by a low Pb concentration inducing that high amounts of As remained in solution. The addition of forest litter modified the quantities and qualities of soil OM. Microbial As transformation was affected by the addition of OM, which increased the concentration of both As(III)-oxidizing and As(V)-reducing microorganisms. The addition of OM negatively impacted the As(III) oxidizing rate, however As(III) oxidation was still the dominant reaction in accordance with the formation of arsenate-bearing minerals.

**Keywords:** Arsenic, metal, mineral stability, microbial arsenic transformation, organic matter, dry/wet cycles, mesocosm study

# 1 **1. Introduction**

2 At the end of the First World War (1914-1918), the former combatants had large stockpiles of unfired munitions.  
3 These unspent weapons were dismantled to recycle reusable material, then destroyed or abandoned by ocean  
4 dumping or land burial. Chemical weapons containing nitroaromatic, chlorine, bromine or arsenical compounds  
5 were destroyed primarily by burning because of their hazardous nature. The site called “Place-à-Gaz”, located  
6 northeast of Verdun (France), is one of many sites along the western front line where chemical shells were  
7 destroyed. Between 1920 and 1928, approximately 200,000 shells containing organo-arsenic compounds were  
8 subjected to simple thermal treatment (Bausinger et al., 2007). The burnt munitions were primarily “blue cross  
9 shells” filled with arsenic-bearing vomiting agents CLARK I (diphenylchloroarsine) and CLARK II  
10 (diphenylcyanoarsine).

11 Thermal treatment resulted in severe As and heavy metal contamination of the upper 10 - 40 cm of topsoil at the  
12 site (Bausinger et al., 2007; Thouin et al., 2016). The surface layer where the inorganic contaminants are  
13 concentrated corresponds to the combustion residues of the munitions. This layer, composed of slag, scoria, various  
14 munitions elements, and large amounts of ash and charcoal from the firewood used for burning, is black in color  
15 (Bausinger et al., 2007; Thouin et al., 2016). The central part of the site was heavily contaminated with As, Zn,  
16 Cu, and Pb; concentrations of these elements reached 72,820 mg.kg<sup>-1</sup>, 90,190 mg.kg<sup>-1</sup>, 9,113 mg.kg<sup>-1</sup> and  
17 5,777 mg.kg<sup>-1</sup>, respectively (Thouin et al., 2016). Most organo-arsenic agents/compounds were oxidized during  
18 combustion, resulting in the release of inorganic arsenic As<sub>2</sub>O<sub>3</sub> and As<sub>2</sub>O<sub>5</sub> (Bausinger et al., 2007). A previous  
19 study by Thouin et al., (2016) showed that As was principally present as the pentavalent form (As(V)) within the  
20 solid phases (about 98 % of arsenate and 2 % of arsenite (As(III))) and that several arsenate minerals (adamite-  
21 olivenite series and pharmacosiderite) crystallized as the material cooled. An amorphous phase rich in Fe, Zn, Cu,  
22 and As and presenting a vitreous texture was also observed, highlighting the association of this unusual mineral  
23 assemblage with thermal treatment.

24 Microorganisms actively contributing to the metabolism of carbon and arsenic were detected at the site despite  
25 low organic matter (OM) bioavailability (Thouin et al., 2016). Microbial activity plays a major role in As  
26 speciation in soils (de Mello et al., 2007; Yamamura et al., 2009). For example, in mining environment, microbial  
27 As-transforming activity was used in biomining of As-bearing minerals and to clean up post mining contamination  
28 (Drewniak and Sklodowska 2013). Several bacterial mechanisms are responsible for As(III) oxidation or As(V)

29 reduction (Battaglia-Brunet et al., 2002; Bachate et al., 2012; Zobrist et al., 2000; Stolz et al., 2002), thus driving  
30 As mobility and bioavailability. Changes in environmental conditions, such as modifications of Eh or pH, are  
31 likely to modify these microbial activities. Moreover, the concentration and the composition of soil OM affect a  
32 microorganism diversity and biomass (Tiedje et al., 1999) and may impact bacterial As transformation (Bachate  
33 et al., 2012; Lescure et al., 2016). The cyclic saturation of soils was shown to induce the mobility and change of  
34 speciation of arsenic in relation with bacterial activities. Fe and As reduction and release were observed during  
35 flooding of contaminated soil (Weber et al., 2010) or redox oscillations (Couture et al., 2015). As-transforming  
36 microbes contribute actively to the transformation of As species in frequently saturated soils such as paddy fields  
37 (Xiao et al., 2016). However, up to now, the activity of As(III)-oxidizing and As(V)-reducing microorganisms has  
38 not been evaluated in highly polluted environments presenting the particular structure and composition of the  
39 weapon burning sites, and submitted to fluctuating water regimes.

40 The contaminated soil at the “Place-à-Gaz” site is regularly subjected to partial water saturation during periods of  
41 high precipitation and runoff because of the underlying clayey formation. Moreover, the margin of the site near  
42 the oak forest is exposed to natural deposition of litter that provides bioavailable OM. These environmental  
43 variations are capable of altering the carriers phases of As and affect bacterial As transformation activities, thereby  
44 changing As mobility at the site.

45 With the aim to better understand the cycle of As in this highly polluted material submitted to environmental  
46 changes, an eight-month experiment was performed in a 1 m<sup>3</sup> mesocosm filled with contaminated material that  
47 was subjected to water saturation episodes and input of bioavailable OM. Monitoring of interstitial water  
48 composition at different/various depths in the mesocosm and of leachate (outlet water) provided information on  
49 the processes that affect the fate of As and other inorganic contaminants and their transfer towards surrounding  
50 environmental compartments (Thouin et al., 2017). After Zn, As was the most mobile inorganic contaminant in  
51 the soil water, with concentrations ranging from 20 to 110 µM. The present study was focused on the evolution of  
52 the solid compartment: mineral phases and arsenic-associated microbial parameters. The goal work was to analyze  
53 changes in mineral phases and variations in arsenic-associated microbial parameters to increase our understanding  
54 of the As cycle in this highly-polluted material that is undergoing environmental exposure.

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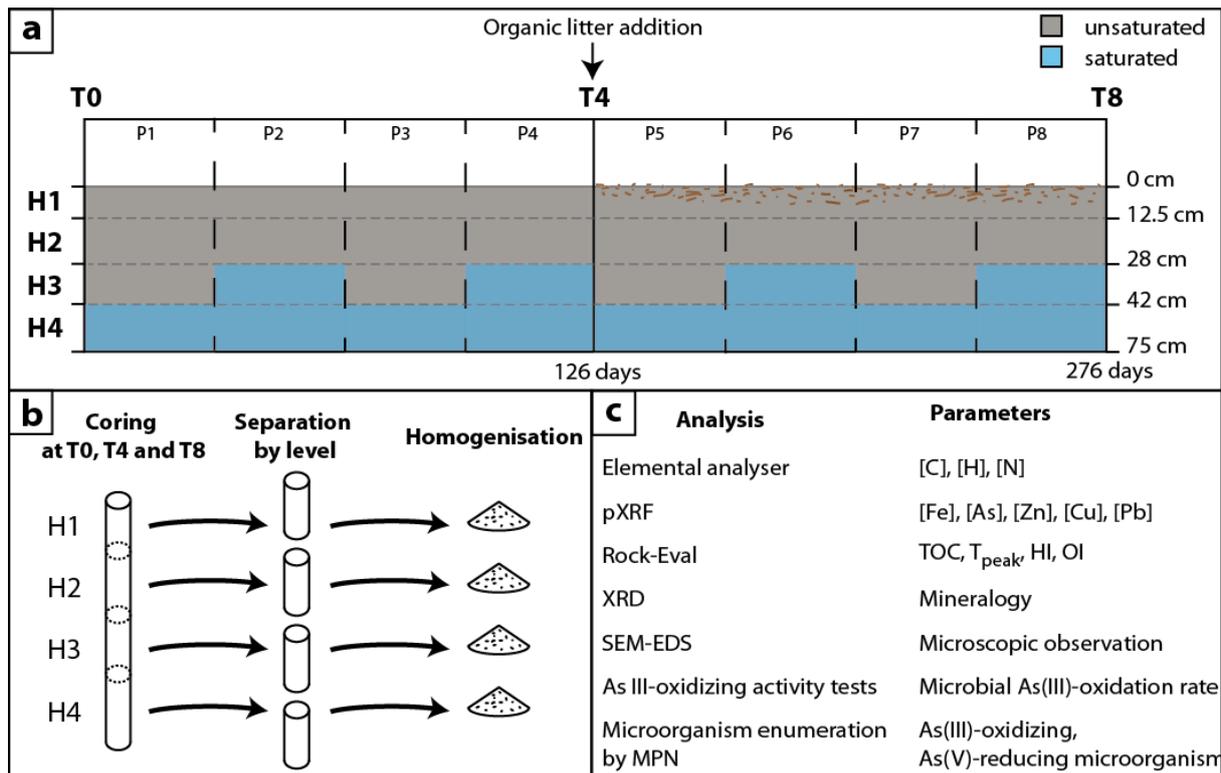
56

