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1 **Origin, fate and ecotoxicity of manganese from legacy metallurgical wastes.**

2

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26 **Abstract**

27 Over the course of history, mining and metallurgical activities have influenced the
28 socioeconomic development of human populations. However, these past and current activities
29 can also lead to substantial environmental contamination by various metals. Here, we used an
30 interdisciplinary approach (incorporating archaeology, mineralogy, environmental chemistry
31 and ecotoxicology) to investigate the origin, fate and potential ecotoxicity of anomalous
32 manganese (Mn) concentrations detected in the ancient mining district of Berthelange
33 (medieval period, eastern France). Mineralogical investigations of slag samples showed that
34 smelting temperature conditions in medieval bloomeries led to the production of slags mainly
35 composed of Fe- and Mn-rich olivine, *i.e.*, fayalites. Further mineralogical analyses of bulk
36 soil and clay fractions allowed us to identify the presence of serpentine. This evidence of
37 olivine weathering can account for the release of Mn from slags into the soil. In addition,
38 chemical analyses of total and available (exchangeable and reducible) Mn concentrations in
39 soil samples clearly showed the contribution of slags deposited 1000 years ago to soil
40 contamination. A complementary ecotoxicity bioassay performed on soils from a slag heap
41 using the land snail *Cantareus aspersus* confirmed that a significant fraction of the Mn
42 detected in soils remains available for partitioning with the soil solution and transfer to soil
43 organisms. Although no growth inhibition of snails was observed after 28 days of exposure,
44 the animals accumulated quite elevated Mn concentrations in their tissues. Our study
45 emphasizes the environmental availability and bioavailability of Mn from ancient
46 metallurgical wastes to soil-dwelling invertebrates, *i.e.*, snails, even one millennium after their
47 deposition. Hence, as for more recent industrial sites, past mining ecosystems must be a cause
48 of concern for the scientific community and public authorities.

49 **Keywords (x6)**

50 slags; weathering; metals; chemical extraction; bioavailability; snails

51 **1. Introduction**

52 Throughout human history, and especially since the Bronze Age, socioeconomic
53 development has been strongly influenced by the exploitation of mineral resources, such as
54 copper (Cu), iron (Fe), lead (Pb) or silver (Ag) (Nriagu, 1996; Tylecote, 1987). However,
55 mineral-related activities also constitute the most ancient and significant source of waste
56 production (Macklin et al., 1997; Pyatt et al., 2005). These types of wastes, originating from
57 mining (ore dumps, ore-washing sediments) or metallurgical (smelter dusts, slags) operations,
58 are currently considered archaeological clues. Until quite recently, slags were mostly
59 considered chemically inert because of the strong association of metals with glass and mineral
60 phases, limiting their lability (Wilson et al., 1994). However, a growing body of evidence
61 shows that slags are affected by weathering and may be able to release metals into aquatic and
62 terrestrial compartments (Ettler, 2002 and 2016; Gee et al., 1997; Lottermoser, 2002; Parsons
63 et al., 2001; Piatak et al., 2004; Seignez et al., 2006).

64 In addition to the modification of the geochemical cycles of metals, this contamination
65 may be responsible for the deterioration of water and soil quality and the impairment of
66 biodiversity (Ettler et al., 2009; Lottermoser and Cairns, 2005). For instance, Monna et al.
67 (2011) showed that wild brown trout living in past mining areas located in the Cevennes
68 (France) were contaminated by Pb originating from historical activities (15th and 20th
69 centuries). More recently, Mariet et al. (2016 and 2020) highlighted that metals originating
70 from mining operations (Pb and Ag) during the end of the Middle Ages in the Vosges
71 Mountains (France) remain bioavailable to soil invertebrates. However, the older the sites are,
72 the scarcer the data are. In the Franche-Comté region (eastern France), Fe mining and
73 metallurgical activities began in the Early Middle Ages (5th century), with particular intensity
74 in the district of Berthelange (Forlin and Laurent, 2014; Laurent, 2016). Until the 15th century,
75 a direct ore-reducing process was conducted within clay-made blast furnaces. In these

76 bloomeries, temperatures exceeded 1000°C, allowing, with the addition of charcoal, the
77 reduction of Fe oxides (Fe₂O₃). At the end of the smelting process, a heterogeneous block of
78 Fe (sponge) was kept for further purification, and metal-rich residues, *i.e.*, slags, were dumped
79 on site as heaps. Currently, more than 1000 years later, the environmental effects of potential
80 metal releases have, to our knowledge, not been evaluated.

81 Therefore, this study aims to assess the effect of very ancient slag deposits on soil
82 quality. Because a single chemical analysis of total metal concentrations in soils may be
83 insufficient for ecological risk assessment purposes (Peijnenburg, 2020), we combined
84 physical, chemical and biological tools in a holistic approach. We operationally investigated
85 the metal distribution in and transfer between various matrices (slags, soil and invertebrates)
86 to reveal the origin, fate and effects of legacy contamination.

87

88 **2. Materials and methods**

89 *2.1. Study area and sampling design*

90 This study was performed in the Bourgogne Franche-Comté region of eastern France,
91 where Fe mining and metallurgical exploitation were important activities during the Middle
92 Ages (Jacob and Mangin, 1990). Over this period, Fe mining and metallurgical activities were
93 particularly intense in the district of Berthelange (140 km², 20 km west from Besançon). The
94 study area is composed of Jurassic limestones and marls (from Toarcian to Bajocian) partly
95 covered by clay and sandy alluvial Pliocene deposits (Dreyfuss and Kuntz, 1967). Here, more
96 than 120 direct Fe ore-reducing process sites were identified (Forlin and Laurent, 2014)
97 because of the presence of slag repositories (from 10 to 20 m in diameter) (Figure 1A). In this
98 study, eleven slag repositories, located in forested regions and well preserved, were
99 investigated for the detection of potential anomalous metal concentrations in soils (Figure 1A,

100 Table 1). Among them, the Antorpe 9 heap (Figure 1B), containing slags produced during the
101 11th century (Forlin and Laurent, 2014), was selected for further investigations. More
102 precisely, slags and soils were sampled using a transect method following four directions (NE,
103 SE, SW and NW) from the centre of the slag heap (sampling point, SP, 4) according to Figure
104 1C. The reference site SP10 was located 100 m west of the heap. This remote station was
105 selected to represent the pedogeological background of the area studied, *i.e.*, the same
106 geological substrate and same vegetation cover, but without any smelting activities or slag
107 deposit. At each sampling point, three soil cores (0-100 cm) were taken (after removing
108 surface vegetation and humus) using a soil auger.

109

110 2.2. Soil analyses

111 2.2.1. Physico-chemical characterisation

112 The soil samples were primarily sieved (2 mm mesh; slag fragments were sorted for
113 further analyses), dried at 40°C and homogenized. The soil pH was measured using a pH
114 metre (WTW, pH/ION 3310) after extraction with demineralized water (1:5, v:v; ISO 10390,
115 2005), and the organic matter (OM) content was determined via the loss of ignition (LOI)
116 method according to NF EN 15935 (AFNOR, 2013). Total metal concentrations were
117 determined after hot mineralization in *aqua regia* (1:2.5 HNO₃: HCl, v:v) using a block
118 digestion system (DigiPREP) and filtration (1 µm) by inductively coupled plasma atomic
119 emission spectroscopy (ICP-AES) (Thermo Scientific, iCAP 6000 Series). The reliability of
120 the analyses was assessed with certified reference material (calcareous loam soil: CRM
121 n°141R from BCR). The recovery rate was on average 107±8% for all the metals analysed.

