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1 *In situ* assessment of phyto and zooavailability of
2 trace elements: a complementary approach to
3 chemical extraction procedures

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22 Key words: trace element, bioavailability, risk assessment, coupling bioindicators, soil parameters,
23 contamination sources

24 **Abstract**

25 For an accurate risk assessment of sites contaminated by trace elements (TE), measurements of
26 bioavailability must be performed. This is routinely achieved using the standardized 0.01 M CaCl₂
27 method. However, the suitability of chemical extractions as proxies of bioavailability is questionable.
28 We analyzed the correlations between chemically estimated TE bioavailability and TE actually
29 accumulated by coupling plant and snails bioindicators. Results showed a better correlation between
30 plant TE contents and CaCl₂ fraction while total soil concentration better explained snail TE contents.
31 However in both cases chemical measures were not suitable to predict TE accumulation and
32 bioavailability. Considering the soil properties only improve the estimation of Cr, Ni and Pb
33 accumulation by plants while for snails, TE contents in viscera were dependent both on soil and plant
34 contents and soil properties. It highlights the complementarities of biomonitoring methods to assess
35 bioavailability. This dual approach allows a “physiologically defined” evaluation of bioavailability.

36 **1. Introduction**

37 Since a few decades, soil degradation (*e.g.*, erosion, loss of organic matter, sealing, pollution, ...) is an
38 increasing problem worldwide. Contamination by trace elements (TE) is considered as one of the main
39 threats (Jeffery *et al.*, 2010) because of human health issues (Qingdong *et al.*, 2007 and Science
40 Communication Unit, 2013), and also as they may cause severe ecological disturbances to both
41 organisms and their habitats (Moriarty, 1999). For these reasons, various protective thresholds for total
42 contents in soils have been proposed for different TE (Carlon, 2007). However it is largely
43 acknowledge today that the toxicity of TE rather depends on their bioavailability than on their total
44 contents in soils (Van Gestel *et al.*, 2009). Consequently, protective thresholds based on total TE
45 contents in soils are only coarse indications of the potential hazard, and should be completed by
46 methods allowing to assess TE's bioavailability (ISO, 17402, 2008).

47 However, although the term “bioavailability” can be easily understood as how much of a coowever,
48 although the term “bioavailability” can be easily understood as how much of a contaminant is
49 available for living organisms, the underlying concept is much more complex and a great number of
50 definitions, and assessment methods, have been proposed so far (Harmsen, 2007, Naidu *et al.*, 2008
51 and Semple *et al.*, 2004). In an effort to offer a clear working definition of this concept, Semple *et al.*
52 (2004) proposed to distinguish the part of the contaminant which is “bioaccessible” (*i.e.*, “which is
53 available to cross an organism's cellular membrane from the environment, if the organism has access
54 to the chemical”), from that which is actually “bioavailable” (*i.e.*, “which is freely available to cross an
55 organism's cellular membrane from the medium the organism inhabits at a given time”). However this
56 distinction has not been retained in the definition adopted in ISO 17402 (ISO, 17402, 2008) which
57 simply states that “bioavailability is the degree to which chemicals present in the soil may be absorbed
58 or metabolized by human or ecological receptors or are available for interaction with biological
59 systems”. Whatever the terms and definitions considered, there is however a consensus today to regard
60 bioavailability as a multi-level concept involving three distinct notions: the environmental availability,
61 the environmental bioavailability and the toxicological bioavailability (Gimbert *et al.*, 2006, ISO
62 17402, 2008, Lanno *et al.*, 2004 and Peijnenburg *et al.*, 1997).

63 The environmental availability depends on multiple physico-chemical processes governing metal
64 partition between the solid and liquid phases of the soil. Environmental availability is generally
65 assessed as the so-called “available” or “easily exchangeable” TE fractions, using more or less weak
66 chemical extractants, such as neutral salt solutions at low concentration or diluted weak acids. Because
67 these chemical extraction methods are easy to use, reproducible and based on an easily understandable
68 concept (the more the TE are easy to extract, the more they can interfere with living organisms), they
69 are routinely used for risk assessment of contaminated sites. However numerous studies (Meers *et al.*,
70 2007, Pauget *et al.*, 2012, Peakall and Burger, 2003 and Van Gestel, 2008) have shown that the level
71 of “available” elements, as determined by chemical extraction methods, is often a poor proxy of the
72 fraction of TE that actually interacts with living organisms. By contrast, the environmental