## 57 **2. Materials and methods**

### 58 **Experiment and soil sampling**

59 Soil was collected at the site named “Place-à-Gaz” (Spincourt Forest, 20 km from Verdun, France) and  
60 characterized by Bausinger et al. (2007) and Thouin et al. (2016). It contains slag, coal ash, and residues from  
61 ammunition resulting in high concentrations of Zn, As, Cu, and Pb and high organic content (25.9 %).

62 All soil samples described here come from the instrumented mesocosm experiment presented in Thouin et al.  
63 (2017) and shown in supplementary material (SM1). Water and solute fluxes of polluted soil were monitored for  
64 276 days using the experimental device, which consisted of a closed stainless steel column (1 m in diameter and  
65 120 cm high), filled with 610 kg of homogenized contaminated soil. After three months of stabilization, the soil  
66 was subjected to dry/wet cycles (over a period of about eight (8) months) and to the addition of organic forest litter  
67 at the top of the surface soil at the midpoint of the experiment (after four (4) months at T4)(Fig.1.a). The dry period  
68 was characterized by a saturation limited to the mesocosm bottom, and by the addition of around 12 L of Mont  
69 Roucous mineral water once a week. The wet period was characterized by the elevation of the water level and by  
70 the addition of 6 L of water every two days. Rainfall was simulated by a sprinkler system. In order to obtain the  
71 data previously presented in Thouin et al. (2017), soil solution was sampled at four sampling levels (H1, H2, H3  
72 and H4; Fig.1.a) thanks to inert porous probes. H1 and H2 levels were permanently unsaturated, H4 level was  
73 permanently saturated and H3 level was not saturated during dry periods but was saturated during wet periods.



74

75 **Fig.1.** a. Experiment design. P1, P3, P5, P7 and P2, P4, P6, P8 were respectively dry and wet periods. From the  
 76 beginning of P5 forest litter was added at the top of the contaminated soil. H4 was permanently saturated; H2 and  
 77 H1 were never saturated. The H3 level was not saturated in the dry periods and saturated in the wet periods  
 78 (modified from Thouin *et al.*, 2017). b. Coring and sampling of solid material at T0 (at the beginning of the  
 79 experiment), T4 (after 4 months just before the addition of organic litter) and T8 (at the end of this experiment,  
 80 i.e. after 8 months). c. Analytical methods and associated parameters performed on each soil sample.

81 Coring was performed in the mesocosm using 5 cm diameter stainless steel pipes, at three steps of the experiment:  
 82 at the beginning (T0), before the addition of fragmented forest litter at the end of month 4 (T4), and at the end of  
 83 month 8 (T8). After sampling, the pipes were clogged and put back in place in the soil to fill the empty space. Each  
 84 core was separated into four samples, H1 at a depth of 0-12.5 cm, H2 at 12.5-28 cm, H3 at 28-42 cm, and H4 at  
 85 42-75 cm, corresponding to the soil water sampling levels (Fig.1.b). These samples were homogenised and  
 86 collected in sterile glass jars that were tightly closed and stored at 5°C.

### 87 Analytical techniques

88 For chemical analyses, soil samples were dried and ground to 70 µm. Total carbon, nitrogen, and hydrogen were  
 89 quantified in powdered samples, using an elemental flash pyrolyser analyser (Flash 2000, Thermo Fischer  
 90 Scientific). Total organic carbon (TOC), hydrogen index (HI), oxygen index (OI), and maximum temperature  
 91 of pyrolysable OM (T<sub>peak</sub>) were determined by Rock-Eval pyrolysis (Rock-Eval 6 Turbo, Vinci Technologies).  
 92 The operating principles of this apparatus are described in Lafargue *et al.* (1998). HI can be used as a maturation

93 indicator and is calculated from the amounts of hydrocarbons generated through thermal cracking of nonvolatile  
94 organic matter relative to the amount of organic carbon. OI represents the amount of hydrogen relative to the  
95 amount of organic carbon present in a sample. It is calculated from the amount of CO<sub>2</sub> and CO released during  
96 pyrolysis of the sample. T<sub>peak</sub> is the accurate temperature experienced by the sample when producing the maximum  
97 amount of hydrocarbons. Total concentrations of As, Cu, Zn, Pb, and Fe were determined using an XL3t800  
98 NITON<sup>®</sup> portable X-ray fluorescence field apparatus (pXRF). The correct correlation of the metal  
99 concentrations determined by this technique and by ICP-MS was previously checked in the same soil (Thouin  
100 et al., 2016).

101 The mineralogical composition of the bulk samples was determined by powder X-ray diffraction (XRD). XRD  
102 patterns were recorded using an INEL CPS120 diffractometer montage transmission (Debye-Scherrer geometry)  
103 equipped with a Co anode (Co K $\alpha_1$  = 1.78897 Å) and operating at 35 kV and 35 mA. Scans were recorded from  
104 5° to 90° (2 $\theta$ ) with angular steps of 0.03° and a total acquisition time of 210 min.

105 Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were used to study the  
106 structural evolution of As and metal carrier phases during the experiment and to identify possible new precipitated  
107 phases. The samples were air dried, deposited on a carbon adhesive and directly analyzed. All types of grains were  
108 investigated for morphological observation and elemental distribution. SEM was performed on a TM 3000  
109 (Hitachi) operating at 15 kV accelerating voltage. The SEM was coupled to a Swift ED 3000 X-Stream module  
110 (Hitachi). The acquisition time of EDS point analyses was 300 seconds.

### 111 **As(III)-oxidizing activity tests and bacterial enumeration**

112 Soil samples were incubated at 25°C for 72 h before starting the As(III)-oxidizing activity tests and the  
113 enumeration of As(III)-oxidizing and As(V)-reducing microorganisms. It was previously shown that this step  
114 allows restoring As-related microbial activities in soil and sediment samples that were stored at 5°C (Lescure et  
115 al., 2013; 2016). The As(III)-oxidizing tests were performed in triplicate in 250 mL Erlenmeyer flasks filled with  
116 100 mL of CAsO1 medium (Bachate et al., 2002) supplemented with 1 mM As(III) and inoculated with a mass of  
117 material equivalent to 0.2 g of dry weight. Flasks were plugged with cotton to retain oxidizing conditions and were  
118 incubated at 25° under agitation (100 rpm). Samples were filtrated (0.45  $\mu$ m) and stored at -20°C until analysis.  
119 As(III) and As(V) were separated using the PDC/MIBK method (Battaglia-Brunet et al., 2002). Separated neutral  
120 fraction, attributed to As(V) was then quantified by Flame Atomic Absorption Spectrometry (FAAS) (Varian, Palo

121 Alto, CA, USA). As(III)-oxidizing rates were determined by linear regression fitting of the As(V) concentration  
122 versus time line, during the reaction.