122 To identify potential decay products of slag minerals, mineralogical analyses of soil
123 samples (bulk and clay fraction) from different depths (0-20 cm, 20-40 cm, 40-60 cm, 60-80

124 cm and 80-100 cm) were obtained using X-ray diffraction (XRD) characterization and a D8
125 Advance Bruker diffractometer equipped with a LinxEye detector (CuK α radiation at 1.54184
126 Å, 40 kV/40 mA) hosted at the Utinam Institute (University Bourgogne Franche-Comté,
127 France). Powdered bulk rock samples were treated between 2θ values of 3° and 65° (divergent
128 slit) with a step scan of 0.019° (2θ) and a scan rate of 1 s/step. The EVA software package
129 was used for data processing and phase identification. The extraction of clay fractions
130 required prior OM destruction by successive addition of 1 cm³ hydrogen peroxide (33% H₂O₂,
131 VWR Chemicals) to hydrated soil samples previously blended with water (1:5; w:v). To
132 increase the reaction rate (Holtzapffel, 1985), this mixture was heated at 60°C on a digital
133 hotplate (Stuart SD500). The samples were then subjected to repeated washing (n=4) using
134 ultrapure water (UP) and centrifugation (10 min, 8,000 rpm). Afterward, clay suspensions
135 were shaken in an end-over-end shaker for 10 min at 10 rpm. Finally, two clay fractions (<16
136 μm and <2 μm) were obtained by the sedimentation process according to Stoke's law, which
137 links the settling rate to the particle size, and mounted on glass slides for XRD analyses.
138 Before successive XRD analyses, the oriented preparations were sequentially air dried,
139 glycolated and heated for 4 h at 490°C for identification of the interlayer of clays, *i.e.*, the
140 (001) basal reflection positions after each treatment. The XRD patterns were obtained
141 between 2° and 32° (2θ) with a step scan of 0.009° (2θ) and a scan rate of 1.5 s/step.

142 2.2.2. Environmental availability

143 The environmental availability was estimated using a chemical extraction method
144 chosen to quantify the potential of metals for leaching or uptake by plants (Abedin et al.,
145 2012). The extraction solution consisted of 1 mol.L⁻¹ ammonium acetate (NH₄OAc, purity
146 >99%) added to a solution containing 2 g.L⁻¹ hydroxylamine hydrochloride salt (NH₂OH,
147 HCl, purity >99%) and buffered at pH=7 \pm 0.05 using ammonium hydroxide (NH₄OH, purity
148 >99%). Soil suspensions were obtained after shaking 37.5 mL of extraction solution and 1.5 g

149 of dried soil in an end-over-end shaker for 2 h at 10 rpm. Suspensions were then centrifuged
150 at 3000 rpm for 10 min and filtered through a 0.45 µm cellulose acetate disk filter to obtain
151 the extracts (AFNOR, 2002). To ensure complete extraction, the soil residues were subjected
152 to four successive extractions of 2 h each. Finally, metal concentrations were determined in
153 the total extract solutions by ICP-AES.

154

155 *2.3. Slag analyses*

156 2.3.1. Total metal concentrations

157 Total metal concentrations in slags (bulk) were determined according to the
158 international standard ISO 14869-1 (2001). Briefly, 0.250 g milled slag samples (n=3) were
159 digested in PTFE dishes on a hot plate (150°C) after the addition of 5 mL of hydrofluoric acid
160 (47% HF) and 1.5 mL of perchloric acid (65% HClO₄). The mixture was allowed to evaporate
161 to near complete dryness, and the residue was dissolved in diluted nitric acid (HNO₃) before
162 analysis by ICP-AES. The reliability of the analyses was assessed with certified reference
163 material (calcareous loam soil: CRM n°141R from BCR). The recovery rate averaged
164 104±6% for all the metals analysed.

165 2.3.2. Mineralogical characterisation

166 Slags were first ground, and the whole-rock powders were used for XRD analysis.
167 XRD patterns were obtained using the same method as for soil mineralogical characterisation
168 and allowed assessment of potential slag decay within soil through mineralogical analysis
169 (comparison of similar mineralogical phases within soil and slag samples).

170 Then, hand samples of slags were cut to prepare a 30 µm-thick polished thin section.
171 Optical investigations using scanning electron microscopy (SEM) in back-scattered electron
172 (BSE) mode were conducted at the Femto-ST Institute (University Bourgogne Franche-

173 Comté, France). Observations were complemented by energy-dispersive spectrometry (EDS)
174 mapping obtained at the University of Lausanne (Switzerland) using a Tescan Mira LMU
175 SEM equipped with a field emission gun (FEG). The composition of slag-forming minerals
176 and glass were obtained by electron probe microanalysis (EPMA) at the University of
177 Lausanne using a JEOL 8200 Superprobe equipped with five wavelength dispersion
178 spectrometers (WDS). Carbon-coated polished thin sections were analysed with a 15 kV beam
179 and a current of 15 nA. The resolution was ca. 1 μm . Oxides and silicates were used as
180 standards. In addition, X-ray intensity mapping was carried out to reveal the specific textures
181 caused by the chemical features of minerals.

182

183 *2.4. Ecotoxicity test: snail growth inhibition and bioaccumulation*

184 2.4.1. Animals

185 Land snails (*Cantareus aspersus*, Müller 1774, syn. *Helix aspersa*) were reared under
186 controlled conditions according to Gomot-de Vaufleury (2000). For the bioassay, juvenile
187 snails (n=105) were reared for 4 weeks and weighed 1.05 ± 0.15 g prior to exposure.

188 2.4.2. Exposure modalities

189 Following the standard ISO 15952 (2018), the juvenile snails were exposed for 28
190 days to three surface soils (0-20 cm) originating from increasing distance to the centre of the
191 smelting waste repository, *i.e.*, presenting a gradient in metal contamination (SP10, 8 and 4),
192 and to a control treatment (C) in which snails were sustained over dampened absorbent paper.
193 For each treatment, five transparent polystyrene containers of 3200 cm^3 (24 \times 21 \times 8 cm; ref.
194 E1DBBAC001, Charles River IFFA-CREDO, 69 L'Arbresle) were used. Each container was
195 filled with a 1.5 cm layer (200 g dry weight, DW) of soil or an absorbent paper layer, and five
196 snails were introduced. The snails were fed ad libitum with uncontaminated Helixal® snail

197 food placed in a Petri dish left on the bottom of the container. The day/night cycle
198 photoperiod was 18/6 h, the temperature was $20\pm 1^{\circ}\text{C}$, and the air relative humidity was
199 $90\pm 5\%$. Three times a week, the containers were cleaned, the faeces were removed, and the
200 food was renewed.

201 2.4.3. Measured endpoints

202 Each week, individual snail fresh mass (whole-body) and shell diameter were
203 monitored as biological endpoints for the assessment of sublethal effects.

