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Original

Insights into the fate of antimony (Sb) in contaminated soils: ageing influence on Sb mobility, bioavailability, bioaccessibility and speciation / Castaldi, Paola; Garau, Giovanni; Albert, Juhasz; Diquattro, Stefania; Ritch, Susie; Enzo, Lombi; Brunetti, Gianluca; Scheckel Kirk, G. - In: SCIENCE OF THE TOTAL ENVIRONMENT. - ISSN 0048-9697. - 770:(2021). [10.1016/j.scitotenv.2021.145354]

Availability:

This version is available at: 11566/315449 since: 2024-05-13T15:24:39Z

Publisher:

Published

DOI:10.1016/j.scitotenv.2021.145354

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(Article begins on next page)

1 **Insights into the fate of antimony (Sb) in contaminated soils: ageing influence on**
2 **Sb mobility, bioavailability, bioaccessibility and speciation**

3

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18

19

20 **Abstract**

21

22 The effect of long-term ageing (up to 700 days) on the mobility, potential
23 bioavailability and bioaccessibility of antimony (Sb) was investigated in two soils
24 (S1: pH 8.2; S2: pH 4.9) spiked with two Sb concentrations (100 and 1000 mg·kg⁻¹).

25 The Sb mobility decreased with ageing as highlighted by sequential extraction, while
26 its residual fraction significantly increased. The concentration of Sb (C_{DGT}), as
27 determined by diffusive gradients in thin films (DGT), showed a reduction in
28 potential contaminant bioavailability during ageing. The DGT analysis also showed
29 that Sb- C_{DGT} after 700 days ageing was significantly higher in S1-1000 compared to
30 S2-1000, suggesting soil pH plays a key role in Sb potential bioavailability. In-vitro
31 tests also revealed that Sb bioaccessibility decreased over time. Linear combination
32 fitting of Sb K-edge XANES derivative spectra showed, as a general trend, an
33 increase in Sb(V) sorption to inorganic oxides with ageing as well as Sb(V) bound to
34 organic matter (e.g. up to 27 and 37 % respectively for S2-100). The results indicated
35 that ageing can alleviate Sb ecotoxicity in soil and that the effectiveness of such
36 processes can be increased at acidic pH. However, substantial risks due to Sb
37 mobility, potential bioavailability and bioaccessibility remained in contaminated
38 soils even after 700 days ageing.

39

40 *Keywords:* Ageing; Antimony; Potential bioavailability; Bioaccessibility; X-ray
41 absorption near-edge structure (XANES) spectroscopy

42

43 **1. Introduction**

44

45 Antimony (Sb) is a potentially toxic element (PTE), considered as a priority
46 pollutant by the United States Environmental Protection Agency and the European
47 Union (CEC, 1998; USEPA, 2009). Antimony concentration in the environment is
48 related to both natural phenomena such as weathering, biological activity, or volcanic

49 activity and anthropogenic inputs (He et al., 2019). In recent years, Sb concentrations
50 in the environment have considerably increased as a result of mining and smelting
51 operations, waste incinerators, coal and petroleum combustion, spent ammunition,
52 polyethylene terephthalate industries, battery factories, and use of pharmaceuticals
53 and pesticides (e.g. Okkenhaug et al., 2016). In this context, knowledge of Sb
54 mobility, potential bioavailability and bioaccessibility in soil is of primary
55 importance for the assessment of ecological risk and potential human health impacts
56 (Diquattro et al., 2020; Fu et al., 2016; Hammel et al., 2000).

57 Antimony mobility, bioavailability and bioaccessibility in soil depend on its
58 speciation as well as on the pH, redox conditions, amount and type of colloids
59 present and microbial populations (Luo et al., 2014; Wilson et al., 2010). Antimony
60 commonly occurs in the environment as trivalent Sb(III) or pentavalent Sb(V)
61 inorganic species, with antimonite $[Sb(OH)_3]$ and antimonate $[Sb(OH)_6^-]$ the most
62 common Sb(III) and Sb(V) compounds, respectively (Kang et al., 2000). Antimonate
63 is the predominant form present under aerobic and mildly reducing conditions
64 (Filella et al., 2002; Filella et al., 2009; Telford et al., 2008) and its mobility in soil,
65 in the 5.0-8.5 pH range, is generally greater than that of antimonite due to the
66 negative charge of the former species [$pK_a HSb(OH)_6 = 2.55$; $pK_a Sb(OH)_3 = 11.8$]
67 (Herath et al., 2017; Johnston et al., 2020).

68 Binding to soil colloid surfaces (e.g., organic matter, Fe, Al, and Mn oxy-
69 hydroxides) by specific and nonspecific adsorption reactions, as well as structural
70 incorporation into Fe(III)-oxyhydroxide minerals, can have substantial influence on
71 Sb mobility and bioavailability, eventually leading to contaminant attenuation
72 (Wilson et al., 2010). For instance, it was shown that different humic substance

73 functional groups (e.g., -SH and -COOH groups) can interact with Sb(III) (Tella and
74 Pokrovski, 2009). In regards to Sb(V), specific adsorption by humic acids under acid
75 conditions can occur via the establishment of pentagonal or hexagonal rings
76 involving Sb–O–C linkages (Sh et al., 2012; Steely et al., 2007; Tella and Pokrovski,
77 2012). Moreover, cationic metals within humic acids [e.g, Fe(III), Mg(II), and
78 Ca(II)], can act as bridging elements between the negatively charged humic
79 molecules and the antimonate complex (Diquattro et al., 2018). Finally, stable
80 interactions (e.g., inner sphere complexes) between Sb(V) and Fe and Al oxy-
81 hydroxides have previously been reported (Bagherifam et al., 2014; Essington and
82 Stewart, 2016; 2018), as well as structural Sb(V) incorporation into Fe(III)-oxide
83 (e.g. goethite) via heterovalent Sb(V)/Fe(III) substitution (e.g. Burton et al., 2020).
84 Overall, the stability of complexes between Sb(V) and soil colloids decreases as the
85 pH raises, due to the increase of electrostatic repulsion phenomena occurring
86 between the $\text{Sb}(\text{OH})_6^-$ oxyanion and the negatively charged surface groups of Fe and
87 Al oxy-hydroxides and organic matter (Xi et al., 2016). Moreover, the strength of Sb
88 binding to soil colloid surfaces is fundamental in regulating contaminant
89 bioaccessibility which is an important parameter for the prediction of exposure and
90 health risks from incidental ingestion of Sb contaminated soils (Ruby et al., 1996). In
91 particular, bioaccessibility refers to the fraction of a contaminant that is soluble in the
92 digestive phase and as such potentially available for intestinal absorption (Mele et
93 al., 2015).

94 Given that the stability of interactions between Sb and soil colloids, and therefore
95 contaminant mobility, bioavailability and bioaccessibility, can change over time, this
96 should be considered in order to gain a greater understanding of ecotoxicological

97 effects of antimony in soil systems and (more in general) in the environment
98 (Violante et al., 2010). The reduction of PTEs mobility, potential bioavailability and
99 bioaccessibility through specific immobilization mechanisms, which occur as a
100 function of their contact time with soil colloids, is called “ageing” (e.g. Peng et al.,
101 2019; Tang et al., 2006). Several PTEs-fixation reactions such as complexation,
102 surface adsorption, precipitation or diffusion and occlusion into meso and
103 micropores, can occur in soil with time (Jalali and Khanlari, 2008). For instance, lead
104 (Pb), zinc (Zn), cadmium (Cd) and copper (Cu) tend to become non-exchangeable
105 with an increase in residence time, due to the transformation of weak interactions
106 (e.g. outer sphere complexes) into more stable ones (e.g. inner sphere complexes)
107 (Jalali and Khanlari, 2008; Tang et al., 2006). Similarly, a decrease in water-soluble
108 and exchangeable arsenic (As) and selenium (Se), and an increase in PTEs bound to
109 amorphous Fe/Al oxy-hydroxides, or in residual fractions, have been observed
110 during ageing (e.g. Peng et al., 2019; Tang et al., 2007).

111 Despite numerous studies over the last decade investigating the effects of ageing
112 on the mobility and bioavailability of many PTEs (e.g. Diagboya et al., 2015;
113 Martínez and McBride, 1998; Yang et al., 2003), limited knowledge is available for
114 Sb. For instance, Sanderson et al. (2014) studied the bioavailability of Pb, Sb, Zn,
115 nickel (Ni), Cu and As in soil after one year ageing, but the study mainly focused on
116 selected ecotoxicological endpoints, i.e. earthworms, plants and microbial activity.
117 Likewise, Zhang et al. (2020) explored Sb toxicity on barley root elongation after 3-
118 months ageing, while Egodawatta et al. (2018) reported higher Sb bioavailability for
119 water spinach when exposed to recently contaminated soil (14 days) compared to
120 historically contaminated (34 years).

121 In this study we therefore sought to clarify the influence of ageing on the fate and
122 behaviour of Sb in soil by using a combination of well-established and innovative
123 approaches. More specifically, the objective of this work was to investigate the effect
124 of ageing (from 1 day up to 700 days) on the mobility, potential bioavailability,
125 bioaccessibility and speciation of Sb added [as Sb(V)] at two different concentrations
126 (100 and 1000 mg·kg⁻¹) on two distinct soils. In particular, the fate of Sb in soil was
127 studied by sequential extraction to identify labile and relatively immobile Sb pools
128 (Diquattro et al. 2020; Garau et al., 2017; Wenzel et al., 2001). Antimony potential
129 bioavailability was addressed with diffusive gradients in thin-films (DGT) probes
130 (Luo et al., 2010), while Sb bioaccessibility was studied by using simulated gastric
131 and intestinal solutions in *in-vitro* tests (Ruby et al., 1996). Finally, synchrotron
132 based X-ray absorption near-edge structure (XANES) spectroscopy was used to
133 define Sb speciation in soil (Maher et al., 2018; Wang et al., 2017). The experimental
134 design and the combined use of the above-mentioned techniques were expected to
135 provide a deeper understanding of environmental risk posed by Sb and of possible
136 attenuation mechanisms governed by time.

