

1 **Complementary assessment of As, Cu and Zn environmental availability in a**
2 **stabilised contaminated soil using large-bore column leaching, automatic**
3 **microcolumn extraction and DGT analysis**

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13

14 **Abstract**

15 Soil pollution with trace elements is a concerning issue worldwide. Monitoring of soil
16 pollution and remediation protocols need still from valid complementary analytical
17 approaches able to detect changes in speciation and lability of metals in soils (e.g.
18 stabilization or mobility). In this work, we compare three different analytical approaches
19 to assess potential changes in environmental availability of Cu, As and Zn in a
20 Mediterranean polluted soil that was amended with different combinations of iron
21 sulphate and alkaline paper sludge waste. The studied methods were: (i) a standard large-
22 bore flow-through column system (macrocolumn), (ii) an automatic dynamic flow-
23 through microcolumn extraction system, and (iii) the diffusive gradients in thin gels
24 technique (DGT). The three analytical approaches tested showed immobilization of Zn
25 and Cu in contaminated mine soils after co-application of paper sludge and iron sulphate,
26 but they differ quantitatively in terms of As mobility. Interconversion between oxidation
27 states of inorganic As is observed to occur to a larger extent in macrocolumn. Because
28 this may only occur in very specific Mediterranean scenarios (i.e. organic matter
29 application to intermittently flooded mine soils), macrocolumn extraction procedures
30 might not appropriately mimic the environmental availability of As in soils with organic

31 amendments (e.g., paper sludge waste). Microcolumn leaching is the fastest screening
32 tool to ascertain the efficiency of chemical amendments, but DGT is a good alternative
33 with less technical demands.

34

35 **1. Introduction**

36 Soil pollution is a significant issue in the environmental agenda. According to the
37 EU Commission, more than half a million sites throughout Europe are considered to be
38 contaminated (European Union, 2013). The US and Australian Environmental Protection
39 Agencies tracked over 530,000 and 160,000 contaminated sites respectively (US EPA,
40 2013, EPA Australia, 2016) and more than 200,000 sites in China (Li et al., 2017).

41 Soil pollution has been linked to health diseases, ecosystem dysfunctionality or
42 water contamination, evidencing the importance of evaluating the risk of abnormally high
43 levels of trace elements (TE) in soils. In such evaluation, TE geochemistry in the soil
44 must be understood (Kim et al., 2015; Rosende and Miró, 2013): mobility of
45 contaminants, soil properties, rate of desorption, environmental conditions and other
46 influential parameters are critical to perform a good diagnosis. Nowadays, it is widely
47 accepted that the impact of soil TE cannot be evaluated simply with the total metal
48 concentration, because the effect of anthropogenic compounds on ecological systems and
49 biological organisms largely depends on their environmental availability, that is defined
50 by the ISO 14702:2008 as the fraction of contaminants that by physico-chemically driven
51 desorption processes could be made potentially available to organisms (ISO 17402, 2008;
52 Rivera et al., 2016). This term comprises both an actual available fraction of contaminant
53 and a potentially available fraction that can be released under predetermined changing
54 conditions. In such scheme, many recent remediation studies have demonstrated the
55 efficiency of immobilising TE to manage efficiently contaminated sites, for example,
56 applying soil amendments or recycle wastes to the soil for decreasing the readily available
57 fraction of TE in contaminated sites (Alvarenga et al., 2016; Fresno et al., 2016; Manzano
58 et al., 2017). Overall, there is, however, a need to set validated methods to assess TE
59 environmental availability in soil to support land managers in their decisions not only
60 based in current availability (e.g. single batch extractions) but also in potential
61 availability. These assessments need to be ideally cost-effective, fast and replicable and
62 reflect environmental risks under realistic environmental scenarios.

63 Single batch manual extractions (i.e. CaCl₂, NH₄NO₃, EDTA, etc.) or sequential
64 extraction procedures are standard approaches to assess the environmental availability of
65 TE in soils and solid materials (Abedin et al., 2012; Argyraki et al., 2018; Fedotov and
66 Miró, 2008; Groenenberg et al., 2017; Kim et al., 2015; Rivera et al., 2016). They rely on
67 subjecting a known amount of soil to the action of a given number of leaching reagents
68 aimed at releasing particular metal-soil phase associations into the liquid phase under
69 environmentally simulated conditions. As a result, insight is obtained into several
70 fractions of TE, with the differentiation of more reactive and mobile TE (e.g., soluble and
71 exchangeable pools) against more stable and immobilized TE (e.g., associated to minerals
72 and oxides). Despite these approaches are well accepted in environmental risk
73 assessment, they do not fully include some intrinsic parameters of the risk evaluation for
74 long-term environmental management, such as the dynamics of TE solubility over time
75 and how the most reactive fractions can be replenished. As a result, the overall content of
76 environmental available contaminants associated with a nominal soil fraction might be
77 underestimated (Rosende and Miró, 2013; Tian et al., 2008).