204 At the end of the exposure, one snail was randomly sampled in each replicate
205 container for each exposure modality and subjected to a fasting period of 48 h to empty its gut
206 (the faeces were removed after 24 h to prevent their ingestion), then sacrificed by freezing at -
207 80°C . After thawing, the whole soft body was removed from the shell and separated into two
208 parts: the viscera (*i.e.*, the visceral complex containing the posterior gut, digestive gland,
209 kidney, mantle, and part of the reproductive tract) and the foot (containing the foot *sensu*
210 *stricto*, anterior gut, and remainder of the genital tract). The samples were then freeze-dried
211 and digested in HNO_3 (purity 99.9%) before analyses for metal content by ICP-AES. The
212 certified reference material was TORT-2 (lobster hepatopancreas from NRCC-CNRL,
213 Canada), and recovery rates ranged between 90% and 118% of the certified values for all the
214 metals.

215

216 *2.5. Statistics*

217 All data are presented as the mean \pm standard deviation (sd). First, correlation analyses
218 were performed using the Spearman rank correlation test between the slag abundance
219 observed at each sampling point and physico-chemical parameters, such as the pH, OM
220 content and total and extractable metal concentrations.

221 Significant differences among soil parameters, metal concentrations, snail growth and
222 metal bioaccumulation were then analysed by one-way analysis of variance (ANOVA)
223 followed by Tukey's HSD post hoc test for pairwise comparisons. For the case in which the
224 normality of distribution and homogeneity of variances were not verified (using the
225 Shapiro–Wilk and Bartlett tests, respectively), the Kruskal-Wallis test followed by the
226 Kruskalmc post hoc test (pgirmess package) were used. For all statistical analyses, a *p*-value
227 of 5% was used as a significance threshold.

228 All statistical analyses were performed with the free statistical software R (ver 3.4.0)
229 (R Core Team, 2020).

230

231 **3. Results**

232 *3.1. Soil contamination in the Berthelange district*

233 Metal concentrations in soils formed above the slag heaps and in reference soils, *i.e.*,
234 soils that had never been subject to metallurgical activities, are presented in Table 1. In the
235 reference sites, metal concentrations are relatively low in comparison with values measured
236 on slag repositories. Indeed, among the metals that have been analysed, some exceed the
237 maximum permissible concentrations in soils. For instance, low anomalous concentrations are
238 observed for arsenic (As) and zinc (Zn) in Petit-Mercey 25, Antorpe 9 or Ferrières 8. The
239 most marked contamination concerns major elements, such as Fe and Mn, with total
240 concentrations in soils of the latter element reaching more than 8500 $\mu\text{g}\cdot\text{g}^{-1}$ at the Antorpe 9
241 site. Therefore, further results and discussion will focus particularly on this site and this
242 element.

243

244 *3.2. Characterisation of slags*

245 At the macroscopic scale, slags from the Antorpe 9 heap are generally homogeneous
246 in shape, density and porosity. The morphology directly reflects the smelting technology in
247 bloomeries. Slags are generally dark-coloured, ranging from blueish to brownish. Flow
248 texture is frequently observed and is characteristic of slags formed in a molten state. Vesicles,
249 ranging from micrometres to centimetres, are commonly met.

250 Metal concentrations in slags of Antorpe 9 (Table 1) reach an average of
251 approximately 15% Fe and 1.7%, 1.5% and 0.35% Mn, Ca and Mg, respectively. Other
252 quantified trace metal elements present lower concentrations ranging between 0.0003% (for
253 cadmium, Cd) and 0.008% (for Cu). At the mineralogical scale, XRD patterns allowed us to
254 identify fayalite, an olivine group mineral, as the main component of slags (Figure 2A).
255 Petrographic investigations on polished thin sections showed that the phases present in
256 Antorpe 9 slags include olivine, glass, spinel, leucite, wustite and metallic Fe (Figure 3).
257 Olivine group minerals commonly display a spinifex texture and elongated skeletal laths; the
258 WDS analyses revealed a fayalite (Fe_2SiO_4) composition. The glass phase is generally
259 interstitial and contains subhedral spinel crystals (Figure 3B). Locally, wustite occurs as bleb
260 aggregates and is partially replaced by secondary Fe phases. Similarly, metallic Fe fragments
261 are corroded and transformed into oxides and hydroxides (Figure 3D). As shown in Table 2,
262 Mn is heterogeneously distributed between minerals and glass. Fayalite, which may contain
263 up to 5% MnO, is the primary carrier of Mn, while glass and spinel display MnO contents of
264 less than 1.8% (Table 2; Figure 3C). Manganese is almost absent in metallic Fe and secondary
265 Fe phases (Figure 3F).

266

267 *3.3. Influence of the slag heap on soil characteristics*

268 The edges of the heap were delimited using the relative abundance of slags in the
269 surface layer (0-20 cm) of soils collected at each sampling point (Figure 4). Regardless of the

270 transect direction considered, the slag abundance decreases with the distance from the centre
271 of the heap, dropping from approximately 70% at SP4 to less than 5% at the heap foot (SP3,
272 5, 7 and 8).

273 Significant positive correlations were identified between the slag abundance and some
274 physicochemical parameters of soils (Figure 4), such as the OM content ($Rho=0.94$, $p<0.001$)
275 and pH ($Rho=0.54$, $p<0.001$). Indeed, from the edge of the slag heap to its centre, the OM
276 content and pH values increase from 8 to 19% and 4.8 to 5.4, respectively.

277 Variations in the clay composition of soils are also observed by means of XRD
278 analyses of soils (Figure 2B) and clay extract (Figures 2C to G). Mineralogical analyses of
279 clay extract from soil samples allowed us to identify different clay minerals that are present in
280 various proportions depending on the samples: illite, chlorite, kaolinite, serpentine
281 (antigorite/chrysotile), and mixed-layer clay minerals. Kaolinite and serpentine exhibit very
282 similar diffraction patterns, but the absence of tetrahedral substitution in antigorite and
283 chrysotile induces rolling up of the layers (Holtzapffel, 1985; Moore and Reynolds, 1997).
284 This characteristic allowed us to distinguish samples without serpentine (Figure 2E) from
285 samples containing serpentine (Figures 2F and G).

286 The total Mn concentrations in the soils range from $1150 \mu\text{g}\cdot\text{g}^{-1}$ at the reference site
287 (SP10) to approximately $3500 \mu\text{g}\cdot\text{g}^{-1}$ at the heap foot (SP3, 7 and 8) and up to more than 8000
288 $\mu\text{g}\cdot\text{g}^{-1}$ at the centre of the heap (SP4) (Figure 4). Manganese extractable concentrations
289 display a similar pattern to total Mn with increasing values from SP10 ($880 \mu\text{g}\cdot\text{g}^{-1}$) to SP4
290 ($2800 \mu\text{g}\cdot\text{g}^{-1}$), representing extraction yields of 77% and 35%, respectively. Surprisingly, at
291 SP9, the total and extractable concentrations were particularly low, *i.e.*, 400 and $170 \mu\text{g}\cdot\text{g}^{-1}$,
292 respectively.

293

294 3.4. Ecotoxicological bioassay

295 No snail mortality was recorded over the 28-day exposure periods. Concerning
296 sublethal effects, the growth of snails exposed to the four modalities did not show any
297 significant difference either in terms of body mass (Figure 5A) or shell diameter (data not
298 shown). Indeed, the final mass of snails reached 6.9 ± 1.2 g for the control modality and $7.3 \pm$
299 1.4 g, 7.2 ± 1.6 g and 7.2 ± 1.1 g for snails exposed to soils located at 100 m, 5 m and the
300 centre of the heap, respectively. Similarly, the final shell diameter increased 28.12 ± 3.0 mm
301 for the control and 28.85 ± 2.38 mm, 28.92 ± 2.52 mm, and 29.29 ± 1.97 mm for snails
302 exposed to the three previously cited soils.