137

138 **2. Materials and methods**

139

140 *2.1. Soil physical-chemical analysis and microcosm set up*

141

142 Different topsoil samples (0-20 cm depth) were collected randomly from two
143 uncultivated fields located in north-western Sardinia (Sassari), soil 1 (S1):
144 40°43'32.77"N 8°24'48.6"E; soil 2 (S2): 40°56'15.7"N 8°53'30.4"E. These soils were

145 selected because they were not previously used for agricultural or anthropogenic
146 activities, they were distant from possible contamination sources (i.e. to reduce the
147 possibility of external Sb input), and they exhibited different physico-chemical
148 properties (Table S1). Soil samples were pooled in the laboratory according to their
149 origin (i.e., S1 and S2), sieved to < 2 mm and analysed as previously reported
150 (Diquattro et al., 2020). Briefly, the S1 soil (sandy clay loam) was alkaline (pH 8.2),
151 while S2 (loamy coarse sand) was acidic (pH 4.9). S1 had a higher cation exchange
152 capacity (CEC) compared to S2, i.e. 20 vs 13 $\text{cmol}_{(+)}\cdot\text{kg}^{-1}$ soil respectively, while the
153 opposite was found for soil organic matter (SOM), i.e. 1.75 vs 2.19 % and dissolved
154 organic carbon (DOC), i.e. 0.17 vs 0.39 $\text{mg}\cdot\text{g}^{-1}$ soil respectively. The point of zero
155 charge (pH_{PZC}) was 5.7 for S1 and 2.6 for S2. High concentrations of total Fe (16,350
156 and 5,650 $\text{mg}\cdot\text{kg}^{-1}$ respectively) and Al (19,930 and 3,924 $\text{mg}\cdot\text{kg}^{-1}$ respectively) were
157 recorded in S1 and S2, while total Ca was more abundant in S1 (62,500 $\text{mg}\cdot\text{kg}^{-1}$)
158 compared to S2 (2,426 $\text{mg}\cdot\text{kg}^{-1}$). Antimony in S1 and S2 was quantified by
159 Inductively Coupled Plasma Mass Spectrometry (ICP-QQQ-MS) (Agilent 8800)
160 after digestion of soil with aqua regia reverse solution (HNO_3/HCl 3:1 ratio) and
161 microwave mineralisation (MARS-6, CEM) using USEPA method 3051A. A
162 standard reference material (NIST-SRM 2711A) was included for quality assurance
163 and quality control. In both soils, Sb was not detected (< 0.01 $\mu\text{g}\cdot\text{kg}^{-1}$). Further
164 details on the chemical features of both soils can be found in Diquattro et al. (2020).

165 Thirty microcosms (each consisting of 300 g soil) were prepared for each soil type
166 (S1 and S2) in plastic pots (10 cm diameter x 10 cm height). All soils were brought
167 to 60 % of their water holding capacity (WHC) with deionised water (Jury et al.,
168 1991). For each soil type, 15 microcosms were spiked with 100 $\text{mg}\cdot\text{kg}^{-1}$ Sb(V) (S-

169 100), and another 15 with 1000 mg·kg⁻¹ Sb(V) (S-1000). Sb(V) derived from
170 K[Sb(OH)₆] (CAS 12208-13-8; Sigma Aldrich, Saint–Louis, USA) was added as a
171 water solution (K[Sb(OH)₆] solubility in water is 20 g L⁻¹). Antimony concentrations
172 added were selected to mimic a medium-low and a high contamination level based
173 on previous studies (Courtin-Nomade et al., 2012; Diquattro et al., 2020; Garau et al.,
174 2017). Before addition to soil, each Sb(V) solution was adjusted to the same pH of
175 the soil using 0.1 M NaOH or HCl solutions. Microcosms were carefully mixed and
176 then left to age at constant temperature (25 °C) and humidity (60 % WHC; by weight
177 adjustment) for different times: 1, 7, 31, 91 and 700 days (d). The selected time-
178 points were chosen to evaluate both the short and medium to long-term influence of
179 ageing on Sb mobility, bioavailability, bioaccessibility and speciation. At each time-
180 point, triplicate S1 and S2 microcosms (i.e., S1-100, S1-1000, S2-100 and S2-1000)
181 were analysed as follows. Total concentration of Sb was quantified in spiked
182 microcosms (< 2 mm and < 250 µm particle size fractions) at different time-points
183 (Table 1), by ICP-QQQ-MS as previously described. A standard reference material
184 (NIST-SRM 2711A) was included for quality assurance and quality control.

185

186 2.2. *Sequential extraction of soil Sb*

187

188 At each time-point, Sb mobility in the different soils was evaluated through the
189 sequential extraction procedure (SEP) proposed by Wenzel et al. (2001) with minor
190 modifications. This method was originally developed for arsenic but has also been
191 widely used for antimony (e.g. Diquattro et al., 2018; Garau et al., 2017; Ngo et al.,
192 2020) because of the similar chemical characteristics of these PTEs (Wilson et al.,

193 2010). Different soil samples (n=10; 1 g each) were randomly collected from each
194 microcosm and pooled together. Then a representative soil sample (1 g) was
195 collected for Sb sequential extraction. The procedure was repeated 3 times for each
196 microcosm. Triplicate soil samples (1 g) collected from each microcosm at different
197 ageing times (i.e., 1, 7, 31, 91, 700 d) were treated with 25 mL of deionised water
198 and shaken for 2 h at 25 °C to extract water-soluble Sb (Fraction 1, F1). Then, the
199 same soil samples were treated with 25 mL of a 0.05 M (NH₄)₂SO₄ solution and
200 shaken for 4 h at 25 °C to extract non-specifically sorbed Sb (Fraction 2, F2).
201 Finally, the same soil samples were treated with 25 mL of a 0.05 M NH₄H₂PO₄
202 solution and shaken for 16 h at 25 °C h to extract specifically sorbed Sb (Fraction 3,
203 F3). Then, soil samples were digested with reverse aqua regia (HNO₃/HCl 3:1 ratio)
204 and microwave mineralisation (MARS-6, CEM) using USEPA method 3051A, to
205 quantify residual Sb (Fraction 4, F4). After each extraction step, soil suspensions
206 were centrifuged at 1800 g for 10 min. Supernatants were then filtered through a 0.45
207 µm filter and Sb concentrations determined in the liquid phase by ICP-QQQ-MS as
208 previously described. A standard reference material (NIST-SRM 2711A) was
209 included for quality assurance and quality control.

210

211 *2.3. DGT measurements*

212

213 At each time-point, Sb potential bioavailability was assessed in different soils
214 through the DGT[®] technique. This is based on a device (deployed on the soil surface)
215 that accumulates labile soil Sb on a binding gel after its diffusion through a hydrogel,
216 which acts as a diffusion layer (Li et al., 2018). In this study, a titanium oxide

217 (Metsorb) binding gel (DGT Research Ltd, Lancaster, UK) was employed. The
218 exposure area of the binding gel was 3.14 cm² and its thickness was 0.4 mm.
219 Triplicate soil samples (50 g) collected from each microcosm at different ageing
220 times (i.e., 1, 7, 31, 91,700 d) were brought to 100 % of their WHC, then a DGT
221 device was deployed on the soil surface and gently pressed (to favour the contact
222 between soil and device). DGT probes were left in contact with soils for 24 h at 25
223 °C. Afterwards, binding gels (of each DGT device) were collected and eluted for 24
224 h in 2 mL of a 1 M NaOH and 1 M H₂O₂ solution. Eluent solutions were then 10-fold
225 diluted in 2 % ultrapure HNO₃ prior to the analysis of Sb content by ICP-QQQ-MS
226 as previously described.

227 The time-averaged Sb concentration present in the soil solution at the surface of
228 the DGT device (C_{DGT} , expressed as $\mu\text{g}\cdot\text{L}^{-1}$; i.e. a measure of labile or bioavailable
229 soil Sb) was obtained according to the following equation (Eq. 1):

$$230 \quad C_{DGT} = M\Delta g/AtD \quad (\text{Eq. 1}) \quad (\text{Davison and Zhang, 1994})$$

231 where M is the mass (μg) of element accumulated on the binding gel, Δg is the
232 thickness of the diffusive gel (0.8 mm) plus the thickness of filter membrane (0.1
233 mm), D is the element diffusion coefficient in the diffusive layer ($5.46 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$
234 for Sb; Luo et al., 2010), A is the exposed area of the binding gel (3.14 cm²), and t is
235 the deployment time (s). Moreover, the R value, which reflects the ability of soil to
236 resupply antimony to the device interface, was calculated as the ratio between Sb-
237 DGT (C_{DGT}) to Sb concentration in soil pore water (C_{sol}) (Harper et al. 2000).

238

239 *2.4. In vitro bioaccessibility of soil Sb*

240

241 At each time-point, Sb bioaccessibility in spiked soils was determined using the
242 Solubility Bio-accessibility Research Consortium in vitro assay (SBRC) which
243 includes a gastric (SBRC-G) and an intestinal phase (SBRC-I) (Mele et al., 2015).
244 The < 250 μm (Table 1) soil particle size fraction was employed for analysis, since it
245 represents the fraction that adheres to fingers and is available for incidental ingestion
246 (Mele et al., 2015). Triplicate soil samples (0.4 g) collected (as described for SEP)
247 from microcosms at different ageing times (i.e., 1, 7, 31, 91, 700 d) were treated with
248 40 mL of a gastric solution (30.03 $\text{g}\cdot\text{L}^{-1}$ glycine adjusted to pH 1.5 with 37 % HCl)
249 and incubated in agitation (40 rpm on a Ratek rotary suspension mixer) at 37 °C and
250 constant pH (i.e., 1.5). After 1-hour incubation, 4 mL of supernatant was collected,
251 filtered through 0.45 μm filters and stored at 4 °C (SBRC-G phase). The remaining
252 suspension was adjusted to pH 7.0 with a 50 % NaOH solution, and intestinal
253 solution (3 mL) containing 70 mg of bile bovine (Sigma-Aldrich) and 20 mg of
254 pancreatin (Sigma-Aldrich) was added. The intestinal phase was then incubated at
255 constant pH (i.e., 7.0) as previously mentioned. After 4 h, supernatant aliquots (10
256 mL) were collected, filtered through 0.45 μm filters and stored at 4 °C (SBRC-I
257 phase). Soluble Sb in SBRC-G and SBRC-I phases was determined by ICP-QQQ-
258 MS (Agilent 8800), while total Sb concentration in soil was quantified after
259 mineralization (< 250 μm) as previously described. A standard reference material
260 (SRM 2711A) was also included for quality assurance and quality control.