78 To understand the current leachability and the potential resupply of TE to soluble
79 fractions over time the International Standardisation Organisation (2007) endorsed
80 ISO/TS 21268-3:2007, which proposes an harmonized flow-through dynamic (non-
81 equilibrium) extraction method using a large-bore-column design in up-flow mode for
82 continuous renewal of the extracting solution, namely, simulated soil pore water. With
83 this purpose, leaching columns have been used lately in several studies in which the
84 dynamics of TE leaching in contaminated soils, including the kinetics of release of
85 contaminants to extrapolate results in the long term, have been studied (Kossoff et al.,
86 2011; Manzano et al., 2016; Tsang et al., 2014). Water or mild electrolytes are the most
87 frequent extractants although it is possible to change the extracting solutions during the
88 experiment so as to simulate mine-drainage water composition (Gibert et al., 2010; Trois
89 et al., 2007). In TE-laden contaminated soils, the modified NEN 7343 Dutch standard
90 column leaching procedure has been also widely used (Beesley et al., 2010; NEN 7343,
91 1995; Tsiridis et al., 2015) wherein a relatively large amount of soil (usually from 0.1 to
92 1 kg) is introduced into a wide-bore column and connected to a peristaltic pump, alike
93 ISO/TS 21268-3:2007, but acidified water instead of a mild electrolyte solution is used.
94 Finally, the leachate is volumetrically collected and analysed off-line to calculate the TE
95 leached in the time course of the assay. However, the experimental procedure is time

96 consuming (usually lasting from weeks to months) and requires laboratory space and
97 several columns for replication.

98 Another recent approach for leaching tests involve microanalytical column-based
99 flow-through systems. They have been proposed and applied as appealing alternatives to
100 the conventional batchwise counterparts or the large-bore column leaching assessment
101 (Rosende and Miró, 2013). As is the case with the macrocolumn extraction system
102 recommended by ISO/TS 21268-3:2007, fresh portions of leaching reagents are
103 continuously provided to the sample, yet contained in small-scale chambers or mini-
104 columns with the subsequent displacement of the extraction equilibrium to the liquid
105 phase until completion, that is, they operate under worst-case scenarios in order to assure
106 exhaustive extraction conditions as demanded in risk assessment/exposure schemes. In
107 this new dynamic small-scale column extraction approach the amount of soil is reduced
108 to a few grams or hundreds of milligrams and the flow rate of the leaching reagent
109 (usually, 0.5- 3 mL min⁻¹) is increased in comparison with the flow rate of the large-bore
110 column leaching systems, with the consequent reduction of the extraction time from
111 weeks to a few hours. It is worth mentioning that flow-through microextraction methods
112 demonstrated their versatility by resorting to a variety of sample container designs,
113 including stirred flow chambers, cylindrical and dual-conical columns, and different
114 liquid drivers, such as peristaltic pumps, syringe pumps or high pressure pumps,
115 depending on the properties of the sample to be evaluated (Boonjob et al. 2008; Rosende
116 et al. 2011, Rosende and Miró, 2013).

117 Complementarily, the diffusive gels in thin layers (DGT) approach has proven
118 appropriate for the evaluation of the TE lability in soil, which is associated to the concept
119 of environmental availability and (bio)availability, that is, the fraction of contaminant
120 which can be taken up by organisms (Pelfrène et al., 2011a, 2011b). This technique
121 consists of assessing the diffusion of TE through a diffusive gel of known thickness to
122 finally be uptaken by a sorptive material (for more details, see www.dgtresearch.com and
123 Nolan et al. (2005); Song et al. (2018)). Interestingly, when comparing DGT labile TE
124 (in mg L⁻¹) with soluble TE in the soil (e.g. concentration of TE in pore water, in mg L⁻¹)
125 ¹, we can calculate R_{diff} , a parameter that is an indicator of TE resupply to the soluble
126 fraction. This is related to the dynamics of TE mobilisation in soils, but DGT has not been
127 compared to the best of our knowledge to any standard leaching column procedure to
128 date.

129 In this study, a holistic evaluation of how three distinct analytical approaches ((i)
130 up-flow macrocolumn percolation test, (ii) automatic downscaled microcolumn
131 extraction and (iii) DGT analysis) can complement each other in assessing the effect of
132 distinct chemical amendments on the stabilization of an As, Cu, and Zn-contaminated soil
133 is undertaken. Overall, we aim at critically comparing the outcomes from the three
134 methods to obtain relevant environmental information applied to a case study of a
135 contaminated soil after application of different chemical amendments.

136

137 **2. Materials and methods**

138 *2.1. Reagents and solutions*

139 All chemicals were of analytical reagent grade and used without further purification.
140 Ultra-pure water (specific resistivity $\geq 18.2 \text{ M}\Omega\cdot\text{cm}$) obtained from a Milli-Q system
141 (Millipore, Bedford, USA) was employed to prepare all solutions and standards. All
142 glassware and polyethylene containers were previously soaked in 10% (v/v) HNO_3 and
143 rinsed three times with deionized water prior to use.

144 A multi-element standard solution for inductively coupled plasma spectrometry
145 (Multielement standard solution-5, Sigma Aldrich, St. Louis, MO, USA) and a stock
146 standard solution of As (1000 mg mL^{-1}) prepared as per the APHA-AWWA-WPCF
147 recommendations, respectively, were employed for the determination of TE in leachates
148 by external calibration. In other cases, certified standards of As, Cu and Zn were used
149 (Certipur, Merck, Germany). Diluted working solutions were prepared daily in 2% (v/v)
150 HNO_3 .