123 The evolution of the concentration in As(III)-oxidizing and As(V)-reducing microorganisms was followed using  
124 the Most Probable Number method (MPN). The method for As(III)-oxidizing microorganisms is detailed in  
125 Thouin et al. (2016). For As(V)-reducing microorganisms (method adapted from Kuai et al., 2001), fresh soil  
126 (equivalent to 0.2 g dry soil) was placed in a sterile, glass Erlenmeyer flask with 10 mL of sterile physiological  
127 saline (9 g.L<sup>-1</sup> NaCl in demineralized water), shaken for 30 min at 25°C, then sonicated 2 x 20 s at 45 kHz.  
128 Triplicate suspensions were prepared for each sample. Soil suspensions were diluted in sterile physiological saline  
129 solution to a dilution of 10<sup>-6</sup>. CAsO1 basal mineral medium (Battaglia-Brunet et al., 2002) was complemented with  
130 20 mM lactic acid and As(V) (100 mg.L<sup>-1</sup>). The medium was distributed over Microtest TM Tissue culture plates  
131 (96 wells), 250 µL per well. Each well was inoculated with 25 µL of diluted soil suspension. Five wells were  
132 inoculated with each dilution. Culture plates were incubated at 25°C for 10 days in anaerobic jars with Anaerocult  
133 packs (Merck). The presence of As(III) formed in the wells during incubation was revealed by the formation of  
134 As(III)-PyrrolidineDithioCarbamate (PDC), an insoluble white complex: 80 µL of 0.1 M acetate buffer (pH 5) and  
135 40 µL PDC solution (5 g.L<sup>-1</sup>) were added to each well. The number of positive wells for each dilution was  
136 determined, and the most probable number of As(V)-reducing microorganisms was given by the Mc Grady table  
137 for five tubes.

### 138 **Statistical analyses**

139 Statistical tests were carried out using R 3.2.4 ([www.r-project.org](http://www.r-project.org)). Pearson correlations were calculated with the  
140 four soil samples at three time steps *i.e.* 12 observations. Principal component analysis (PCA) was performed on  
141 biogeochemical parameters on the same observations.

## 142 **3. Results and discussion**

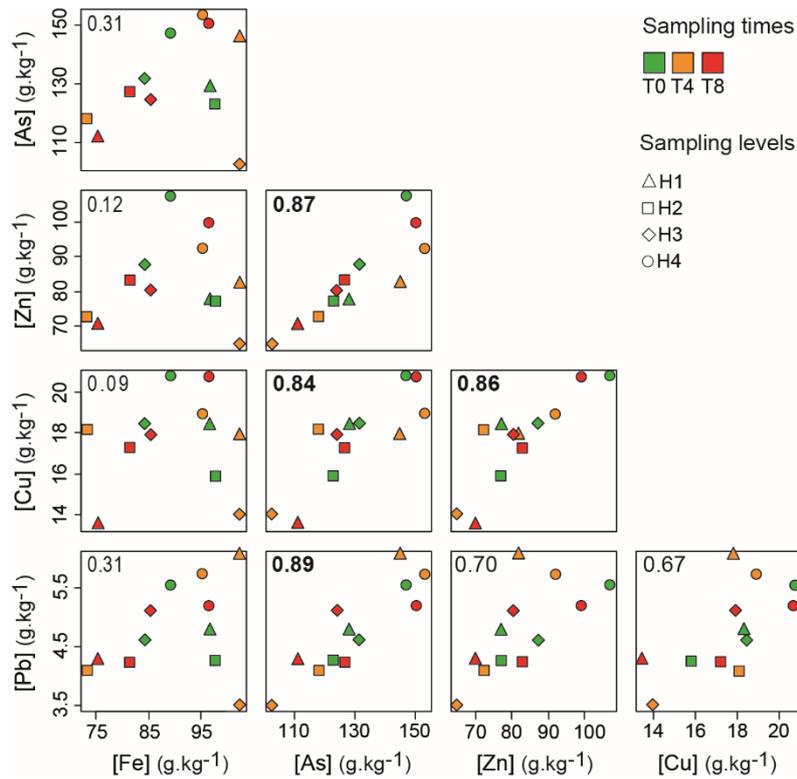
### 143 **3.1. Stability of As bearing mineral phases**

#### 144 **Relationship between As and metals**

145 As and metal concentrations measured/quantified by the pXRF method were high in all soil samples, in the  
146 following decreasing order As > Fe > Zn > Cu > Pb (SM2). Arsenic concentrations ranged from 102,380 to 150,360  
147 mg.kg<sup>-1</sup>, Zn concentrations from 70,040 to 107,400 mg.kg<sup>-1</sup>, Cu concentrations from 13,450 to 20,790 mg.kg<sup>-1</sup>,  
148 and Pb concentrations from 3,490 to 6,030 mg.kg<sup>-1</sup> (SM2). These inorganic contaminant concentrations were very  
149 elevated and in the same range as those measured in other soil samples from the same site (Bausinger et al., 2007;

150 Thouin et al., 2016). Concentration variations were difficult to detect with depth and time because of the high  
151 heterogeneity of the soil. However, at the end of the experiment (T8), metal(loid) concentrations seemed to be  
152 distributed in a pattern showing higher values at the bottom of the mesocosm (H4) (Fig.2; SM2). Moreover, As  
153 and metals were less concentrated at the surface after eight (8) months than at the beginning of the experiment  
154 (Fig.2; SM2). These observations may be the result of the downward vertical transport of metals within the  
155 mesocosm and their accumulation in the saturated soil. Monitoring of metal(oid) concentrations in interstitial water  
156 (Thouin et al., 2017) showed that As was immobilized in the bottom level H4 of the mesocosm as its concentration  
157 values decreased from 60-80  $\mu\text{M}$  in unsaturated soil to 40  $\mu\text{M}$  in the saturated zone. However, this phenomenon  
158 was not observed with other inorganic contaminants. Moreover, the amounts of As released from the liquid phase  
159 in the saturated parts are too low to explain the increase of about 40,000  $\text{mg.kg}^{-1}$  at the bottom of the mesocosm.  
160 It seems more likely that these results highlight the downward particular transport of contaminant-bearing fine  
161 particles during the experiment, even if these results are not sufficient to confirm this hypothesis.

162 Arsenic concentration was significantly correlated with that of metals, as shown by Pearson indices  $> 0.80$  (Fig.2),  
163 with the exception of Fe concentration which was not correlated with any other metal(loid)s. These results suggest  
164 that As, Zn, Cu, and Pb were mainly linked to the same solid phase, as shown in previous characterizations of the  
165 polluted material (Thouin et al., 2016), indicating that the principal As carrier was an amorphous phase containing  
166 high concentrations of metals.