303 Growth measurements were completed with Mn bioaccumulation data in snail foot
304 and viscera after 28 days of exposure (Figure 5B). As snails grew over the 28-day duration of
305 the exposure, we used body burdens rather than tissue concentrations because the latter can be
306 affected by rapid changes in the mass of the organism (Gimbert et al., 2006; Gimbert and de
307 Vaufleury, 2009). Snails significantly accumulate Mn in their viscera following a dose-
308 response pattern according to the Mn concentrations in soils. Viscera internal burdens reached
309 25.2 ± 8.3 μg in the control snails (*i.e.*, snail food exposure only) and 93.4 ± 1.1 μg , $93.5 \pm$
310 29.6 μg and 154.0 ± 34.4 μg in snails exposed to soils from SP10, SP8 and SP4, respectively.
311 Accumulation in the foot is low, and body burdens do not vary regardless of the exposure
312 modality (Figure 5B).

313

314 4. Discussion

315 4.1. Source of Mn contamination in soils

316 Manganese concentrations measured in reference soils from the study area average
317 approximately $800 \mu\text{g.g}^{-1}$ and are in accordance with the Mn pedogeochemical background of

318 soils (Pinsino et al., 2012; Grygo-Szymanko et al., 2016). Varying from 480 to 1150 $\mu\text{g.g}^{-1}$,
319 these background concentrations nevertheless show a spatial heterogeneity that has to be
320 related to the lithological nature of the soils (including within the same geological formation)
321 and to the local soil features (both abiotic and biotic). However, this natural variability is not
322 of the same order of magnitude as what is observed in soils formed on ancient slag
323 repositories. Indeed, Mn concentrations range from 1200 to more than 8000 $\mu\text{g.g}^{-1}$, testifying
324 to the influence of smelting wastes on soil contamination. These values exceed the maximum
325 permissible concentrations in soils; therefore, it is necessary to better understand the origin
326 and fate of this element in past mining ecosystems.

327 In slags, three main mineralogical phases are identified: metallic Fe residues, glass and
328 fayalites belonging to the olivine group. The presence of metallic Fe inclusions in slags attests
329 to the generally moderate extraction yields of Fe during the Early Middle Ages. Glass is
330 ubiquitous in slags from Antorpe 9 and is primarily composed of Si, Ca and Al oxides, as
331 reported in the literature (Piatak et al., 2015). The presence of fayalite and its dendritic
332 shape are related to the temperature in the bloomeries (reaching more than 1000°C)
333 and the slow air-cooling conditions of molten slags at the end of the smelting
334 process (Piatak et al., 2004; Tossavainen et al., 2007). The manganese concentration is
335 1.7% in slag bulk samples and is primarily located in fayalites, where it reaches 4.5
336 wt.% MnO. In their review, Piatak et al. (2015) provided average concentrations of Mn
337 in steel and pre-1900 and modern Fe slags between 1 and 4 wt.% MnO.

338 The weathering of the slag primary phases after abiotic (air or water) or biotic
339 (microorganisms) interactions leads to the production of secondary products that can be used
340 as indicators of the reactivity and alteration of slags (Piatak et al., 2015). In Antorpe 9 soils,
341 three clues of slag alteration have been identified. First, the alkalization of soil (+ 0.6 pH unit
342 between the reference soil and the centre of the heap) may originate from the hydrolysis of
343 CaO and other Ca-Mg oxide and silicate phases of slags and the subsequent release of Ca²⁺
344 and hydroxyl (OH⁻) ions (Riley and Mayes, 2015; Roadcap et al., 2006). This well-known
345 phenomenon led to the use of slags as a liming agent for soil quality restoration (Lopez et al.,
346 1995; Pinto et al., 1995). Second, we identified serpentine minerals ((Mg, Fe, Ni, Mn, Al, Zn)
347 [Si₂O₅] (OH)₄) in soils from Antorpe 9 slag heap. Serpentine is a subgroup of minerals
348 belonging to the kaolinite-serpentine group and usually contains divalent cations in
349 octahedrally coordinated sites. This type of mineral is frequently formed after weathering of
350 olivine in rocks. This serpentinization process is also known for magnesiferous olivines in
351 geological environments (basaltic rock alteration; Muntener, 2010), as well as for zinciferous
352 silicates in surface conditions (Choulet et al., 2016); to our knowledge, the process is rarely
353 demonstrated for ferriferous and manganiferous olivines. Finally, along with alkalization and
354 serpentinization, Mn can be considered a release product of slag weathering leading to soil
355 contamination.

356 From an environmental point of view, this soil contamination raises the question of
357 mobility and potential transfers to abiotic and biotic compartments of the current ecosystem.
358 Chemical extraction using ammonium acetate and hydroxylamine hydrochloride allowed us to
359 estimate the environmental availability of Mn in soils from Antorpe 9. This extractant can
360 extract the exchangeable metal fraction (including water-soluble Mn held on the surface of
361 negatively charged exchange complexes and Mn coprecipitated with the carbonate fraction)
362 and the easily reducible fraction (including oxides) (McAlister and Smith, 1999). This result

363 explains the elevated Mn extraction yields in the reference soil (77%, *i.e.*, 880 $\mu\text{g}\cdot\text{g}^{-1}$). In slag-
364 impacted soils, Mn availability is higher and increases from the edge (approximately 1600
365 $\mu\text{g}\cdot\text{g}^{-1}$) to the centre of the heap (2800 $\mu\text{g}\cdot\text{g}^{-1}$). In contrast, the extraction yields decreased,
366 ranging from 66 to 35%. This result may be related to the fractionation of Mn, which relies on
367 (i) the soil characteristics affected by the slag deposit (*i.e.*, alkaline soil pH, high OM and clay
368 content) (Walna et al., 2010) and (ii) the sequestration of a substantial portion of the total Mn
369 concentrations in a soil fraction refractory to the extraction (*i.e.*, the mineral matrix) (Maskall
370 et al., 1995). We have recently confirmed these findings with an in-depth study of the Mn
371 partitioning in soils from Roman and medieval slag heaps (Amnai et al., 2021). We have
372 indeed demonstrated that Mn was mainly found in the reducible (oxides) and residual (slag
373 micro-fragments) fractions of soils. The anomalously low total and extractable concentrations
374 at SP9 may hence be explained by its localization in the vicinity of a small pond, which
375 modifies the hydromorphic conditions of soil (*i.e.*, decreases the redox potential) and
376 subsequently promotes the leaching of Mn (Walna et al., 2010).