261 In vitro Sb bioaccessibility values (%) were calculated by dividing the Sb
262 concentration determined in SBRC-G or SBRC-I phases by the total Sb
263 concentration in the soil (< 250 μm) as in the following equation (Eq. 2):

264 $BA_{Sb} (\%) = [\text{soluble Sb in SBRC-G or SBRC-I (mg}\cdot\text{kg}^{-1}) / \text{total Sb concentration}$
265 $<250 \mu\text{m (mg}\cdot\text{kg}^{-1})] \times 100$ (Eq. 2).

266

267 2.5. X-ray absorption spectroscopy

268

269 Antimony K-edge X-ray absorption near-edge structure (XANES) spectra were
270 obtained to understand Sb speciation and its interaction with soil components during
271 ageing. XANES spectra were collected at the Materials Research Collaborative
272 Access Team's (MRCAT) beamline 10-BM (Kropf et al., 2010) at the Advanced
273 Photon Source (7 GeV storage ring in top-up mode), Argonne National Laboratory,
274 Argonne, IL (USA). Soil samples, collected at different ageing times (i.e., 1, 31, 700
275 d), were prepared by freeze drying, grinding with an agate mortar and pestle, and
276 pressing 75 mg of the sample in a 7-mm handheld pellet press. Samples from 7 and
277 91 d were not included in XANES analysis since SEP, DGT and SBRC data from
278 these time-points were similar to those at 31 and 700 d respectively. The sample
279 pellet was then encapsulated with Kapton tape and placed on a sample holder for
280 analysis. The beamline optics and setup parameters for the Sb edge XANES
281 measurements included calibration of the monochromator with a metallic Sb foil
282 (30491 eV) and the acquisition of metallic foil spectra with each sample scan for
283 spectral calibration verification. Data collection was conducted in both transmission
284 and fluorescence (4-element Vortex Si-drift detector) modes with gas purged ion
285 chambers for I_0 (80% Ar/20% N₂), $I_{\text{Transmission}}$ (100% Ar), and $I_{\text{Reference}}$ (100% Ar). At
286 least six scans for each sample were collected and it was evident fluorescence data
287 were of higher quality than transmission. Data analysis was conducted using Athena

288 software (Ravel and Newville, 2005). Multiple scans for each sample were aligned,
289 merged, normalized, and calibration was performed by assigning the first inflection
290 point of the reference Sb metal foil to 30491 eV. The relative abundance of Sb-
291 bearing solid-phases was examined by linear combination fitting (LCF) of Sb K-edge
292 XANES derivative spectra relative to known Sb reference samples (fitting range of -
293 30 eV to +70 eV relative to the calibration energy). The χ^2 is a measure of the mean
294 square sum of misfit at each data point and describes the degree of uncertainty in the
295 fitting process (Ravel and Newville, 2005). Antimony reference standards included
296 Sb_2S_3 , Sb_2O_3 , Sb(V)-tartrate, Sb(V)-citrate and $\text{KSb}(\text{OH})_6$ (Sigma Aldrich, used for
297 spike); Sb(V) and Sb(III) sorbed to goethite and Sb(III) sorbed to thiol-functionalised
298 cellulose. Reference standards considered for LCF included Sb(V) sorbed to goethite
299 to represent Sb(V) sorption by inorganic oxy-hydroxides (Essington and Stewart,
300 2018), Sb(V)-citrate to represent Sb(V) bound to organic matter, and $\text{KSb}(\text{OH})_6$ to
301 represent labile (e.g. water-soluble and exchangeable) Sb(V) in soil (Essington and
302 Stewart, 2016). This latter interpretation was based on the high similarity of
303 $\text{KSb}(\text{OH})_6$ and $\text{Sb}(\text{OH})_6^-$ spectra (e.g. Ji et al., 2017).

304

305 *2.6. Statistical analysis*

306

307 All chemical analyses were performed on triplicate soil samples collected from
308 each mesocosm at different ageing times with mean values \pm standard errors reported
309 in tables and figures. The Shapiro-Wilk test was performed in order to evaluate the
310 normality of data distribution. For each soil (S1 or S2) and contamination level (100
311 or 1000 $\text{mg}\cdot\text{kg}^{-1}$), the Fisher's LSD test was used to assess the influence of ageing

312 time on Sb mobility, potential bioavailability and bioaccessibility. Moreover, for
313 each ageing time, comparisons between S1 and S2 at the same contamination level
314 were made by using a Student t-test. Differences were considered statistically
315 significant for $P < 0.05$. Pearson's correlation test was also performed to investigate
316 the relationships between Sb concentrations recovered in the different steps of the
317 SEP procedure, and those detected by DGT (i.e. C_{DGT}) at different ageing times.
318 Correlation values were considered statistically significant for $P < 0.05$. All
319 statistical analyses were carried out using the Sigma Plot Software (SPSS Inc.,
320 Chicago, IL, USA).

321

322 **3. Results and discussion**

323

324 *3.1. Influence of ageing on Sb mobility in contaminated soils*

325

326 To investigate the influence of ageing on the mobility of antimony in
327 contaminated soils, different Sb fractions (or pools) characterised by a diverse degree
328 of lability (i.e., solubility) were quantified at different time points. The water-soluble
329 (i.e., highly mobile) Sb fraction (F1) decreased significantly over time for both soils
330 and at both Sb concentrations (Fig. 1). The highest amounts of soluble Sb were
331 detected at 1 d since spiking (40 and 18 % of total Sb for S1-100 and S2-100 and 24
332 and 26 % for S1-1000 and S2-1000 respectively), as previously reported elsewhere
333 for other PTEs (e.g. Pb, Cu, Zn, Cd) (Jalali and Khanlari, 2008; Tang et al., 2006).
334 Overall, such Sb amounts progressively and significantly reduced with ageing in
335 both soils, even if different trends were observed depending on soil type and level of

336 contamination. In particular, significant reductions in water-soluble Sb occurred in
337 all soils (with the exception of S2-100), after 7 d of ageing (Fig. 1). Interestingly, in
338 S2-100, a significant reduction in soluble Sb occurred only at 91 d (Fig. 1). Such
339 prolonged environmental stress could have favoured the development of an Sb-
340 resistant bacterial community previously observed in this soil at 31 d ageing
341 (Diquattro et al., 2020). The lowest amounts of soluble Sb were detected at 700 d of
342 ageing even if, within each soil, the differences between 91 and 700 d were limited
343 and, in some cases, not significant (Fig. 1). After 700 d ageing, soluble Sb was
344 significantly reduced, compared to 1 d, by approx. 80 % in S1- and S2-100, and by
345 70 and 95 % in S1- and S2-1000 respectively. Taken together, these data indicate
346 that 3 months ageing could be a suitable time for soil to equilibrate with aqueous Sb
347 (i.e. very limited changes occurred after this time) and, consequently, to investigate
348 the environmental fate of the contaminant and its ecotoxicological effects. Moreover,
349 the higher Sb solubility observed in S1 compared with S2, especially after 700 d
350 ageing (6 and 77 mg·kg⁻¹ in S1-100 and -1000 vs 4 and 13 mg·kg⁻¹ in S2-100 and -
351 1000), could support previous research findings (Diquattro et al., 2020; Herath et al.,
352 2017) where pH was identified as a key factor influencing Sb mobility. In particular,
353 competition phenomena between hydroxide (OH⁻) and Sb(OH)₆⁻ anions for Sb
354 retention sites and/or repulsion between Sb(OH)₆⁻ anions and negatively charged soil
355 surfaces, could explain the higher Sb solubility in the alkaline S1 soil (pH 8.2)
356 compared to the acidic S2 (pH 4.9). On the contrary, Fe/Al oxides and soil organic
357 matter, i.e. other important sinks of soil Sb (Herath et al., 2017), seemed to play a
358 limited or negligible role at influencing Sb mobility in these soils (Fe/Al content was
359 much higher in S1, and SOM content was similar in S1 and S2).

360 After 1 d ageing, Sb extracted with $(\text{NH}_4)_2\text{SO}_4$ (F2), i.e. the exchangeable pool,
361 was significantly lower than water-soluble Sb in both soils (Fig. 1). Moreover, Sb
362 extracted in F2 was significantly higher in S1-100 and -1000 (15.4 and 13.7 %)
363 compared to S2-100 and -1000 (8.4 and 8.6 %), in agreement with the results of a
364 recent short-term investigation based on the same soils (Fig. 1; Diquattro et al.,
365 2020). Starting from 7 or 31 d, the amounts of exchangeable Sb significantly and
366 progressively decreased (compared to 1 d) in all soils except S2-100 where a
367 reduction was observed only at 700 d ageing (Fig. 1). Overall these data indicate a
368 decrease of weaker (e.g. outer sphere) complexes and a concomitant increase of more
369 specific (e.g. inner sphere) bonding between Sb and soil mineral and organic surfaces
370 over time (Violante et al., 2010; Wenzel et al., 2001). As for the water-soluble F1
371 fraction, this implied a Sb shift from more mobile (and potentially bioavailable)
372 pools to less mobile (and hardly bioavailable) ones during ageing.