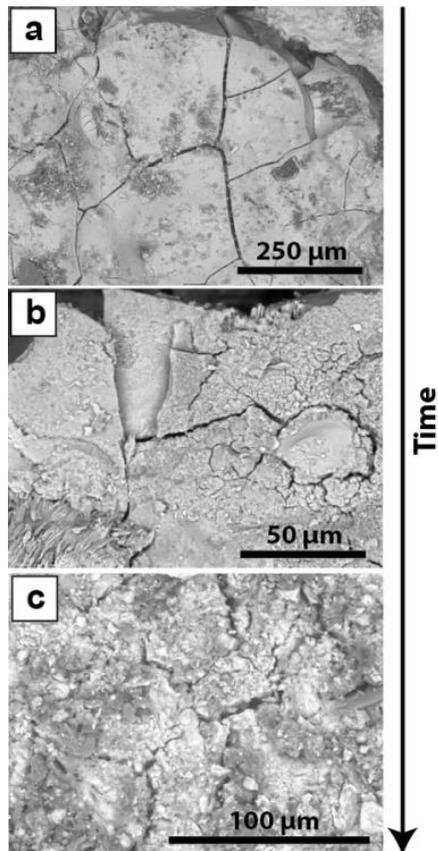
167

168 **Fig.2.** Scatterplot matrix of metal(loid) concentration. Pearson correlation coefficient of variables is specified in  
 169 the upper-left corner of each panel (highest correlations are indicated in bold characters).

170 **Stability of amorphous phases**

171 Morphological evolution of amorphous phases was observed by SEM in various samples, so as to compare their  
 172 stability at different times and under different environmental conditions. In the unsaturated levels, amorphous  
 173 phases did not show evidence of weathering all along the experiment. Figure 2 shows the textural evolution of the  
 174 vitrified amorphous phases in the soil level that was permanently water saturated. At the beginning of the  
 175 experiment (Fig.3 (a)), this phase was characterized by a smooth texture with micro-cracks, similar to the  
 176 morphology previously observed in samples from the site and attributed to the thermal process used to destroy the  
 177 weapons (Thouin et al., 2016). After four (4) months of the mesocosm experiment, the surface of the amorphous  
 178 phases had changed and taken on a rough texture (Fig.3 (b)). Edges of the cracks were altered and new fissure  
 179 networks grew from the previous micro-cracks. At the end of the experiment, after 276 days of water saturation,  
 180 the fissure networks covered the entire surface of the amorphous phases, which had become highly irregular (Fig.3  
 181 (c)). Similar observations have been made during the alteration of a partially vitrified metallurgical waste (Seigneur  
 182 et al., 2007). This morphological evolution may be consistent with dissolution of amorphous phase surfaces in the  
 183 saturated soil, which should be accompanied by the release of As, Zn, Cu, Pb. However, metal(oid)s behaved

184 differently with high As and Zn concentrations in solution, whereas Cu, Pb and Fe were not very mobile (Thouin  
185 et al., 2017).



186

187 **Fig.3.** Backscattered electron images of amorphous phases in the saturated soil. (a), (b) and (c) illustration of three  
188 amorphous phases sampled in the H4 level at T0, T4 and T8 respectively.

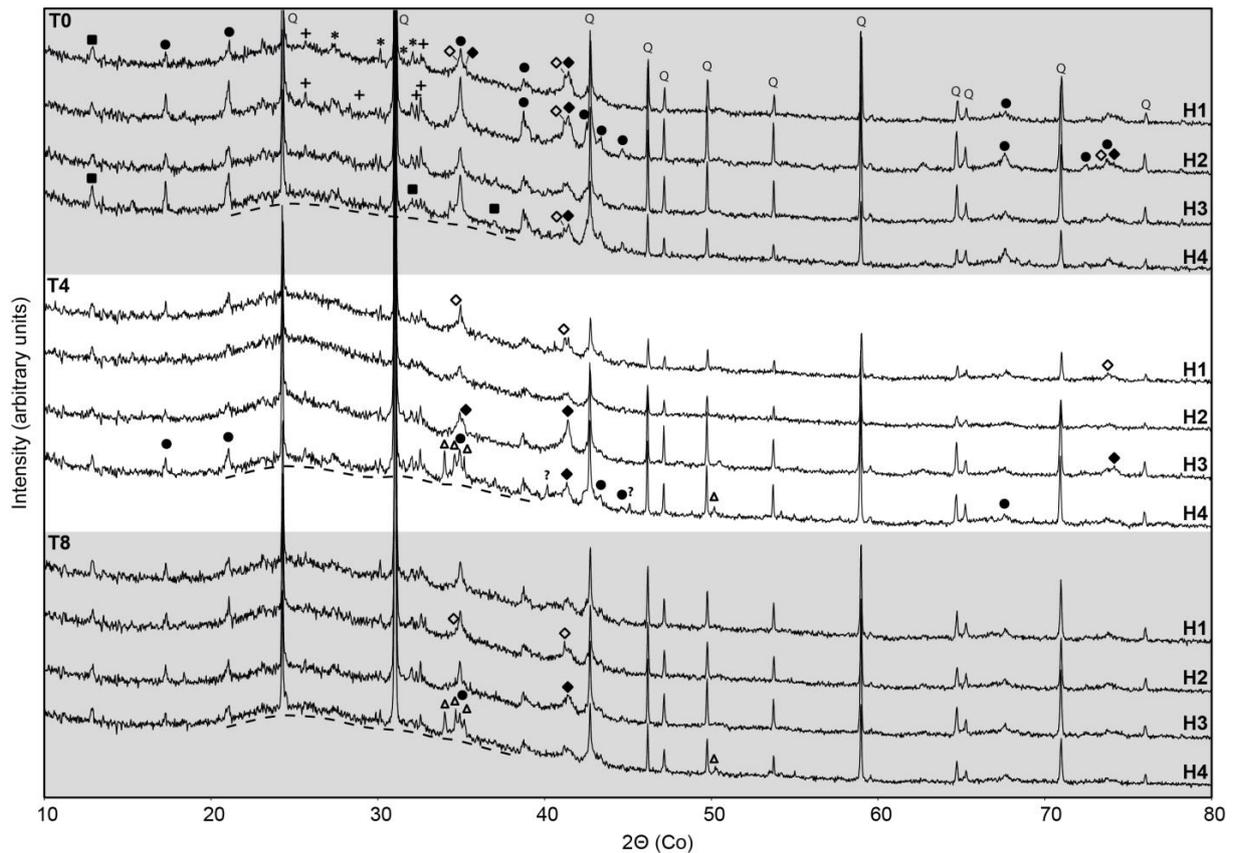
189 Elemental composition of altered amorphous phases was investigated using SEM-EDS analyses (SM3). The  
190 elemental maps of Fe, Zn, As, Pb and Cu on amorphous phases presenting a partially altered surface showed a  
191 different distribution between Fe and Zn. In this study zone Fe content was lower in the smooth amorphous phases  
192 than in the rough surface. An alteration study of partially vitrified metallurgical waste composed primarily of an  
193 iron-silica-lime glass matrix showed that altered glass is characterized by relative enrichments of iron induced by  
194 high calcium depletion (Seigneur et al., 2007). Here the results were similar, with higher iron concentrations in  
195 altered material even though this amorphous material does not have the same chemical composition (no silicium).  
196 Other studies showed that under oxidizing conditions, iron mobilized during dissolution of the vitrified material  
197 precipitated on the surface of the altered zone (Deneele 2002; Ettler et al., 2004). Our results can be explained by  
198 the precipitation of amorphous and poorly crystallized ferric oxides and/or hydrous ferric oxides (HFO) in the  
199 proximity of the amorphous phase, which can bind a portion of As and metals. This hypothesis is consistent with  
200 the low concentrations of mobile Fe ( $< 40 \mu\text{M}$  throughout the experiment, Thouin et al., 2017). The precipitation

201 of iron oxy-hydroxide permits the sorption of Cu and Pb (Swallow et al., 1980) and may also explain the low  
202 mobility of these elements. Moreover, the high adsorption of As(V) by amorphous HFO is a well-known  
203 phenomenon (Dixit and Hering 2003). Thus, precipitation of HFO resulting from the dissolution of amorphous  
204 phases in the water-saturated material may be one of the phenomena inducing the immobilization of As(V) that  
205 was observed in this specific environment of the mesocosm. The Zn depletion observed in the surface of the altered  
206 zone (SM3) seems to show the easier mobilization and the low affinity to HFO of Zn. These assumptions are  
207 consistent with the important Zn concentration in the soil solution, induced by favorable pH conditions (pH range  
208 of 5-6; Thouin et al., 2017), during the experiment.