377

378 *4.2. Manganese bioavailability, transfer and toxicity*

379 The terrestrial snail *Cantareus aspersus* is well recognized as a biological indicator (de
380 Vaufleury, 2015), integrating three contamination routes: respiratory (from atmospheric
381 sources), digestive (from plant and soil sources) and dermal (diffusion of metals through the
382 foot epithelium). In addition to the chemical assessment of metal environmental availability,
383 the analysis of metal bioaccumulation in snail tissues constitutes a way to estimate the
384 fraction of the total concentration in soil that is truly transferable in biota, *i.e.*, the metal
385 bioavailability (ISO 17402, 2008). The snail foot is primarily composed of muscles and does
386 not appear, as for other trace elements, to be an important sink for Mn (de Vaufleury et al.,
387 2006). In viscera, the digestive gland (or hepatopancreas) is the main storage organ of Mn,

388 especially in intracellular granules occurring in large numbers in basophil cells (Mason and
389 Simkiss, 1982). The Mn burdens accumulated in snail viscera are in accordance with
390 extractable concentrations and confirm that a substantial portion of the Mn released by slags
391 is effectively bioavailable to soil organisms and particularly snails. Despite the high total and
392 available Mn exposure concentrations in soils, we did not observe deleterious effects
393 regarding snail survival or growth, as already noticed with artificially contaminated soils (up
394 to 750 $\mu\text{g Mn.g}^{-1}$ soil; Bordean et al., 2014). These results are not obvious with respect to
395 ecotoxicity data available in the literature. Indeed, Kuperman et al. (2004) determined
396 laboratory LC_{50} values (concentrations in the soil causing 50% mortality) of 389 $\mu\text{g.g}^{-1}$, 1970
397 $\mu\text{g.g}^{-1}$ and 2575 $\mu\text{g.g}^{-1}$ for *Enchytraeus crypticus*, *Eisenia fetida* and *Folsomia candida*,
398 respectively. The absence of significant detrimental effects on snails may be related to (i) a
399 misestimation of toxicity metrics estimated using spiked artificial soils (Lock and Janssen,
400 2001); (ii) the presence in field soils of growth-promoting factors for snails, such as humic
401 substances and oligo-elements (Gomot et al., 1989); and (iii) the ability of *C. aspersus* snails
402 to accumulate and detoxify Mn in their tissues using detoxification processes, such as metal-
403 rich granules (Mason and Simkiss, 1982; Simkiss and Taylor, 1994). However, other sensitive
404 biomarkers at the cellular and subcellular levels (*e.g.*, cytotoxicity; Bradley and Runham,
405 1996) deserve to be investigated. At the ecosystem scale, snails, as possible prey for
406 numerous invertebrates and vertebrates, may contribute to the diffusion of metals in trophic
407 chains (Moreno-Jiménez et al., 2011).

408

409 **5. Conclusion**

410 Although slag heaps may be regarded as punctual sources of metal release, their very
411 large number, considerable cumulative volume and spatial coverage make them a significant

412 source of risk (Amnai et al., 2021). The combination of mineralogical, chemical and
413 ecotoxicological approaches allows us to show here that, in addition to the modification of
414 various soil physico-chemical characteristics, such as the pH and OM content, slag
415 weathering over centuries led to the release of metallic contaminants in the surrounding
416 environment. Among them, Mn ranks, based on a combination of its frequency, toxicity, and
417 potential for human exposure, 140th (out of 275) in the substance priority list of the Agency
418 for Toxic Substances and Disease Registry (ATSDR, 2019). We have emphasised not only the
419 environmental availability of this metallic palaeo-pollution but also its bioavailability to soil-
420 dwelling invertebrates, such as snails. Hence, such legacy contaminated sites need to be more
421 clearly identified and included in environmental risk assessment procedures.

422

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431

432

433

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610

611 **Table captions**

612

613 **Table 1.** Metal concentrations ($\mu\text{g.g}^{-1}$) in soils from slag heaps and reference sites of the
614 Berthelange district and in slags (bulk) from the Antorpe 9 repository. ^a: data from
615 Crommentuijn et al. (2000). ^b: ecological soil screening level (Eco-SSL) from the US EPA
616 (2007).

617

618 **Table 2.** Chemical composition (mean \pm standard deviation, SD) of slag-forming minerals
619 and glass obtained by EPMA (% in weight).

620

621 **Figure captions**

622

623 **Fig. 1.** A: map of the Berthelange ancient metallurgical district. Green areas are forests, red
624 dots correspond to archaeologically referenced slag deposits (from Forlin and Laurent, 2014)
625 and white stars correspond to the investigated slag heaps. B: photograph of the Antorpe 9 slag
626 heap. C: sampling map of the Antorpe 9 heap.

627

628 **Fig. 2.** Diffractograms. A: slag; B: bulk soil (SP8, 40-60 cm); C and D: clay fraction in soil
629 (SP8, 40-60 cm and SP4 80-100 cm, respectively); E: photograph of glass slide with clay
630 fraction without serpentine (SP10 0-20 cm); F and G: photograph of glass slide with clay
631 fraction containing serpentine (SP4, 80-100 cm and SP8 40-60 cm, respectively).

632

633 **Fig. 3.** Left (A to C): representative view of mineral-rich slag material from Antorpe 9. Right
634 (D to E): detailed view of a spherical metallic Fe particle trapped within slag from Antorpe 9.
635 A and D: BSE images; B and E: interpretative map of the mineral phases based on WDS and
636 compositional maps, respectively; C and F: Mn X-ray composition map based on WDS and
637 EDS, respectively. Brightness correlates with Mn content.

638

639 **Fig. 4.** Total (black bars) and extractable (white bars) manganese concentrations ($\mu\text{g.g}^{-1}$), the
640 organic matter content expressed in % (dashed line) and the pH value and observed slag
641 content (%) at each sampling point. The NW-SE transect is represented in the left panel (A),
642 and NE-SW is represented in the right panel (B). Different letters correspond to significant
643 differences ($p\text{-value}<0.05$). Code for slag abundance: AA (very abundant, *i.e.*, more than

644 50%), A (abundant, *i.e.*, between 25 and 50%), F (common, *i.e.*, between 20 and 25%), R
645 (rare, *i.e.*, between 10 and 20%), and T (trace, *i.e.*, less than 10%).

646

647 **Fig. 5.** A: Time course of snail growth (fresh weight) during the 4-week exposure to control
648 conditions (C) and to surface soils from site Antorpe 9. No significant difference (*p*-
649 value<0.05) between treatments was identified. B: Mn bioaccumulation in *C. aspersus* foot
650 (white) and viscera (black) (expressed in body burdens, $\mu\text{g}\cdot\text{snail}^{-1}$) after exposure (for 28
651 days) to control conditions (C) and to surface soils from site Antorpe 9. t_0 corresponds to the
652 metal body burdens in snails at the beginning of the experiment. For each snail part, different
653 letters correspond to significant differences (*p*-value<0.05).