373 This was partly supported by Sb extracted with $\text{NH}_4\text{H}_2\text{PO}_4$ (F3), i.e. Sb
374 specifically sorbed by soil colloids (Wenzel et al., 2001). Such Sb pool can be
375 considered as relatively mobile since it can be mobilised following a local pH change
376 and/or a variation in P concentration (Wenzel et al., 2001). Antimony extracted in F3
377 was generally low at day 1 (i.e., 10.2 and 7.6 % of total Sb for S1-100 and -1000, and
378 3.7 and 2.3 % for S2-100 and -1000 respectively) which reduced (e.g. S1-100 and
379 S2-1000) or remained constant (e.g. S1-100) during ageing (Fig. 1). The only
380 exception was S1-1000 where Sb concentration slightly (yet significantly) increased
381 with time, i.e. from 76 (at 1 d) to 81 $\text{mg}\cdot\text{kg}^{-1}$ soil (at 700 d) (Fig. 1).

382 Antimony detected in F4, i.e. residual Sb (e.g. associated with amorphous and
383 crystalline Al and Fe oxy-hydroxides and/or precipitated or occluded), significantly

384 increased over time from 34, 70, 55 and 63 % at 1 d to 82, 79, 88 and 95 % at 700 d
385 ageing in S1- and S2-100, and S1- and S2-1000 respectively (Fig. 1). This was in
386 agreement with previous studies (e.g. Jalali and Khanlari, 2008; Peng et al., 2019)
387 which reported an increase in residual PTEs such as Se, Pb, Cu, Cd, and Zn with
388 ageing. However, the influence of ageing on Sb fractionation has been rarely
389 addressed (e.g. Egodawatta et al., 2018; Zhang et al., 2020).

390 Taken together, SEP results highlighted a re-distribution of Sb from soluble (i.e.,
391 mobile) or weakly bound (easily mobilizable) forms, to less mobile and more stable
392 ones during ageing. From an environmental viewpoint, this implies a time-dependent
393 decrease in (micro)biological-impacting Sb fractions and an increase in less
394 bioavailable forms.

395

396 *3.2. Influence of ageing on Sb bioavailability in contaminated soils*

397

398 The diffusive gradient in thin film technique (DGT) was used to measure the flux
399 of soluble Sb, which is supplied by diffusion through soil solution, as well as that of
400 labile Sb resupplied by the soil solid phase to the DGT device (Letho, 2016). The
401 diffusion of labile soil Sb (free and complexed soluble ions and weakly bound Sb
402 species) into DGT devices has been previously shown to mimic the continuous
403 uptake of PTEs by plant roots (Zhou et al., 2019). This is because, as pointed out by
404 Zhang and Davison (2000), elemental flux to plant membranes and to DGT can be
405 similar from a quantitative viewpoint (Letho et al., 2016). For this reason, the use of
406 the DGT technique to estimate PTEs bioavailability to plants and soil (micro)biota
407 has been steadily growing (e.g. Dai et al., 2018; Gu et al., 2017; Wang et al., 2019).

408 The C_{DGT} measurements showed that bioavailable Sb rapidly decreased at the
409 initial stage of ageing (7-31 d) and reached an equilibrium at 91 d (in agreement with
410 SEP results) in the majority of soils (Fig. 2). At low contamination levels (i.e., 100
411 $\text{mg}\cdot\text{kg}^{-1}$), Sb bioavailability was always higher for soil S2 at all ageing times.
412 However, after 700 d ageing, Sb- C_{DGT} values in the pore water of the two soils were
413 low and similar, i.e. 0.33 and 0.49 $\mu\text{g}\cdot\text{L}^{-1}$ (Fig. 2). This is relevant, as these
414 concentrations are expected to pose limited threats for plants and soil (micro)biota
415 (Geng et al., 2020; Sh et al., 2012). Nonetheless, it should be noted that much higher
416 Sb- C_{DGT} concentrations were detected during the previous time-points and this could
417 have produced substantial perturbations of soil (micro)biological and biochemical
418 features (e.g. Diquattro et al., 2020) whose effects could be still present after 700 d.
419 In soils containing higher Sb concentrations (i.e., 1000 $\text{mg}\cdot\text{kg}^{-1}$), Sb bioavailability
420 was significantly higher for soil S1 at all ageing times, with the exception of day 1
421 (Fig. 2). After 700 d ageing, the Sb- C_{DGT} in S1 was still 4.5-fold higher than in S2,
422 and this supports higher Sb mobility detected by the SEP procedure in soil S1 (Fig.
423 1). As previously mentioned, this could be attributed to the alkaline pH of soil S1
424 and/or to the increased formation of stable Sb precipitates (e.g. FeSbO_4 and AlSbO_4)
425 in the acidic S2 (Dousova et al., 2018; Herath et al., 2017). It should be noted that, at
426 low Sb contamination level (i.e., 100 $\text{mg}\cdot\text{kg}^{-1}$), this was not particularly evident as
427 highlighted by both SEP and DGT results (Figs. 1-2). Overall, these results clearly
428 indicate a steady reduction in Sb bioavailability during ageing, which also suggests a
429 parallel increase in stable Sb-soil interactions over time, which was supported by
430 SEP results.

431 The resupply of Sb from the solid phase to the soil pore water, i.e. R value ($C_{DGT} /$
432 C_{sol}), was low in both soils for all ageing times (i.e., $R < 0.12$ and < 0.05 for S-100
433 and S-1000 soils; Table 2) indicating that diffusion was the main process supplying
434 Sb to the DGT device (Ma et al., 2020; Netho, 2016; Peng et al., 2019). Since the
435 selected Sb fractions extracted by SEP are often correlated with the most labile (and
436 potentially bioavailable) fractions in soil, i.e. those readily taken up by plants and
437 soil microorganisms (e.g. Castaldi et al., 2018; Diquattro et al., 2018, 2020; Garau et
438 al., 2017), the correlation between Sb- C_{DGT} and that recovered in F1-F4 was tested
439 (Table 3). Significant positive correlations were observed between F1 (0.786 –
440 0.959; $P < 0.01$), and F1+F2 (0.683 – 0.979; $P < 0.01$), and Sb- C_{DGT} while weaker
441 relationships were recorded for F2 (Table 3). No significant relationship between Sb-
442 C_{DGT} and F3 was found, whereas significant negative correlations were observed
443 between F4 (i.e., the less mobile and less bioavailable Sb) and Sb- C_{DGT} (Table 3).

444 Taken together, these data highlight a good agreement between DGT and SEP
445 results and confirmed the suitability of both approaches for the assessment of Sb
446 mobility and potential bioavailability in soil. Overall, Sb- C_{DGT} values showed that
447 ageing had a marked influence on the potential bioavailability of Sb. This latter
448 steadily decreased with time due to the conversion of Sb labile fractions into non-
449 labile ones as indicated by SEP results.

450

451 *3.3. Influence of ageing on Sb bioaccessibility*

452

453 To investigate the influence of ageing on potential exposure for humans and
454 animals related to incidental ingestion of Sb-contaminated soil, gastric and intestinal

455 phase Sb bioaccessibility was determined at different ageing times using the in vitro
456 SBRC assay. Antimony bioaccessibility varied over time, and different trends were
457 observed depending on soil type and Sb concentration (Fig. 3). When assessed using
458 SBRC-G, Sb bioaccessibility significantly decreased with increasing ageing times
459 with the exception of S1-100 when no variation in Sb bioaccessibility was recorded
460 (Fig. 3). After 700 d ageing, Sb bioaccessibility in the SBRC-G phase was ~30, 26,
461 39 and 9 % (of total Sb present in the < 250 μm soil fractions) in S1- and S2-100,
462 and S1- and S2-1000 respectively.

463 Considering that elemental absorption takes place in the intestinal compartment
464 (Hamel et al., 1998), SBRC-I data are of particular interest. Antimony
465 bioaccessibility in the SBRC-I phase quickly declined with ageing in the majority of
466 soils (Fig. 3). In particular, significant reductions in bioaccessible Sb were recorded
467 after 7 d for S2-1000 and after 31 d for S2-100 and S1-1000. However, it should be
468 noted that after one day, Sb bioaccessibility in the SBRC-I phase was 46 % (of total
469 Sb present in the < 250 μm soil fraction; Table 1) in S1-100 and between 61 and 72
470 % in the remaining soils (Fig. 3), that means an approx. 55 – 1800 mg Sb·kg⁻¹ soil.
471 Nonetheless, ageing had a clear influence on Sb bioaccessibility (SBRC-I) which
472 declined, compared to day 1, by a minimum of 15 % in S1-100, up to 60 % in S2-
473 1000 (Fig. 3). Again, this supports a time-dependent increase of stable bonds
474 between Sb and soil components (e.g. Fe oxy-hydroxides and organic matter), able to
475 resist dissolution processes which take place during digestion (Denys et al., 2008).
476 However, this was more pronounced for S2 soils where Sb binding to Fe/Al oxy-
477 hydroxides and organic matter was likely favoured by the lower pH. Indeed, the
478 acidic pH of the gastric phase commonly promotes the solubilisation of mineral

479 phases and the release of bonded PTEs, while the higher concentration of OH^- in the
480 intestinal phase can prevent anion sorption/adsorption [e.g. $\text{Sb}(\text{OH})_6^-$] by soil
481 colloids which favours the release of further Sb into solution (Udovic and McBride,
482 2012).

483 Even if these results indicate a decreased Sb bioaccessibility over time,
484 nonetheless, absolute Sb concentrations recorded in the SRBC-I phase at 700 d (i.e.,
485 43 and 511 $\text{mg}\cdot\text{kg}^{-1}$ for S1-100 and S1-1000 respectively, and 90 and 367 $\text{mg}\cdot\text{kg}^{-1}$
486 for S2-100 and S2-1000) highlighted substantial potential health risks in both
487 contaminated soils (Filella et al., 2002).