151

152 *2.2. Soil and amendment preparation*

153 Uncontaminated soil collected from the surrounding of an arsenopyrite mine, so-called
154 Monica mine, in Bustarviejo, NW Madrid, Spain (40.866246, 3.730903) and a heavily
155 contaminated material coming from the dumping tailings of the abovementioned mine
156 were used as samples in this study. In the laboratory, both materials were dried at room
157 temperature, sieved to 4 mm, and mixed in a rate 60:40 (w:w) uncontaminated soil:
158 dumping material to obtain a composite soil sample.

159 A de-inking paper sludge waste (PS), obtained from Holmen Paper (Fuenlabrada, Madrid,
160 Spain), and commercially available FeSO_4 (Sigma-Aldrich,) were used as chemical soil

161 amendments. The treatments were added to the composite soil in four variable amounts
162 in weight, a) 0.75% FeSO₄ and 3% paper sludge (*Fe0.75:PS3*); b) 1% FeSO₄ and 3%
163 paper sludge (*Fe1:PS3*); c) 1.5% FeSO₄ and 3% paper sludge (*Fe1.5:PS3*); and d) 1%
164 FeSO₄ and 1% paper sludge (*Fe1:PS1*). The TE leachability of the control soil and the
165 four amended soils was evaluated using the large-bored column and the small-scale
166 column flow-through extraction methods and the DGT assays in pots.

167 The composite soil and paper sludge amendment were physicochemically
168 characterized by the determination of pH, organic matter, conductivity, soil texture (only
169 for soil), total and (NH₄)₂SO₄-extractable TE or water-soluble TE as listed in Table 1.
170 Soil and paper sludge pH values were measured in a solid to deionised water suspension
171 of 1:2.5 and 1:5, respectively. Suspensions were shaken for 30 mins and left to rest for 10
172 min before measuring the pH in the supernatant with a combined pH electrode. Electrical
173 conductivity (EC) of soils was determined in a soil to deionised water ratio of 1:5,
174 followed by shaking the mixture for 10 min, and analysing the EC after stopping agitation
175 for 10 min using a conductimeter. Organic matter was determined as the dichromate-
176 oxidizable sample fraction. Particle size distribution was determined by the Bouyoucos
177 hydrometer method (MAPA, 1994).

178 For the determination of the pseudo-total concentrations of As, Cu and Zn in the
179 composite soil and paper sludge, the samples were digested using a mixture of
180 HNO₃:H₂O₂ according to Wenzel et al. (2001). Extractable elements in the composite soil
181 were obtained by shaking the soil with 0.1M (NH₄)₂SO₄ in a soil to solution ratio of 1:10
182 for 4 h at 25 °C. The soil suspension was then filtered, and the filtrates were acidified
183 with HNO₃ (0.1% v/v) to preserve samples (Vazquez et al. 2008). The water soluble
184 fraction of the de-inking paper sludge was prepared by shaking the material (dried and
185 sieved to 2 mm) under constant agitation for 24 h at room temperature in a soil-to-liquid
186 ratio of 1:10 (w/v) with deionised water as per DIN 38414-S4 (1984). The leachates were
187 filtered through a membrane filter (0.45 µm). The arsenic in the pseudo total and the
188 water-soluble extracts was determined by atomic fluorescence spectrometry and the
189 metals by flame atomic absorption spectrometry, with the same conditions as reported in
190 section 2.3.

191

192 *2.3.Up-flow macrocolumn percolation test: Instrumentation and procedure*

193 The long-term leaching test is based on the Dutch standardised method (NEN
194 7343, Netherlands Standardisation Institute 1995), but using distilled water as extractant
195 (Hartley et al., 2004) according to DIN 38414-S4 (1984). Columns XK 50/30 (Pharmacia
196 Biotech, GE) consisted of a 30 cm long and 5 cm diameter cylinder of borosilicate acrylic
197 material. The columns were sealed at both ends with two reinforced acetal plastic adapters
198 that contained a nylon mesh (pore size: 10 μm). The upper adapter included a plunger to
199 adjust the effective height of the column. This upper part was connected to a 50 cm long
200 and 1.2 mm ID tube while the lower adapter was connected to a 30 cm long and 1.2 mm
201 ID tube, working as outlet and inlet of the extractant, respectively (see Fig. SM1).

202 The columns were filled with 800 g of the composite soil treatments, sealed with
203 both plungers and subjected to a continuous flow of distilled water maintained by a
204 multichannel peristaltic pump (0.3 mL min^{-1}) from the bottom of the column upwards in
205 order to prevent the formation of channels by gravity. Once columns were saturated with
206 the extractant, nine fractions were collected, with two replicates per treatment. A total of
207 3150 mL of deionised water passed through each column, so as to affix a soil:water ratio
208 of ca. 1:4 (g:mL). A portion of every sample was collected, filtered to $0.45 \mu\text{m}$, acidified
209 with 0.1% (v/v) HNO_3 and stored at 4°C pending analysis. Arsenic in the leaching
210 solutions was determined by atomic fluorescence spectrometry (Millennium Excalibur,
211 PS Analytical), using NaBH_4 in NaOH as a reducing agent and HCl-KI -ascorbic acid as
212 a carrier with analysis of the evolved arsine in an H_2 flame at 197.3 nm. Metal
213 concentration was determined by flame atomic absorption spectrometry (AA800 Perkin
214 Elmer), using the spoiler as nebuliser and a flame of acetylene, with measurements at:
215 213.9 nm for Zn and 324.8 nm for Cu.