### 209 **Evolution of the mineralogical association**

210 XRD analysis of soil samples from different depths was used to investigate the mineralogical evolution of the  
211 polluted material during the experiment (Fig.4). First, the high background of X-ray diffractograms confirmed the  
212 abundance of amorphous phases in the soil. Quartz and potassium and sodium feldspars were identified in each  
213 sample with a relative peak intensity that evolved slightly over time and depth. These silicates, which had already  
214 been observed in samples from the polluted site (Thouin et al., 2016), were attributed to the substrate, *i.e.* the  
215 clayey Woëvre formation.

216 Magnetite ( $\text{Fe}_3\text{O}_4$ ) and franklinite ( $\text{ZnFe}_2\text{O}_4$ ) were detected in most samples. Magnetite and franklinite exhibit a  
217 spinel structure with similar lattice parameters that result in an overlapping of their corresponding XRD peaks, so  
218 it is difficult to ascertain the presence of franklinite with XRD spectra. However, the Zn concentration in solids  
219 and the common occurrence of the magnetite-franklinite mineral association in metallurgical furnace wastes  
220 (Pisciella et al., 2001; Juillot et al., 2003; Dutra et al., 2006; Puziewicz et al., 2007; Seignez et al., 2007; Vereš  
221 2014) and in vitrified products of electrostatic precipitator ash from municipal solid wastes (Le Forestier and  
222 Libourel 2008) suggest that magnetite-franklinite solid solutions are present as a result of the thermal destruction  
223 process. The presence of zincite in site soil samples (Thouin et al., 2016) and in metallurgical wastes, together  
224 with magnetite and franklinite (Dutra et al., 2006; Puziewicz et al., 2007; Vereš 2014), confirm the inherited  
225 character of these minerals in this context. Although not detected by XRD in the mesocosm samples, zincite, as  
226 well as magnetite and franklinite, were most probably present but in insufficient amounts for appropriate detection  
227 due to soil heterogeneity. No significant effect of dry/wet cycles or the addition of OM on the stability of these Fe  
228 and Zn minerals was detected.



229

230 **Fig.4.** X-ray diffractograms of bulk soil samples. Q: quartz (ICDD-1045); \* : sodium feldspar (ICDD 19-1184);  
 231 + : potassium feldspar (ICDD 31-0966); ● :adamite (ICDD 39-1354); ■ :Na-Pharmacosiderite (ICDD 38-0388);  
 232 ◆ : magnetite (ICDD 19-0629); ◇ : franklinite (ICDD 22-1012); ▲ : mimetite (ICDD 19-0683); and ? : unidentified  
 233 mineral.

234 The Zn, Cu, and Fe arsenates previously observed in this soil (Thouin et al., 2016), adamite ( $Zn_2AsO_4(OH)$ ),  
 235 olivenite ( $Cu_2AsO_4(OH)$ ), and pharmacosiderite ( $(K,Na,Ba)Fe_4(AsO_4)_3(OH)_5 \cdot 5H_2O$ ) were again identified in all  
 236 samples (Fig.4). Adamite and olivenite are characterized by the same structure so they have similar XRD patterns,  
 237 but SEM-EDS showed that crystals were mostly a solid solution of adamite and olivenite (SM4.a). Indeed,  
 238 adamite-olivenite crystals were easily recognized because of their prismatic and acicular texture, and the ability to  
 239 form large crystals that may grow on grain surfaces. Pharmacosiderite with a cubic structure was also observed  
 240 (SM4.b). No evidence of weathering was observed on these arsenate minerals, which were present all along the  
 241 soil profile.

242 Mimetite ( $Pb_5(AsO_4)_3Cl$ ), a lead arsenate chloride, was detected exclusively in the water-saturated H4 level at T4  
 243 and T8 (Fig.4). Mimetite precipitation was proposed to be part of a soil treatment technology allowing the removal  
 244 of arsenate ions from solution (Twidwell et al., 1994 ; Bajda et al., 2006). Indeed, mimetite precipitates under  
 245 conditions of low Pb and arsenate concentrations and has a stability domain that covers the pH range of natural

246 waters (Magalhães and Silva 2003; Bajda 2010). Arsenate and chloride ions, which were present in high  
247 concentrations in interstitial water throughout the experiment, and the pH range of 5.5-6.0 (Thouin et al., 2017)  
248 were optimal for mimetite precipitation. The factor that limited mimetite precipitation in the soil was the low  
249 amount of dissolved Pb concentration. Mimetite precipitation in the saturated part of the soil means that Pb was  
250 previously released into solution under these conditions; this confirms the hypothesis of progressive dissolution of  
251 metal-bearing amorphous phases throughout the experiment. Mimetite was not observed with SEM, but  
252 micrometric grains with high lead and other metal concentrations were observed in the saturated depth level  
253 (SM4.c). This observation confirms that lead was released and then re-precipitated. Mimetite precipitation,  
254 promoted by soil water saturation, was probably one of the phenomena that caused the previously observed  
255 immobilization of Pb and As(V). Due to mimetite's broad pH stability range (down to pH = 2), this mineral  
256 represents a durable trap for As and Pb. However, the very low dissolved Pb concentration limited mimetite  
257 precipitation and relatively high amounts of As remained in solution.

### 258 **3.2. Modification of the soil organic matter**

259 Characterization of soil OM present in the mesocosm was performed using an elemental flash pyrolyser analyser  
260 and Rock-Eval pyrolysis. Results are represented in table 1. Total carbon (C) ranged from 14.7 to 27.8 %, and  
261 total hydrogen from 1.6 to 2.4 % in the mesocosm soil. The N content of soil ranged from 0.3 to 0.8 %. TOC  
262 ranged between 14.1 and 25.3 %. HI results were low for all mesocosm samples ( $< 0.18 \text{ mg HC. g}^{-1} \text{ TOC}$ ) except  
263 for sample H1 – T8. The proportion of O containing OM (OI) ranged between 125 and 200  $\text{mg O}_2. \text{g}^{-1} \text{ TOC}$ . All  
264  $T_{\text{peak}}$  values were higher than 440 °C, and were higher than 500 °C for two samples (H3 – T4 and H4 – T8). The  
265 high organic carbon content together with the HI and OI values are quite different from those expected in soil OM  
266 (Disnar et al., 2003), but instead show chemical signatures of charcoals (Wolf et al., 2013; Saenger et al., 2015).

267 Previous studies suggest that physical and chemical charcoal properties depend on the temperature of the fire  
268 (Schneider et al., 2010; Wolf et al., 2013). Charcoal formation begins with the loss of easily oxidizable OM (such  
269 as aliphatic and carboxylic compounds), thus with increasing temperature organic compounds progressively  
270 aromatize. High temperature combustion ( $> 700^\circ\text{C}$ ) may cause complete carbonization of OM and may form  
271 polyaromatic crystallites or graphite-like structures (Keiluweit et al., 2010). This restructuring of OM is expressed  
272 by an increase in TOC and a decrease in OI and HI. Wolf et al., (2013) studied the relationship between charcoal  
273 signatures and burning conditions. According to their data, our OM signature (HI/OI,  $T_{\text{peak}}$ ) would correspond to  
274 a fire temperature ranging between 300 and 400 °C. However, this temperature seems to be relatively low for

275 melting metals (Fe, Cu, Zn, Pb) from shell parts, thus to form the amorphous phases. Wood was placed on top of  
 276 the shell dumps during destruction. The temperature was probably higher in the center of the fire, thus explaining  
 277 the difference between the fire temperature signal of charcoal and the total range of temperatures reached during  
 278 combustion.