488

489 *3.4. Influence of ageing on Sb speciation*

490

491 Biogeochemical redox processes strongly influence Sb speciation, its interactions
492 with soil colloids (and hence mobility and stability) as well as its toxicity. In this
493 sense, spectroscopic techniques such as electron spin resonance (ESR) and X-ray
494 absorption spectroscopy (XAS, including XANES) have been useful for the
495 identification of metal and metalloid speciation and their complexes at surfaces of
496 Al, Fe or Mn oxy-hydroxides, silicate clays and soil organic matter (Violante et al.,
497 2010).

498 Derivative XANES spectra of Sb reference materials considered for LCF and of
499 S1 and S2 soils are shown in Fig.s S1 and S2 respectively. LCF results for S1 and S2
500 samples (Table 4, Fig. S2) show the presence of labile Sb(V) (i.e. $\text{KSb}(\text{OH})_6$ spectra)
501 at varying degrees. Importantly, Sb(V) was the only redox state identified in XANES
502 data. Labile Sb(V) concentration was higher for S1 samples than S2. Again, this

503 could be due to the influence of pH (8.2 vs 4.9, respectively) given the similar SOM
504 content of S1 and S2 (1.75 and 2.19 %), and the higher Fe and Al content in the
505 former soil (~ 3 and 5-fold respectively compared to S2). A similar influence of pH
506 on Sb sorption by ferrihydrite was recently reported by Garau et al. (2019).
507 Consistently, higher Sb-organic interactions (Sb(V)-citrate) and Sb sorbed to
508 inorganic oxides (Sb(V)-goethite LCF reference) were recorded in S2 relative to S1
509 over time (Table 4). XANES spectroscopy is often not sensitive enough to
510 distinguish if elements are sorbed by Fe or Al oxy-hydroxides in a complex soil
511 matrix; however, spectra obtained are unique enough to distinguish them from
512 organic complexes.

513 The initial concentration of Sb in each soil influenced final speciation results. S1-
514 100 at 700 d resulted in Sb speciation components of 57 % KSb(OH)_6 (which is
515 indicative of labile Sb(OH)_6^-), 18 % organic bound Sb(V), and 24 % sorbed to
516 inorganic oxides; whereas S1-1000 at 700 d observed 81 % KSb(OH)_6 , 14 % organic
517 bound Sb(V), and 5 % sorbed to inorganic oxides. A similar trend was noted for the
518 S2 concentration series where the KSb(OH)_6 fraction was larger for the higher
519 concentrated spiked sample.

520

521 **4. Conclusions**

522

523 Ageing had a clear influence on Sb mobility, bioavailability and bioaccessibility
524 in contaminated soils. Labile (i.e., more soluble) Sb fractions progressively reduced
525 during ageing indicating a decrease of weaker (e.g. outer-sphere) complexes and a
526 concomitant increase of more specific (e.g. inner-sphere) bonding between Sb and

527 the soil components over time. This was accompanied by a parallel decrease of
528 bioavailable and bioaccessible Sb which implied: i) a time-dependent increase of
529 stable bonding between Sb and soil components and ii) a reduced ecotoxicological
530 Sb impact on soil (micro)biota and humans with ageing. XANES LCF results
531 indicated that Sb(V) was the only redox state present in soils during the almost 2-
532 years experiment and that soil organic matter and Fe/Al oxy-hydroxides were the
533 main sink of Sb during ageing. In this regard, soil pH appeared as a key parameter
534 influencing the fate of Sb in our soils, i.e. Sb mobility, bioavailability and
535 bioaccessibility were overall higher in the alkaline S1 soil compared to the acidic S2.
536 Consistently, Sb fixation by soil organic matter and Fe/Al oxy-hydroxides was
537 quantitatively less in S1 compared with S2 at all time-points considered.
538 Nevertheless, absolute Sb concentrations recorded in the intestinal phase at 700 d
539 highlight substantial potential health risks in both contaminated soils.

540

541 **Credit Author Statement**

542

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544 Original draft preparation. **Paola Castaldi:** Conceptualization, Formal analysis,
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551

552 **Acknowledgements**

553

554 The financial support of the University of Sassari (Fondo di Ateneo per la Ricerca
555 2019) is gratefully acknowledged.

556 MRCAT operations are supported by the Department of Energy and the MRCAT
557 member institutions. This research used resources of the Advanced Photon Source, a
558 U.S. Department of Energy (DOE) Office of Science User Facility operated for the
559 DOE Office of Science by Argonne National Laboratory under Contract No. DE-
560 AC02-06CH11357. Although EPA contributed to this article, the research presented
561 was not performed by or funded by EPA and was not subject to EPA's quality system
562 requirements. Consequently, the views, interpretations, and conclusions expressed in
563 this article are solely those of the authors and do not necessarily reflect or represent
564 EPA's views or policies.

565 The Future Industries Institute, University of South Australia is acknowledged for
566 supporting this project.

567

568 **Appendix A. Supplementary data.**

569 Supplementary material related to this article is supplied as Table S1, Fig.s S1 and
570 S2.

571

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774

775 **Figure captions**

776

777 **Fig. 1.** Sequential extraction of antimony from S1 and S2 soils spiked with 100 (S-
778 100) and 1000 (S-1000) mg Sb kg⁻¹ soil at different ageing time. For each Sb fraction
779 (F_x), different letters on top of each bar (e.g. a, b, c, d) denote ageing-dependent
780 statistical differences (Fisher's LSD, $P < 0.05$). For each Sb fraction and ageing time,
781 asterisks denote statistical differences between S1-100 and S2-100, and between S1-
782 1000 and S2-1000 soils (Student t-test, $P < 0.05$).

783

784 **Fig. 2.** Antimony concentrations as obtained by DGT (Sb-C_{DGT}) in S1 and S2 soils
785 spiked with 100 (S-100) and 1000 (S-1000) mg Sb kg⁻¹ soil at different ageing time.
786 For each soil, different letters on top of each bar (e.g. a, b, c, d) denote ageing-
787 dependent statistical differences (Fisher's LSD, $P < 0.05$). For each ageing time,
788 asterisks denote statistical differences between S1-100 and S2-100, and between S1-
789 1000 and S2-1000 soils (Student t-test, $P < 0.05$).

790

791 **Fig. 3.** *In vitro* bioaccessibility of Sb in S1 and S2 soils spiked with 100 (S-100) and
792 1000 (S-1000) mg Sb kg⁻¹ soil at different ageing time. For each soil, different letters
793 on top of each bar (e.g. a, b, c, d) denote ageing-dependent statistical differences
794 (Fisher's LSD, $P < 0.05$). For each ageing time, asterisks denote statistical
795 differences between S1-100 and S2-100, and between S1-1000 and S2-1000 soils
796 (Student t-test, $P < 0.05$). SBRC-G= bio-accessible Sb in the gastric phase; SBRC-I
797 = bio-accessible Sb in the intestinal phase.

798

799 **Table S1.** Selected physico-chemical characteristics of S1 and S2 soils.

800

801 **Fig. S1.** Derivative XANES spectra of antimony reference materials considered for
802 linear combination fitting.

803

804 **Fig. S2.** Derivative XANES spectra of antimony from S1 and S2 soils spiked with
805 100 (S-100) and 1000 (S-1000) mg Sb kg⁻¹ soil at different ageing time. Black curves
806 represent sample data while red curves represent LCF results. Processed results are
807 presented in Table 4.

Table 1Concentration of total Sb in < 250 μm particle size fractions.

Time	Sb concentration (mg kg^{-1})	
	<i>S1-100</i>	<i>S1-1000</i>
1 d	121.1 ± 2.51	1167 ± 19.3
7 d	159.2 ± 6.86	1384 ± 9.64
31 d	119.0 ± 5.99	1326 ± 24.0
91 d	156.3 ± 1.65	1321 ± 22.3
700 d	139.0 ± 4.25	1403 ± 71.9
	<i>S2-100</i>	<i>S2-1000</i>
1 d	234.2 ± 9.13	2500 ± 47.2
7 d	237.9 ± 7.94	2954 ± 438
31 d	254.2 ± 1.21	2734 ± 250
91 d	297.0 ± 30.2	2950 ± 268
700 d	252.1 ± 5.80	2956 ± 287

Table 2Concentration of Sb in soil solution (C_{sol}) and R values in soils during ageing.

Ageing time (d)	C_{sol} ($\mu\text{g}\cdot\text{L}^{-1}$)				R value ($R = C_{\text{DGT}}/C_{\text{sol}}$)			
	S1-100	S2-100	S1-1000	S2-1000	S1-100	S2-100	S1-1000	S2-1000
1	88.88	38.06	535.6	596.6	0.049	0.119	0.045	0.050
7	28.83	37.44	300.3	91.2	0.039	0.068	0.035	0.049
31	27.64	39.11	260.3	72.2	0.036	0.046	0.033	0.039
91	13.33	16.73	212.3	35.3	0.039	0.061	0.030	0.038
700	15.76	8.84	173.0	26.6	0.021	0.056	0.028	0.041

Table 3

Pearson correlation coefficients (r) between the Sb fractions detected by SEP and those quantified by DGT (C_{DGT}) in S1 and S2 soils [†].

	S1-100	S1-1000	S2-100	S2-1000
	C_{DGT}	C_{DGT}	C_{DGT}	C_{DGT}
F1	0.959**	0.946**	0.786**	0.947**
F2	0.853**	0.911**	0.297 ^{NS}	0.780**
F1+F2	0.967**	0.979**	0.683**	0.966**
F3	0.425 ^{NS}	-0.106 ^{NS}	-0.041 ^{NS}	-0.032 ^{NS}
F4	-0.958**	-0.921**	-0.537*	-0.963**

[†]NS not significant ($P > 0.05$); * statistically significant at $P < 0.05$; ** statistically significant at $P < 0.01$.

Table 4

Linear Combination Fitting (LCF)–XANES analysis of Sb speciation.