216 Several aliquots from the leaching procedure were preserved in 10 mM EDTA to
217 speciate As in the leachates. The speciation was accomplished by HPLC-HG-AFS (HPLC
218 Agilent 1260 Infinity, Agilent, Santa Clara, USA, and HG-AFS PS Analytical 10.055,
219 Millenium Excalibur). To this end, 100 μL aliquots of the filtered extracts were injected
220 into the HPLC system. The isocratic separation was carried out with a Hamilton PRP-
221 X100-10 μm anion-exchange column (Hamilton, Reno, USA). An aqueous solution
222 consisting of 20 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ adjusted to pH 6.25 was used as mobile phase
223 at 1 mL min^{-1} flow rate. Aqueous standard solutions of As(V), As(III), DMA and MMA,
224 prepared by dissolving appropriate amounts of $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, NaAsO_2 ,
225 dimethylarsinic acid (DMA) and monosodium acid methane arsonate sesquihydrate

226 (MMA) (Sigma-Aldrich), were used for external calibration within the concentration
227 range of 0–500 $\mu\text{g L}^{-1}$.

228

229 *2.4. Downscaled microcolumn flow-through extraction: Instrumentation and* 230 *procedure*

231 The miniaturized dynamic flow-through extraction system employed in this work (Fig.
232 SM2) consisted of a micro-sequential injection setup (μSI , FIALab Instruments, Bellevue,
233 WA, USA) equipped with a 3000-step bi-directional syringe pump (SP, Cavro,
234 Sunnyvale, USA) for automatic handling of the extractant, a small-scale extraction
235 microcolumn, and an autosampler collector. The SP was furnished with a 5-mL glass
236 syringe and was equipped with a three-way distribution valve at its head, which allowed
237 connection with the extraction microcolumn or the extractant reservoir. The extraction
238 micro-column was made of light-brownish transparent polysulfone (PSU). It was
239 composed of a central cylindrical sample container with an internal volume of 4.1 mL (23
240 mm long, 15 mm ID) and was equipped with two filter housing caps at both ends. The
241 membrane filters (Fluoropore TM, 25 mm diameter, 1.0 μm pore size, Millipore) were
242 placed on perforated supports that allowed leachates to flow freely yet retaining
243 particulate matter. O-rings and silicone gaskets served for the column components to be
244 screwed tightly to avoid leaking problems (Fig. SM2). An XYZ autosampler (AIM1250,
245 Aim Lab Automation Technologies, Brisbane, Australia) permitted the unsupervised
246 collection of the leachates. The entirely enclosed flow system was assembled using
247 polytetrafluoroethylene (PTFE) connectors, polyetheretherketone (PEEK) ferrules and
248 rigid PTFE tubing of 1.5 mm ID from Omnifit (Cambridge, United Kingdom). All the
249 programmable flow sequences were executed by the FIALab software (FIALab
250 instruments) run under Windows environment (Microsoft, Redmond, WA, USA).

251 Prior to starting of the microcolumn flow-through dynamic extraction test, the
252 miniaturized column was partially filled with 1.0 g of composite soil or the amended soil
253 mix. The SP was thereafter set to aspirate 5 mL of distilled water at 10.0 mL min^{-1} from
254 the reservoir, which were whereupon backward dispensed to the soil containing column
255 at 5.0 mL min^{-1} , thus allowing the leaching of TE to take place. The entirely procedure
256 was repeated twice in order to obtain a 10 mL-extract, which were automatically collected
257 into 12-mL plastic containers from the XYZ autosampler. The above analytical protocol
258 was repeated 25 times, thus totally amounting to 25 subfractions of 10 mL each for a
259 complete set. Each subfraction was preserved in 2% HNO_3 (v/v) to avoid metal

260 hydrolysis, and stored at 4°C. It should be noted that the partially-filled miniaturized
261 column was oriented in upright configuration, as indicated in Fig. SM2, to allow the
262 formation of fluidized bed extraction conditions, which are deemed essential for uniform
263 exposition of the soil particles to the extractant (Hooda et al., 1999; Rosende et al., 2010).
264 The overall content of environmentally available elements was calculated as the sum of
265 the TE released in every individual subfraction. Determination of TE in the leachates of
266 the amended soils and raw material was performed by ICP OES (OPTIMA 5300 DV,
267 Perkin Elmer, MA, USA) equipped with an ultrasonic nebulizer, U-6000AT+. The ICP
268 OES plasma torch operated with an RF power and frequency of 1300 W and 40 MHz,
269 respectively, a coolant argon flow rate of 15 L min⁻¹, an auxiliary argon flow rate of 0.2
270 L min⁻¹, a nebulizer flow rate of 0.5 L min⁻¹ and a carrier flow rate of 1.5 mL/min, with
271 simultaneous recording of analytical signals at 188.979 nm for As, 327.393 nm for Cu,
272 and 206.199 nm for Zn. Finally, the concentration of metals was given as mg kg⁻¹ by based on
273 the concentration in the lechate (mg L⁻¹) and the volume of extractant passed through the column
274 in each fraction (L) and referred to the total amount of soil weighed inside the column (kg).