654

655 **Table 1.**

656

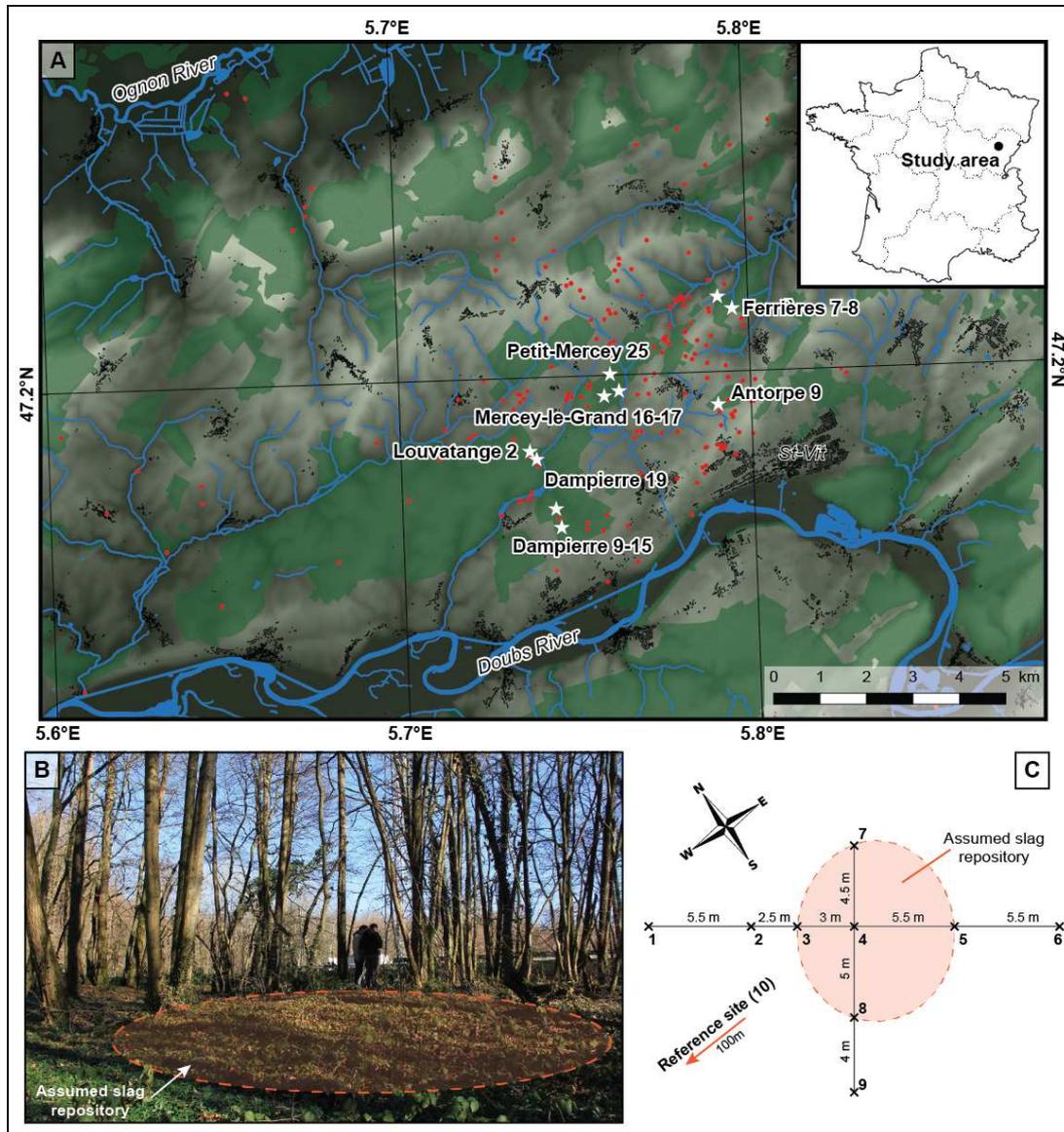
	As	Ca	Cd	Co	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Ref 1	13.3	268.6	<DL	8.3	11.7	16,811.6	1402.7	483.5	12.4	41.4	33.2
Ref 2	26.2	403.7	<DL	16.8	20.3	32,814.5	1390.4	789.2	21.3	48.9	62.9
Ref 3	19.2	335.4	<DL	16.4	15.4	25,574.8	1849.5	1146.5	18.5	28.2	47.6
Mercey-le-Grand 16	21.9	995.7	<DL	14.4	19.7	36,936.9	1663.0	1172.4	20.6	31.0	69.1
Ferrières 7	23.3	775.9	<DL	22.4	19.0	35,746.0	1850.6	1208.2	25.9	26.6	195.7
Dampierre 19	23.1	5461.2	0.6	15.0	21.3	27,745.6	1622.5	1422.5	24.4	29.8	61.2
Ferrières 8	35.4	797.4	0.3	22.5	21.0	40,902.3	1704.1	2088.4	32.3	31.6	135.7
Mercey-le-Grand 17	25.0	573.8	<DL	18.3	25.0	57,745.1	1391.5	2104.3	23.4	30.7	91.6
Petit-Mercey 25	57.5	3807.3	<DL	24.0	29.5	60,975.6	1600.6	2236.7	31.0	40.3	104.1
Louvatange 2	20.0	923.2	0.8	18.1	25.3	38,756.5	1294.2	2602.0	24.6	28.6	78.0
Dampierre 15	9.5	1075.6	<DL	14.3	16.9	32,110.0	1051.2	3165.8	16.9	31.2	70.2
Antorpe 22	30.1	3369.2	0.2	20.4	38.8	82,160.3	2027.5	3207.0	36.4	38.3	121.0
Dampierre 9	16.8	2152.3	<DL	16.4	26.3	52,950.5	1383.0	4709.2	20.3	55.2	87.2
Antorpe 9	51.8	6618.9	2.2	37.9	65.4	137,095.6	1553.7	8575.6	64.8	41.7	244.8
Maximum Permissible Concentration ^a	34.0		1.6	20.0	40.0			450 ^b	30.0	50.0	50.0
Antorpe 9 Slag #1	<DL	12,665.0	3.3	6.9	86.3	149,243.0	3296.6	17,590.5	27.3	11.5	27.2
Antorpe 9 Slag #2	<DL	14,899.2	3.3	10.8	87.4	154,248.9	3759.9	22,070.9	31.2	13.3	36.0
Antorpe 9 Slag #3	<DL	18,485.5	2.9	6.2	76.4	137,505.3	3436.2	11,135.5	25.0	9.0	28.8

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	<i>SiO2</i>	<i>K2O</i>	<i>Na2O</i>	<i>P2O5</i>	<i>MnO</i>	<i>Al2O3</i>	<i>CaO</i>	<i>MgO</i>	<i>TiO2</i>	<i>FeO</i>	<i>Cr2O3</i>	<i>NiO</i>	<i>Total</i>
Glass (n=4)													
mean	40.16	3.12	0.25	1.55	1.69	20.23	9.07	0.02	1.22	22.35	0.01	0.02	99.68
SD	2.68	0.73	0.05	0.20	0.08	2.85	1.46	0.02	0.10	2.30	0.01	0.03	0.21
Fayalite (n=7)													
mean	29.24	0.07	0.01	0.52	4.51	0.59	0.51	1.00	0.13	63.72	0.02	0.01	100.32
SD	0.43	0.11	0.02	0.38	0.88	0.76	0.11	0.60	0.07	1.25	0.02	0.01	0.60
Leucite (n=3)													
mean	55.10	18.65	0.12	0.15	0.14	25.52	0.11	0.02	0.06	2.15	0.01	0.00	102.02
SD	1.85	1.10	0.03	0.18	0.14	0.37	0.12	0.01	0.03	2.31	0.01	0.00	0.50
Spinel (n=4)													
mean	3.01	0.13	0.02	0.02	1.56	49.23	0.08	0.25	2.41	44.60	1.02	0.00	102.32
SD	1.94	0.15	0.03	0.02	0.07	2.57	0.02	0.11	0.85	3.23	0.59	0.01	1.07
Wustite (n=2)													
mean	3.47	0.04	0.02	0.73	0.68	7.65	0.55	0.08	0.59	87.11	0.02	0.01	100.95
SD	2.19	0.01	0.02	0.58	0.06	7.93	0.39	0.02	0.18	5.13	0.01	0.01	1.83
Metallic Fe (n=2)													
mean	0.87	0.03	0.00	1.46	0.13	1.49	0.08	0.03	0.05	125.06	0.08	0.05	129.30
SD	0.39	0.03	0.00	1.91	0.15	2.10	0.10	0.02	0.06	1.89	0.12	0.07	1.54
Fe oxides (n=4)													
mean	2.21	0.01	0.04	0.09	0.05	0.01	0.02	0.02	0.01	87.65	0.01	0.02	90.12
SD	1.11	0.01	0.05	0.18	0.02	0.01	0.01	0.02	0.01	1.66	0.01	0.01	0.79
Fe hydroxides (n=4)													
mean	6.53	0.07	0.09	0.64	0.29	0.55	0.11	0.03	0.00	73.32	0.01	0.03	81.65
SD	4.08	0.04	0.10	0.77	0.23	1.06	0.04	0.03	0.01	2.86	0.01	0.03	1.45