73 bioavailability refers to the fraction of contaminant that is actually taken up by biological receptors. It
74 depends on complex species-specific physiological processes, controlling desorption of contaminants
75 from the solid matrix and their assimilation (absorption and excretion) by the organism. To assess the
76 environmental bioavailability of TE in soils, the use of accumulation bioindicators is obviously highly
77 relevant (Peakall and Burger, 2003). Indeed, during the last two decades, plants (Le Guédard *et al.*,
78 2012, Remon *et al.*, 2013 and Vergé *et al.*, 2002) or soil organisms, such as snails or earthworms
79 (Dallinger and Berger, 1992, Gimbert *et al.*, 2008a, Pauget *et al.*, 2013, Rabitsch, 1996 and Scheifler
80 *et al.*, 2003), have been proposed as accumulation bioindicators. However, the transfer of TE and their
81 accumulation by living organisms and in food webs depends both on the species, its trophic level and
82 exposure pathways. It is thus questionable to use a single species or to consider a single trophic level
83 for assessing bioavailability, as this may lead to misinterpretations.

84 In this work we hypothesized that considering different organisms, representative of different
85 trophic levels (primary producers and primary consumers), could be a relevant approach to get
86 information on the environmental bioavailability of TE in soils. Transfer to primary producers was
87 estimated by analyzing TE content in composite plant samples, as recently proposed by Remon *et al.*
88 (2013). This passive biomonitoring approach informs on the phytoavailability of TE for a plant
89 community, considered as a whole. Transfer to primary consumers was assessed by active
90 biomonitoring with garden snails, informing on the zooavailability of TE (Fritsch *et al.*, 2011 and
91 Gimbert *et al.*, 2008a). We also assumed that the transfer of TE from soil to organisms at various
92 spatial scales, could be partly conditioned by their environmental availability or/and by the soil
93 properties. To address these issues, we performed an extensive study on 25 experimental plots (7
94 geographical sites) exemplifying different land use and contamination levels. We analyzed (i) the
95 correlations between environmental availability and environmental bioavailability, as determined by
96 chemical and biological methods, (ii) the influence of soil properties on TE accumulation by snails and
97 plants and (iii) the relationship between TE concentrations in plants and TE accumulation by snails.

98 2. Materials and Methods

99 2.1. Selected sites and studied soils

100 Seven sites (hereafter named Andra, Auzon, GISFI, Metaleurop, RENECOFOR, SHSE and Yvetot,
101 S1) were selected throughout France (Fig. 1), among those studied in the French national research
102 program “Bioindicators 2”; <http://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicateur>. Each site was
103 subdivided into two to six 100 m² (10 m x 10 m) experimental plots, according to the local typology
104 and/or land use. The Auzon (6 plots), Metaleurop (4 plots), SHSE (3 plots) and GISFI (2 plots) sites
105 were industrial landfills or peri-industrial woodlands and grasslands more or less impacted by
106 industrial activities; the RENECOFOR site (4 plots) belonged to a network of French forests
107 (<http://www.onf.fr/renecofor>); the Yvetot site (4 plots) was a cultivated grassland (Plassart *et al.*,
108 2008) and the Andra site presented two plots, one in a forest and one in a grassland. All these sites and
109 experimental plots have been previously described and located in Pérès *et al.* (2011) and in Pauget *et*
110 *al.* (2013).

111 Samples of each plot were taken on a grid (10 m × 10 m) subdivided into 4 sampling-zones (5 m × 5
112 m). In each 25 m² sampling zone, 12 randomized soil samples were taken (0–15 cm depth after
113 removal of the humus) and pooled to characterize soil physico-chemical parameters.

114 Soils from the 25 plots studied were analyzed for their pedological characteristics, as well as for
115 their total and “available” TE contents. All analyses were performed by the Laboratory for Soil
116 Analyses of the National Institute for Agronomic Research (INRA Arras, France), which benefits from
117 the COFRAC (French Accreditation Committee) accreditation n°1-1380 (available at www.cofrac.fr)
118 for its analytical insurance in soil metal(loid) measurements. Briefly, total metals (Cd, Cr, Cu, Ni, Pb
119 and Zn) in soil samples (routinely 250 mg dry soil, ground at < 250 µm) were extracted using
120 hydrofluoric (HF) and perchloric (HClO₄) acids, according to the NF X 31-147 procedure (AFNOR,
121 1996). For the determination of total As, soil samples were extracted with a mixture of sulfuric acid
122 (H₂SO₄)/nitric acid (HNO₃) (2/1, V/V) in the presence of vanadium pentoxide (V₂O₅) at 100 °C for 3

123 h. For the determination of “available” metal(oids), extractions were performed with 0.01 M calcium
124 chloride (CaCl₂), according to the NEN 5704 procedure (NEN 5704, 1996). Metal concentrations in
125 the various extracts were measured using inductively coupled plasma atomic emission spectrometry
126 (ICP-OES). Selected pedological characteristics of the 25 plots studied and their total and CaCl₂
127 extractable TE contents are given in Supplementary Material S2 and S3.