275

276 *2.5. Pore water and DGT devices: Operational procedures*

277 500 g of the mixture of uncontaminated soil and material from the tailing dumps
278 of the arsenopyrite mine (60:40 w/w) and their respective amendments were transferred
279 to 0.5 L containers and saturated at their 60% water holding capacity (WHC, previously
280 determined as g of water retained in a g of soil subjected to gravity). Rhizon soil samplers
281 (Eijkelkamp, Agrisearch Equipment, The Netherlands) made of a hydrophilic porous
282 poly-ether-sulfone (10 cm long, 2.5 mm diameter) were inserted horizontally into
283 containers covering their sampling area. The system was left to equilibrate for 3 weeks
284 and water losses were replenished during this period. One day prior to pore water
285 sampling and DGT extraction, soils were saturated at 100% WHC. A picture of the
286 experimental setup for DGT analyses is shown in Fig. SM3. Pore water was obtained by
287 subjecting the saturated soil to vacuum and collecting the pore water by the Rhizon
288 samplers. The DGT devices for deployment in soil were composed of by a diffusive gel,
289 a Chelex or ferrihydrite for metals and As-based binding layers, respectively, and a
290 cellulose nitrate filter (0.45 µm). DGT assays were performed in the darkness at a steady
291 temperature of 21 ± 2 °C. Deployment time for the raw soil and the Fe1:PS1 amendment
292 were 3h and 4h, respectively. In the other cases, deployment time was fixed to 8 h using

293 both binding layers. The varied time of deployment is a consequence of the fact that the
294 control and the Fe1:PS1 soils were expected to have more soluble As, Zn and Cu than the
295 other treatments. After deployment, the devices were rinsed with Milli-Q water and
296 disassembled to retrieve the binding layer. Chelex and ferrihydrite binding layers were
297 transferred to tubes containing 1 mL of 1M HNO₃ for 24 hours. After that, 5 mL of Milli-
298 Q water were added to the eluate. All liquid extracts, viz., DGT extracts and pore water,
299 were analysed by ICP-MS. The measurement was performed by ICP-MS (Elan 9000,
300 DRCE, Perkin Elmer, MA, USA) equipped with a Mira Mist nebulizer. The plasma torch
301 operated with an RF power of 1250 W, a coolant argon flow rate of 15 L min⁻¹, an
302 auxiliary argon flow rate of 0.7 L min⁻¹, a nebulizer flow rate of 1.0 L min⁻¹, with
303 simultaneous recording of analytical signals at ⁷⁵As, ⁶⁵Cu and ⁶⁶Zn, using ¹¹⁵In as the
304 internal standard. The DGT data were processed according to DGT research guidelines
305 (Hooda et al., 1999) to obtain the concentration of environmental available TE in the soil
306 materials (C_{DGT} for each metal).

307 *2.6. Data processing and statistical analyses*

308 Data from each assessment were processed with Excel 2016 and SigmaPlot 11.0.
309 The statistical analyses were performed with IBM SPSS Statistics 21. First, all data were
310 checked for normality and homoscedasticity. Secondly, the effect of soil chemical
311 treatment on TE leaching was evaluated by ANOVA and the differences between groups
312 were ascertained using the Tukey's HSD test.

313

314 **3. Results**

315 *3.1. Large-bore column extraction system for dynamic environmental availability* 316 *assays*

317 The leaching of Zn and Cu from the large-bore (macrocolumn) device followed
318 an asymptotic trend, and thus a steady-state value was reached in most cases (Fig. 1).
319 Both metals were leached in greater amounts from the Control while the rest of treatments
320 decreased leaching, including Fe1:PS1, the treatment providing lower pH (Fig SM4),
321 which led to a decrease of around 40% and 65% for the environmental availability of Zn
322 and Cu, respectively. The most efficient treatment is Fe 0.75:PS3, with a 76% reduction
323 in Zn and 93 % reduction in Cu environmental availability. The maintenance of a high

324 pH is known to be effective in immobilising TE (Manzano et al., 2014; Pardo et al., 2014).
325 But, iron sulphides or oxides might also foster the decrease of TE environmental
326 availability observed in Fig. 1. Fe(II) provided by the amendment may have formed iron
327 sulphide precipitates with sulphur from the dumping material, contributing to metal
328 immobilization through co-precipitation or re-adsorption on the secondary sulphide
329 precipitates (Lee, 2006), although our study did not investigate *in situ* Fe mineral
330 formation. Fe(II) must be added with caution because of this might lead to a pH decrease
331 with the potential increase of the environmental availability of TE. This effect has
332 however not been observed in our experimental conditions.