661 **Figure 1.**

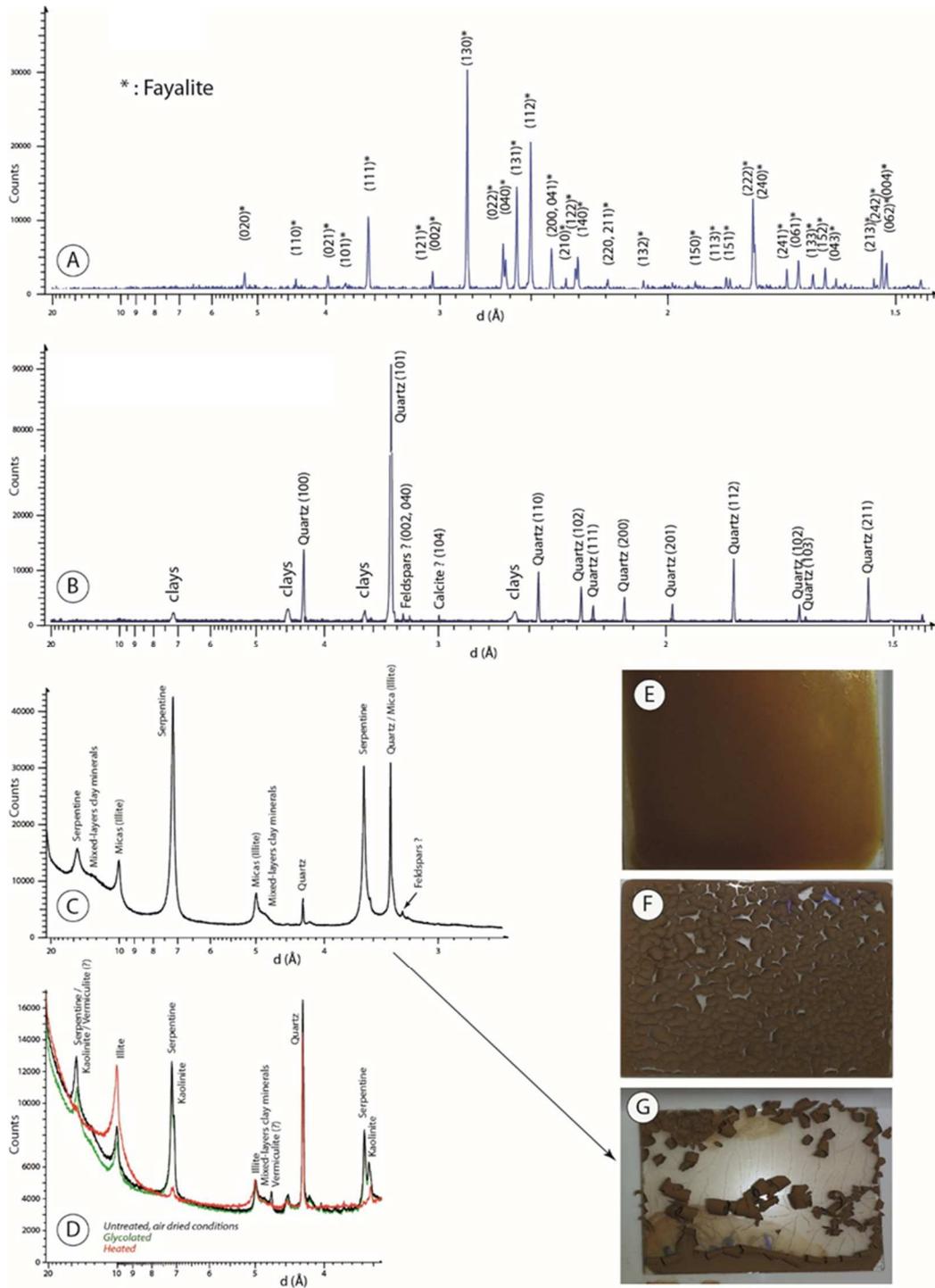
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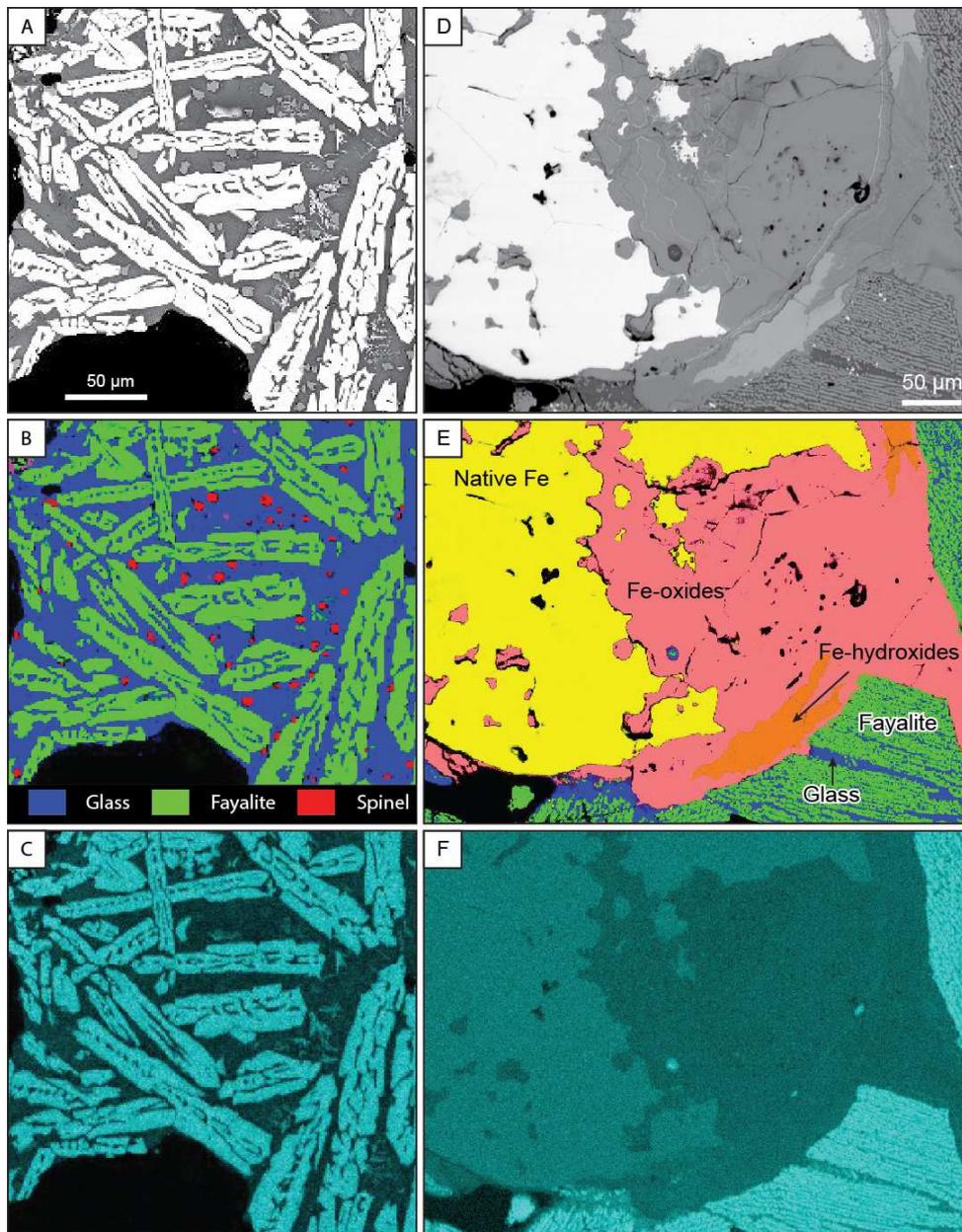
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670 **Figure 3.**