128 2.2. Biological material and sampling protocol

129 2.2.1. Plants

130 To get a general insight into TE transfer toward plants, and to avoid species-specific responses,
131 metal analyses were performed at a plant community level, *i.e.*, using composite plant samples
132 (Remon *et al.*, 2013). The basic assumption of this approach is that TE concentrations in leaves of an
133 ensemble of species colonizing a site, is a relevant indicator of TE phytoavailability in its whole.

134 Composite plant samples were prepared by collecting green leaves (during the months of June to
135 August) from the most abundant species identified at each site, *i.e.*, those species which were
136 representative of the plant community in place. Ten to 15 g fresh weight (FW) of leaves was taken
137 from four to five different species collected in the same area (approx. 5 m²) This elementary sampling
138 was repeated five times in each plot (at each of the corners and in the center), with different species for
139 each replica, when possible.

140 For each elementary sampling, specimens of each species were washed thoroughly with tap water
141 and rinsed with distilled water. They were then dried at 40 °C to constant weight and individually
142 ground up to pass through a 2-mm sieve. A composite sample (“pool”) was then prepared by mixing
143 the same quantity (routinely 100 mg DW) of each species constituting the pool. Five pools of plants
144 were prepared in this way, for each plot studied.

145 2.2.2. Snails

146 Garden snails (*Cantareus aspersus*), were obtained from our laboratory breeding. They were fed
147 with commercial snail meal (Helixal®, Antigny Nutrition S.A., France) and reared under controlled

148 conditions until the age of 7–9 weeks, as described by Gomot-de Vaufleury (Gomot-de Vaufleury,
149 2000). To avoid strong modification of mass during the exposure time that could lead to
150 misinterpretation of internal concentration variations (Gimbert *et al.*, 2008b), only sub-adults weighing
151 5.0 ± 0.6 g ($n = 1230$) were used. At the beginning of the exposure, TE concentrations in the viscera
152 were 0.33 ± 0.11 kg⁻¹ for As DW, 0.73 ± 0.10 mg kg⁻¹ Cd DW, 2.19 ± 0.48 mg kg⁻¹ Cr DW, $139 \pm$
153 40.1 mg kg⁻¹ Cu DW, 0.59 ± 0.26 mg kg⁻¹ Pb DW, and 881 ± 182 mg kg⁻¹ Zn DW (mean \pm SD, $n =$
154 10).

155 For in situ exposure, snails were caged in microcosms (25×25 cm stainless steel cylinders), during
156 a 28-days period (during the months of June to August), as described by Fritsch *et al.* (2011). They
157 were exposed to the soil and vegetation of each plot, under natural climatic conditions, from June to
158 August, in 2009 (for Metaleurop, GISFI, F08 and F57), 2010 (for Auzon, SHSE, Yvetot, F63 and F76)
159 and 2011 (for ANDRA). Fifteen snails were placed in each microcosm. On forest and grassland sites,
160 one microcosm per plot was used and 6 individuals were sampled for TE analysis after 28 days. On
161 sites contaminated by industrial activities, 3 microcosms per plot were used to account for the
162 heterogeneity in soil contamination, and two snails per microcosm (= 6 snails) were sampled for TE
163 analysis after 28 days.

164 2.3. Analyses of trace elements

165 2.3.1. Plants

166 Ground composite samples (“pools”) were dried overnight at 40 °C, before processing. Samples
167 were then weighted (500 mg DW) into clean, dry PTFE (Teflon®) screw cap digestion tubes, and 4 ml
168 concentrated nitric acid (65% HNO₃, analytical grade) were added. Open tubes were heated up to 50
169 °C for 1 h, and kept overnight at room temperature. Three ml hydrogen peroxide (36.5% H₂O₂,
170 analytical grade) were then added and tubes were stand for 30 min at room temperature; they were
171 then heated to 70 °C and allowed to evaporate until about 1 ml. A final aquae-regia digestion was then
172 performed, by adding 2 ml concentrated HNO₃ and 4 ml concentrated hydrogen chloride (37% HCl,

173 analytical grade); the tubes were hermetically closed and heated up to 150 °C for 2 h. Samples were
174 then cooled, tubes were opened, and kept at 70 °C until complete evaporation. Dry samples were
175 finally solubilized in 10 ml HCl (2 M) by heating for 1 h at 100 °C in closed tubes. Solutions were
176 cooled to room temperature and metal concentrations in the extracts were determined by ICP-OES
177 (Jobin-Yvon, Activa).