Sb speciation distribution (%)				
	KSb(OH) ₆	Sb(V)-Citrate	Sb(V)-Goethite	χ^2
S1-100 1 d	88	12	0	0.0059
S1-100 31 d	63	19	18	0.0242
S1-100 700 d	57	18	24	0.0063
S2-100 1 d	55	32	13	0.0088
S2-100 31 d	43	36	21	0.0150
S2-100 700 d	36	37	27	0.0091
S1-1000 1 d	88	12	0	0.0022
S1-1000 31 d	85	12	3	0.0021
S1-1000 700 d	81	14	5	0.0034
S2-1000 1 d	62	25	13	0.0037
S2-1000 31 d	59	23	18	0.0014
S2-1000 700 d	58	21	21	0.0543

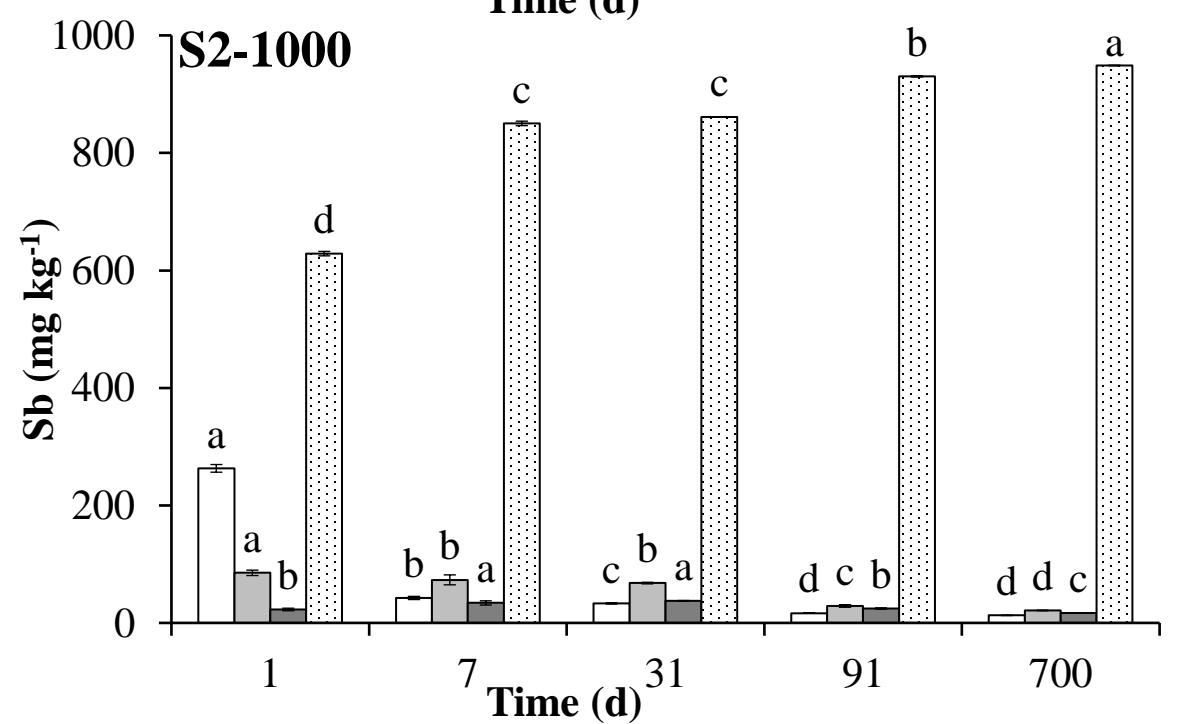
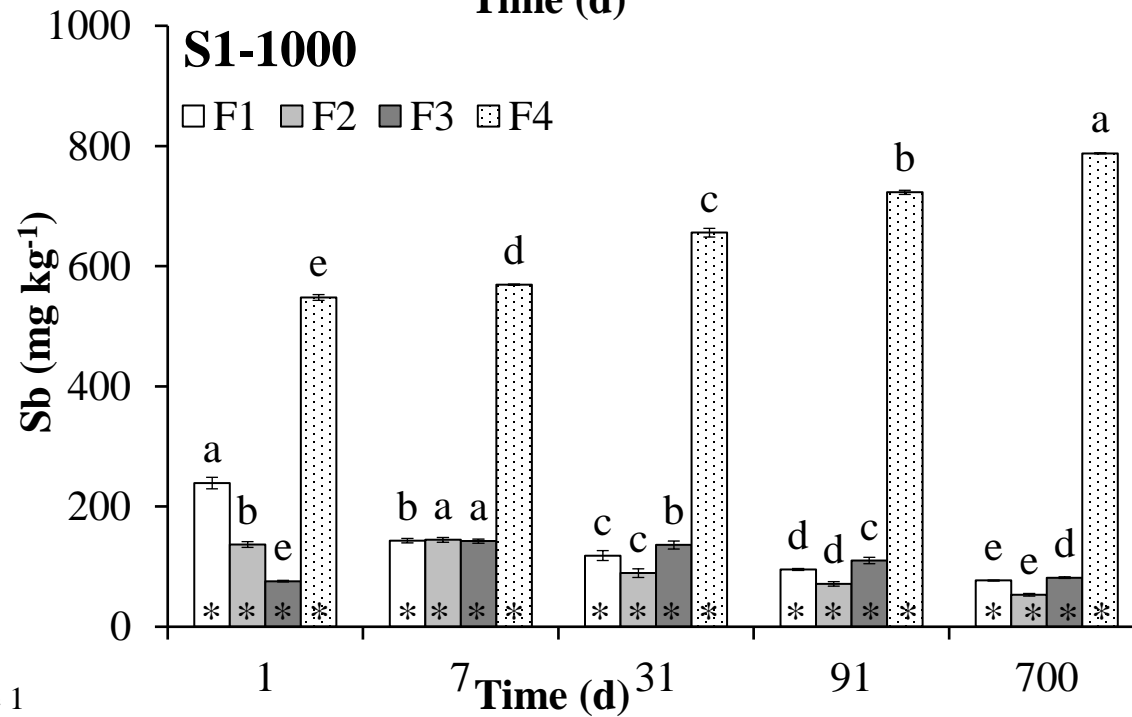
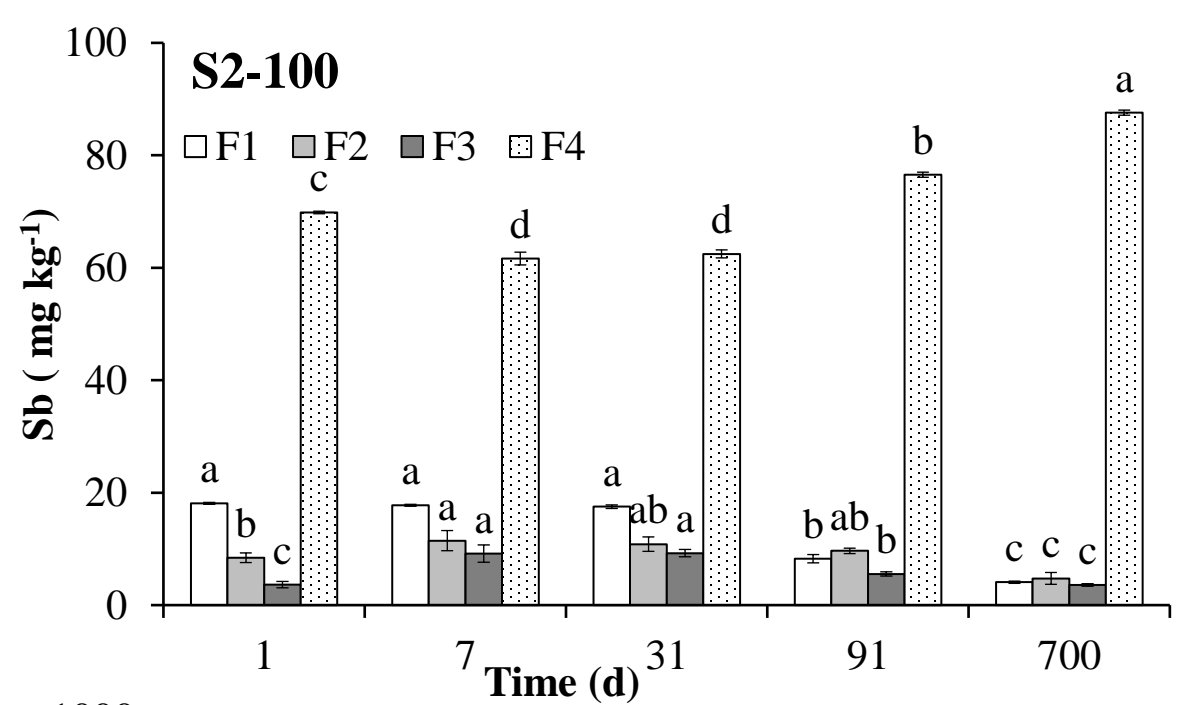
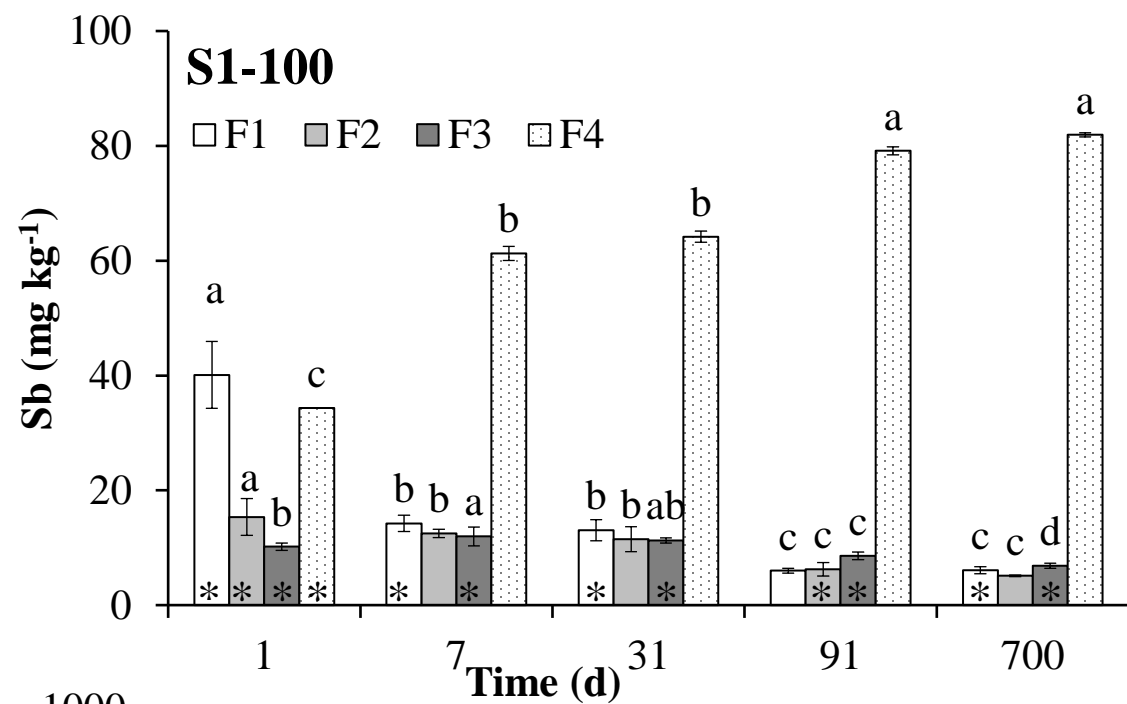