279 **Table 1** Parameters related to OM

Time	C <sup>a</sup>	H <sup>a</sup>	N <sup>a</sup>	C/N <sup>a</sup>	TOC <sup>b</sup>	T <sub>peak</sub> <sup>b</sup>	HI <sup>b</sup>	OI <sup>b</sup>	HI/OI <sup>b</sup>
	%	%	%		%	°C	mg HC. g <sup>-1</sup> TOC	mg O <sub>2</sub> . g <sup>-1</sup> TOC	
H1	21.1	1.8	0.6	36.3	16.1	441	13	161	0.08
H2	23.8	2.1	0.7	36.2	24.9	446	11	125	0.09
H3	25.9	2.3	0.7	36.6	18.5	443	17	161	0.11
H4	27.8	2.4	0.8	37.0	14.6	445	15	200	0.08
H1	27.1	2.4	0.7	38.1	15.3	446	15	184	0.08
H2	25.9	2.2	0.6	40.5	18.3	452	16	147	0.11
H3	14.7	1.5	0.3	53.5	14.1	542	18	157	0.11
H4	15.1	1.6	0.3	45.8	19.4	440	14	147	0.10
H1	27.8	2.4	0.8	37.0	25.3	439	37	169	0.22
H2	22.6	1.8	0.4	53.2	23.2	445	16	151	0.11
H3	22.8	1.7	0.5	49.5	22.8	446	18	150	0.12
H4	17.1	1.6	0.4	46.2	17.5	559	18	154	0.12
Litter	38.5	4.7	1.8	21.4	30.5	369	268	205	1.31

280 a : Flash pyrolyser analyser; b : Rock-Eval 6

281 The forest litter added at T4 had a very different signal from that of the soil. It has higher organic carbon (30.5 %)  
 282 and nitrogen (1.8 %) concentrations, higher HI (268 mg HC. g<sup>-1</sup> TOC) and OI (205 mg O<sub>2</sub>. g<sup>-1</sup> TOC) values, and a  
 283 lower T<sub>peak</sub> (369 °C). Disnar et al. (2003) determined that fresh or fragmented litter normally has high TOC values  
 284 (10-40%) and HI values higher than 300 mg HC. g<sup>-1</sup> TOC. The T<sub>peak</sub> of 360-370 °C is mostly attributed to cellulose  
 285 and/or lignin, two major components of woody tissues frequently observed in litters. These results confirmed the  
 286 immature nature of the organic litter added to the top soil of the mesocosm.

287 The intrinsic OM of the “Place-à-Gaz” soil, with a high C/N and low HI/OI ratio has characteristics of a barely  
 288 biodegradable material (Tab.1 and SM5). However, the addition of fragmented forest litter enhanced the proportion  
 289 of biodegradable OM in the top soil. The effect of forest litter was visible on surface soil sample H1 – T8, which  
 290 had a higher organic carbon content (25.3 %) and a higher HI/OI ratio (0.22; Tab.1 and SM5), but not a higher

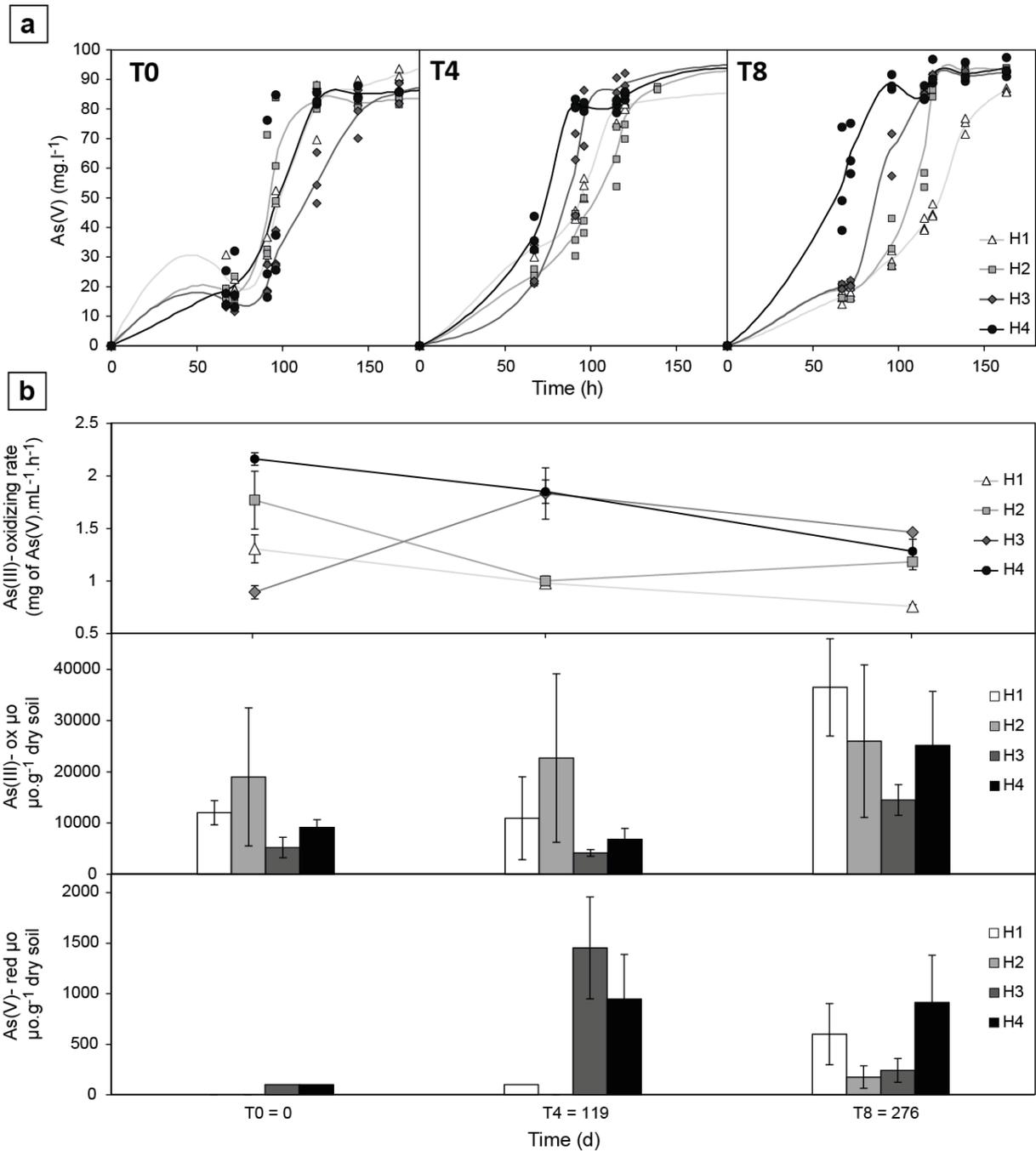
291 C/N ratio. Addition of organic litter increased the carbon mineralization rate in the mesocosm (Thouin et al., 2017),  
292 as a result, it was supposed that the addition of litter increased microbial activity.