178 The detection limits for metal analysis by ICP-OES were 0.11, 0.060, 0.013, 0.080, 0.064, 0.341 and
179 0.028 mg/kg DW, for As, Cd, Cr, Cu, Ni, Pb and Zn respectively. The analytical precision was
180 checked by measuring in triplicate about 20% of the samples. The relative standard deviation routinely
181 was between 1 and 8%, and never higher than 10%. For the quality assurance of plant analysis, the
182 certified reference CTA-OTL-1 (Oriental Tobacco Leaves from the Bulgarian Institute for Plant
183 Protection) was employed. Average recoveries (n = 6) were 91, 87, 66, 98, 85, 58, and 79% for As,
184 Cd, Cr, Cu, Ni, Pb and Zn, respectively.

185 2.3.2. Snails

186 The snails sampled were fasted for 48 h (the feces were removed after 24 h) and then weighed and
187 sacrificed by freezing at – 80 °C. After thawing, the whole soft body was removed from the shell and
188 the foot was separated from the viscera. The viscera were studied because they are the main site of
189 metal accumulation in snails (Hopkin, 1989). The viscera were oven-dried at 60 °C until they reached
190 a constant weight (~ 0.2/0.3 g DW), digested in HNO₃ (65% HNO₃, Carlo-Erba analytical quality) as
191 previously described (Pauget *et al.*, 2011) and analyzed by ICP-MS. The validity of the analytical
192 methods was checked by analyzing standard biological reference material (TORT-2, lobster
193 hepatopancreas; National Research Council of Canada-Institute for National Measurement Standard,
194 Ottawa, ON, Canada). The quantification limits for metal analysis by ICP-MS were 0.081, 0.003,
195 0.104, 0.391, 0.065, 0.009 and 0.853 µg/L, for As, Cd, Cr, Cu, Ni, Pb and Zn respectively.

196 2.4. Statistical analyses

197 In a first approach, simple linear regressions were performed to assess the ability of soil chemical
198 extraction methods (*i.e.*, total concentrations and CaCl₂ extraction for “available” concentrations) to
199 predict TE accumulation by plants and snails.

200 Then multiple linear regression (MLR) models were established to better estimate the influence of
201 the soil's characteristics on TE accumulation in plant and in snails (Eq. (1)):

202
$$Y = x*A + y*B + , \dots z \quad (1)$$

203 The dependent variables (Y) were the medians of concentrations (C) of each metal(loid) in the
204 organisms studied, after log (C + 1) transformation. The explanatory variables (A, B, ...) were the
205 main soil's related-parameters, *i.e.*, pH, cation exchange capacity (CEC), organic carbon (C_{org}) content,
206 clay content, sum of exchangeable cations (SEC : Al, Ca, Fe, Mg, Mn, Na.), sands, silts, and total
207 metal concentrations (some parameters are compositional in nature, but some were not (*e.g.*, pH,
208 CEC); thus MLR were used because they allow the specific influence of each soil parameter on the
209 biological response to be taken into account). All values, except pH, were log (a + 1) transformed
210 before data processing and x, y, ... represent the coefficients of the explanatory variables.

211 Lastly, another set of MLR analyses was performed, by only considering the internal metal contents
212 in snails as the dependent variables, and by adding the metal contents in plants as supplementary
213 independent variables. The goal of this last approach was to integrate the trophic level of snails
214 (herbivorous) to better assess metal accumulation in this species.

215 For each set of regressions, the best model (*i.e.*, the one providing the best adjusted coefficient of
216 determination, with the lowest number of independent variables) was chosen using corrected Akaike
217 criterion –AICc– (Burnham and Anderson, 2004). All calculations were performed using the R
218 program (version 2.15.2) (R Development Core Team, 2011).