Figure 1

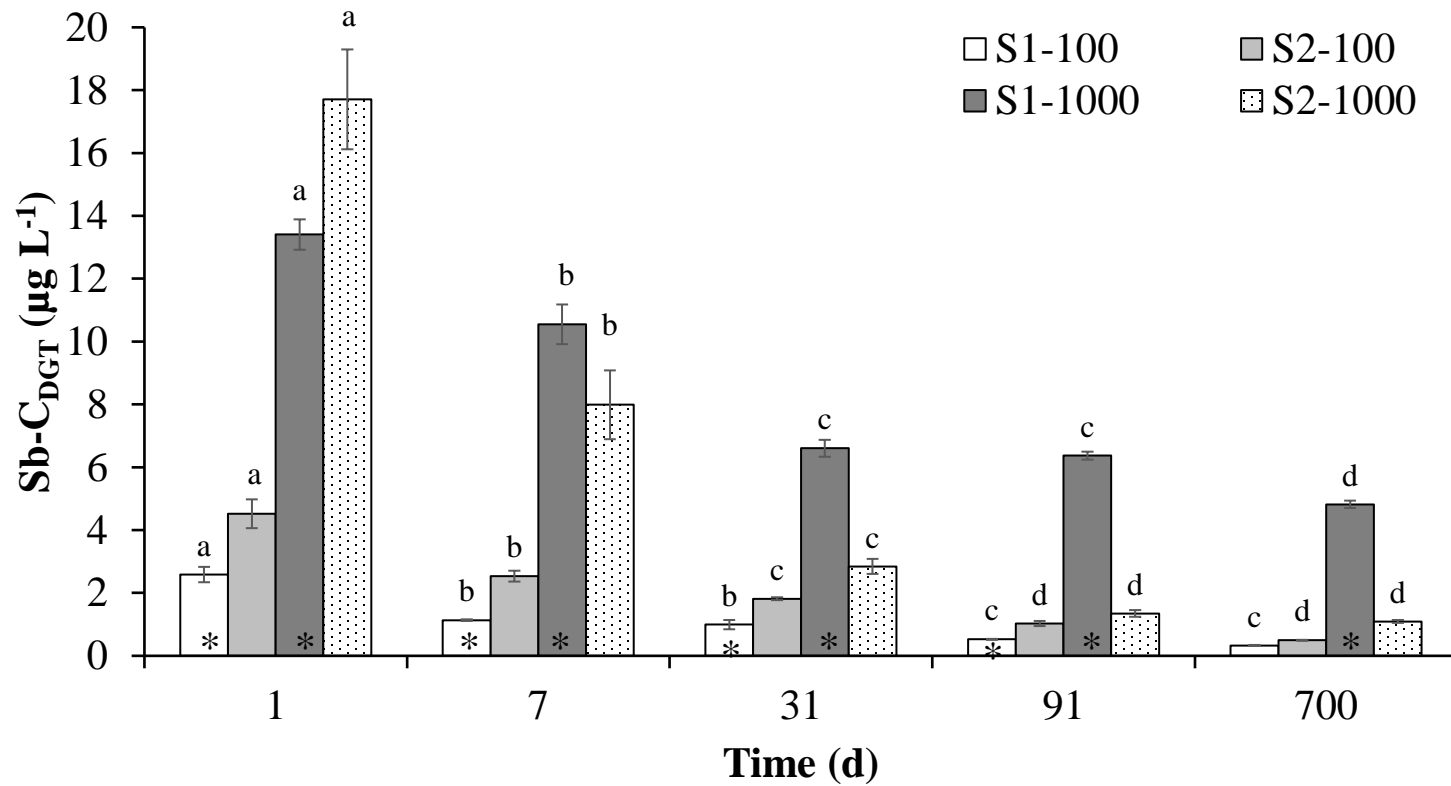


Figure 2

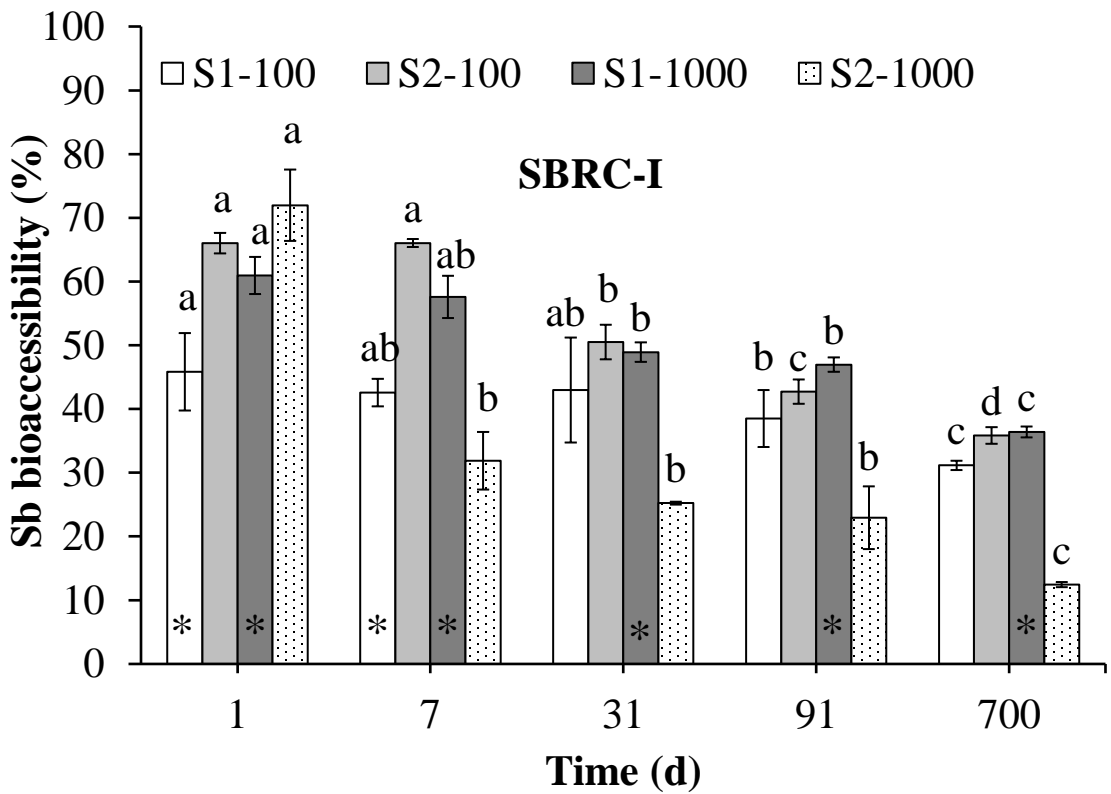
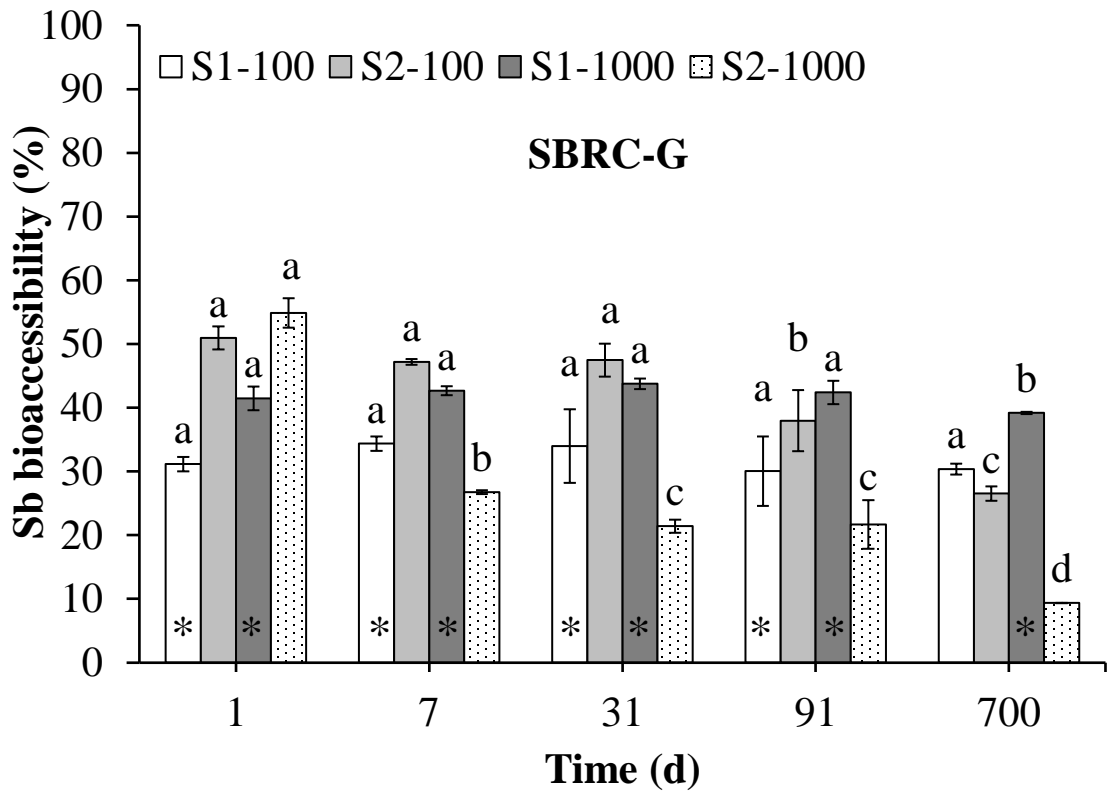


Figure 3

1 **Table S1.** Selected physico-chemical characteristics of S1 and S2 soils.