### 293 **3.3. Biogeochemistry of arsenic**

#### 294 **Microbial arsenic transformation**

295 As(III)-oxidizing activity tests were used to evaluate the potential for microbial As(III) oxidation during the  
296 experiment (Fig. 5). These global activity tests integrate the diversity and abundance of microflora and  
297 environmental soil conditions (Lescure et al., 2016). With samples collected at T0, As(III) oxidation started after  
298 70 and 90 hours of lapsed time at all levels. The results of microbial As(III) oxidation activity tests at T0 were  
299 consistent with results previously obtained with soil sampled from the most polluted zone of the “Place-à-Gaz”  
300 site (Thouin et al., 2016), with identical latency of about 100 h. Here, the lapsed time decreased progressively  
301 during the mesocosm experiment, particularly in the water-saturated levels. At T8, the As(III) oxidation kinetics  
302 of each sample were well separated temporally, with complete As(III) oxidation achieved in time periods that  
303 increased as follows: H4 < H3 < H2 < H1. The rate of microbial As(III) oxidation was consistently higher in  
304 saturated or alternatively saturated/unsaturated levels H4 and H3 than in unsaturated levels H1 and H2 (except for  
305 H3 T0) (Fig.5.b). The rate of microbial As(III) oxidation decreased over time, mostly in H1 and H4, whose As(V)  
306 values decrease from 1.3 and 2.2 mg of As(V).mL<sup>-1</sup>.h<sup>-1</sup> at T0 to 0.8 and 1.3 mg of As(V).mL<sup>-1</sup>.h<sup>-1</sup> at T8.

307



308

309 **Fig.5.** Microbial As(III)-oxidizing and As(V)-reducing activities. a: Evolution of As(V) concentration during the  
 310 As(III)-oxidizing activity test for the four soil samples at T0, T4 and T8. b: Plot of As(III) oxidation rates  
 311 corresponding to the activity tests in "a". Concentration of As(III)-oxidizing and As(V)-reducing microorganisms  
 312 evaluated by MPN. Errors bars represent the standard deviation of the mean of three replicates for the four soil  
 313 samples. For As(V)-reducing microorganisms a lack of value means that results from the two or three replicates  
 314 were < 50 μo.g<sup>-1</sup> dry soil. (ox: oxidizing; red: reducing; μo: microorganisms).

315 The evolution of abundance of As(III)-oxidizing and As(V)-reducing microorganisms was followed by MPN  
 316 (Fig.5.b). Between T0 and T4, As(III)-oxidizing microorganism concentrations were stable with higher values in  
 317 H2 and lower in H3.

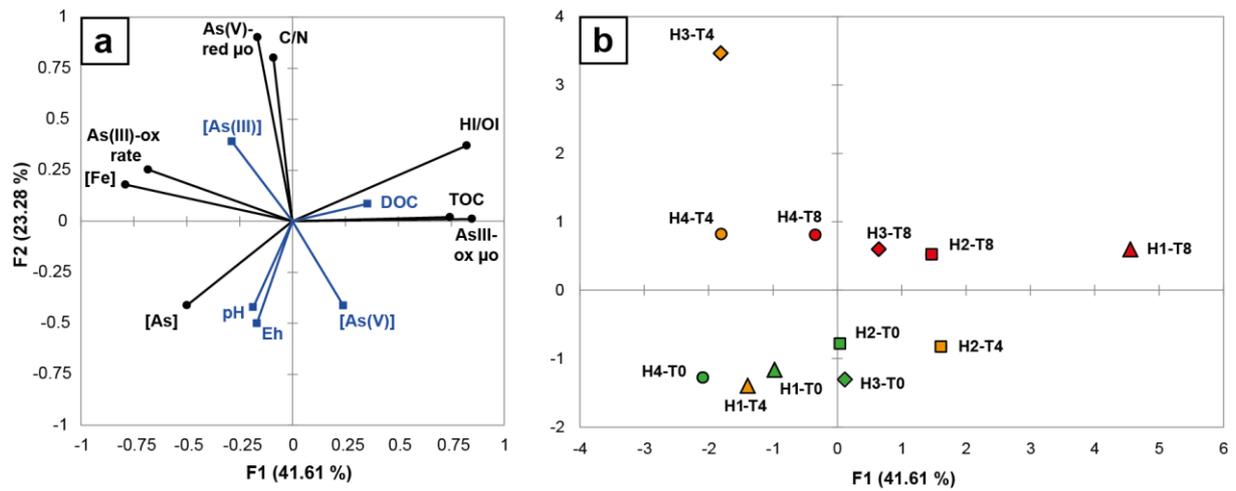
318 After the addition of fragmented forest litter, the concentration of As(III)-oxidizing microorganisms increased in  
319 all levels, and particularly in H1. As(V)-reducing microorganisms abundance that could be estimated by the  
320 selected MPN method were significantly lower than As(III)-oxidizing microorganisms (one order of magnitude  
321 less). At T0, less than 100 As(V)-reducing microorganisms per gram of dry soil were observed. After four (4)  
322 months of the experiment, As(V)-reducing microorganism concentrations increased in the saturated and  
323 alternatively saturated/unsaturated levels. At T8, the concentration of As(V)-reducing microorganisms increased  
324 in H1 and H2, decreased in H3, and remained constant in H4. Thus, both water saturation (for H3 and H4) and  
325 OM supply (for H1 and H2) seemed to promote the growth of As(V)-reducing micro-organisms.

#### 326 **Effect of water saturation and addition of litter on arsenic biogeochemical cycle**

327 The impact of water saturation levels and redox oscillation on As solubility has been described in many studies  
328 (Kumpiene et al., 2009 ; Weber et al., 2010 ; Parsons et al., 2013 ; Couture et al., 2015) where it was shown that  
329 soil saturation increased As solubility by the way of reduction of Fe oxides together with more efficient microbial  
330 reduction of As(V). Increased As(III) concentration in soil solution previously observed in the saturated level H4  
331 of the mesocosm (Thouin et al., 2017) could be related to increased As(V)-reducing microorganism concentrations  
332 in the saturated zones. The stimulation of microbial As(V)-reducing activity induced by soil saturation may have  
333 negatively impacted the overall As(III) oxidation rate in water-saturated levels, but could not explain a decreased  
334 As(III) oxidation rate in unsaturated levels H1 and H2 (Fig.5.a T8).

335 OM may also affect microbial As(III) oxidizing activity. For example, high concentrations of organic substrates  
336 may inhibit this activity (Challan-Belval et al., 2009 ; Bachate et al., 2012 ; Lescure et al., 2016) and may also  
337 stimulate aerobic As(V)-reducing activity of soil microorganisms (Yamamura et al., 2009). The As(III) oxidation  
338 rate results from the overall activity of all microorganisms involved in As speciation, because microbial As(III)  
339 oxidation and As(V) reduction can occur simultaneously, even under aerobic conditions.

340 To describe more precisely the impact of the addition of litter on the available microbial parameters, a principal  
341 components analysis (PCA) was built (Fig.6), including variables that describe OM quantities and qualities (TOC,  
342 DOC, HI/OI, C/N), microbial As transformation parameters (As(III)-oxidation rate, concentrations of As(III)-  
343 oxidizing microorganisms and As(V)-reducing microorganisms), and the variables related to the water phase of  
344 the mesocosm, given in Thouin et al., (2017): As speciation and mobility in the interstitial water ([As], [As(III)],  
345 [As(V)]), and physicochemical parameters of water (pH, Eh).



346

347 **Fig.6.** PCA using biogeochemical parameters of soil samples (black) with geochemical parameters of soil solutions  
 348 (from Thouin *et al.*, 2017) as supplementary data (blue). a: Correlation circle showing variable relationships. b:  
 349 Factorial plan showing samples. (ox: oxidizing; red: reducing;  $\mu$ : microorganisms). Pearson correlation matrix  
 350 was presented in SM6.