333 As opposed to the metal species, As showed an exponential-like curve of leaching
334 in all of the treatments, which is not what one would expect in standard leaching protocols
335 in a dynamic format because iron oxides should hinder As leaching, although some
336 authors have found increasing leaching patterns of arsenic in column experiments
337 (Khodadoust et al., 2011). In contrast with the rest of treatments, As was leached linearly
338 in the control soil. All amendments reduced the environmental availability of As until
339 around 2000-2500 mL of leachate collected compared to control, but afterwards all
340 treatments with 3% of PS increased the leaching rate of As. In contrast, decreased
341 environmental availability for 1% of Fe(II) and 1% PS was observed independently of
342 the volume of leachate collected, accordingly with the lower pH values of this amendment
343 (Fig SM4), even below the pH of the control treatment. Arsenic is a redox-sensitive
344 metalloid, having two contrasting chemical redox species in soils, As(V), slowly
345 mobilizable, and As(III), rapidly mobilizable. The leaching curves of the amendments
346 (Fig. 1) pointed out that As may have been reduced to As(III) in the time course of the
347 macrocolumn-based extraction. Under moderately reducing conditions As(III), As(V),
348 Fe(II) and Fe(III) species can coexist. To this end, arsenic speciation was assessed in the
349 leaching solutions (Table 2). As(V) was the predominant arsenic species in the control,
350 while As(III) amounted more than 60% in all chemical treatments (last fractions, volume
351 collected > 1500 mL). The combination of organic matter from the PS and the saturated
352 conditions during leaching seems to trigger As reduction that, in turn, causes arsenic
353 mobilisation. This is in good agreement with previous studies by Miretzky and Cirelli
354 (2010). Iron is also intimately linked to arsenite and organic matter in flooding conditions.
355 Depending on the pH, Fe(II) is complexed with organic matter forming bidentate
356 complexes (Catrouillet et al., 2014). In this work, at moderately acid and neutral pH
357 values, Fe(II) could be strongly bound to the carboxylic groups of the organic matter from

358 PS, thus hindering the formation of secondary minerals like goethite or magnetite that
359 might have accounted for the immobilization of arsenic forms. Thus, under the
360 experimental conditions of the large-bore column leaching assay for 3% PS, Fe(II)
361 hydrolysis or oxidation would have been prevented, and the binding of Fe(II) to organic
362 matter facilitated As mobility (Catrouillet et al., 2016). As(III) might be indirectly bound
363 and mobilized as a ternary complex to organic matter via Fe(II) ions, this association with
364 Fe(II) being relatively important in flooded soils, or directly through thiol moieties of the
365 organic matter. Such flood and reducing conditions are however unlikely occurring in the
366 contaminated mine scenario from where the soils were collected.

367

368 *3.2.Small-scale column system for automatic TE environmental availability assays*

369 The most salient feature of miniaturized flow-through leaching tests in automatic
370 flow systems is the high temporal resolution of the leachates collected for appropriate
371 assessment of the kinetics of TE environmental availability. The average cumulative
372 leaching profiles of As, Cu and Zn for the control soil and the different amendments are
373 depicted in Fig. 2. As is the case with the up-flow macrocolumn extraction system for Cu
374 and Zn, the leaching of TE in downscaled systems also follows asymptotic leaching
375 profiles. In fact, 250 mL of water proved to suffice for reaching exhaustive extraction (so-
376 called ‘worst-case extraction scenario’) or a steady regime in which the increase of TE
377 extracted in five consecutive fractions (50 mL) was below 10% (8.9% and 2.5% for Cu
378 and Zn, respectively) of the cumulative leached amounts.

379 Alike the macrocolumn percolation system, the four chemical amendments served
380 to immobilize Zn with a decrease in environmental availability against the control soil by
381 17, 87, 84, and 91 % for Fe1:PS1, Fe0.75:PS3, Fe1.5:PS3, Fe1:PS3, respectively.
382 Treatments with 3% PS afforded statistically equivalent Zn immobilization at the 0.05
383 significance level regardless the content of Fe(II), which is attributed to the increase of
384 the leachate pH up to ca. 6.3 (Fig SM4), occasioned by portlandite and calcite in the PS.
385 Regarding the kinetics of metal stabilization by the various amendments, immediate
386 immobilization of Zn from the very first subfraction (10 mL) is observed for Fe1:PS3, Fe
387 1.5:PS3, Fe 0.75:PS3, which signals a favorable decrease of leachability in the short term.
388 On the other hand, the soil treated with 1% of PS showed a leaching pattern on a par with
389 that of the control soil up to fraction 7 (i.e.,70 mL), which suggested Zn immobilization
390 might occur in the long term, as was also observed for macrocolumns.