219 3. Results

220 3.1. Accumulation of TE in plants and snails

221 The main distribution parameters of TE contents in plants and snails sampled on the 25 plots studied
222 are given in Table 1. The median concentrations in Cd, Cu, Pb and Zn were 7- to 21-fold higher in
223 snail viscera than in plant leaves. For Cr and Ni the median concentrations in snails and plants were
224 about the same, while for As the measured concentrations were about 2-fold higher in plants than in
225 snails. However there was a great variability in TE contents, both in plants and snails, as shown by the
226 wide ranges of the data, and the median absolute deviation (MAD) values which were often close to
227 the medians. This was obviously due to the large diversity of the studied sites, in terms of pedological
228 characteristics and contamination levels (see S2 and S3). Consequently, we further examined the data
229 in more details by separating values from the different sites and plots studied.

230 TE contents in plants and snails for each individual plot are given in Fig. 2. Comparison of
231 accumulation profiles allowed to easily distinguishing some plots, where both plant and snail TE
232 contents were distinctly above those measured at the other plots. For instance, at the four plots from
233 the Metaleurop site (RW, LW, IW and IW), the Cd content in plants and snails was clearly higher. On
234 the same site, the Pb content in both types of organisms was also abnormally high for plots LW, IW
235 and HW. Likewise, the three plots (LCV, ICV and HCV) from the industrial SHSE site were
236 associated with higher Cr contents in plants and snails. Lastly at the LCV (SHSE site) and CoWH
237 (Auzon site) plots, the Ni contents were particularly high, both in plants and snails. It must be noticed
238 that the above mentioned sites (*i.e.*, Metaleurop, Auzon and SHSE) were all considered as highly
239 contaminated (see S2); thus high levels of Cd, Pb, Cr and/or Ni in plants and snails reflected the soil
240 contamination. Nevertheless, soil contamination was not systematically associated with high
241 accumulation of TE in living organisms. Indeed at the plots GHM and GHF (GISFI site) both plants
242 and snails showed low TE contents, despite a quite high soil contamination. Taken together these
243 results highlighted that measuring TE contents in plants and snails, could clearly discriminate the plots
244 studied, with respect to phyto- and zoo-availability.

245 However in several cases there were some discrepancies between TE contents in plants and
246 snails. For example, the As content in snails exposed on the CoWW plot (Auzon) was very high, but
247 no anomaly was observed in plant As content on the same plot. Likewise at the two Pa and Fo plots
248 (ANDRA) the Cr content in snails was particularly high, while concentrations measured in plants were
249 quite low. At the opposite plant contents in Cd and Pb were very high at the CoWH (Auzon) and F57
250 (RENECOFOR) plots respectively, while no anomaly in snail contents were evidenced. Thus, on these
251 plots, there was no clear cut relationship between metal contents in plants and snails. This suggested
252 that the levels of metals accumulated in both type of organisms probably depend on complex
253 environmental factors. Consequently, to go a step further in the understanding of metal accumulation
254 in plants and snails, we performed regression analyses taking into account the soil's parameters.

255 3.2. Relationships between TE contents in soil and TE accumulation in plants and snails.

256 A synthetic representation of TE contents in soil (total fractions extracted with HF + HClO₄ and
257 “available” fractions extracted with CaCl₂), in plants and in snails on the 25 plots studied is given in
258 Fig. 3. This representation illustrated a complex pattern where no obvious relationship between TE in
259 soil and in living organisms was distinguished. Consequently in order to better explain the observed
260 TE levels in plants and snails, we first performed simple linear regression analyses and correlation
261 studies using total and “available” TE contents in soil as explanatory variables, respectively. Results
262 are shown in Table 2 and Table 3.

263 When total TE contents in soil were considered as the explanatory variables (Table 2) for
264 accumulation in plants, significant correlations were only evidenced for As, Cr, Ni and Pb. However,
265 the coefficients of correlation were relatively low. The highest value was observed for Cr ($r^2_{\text{adj}} = 0.49$)
266 while for As, Ni and Pb the r^2_{adj} values were comprised between 0.15 and 0.35. For Cd, Cu and Zn no
267 significant correlations between total TE in soil and plant contents were found. In contrast for snails,
268 internal TE concentrations were significantly correlated with total contents in soil for all elements,
269 excepted for Cu (Table 2). The highest coefficient of correlation was observed for As ($r^2_{\text{adj}} = 0.63$), the
270 r^2 values for the other elements ranging between 0.14 and 0.45.