351 As previously observed, the As(III) oxidation rate seems to be negatively correlated with As(III)-oxidizing  
 352 microorganism concentrations ( $p = -0.50$ ) and with DOC ( $p = -0.53$ ) and HI/OI ( $p = -0.50$ ) (Fig.6.a). This  
 353 distribution was brought by principal component F1, which represented about 40% of total variability. For this  
 354 component, the surface soil (H1-T8), as the end member, is pulled to the right (Fig.6.b) due to the combined effect  
 355 of DOC, TOC, HI/OI, and As(III)-oxidizing microorganism concentrations. This level was the most impacted by  
 356 the addition of forest litter. The sample distribution along the principal component F1 shows the overall effect of  
 357 the litter on the entire mesocosm, with an enhanced influence towards the right from T4 to T8. Moreover, the  
 358 distribution of T8 samples clearly depends on their depth because, as previously discussed, the deeper level was  
 359 less impacted by the addition of OM.

360 Principal component F2 accounts for 23% of total variability. The concentration of As(V)-reducing  
 361 microorganisms and the C/N ratio were correlated ( $p = 0.59$ ) and were the primary contributors to F2. The  
 362 previously mentioned positive effect of soil saturation on As(V)-reducing microorganisms is expressed by the  
 363 upward translation of samples H3 and H4 between T0 and T4 along the F2 axis (Fig.6.b). Bioavailable OM may  
 364 stimulate the As(V)-reducing activity of soil microorganisms under aerobic conditions (Yamamura *et al.*, 2009)  
 365 because the As(V)-reducing mechanism linked to the As resistance system consumes energy. In our study,  
 366 concentrations of As(V)-reducing microorganisms increased in the non-saturated soil after the addition of  
 367 fragmented litter. However, As(V)-reducing microorganisms were correlated to the C/N ratio ( $p = 0.59$ ) but were  
 368 not correlated to total OM concentrations (TOC,  $p = -0.18$ ; DOC,  $p = -0.09$ ). The relationship between As(V)-

369 reducing microorganism concentrations in the soil and OM qualities and quantities cannot be confirmed here.  
370 Finally, the quantity of As(V)-reducing microorganisms was correlated with As(III) concentrations in soil  
371 interstitial water ( $p = 0.76$ ) suggesting that microbial As-reducing activity was promoted by soil saturation.  
372 However, the overall proportion of As(III) in interstitial water remained lower than that of As(V) (10 to 20%, Thouin  
373 et al., 2017).

374 The present experiment differs from previous studies by two main aspects: (1) the extreme level of metals and As  
375 concentrations, that resemble those found in mining wastes (Drewniak et al., 2008) but without the high  
376 concentration in sulfur species, and (2) the fact that in spite of water saturation and addition of OM, the redox  
377 potential did not reach very low values. Weber et al. (2010) observed a decrease of redox potential down to 0 mV  
378 in the porewater of their contaminated surface soil after 20 days of flooding with artificial river water, with a  
379 simultaneous strong reduction of As(V), whereas in our experiment, the redox potential always remained higher  
380 than +100 mV (Thouin et al., 2017). As a fact, most of the reported studies about redox dynamic were performed  
381 with soils or sediments less contaminated than the “Place-à-Gaz” material: 300 ppm As in a polluted floodplain  
382 soil (Weber *et al.*, 2010), 375 ppm As in an artificially polluted soil (Couture et al., 2015), 63 ppm As in a  
383 contaminated sediment (Moon et al., 2017) compared with 100 000 ppm As in our soil. Thus, the present  
384 experiment provides information about microbial As transformation in a biogeochemical environment that was  
385 not previously explored in variable redox conditions. Thouin et al. (2016) found that elevated toxic element  
386 concentrations (Cu, Pb, Zn, As) seemed to have exerted a selective pressure on the microbial communities, with  
387 higher As(III)-oxidizing rates and lower microbial activity involved in carbon mineralization of the microbial  
388 populations from the most polluted zone of the site. Thus, phenomena linked to high concentrations of metals and  
389 arsenic may have attenuated the decrease of redox potential linked to oxygen consumption in the saturated level,  
390 while OM was provided, compared with less polluted environments. Up to now, all As(III)-oxidizing bacteria  
391 isolated from soils were heterotrophs or facultative autotrophs (Inskeep et al., 2007; Bachate et al., 2012; Bahar et  
392 al., 2013; Dong et al., 2014). The increasing amount of OM and its quality therefore promoted the growth of  
393 As(III)-oxidizing microorganisms. However, several studies have shown that elevated concentrations of  
394 bioavailable OM negatively impact As(III)-oxidizing bacterial activity (Challan-Belval et al., 2009; Bachate et al.,  
395 2012; Lescure et al., 2016). The opposite trend of overall As(III) oxidizing rate with increasing As(III)-oxidizing  
396 microorganisms concentration ( $p = -0.50$ ) and with the quality and quantity of OM seems to confirm the inhibiting  
397 effect of OM on the specific activity of As(III)-oxidizing microbes. However, globally, As(III) oxidizing  
398 microorganisms remained active in presence of OM, even in the saturated zones of the mesocosm, in accordance

399 with the predominance of As(V) in the interstitial water all along the experiment and with the formation of  
400 mimetite. This original phenomenon could result from the particular balance between As and OM microbial  
401 transformations in the presence of extremely high concentrations of toxic contaminants.

#### 402 **4. Conclusion**

403 The mesocosm experiment provided important information about the mineralogical evolution and stability of As  
404 and heavy metal mineral carriers present in the highly-polluted soil at the “Place-à-Gaz” site, as it underwent  
405 environmental changes. As, Cu, and Zn-bearing amorphous phases were altered under saturated conditions.  
406 Related to this alteration process, SEM results suggest a mechanism of dissolution and re-precipitation of iron in  
407 the form of amorphous oxides that may be a secondary sink for As and metals. In contrast, no traces of alteration  
408 were observed on crystallized arsenate minerals present in the soil at the beginning of the experiment. However,  
409 the formation of mimetite, a lead arsenate chloride mineral, was detected in water saturated zones. This  
410 phenomenon contributed to the immobilization of As(V) and Pb, but was limited by the low Pb concentration of  
411 the soil solution.

412 The input of OM contributed to the growth of both As(III)-oxidizing and As(V)-reducing microorganisms and  
413 negatively impacted the overall As(III) oxidation efficiency. This last phenomenon may be related to both the  
414 inhibition of microbial As(III)-oxidizing activities and the development of As(V)-reducing microorganisms.  
415 However, As(III)-oxidizing activity remained the major microbial-related phenomenon even in the saturated  
416 zones. Altogether, results from the mesocosm experiment show that water saturation of the “Place-à-Gaz” soil  
417 during high precipitation episodes is likely to promote the release of Zn and As into the water compartment.  
418 However, microbial As(III) oxidation activities, adsorption of As(V) on HFO and precipitation of mimetite are all  
419 processes observed during the experiment that contribute to partial immobilization of As, i.e. a decrease of total  
420 soluble As. The elucidation of these geochemical and biogeochemical processes at the mesocosm scale will  
421 contribute to more precise evaluations of the risks associated with these types of polluted sites and to better design  
422 management strategies.

#### 423 **Acknowledgments**

424 This work was co-funded by the Région Centre Val-de-Loire and BRGM (convention 00087485) and the Labex  
425 Voltaire (ANR-10-LABX-100-01). The authors wish to thank Philippe Penhoud, Marielle Hatton, and Rachel  
426 Boscardin (ISTO) for XRD, CHNS and Rock-Eval results, Pascal Auger (BRGM) for Niton analyses, and Daniel  
427 Hube (BRGM) who introduced the environmental issue and provided the polluted material.

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