391 A different behavior was here observed for Cu. For the three treatments with the
392 higher concentration of PS a minimal immobilization of Cu (only 1.2, 2.1 and 1.6-fold
393 decrease for Fe 0.75:PS3, Fe1:PS3, and Fe1.5:PS3, respectively, against the control soil)
394 was detected. Previous authors reported that the use of Fe oxides for remediation of As
395 contaminated soils might result in the mobilization of other TE because the occurrence of
396 soluble Fe(II), even if small, might participate in cation exchange and displacement
397 reactions of soil containing transition metals (Hartley et al., 2004). All seems to indicate
398 that FeSO₄ may exert an antagonistic effect in the remediation of soil contaminated by
399 Cu. Further, the reduction of PS down to 1% (w:w) in the presence of FeSO₄ (1%),
400 favored the mobilization of Cu from 1.3 ± 0.1 mg/kg (control) to 2.6 ± 0.4 mg/kg. This is
401 explained by the fact that the acidity of the mining soil is not neutralized sufficiently with
402 1% PS, hindering iron oxides precipitation (pH ≤ 5.5 for 1% PS, compared to pH ≤ 6.3
403 of soils with amendments of 3% PS).

404 In the evaluation of As immobilization using the flow-through microcolumn
405 system, an identical leaching behavior was observed for all of the chemical treatments,
406 that is, the environmental availability of As in the control soil is almost equal to that of the
407 amended soils. The addition of PS on acidic mining soils with the purpose of liming seems
408 to have the same effect on microcolumn leaching of As than that of large-bore column
409 (see above). However, the HPLC-AFS speciation analysis of microcolumn leachates (last
410 fractions) indicate that the predominant As form in this case is As(V) (Table 2) with
411 negligible amounts of organic As species. The leaching profiles from microcolumn
412 extraction suggest that the neutralization of acidic soil by PS triggers the solubility of
413 As(V) in water, most likely due to the generation of more negatively charged species,
414 with the subsequent electrostatic repulsions with soil colloids (pK_a arsenate: pK_{a1} = 2.1,
415 pK_{a2} = 6.7, and pK_{a3} = 11.2).

416

417 *3.3. Diffusive Gradients in Thin Gels (DGT) technique*

418 The metal/metalloid DGT assessment (C_{DGT} in mg L⁻¹) gives a proxy of the lability
419 and the availability of species from the soil, while the R_{diff} parameter (as $C_{DGT}/C_{porewater}$
420 for each metal) indicates the rate of TE resupply, which serves to discriminate fully
421 against poorly sustainable cases, the former bearing the higher environmental risk to
422 biota. The control soil showed the largest ZnDGT concentration, but 1% PS and 1% Fe(II)

423 showed increased ZnDGT than those of the three treatments with 3% PS (Fig. 3). R_{diff} for
424 Zn was maximum for the treatments of 3% PS with 0.75 and 1.5% Fe(II), suggesting that
425 the Zn might be mobilized in a labile form in the long term. The treatment with 1% Fe(II)
426 and 3% PS was the most efficient for immobilising Zn (low ZnDGT and low R_{diff} for Zn).
427 AsDGT resembled ZnDGT, but R_{diff} was lower in all the treatments with 3% PS, yet
428 comparable to the control. Copper showed a similar trend than those of Zn and As, with
429 the only difference that maximum CuDGT was observed with 1% Fe(II) and 1% PS.
430 Based on Fig. 3, 1% Fe(II) and 3% PS afforded minimum ZnDGT and AsDGT values
431 and a low R_{diff} for the three contaminants, while 1% PS was deemed insufficient to
432 minimize risk exposure of TE contamination.

433

434 *3.4. Critical comparison between analytical systems for ascertaining TE* 435 *environmental availability in amended soils*

436 Flow-through column extraction systems are common approaches to evaluate the
437 potential leaching of toxic elements from soils and sediments. The macrocolumn
438 experiment is usually regarded as a simple approach to mimic environmental conditions,
439 while the microcolumn counterpart is fast and easily replicable. DGT is being
440 increasingly used to analyse soils, in particular to assess “available fractions”, and is easy
441 to deploy despite the skills required for processing the analytical data (see
442 www.dgtresearch.com and Nolan et al. (2005); Song et al. (2018)).

443 Both column systems evaluated in this work signalled that amendments with 3% PS
444 afforded significant decrease in the environmental availability of Zn and Cu (Table 3).
445 Although slightly higher concentrations of mobile Cu and Zn were observed as the Fe(II)
446 dose increases, comparing the three treatments with 3% PS, no statistically significant
447 differences were found for the environmental availability of Cu and Zn using different
448 concentrations of Fe(II). This increasing trend in mobility as the ratio Fe/PS increases is
449 confirmed by the results for the Fe1:PS1 treatment, that afforded the highest
450 environmental availability for Cu and Zn in both macro- and microcolumn setups, even
451 exceeding that of the control for Cu throughout the microcolumn-based assays. DGT
452 results also corroborated the trend. This fact suggests that Fe1:PS1 is the least suitable
453 amendment to test in real scenarios for short-term TE immobilization, as it afforded the
454 lowest environmental availability reductions (or even increase) for the three approaches.