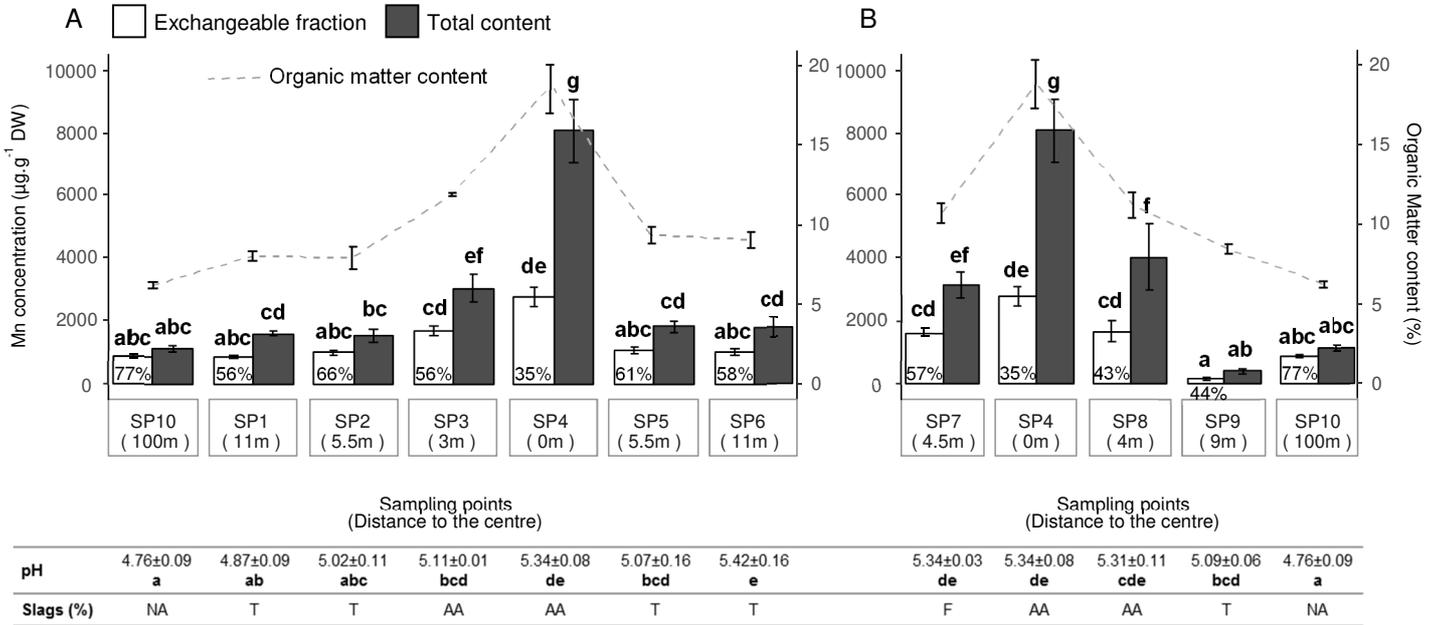
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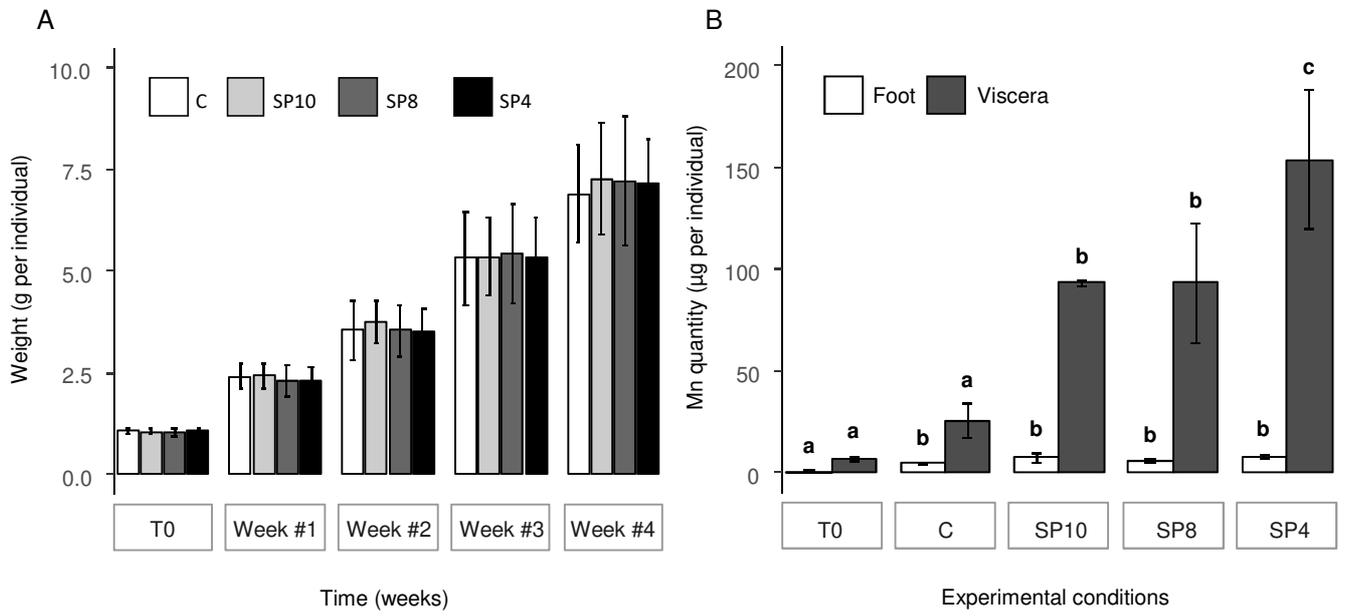


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679 **Figure 5.**



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Graphical Abstract