Physico-chemical parameters	S1 soil	S2 soil
pH _(H₂O)	8.2 ± 0.1	4.9 ± 0.2
DOC (mg g ⁻¹)	0.17 ± 0.00	0.39 ± 0.02
SOM (%)	1.75 ± 0.1	2.19 ± 0.2
CEC (cmol ₍₊₎ kg ⁻¹)	20 ± 0.8	13 ± 2.5
pH _{PZC}	5.7	2.6
Texture (USDA)	Sandy clay loam	Loamy coarse sand
<i>Total metal(loid)s (mg kg⁻¹)</i>		
Fe	16,350 ± 1,061	5,650 ± 71
Ca	62,500 ± 2,828	2,426 ± 145
Al	19,930 ± 1,018	3,924 ± 749
Sb	n.d.*	n.d.

2 *n.d., not detected (< 0.01 µg·kg⁻¹).

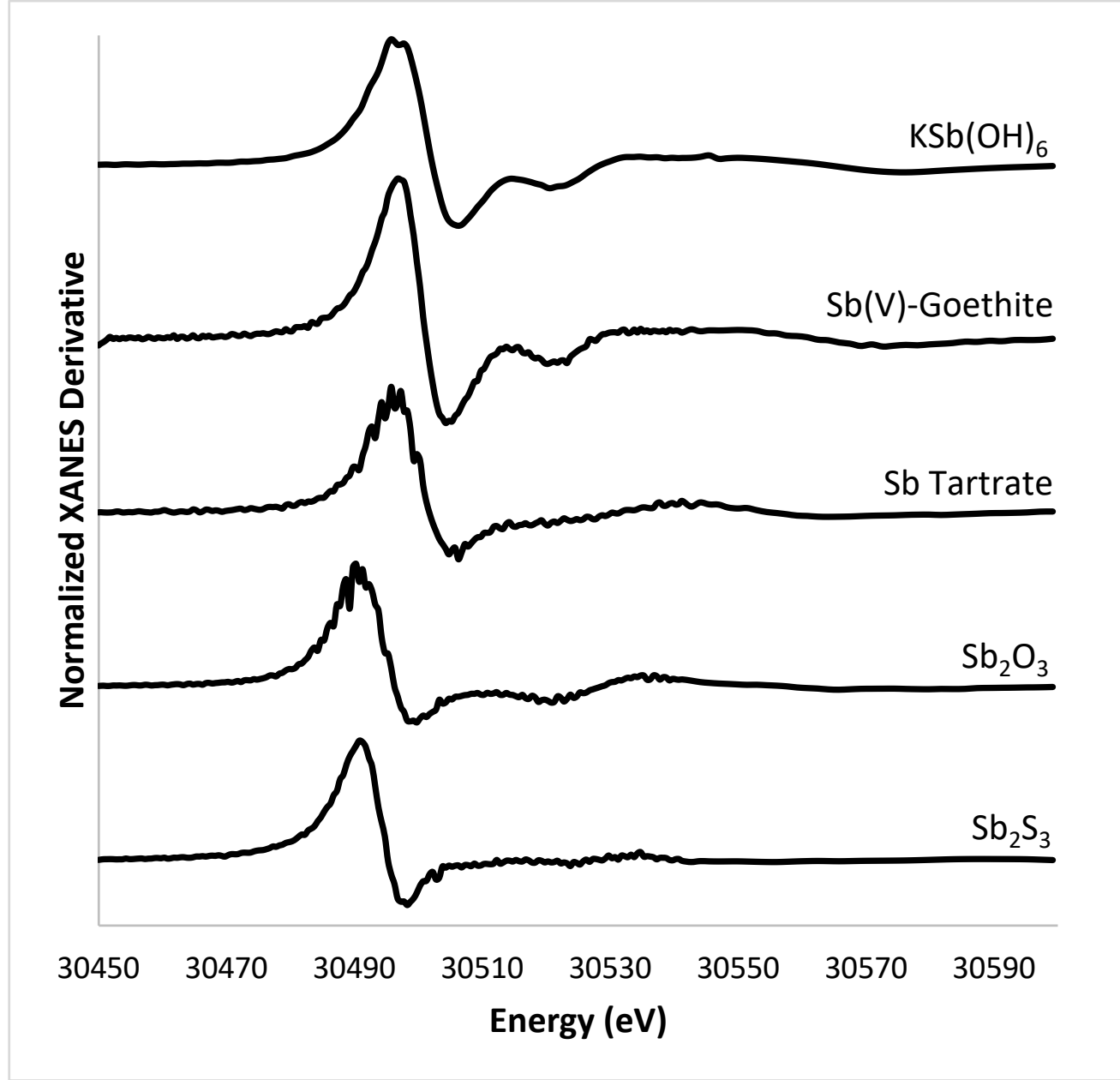


Figure S1. Derivative XANES spectra of antimony reference materials considered for linear combination fitting.

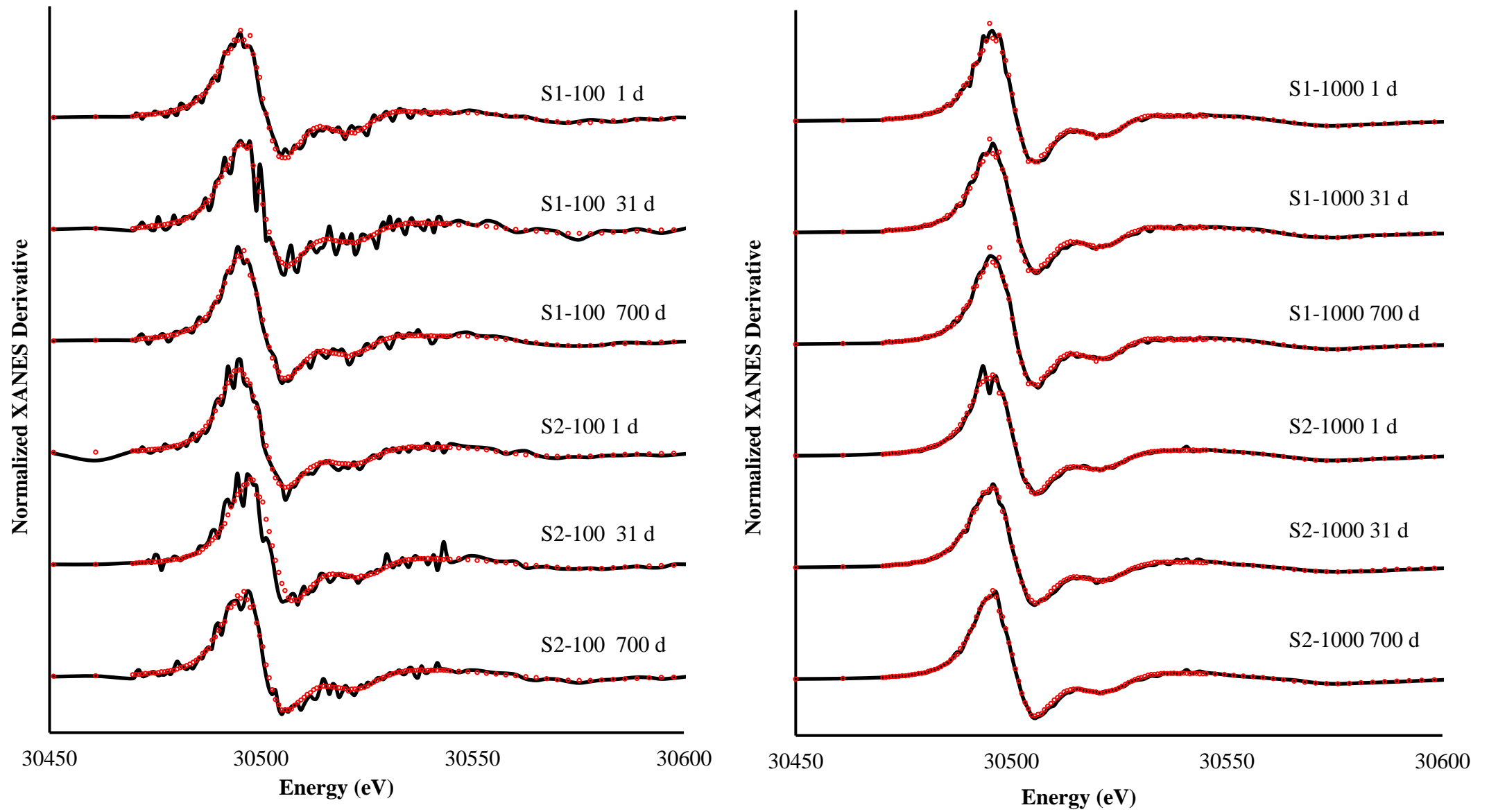


Figure S2. Derivative XANES spectra of antimony from S1 and S2 soils spiked with 100 (S-100) and 1000 (S-1000) mg Sb kg⁻¹ soil at different ageing time. Black curves represent sample data while red curves represent LCF results. Processed results are presented in Table 4.