455 Regarding As, environmental available concentrations were scarcely comparable
456 across column systems. For example, there were no significant differences in As leaching
457 for any of the amendments assayed by microcolumn extraction experiments (in line with
458 the small differences in pH values (in all instances $\text{pH} < 6$) obtained for this setup among
459 treatments), while higher As amounts were leached from macrocolumns with the 3% PS
460 treatments in comparison with the control and Fe1:PS1 treatments, according to the
461 increase of the pH values (above 6) recorded in this experimental setup. Also, only minute
462 amounts of As were leached by macrocolumn setups as compared to the miniaturized
463 counterpart (Table 3). As macrocolumn-based tests operate with significantly lower water
464 to soil ratios, our results could indicate that the solubility of As is thus limited and that
465 the environmental available pools identified by classical large-bore column methods
466 might be underestimated from the point of view of the “worst case scenario” (Thomsen
467 et al., 2012). The lack of As(V) reduction throughout microcolumn tests compared to the
468 macrocolumn counterpart (Table 2) might be attributed to the minimal residence time of
469 the extractant in the soil column per eluate fraction. A water flow of 5 mL min^{-1} across 1
470 g of soil was set for the microcolumn test against 0.3 mL min^{-1} and 800 g of soil for the
471 macrocolumn extraction system, so $>10^4$ times higher extractant flow per unit of soil mass
472 is observed in the case of microcolumn extraction. In previous dynamic experiments, the
473 higher the residence time of the extractant the lower was the desorption rate by ligands
474 for both inorganic arsenic species, viz., arsenate and arsenite (Caporale et al., 2013, 2011;
475 O’Reilly et al., 2001). Arsenic availability according to the DGT method, on the contrary,
476 showed significant reductions for the 3% PS treatments, that resembles macrocolumn
477 behaviour for the fractions below 2000 mL. According to the overall results of the three
478 methods, none of the tested amendments could be clearly recommended for As
479 immobilization in the long term, at least based in a single application.

480 As is the case with arsenic, Cu leaching was significantly reduced in macrocolumn
481 settings as compared to microcolumn designs, probably due to solubility limitations and
482 potential channelling across the packed soil body. This was not observed for Zn and both
483 column systems afforded environmental available concentrations within the same order
484 of magnitude (as well as DGT results), which accounts for the fact that Zn is more readily
485 solubilizable than As and Cu in soils. Our results indicate that the cumulative extracted
486 Zn reaches steady-state regime in both column systems well before Cu, at least for the
487 3%PS treatments (see Fig. 1 and 2). However, it seems that the overall environmental

488 available Cu does not reach equilibrium conditions in macrocolumn settings at
489 concentration values close to those found in microcolumn extractions (Table 3). DGT and
490 microcolumn results for Cu are much more similar.

491

492 **4. Conclusions**

493 The three analytical approaches herein presented are able holistically to assess the
494 extent of immobilisation of Zn and Cu in contaminated acidic mine soils after co-
495 application of paper sludge and iron sulphate. Interconversion between oxidation states
496 of inorganic As is observed to occur to a larger extent in large-bore column settings.
497 Because this may only occur in very specific Mediterranean scenarios (i.e. organic matter
498 application to intermittently flooded mine wastes), macrocolumn extraction procedures
499 might not appropriately mimic the leachability of As(V) in soils with organic amendments
500 (e.g., paper sludge waste). Overall, the automatic small-scale column system is the fastest
501 screening tool to select amendments because the results can be obtained just in a single
502 day as compared to weeks/months in the large-scale column counterparts. On the other
503 hand, recommendations based only on this method can overestimate the real pollution
504 risk. As an alternative, DGT is less demanding in terms of technical equipment and
505 permits a good discrimination across treatments for efficient immobilization of TE based
506 on environmental availability data.

507

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515

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682

683

684 **Figure caption**

685 **Figure 1.** Cumulative extracted (bioaccessible) pools of Zn, As and Cu (in mg kg⁻¹ soil)
686 from a contaminated soil amended with different combinations of Fe(II) (Fe) and paper
687 sludge waste (PS) using a dynamic flow-through large-bore column extraction system.
688 Control was not amended, and chemical treatments consisted of (in %, w:w): 0.75% Fe
689 and 3% PS; 1% Fe and 3% PS; 1.5%Fe and 3%PS; 1% Fe and 1%PS. Results are
690 expressed as Mean ± SE (n=2). For As, the starting points were magnified in the inset
691 (dotted upper-left square).

692 **Figure 2.** Cumulative extracted (bioaccessible) pools of Zn, As and Cu (in mg kg⁻¹ soil)
693 from a contaminated soil amended with different combinations of Fe(II) (Fe) and paper
694 sludge waste (PS) using a dynamic flow-through small-scale column extraction system.
695 Control was not amended, and chemical treatments consisted of (in % w:w): 0.75% Fe
696 and 3% S; 1% Fe and 3% PS; 1.5%Fe and 3%PS; 1% Fe and 1%PS. Results are expressed
697 as Mean ± SE (n=3).

698 **Figure 3.** Zn, As and Cu labile concentration as obtained by DGT (columns, left axis, in
699 mg L⁻¹) and R_{diff} (triangles, right axis, unitless) in contaminated soil amended with
700 different combinations of Fe(II) (Fe) and paper sludge waste (PS). Control was not
701 amended, and chemical treatments consisted of (in % w:w): 0.75% Fe and 3% PS; 1% Fe
702 and 3% PS; 1.5%Fe and 3%PS; 1% Fe and 1%PS. Results are expressed as mean ± SE
703 (n=4).

704