271 When “available” TE contents in soil were considered as the explanatory variables for accumulation
272 in plants and snails (Table 3), quite different results were obtained. For plants, significant, albeit
273 relatively low ($0.23 < r^2_{\text{adj}} < 0.54$), correlations were observed for As, Cd, Ni and Pb, while no
274 significant correlation was evidenced for Cu and Zn. For snails, significant correlations between
275 “available” TE in soil and concentrations in viscera were only found for As and Cd ($r^2_{\text{adj}} = 0.60$ and
276 0.20, respectively); for the other elements studied (Cr, Cu, Ni, Pb and Zn) no significant correlations
277 were observed.

278 3.3. Influence of soil properties on TE accumulation by plants and snails

279 Because simple regression models solely based on TE contents in soil poorly predicted the observed
280 TE contents in plants and snails, we further performed MLR by adding the main soil characteristics as
281 supplementary explanatory variables. Results are given in Table 4.

282 For plants, the addition of the soil parameters in the regression models greatly improved the
283 estimation of Cr and Ni accumulation. The main variables influencing the accumulation of these
284 metals were the SEC for Cr, the pH for Pb and pH coupled with silt content for Ni. On the other hand
285 for As multiple regression model did not improve the estimation of accumulation in plants, as
286 compared with simple models (using either total or “available” contents in soil). Lastly, for Cd, Cu
287 and Zn no statistically significant regression was found, suggesting that accumulation of these metals
288 in plants was not dependent on the soil parameters for the set of plot studied.

289 For snails, adding the soil characteristics in the regression models improved the assessment of metal
290 accumulation in viscera for Cd, Cr, Ni and Zn. This increased the adjusted coefficients of correlation
291 of approx. 10%, 22%, 12% and 10% for Cd, Cr, Ni, Zn respectively. Beside total element contents in
292 soils, the main parameters that modulated metal accumulation were the SEC (for Ni and Zn), the
293 organic carbon content (for Cd) and the pH and CaCO_3 content (for Cr). On the other hand, for As and
294 Pb no significant influence of the soil parameters was observed. Lastly for Cu, no significant model
295 was found.

296 3.4. Influence of plants on TE accumulation by snails

297 In order to assess the impact of the different potential sources of contamination (*i.e.*, plants and
298 soils) on snails' TE contents, we performed a last set of simple and multiple linear regressions. Firstly,
299 TE contents in snails were assessed with simple regression models, using plants contents as the sole
300 source of contamination. Secondly, we performed multiple regressions using TE concentrations in
301 plants and in soils, as explanatory variables. Lastly, TE contents in snails were estimated taking into
302 accounts both contamination sources and soil parameters. Results are shown in Table 5.

303 Simple regression analyses using plant TE contents as explanatory variables showed significant
304 correlations with TE in snails' viscera for all the elements but Cu, with adjusted correlation
305 coefficients ranging from 0.21 for Cr to 0.56 for Pb. Considering both sources of contamination (TE
306 contents in soil and in plants) did not improve the simple regression models, except for Cd where both
307 soil and plant TE content were significantly correlated with internal contents in snails. For Ni, Pb and
308 Zn, the plant contents were the best predictors of snail's contents, while for As it was the soil total
309 content that gave the best r^2_{adj} . For Cr, considering either the soil or the plants as explanatory variable
310 gave comparable r^2_{adj} .

311 Lastly using more complex multiple regression models, taking into account the two sources of
312 contamination plus the soil parameters (Table 5), only improved the assessment of Cd, Cr and Pb
313 accumulation. For Cd, both soil content, plant content and soil organic carbon significantly influenced
314 the concentration in snail's viscera. For Cr, the observed increased in the r^2_{adj} was only due to the soil's
315 parameters (pH and CaCO_3). For Pb, the concentration in snail's viscera was significantly influenced
316 by plant content and soil parameters (*i.e.*, pH) but not by total content in soil. For the other TE (As,
317 Cu, Ni and Zn), complex multiple regression models did not improve the r^2_{adj} values, as compared with
318 those using only soil or plant TE contents as explanatory variables.

319 **4. Discussion**

320 Although separating bioavailability into three operational insights (*i.e.*, environmental availability,
321 environmental bioavailability and toxicological availability) allows to clarify the concept and to
322 propose various measurement methods, the underlying physical, chemical and biological factors that
323 ultimately govern the transfer of TE from a contaminated habitat to living organisms are still poorly
324 understood. In particular the question remains to know the relationships between environmental
325 availability and environmental bioavailability and, ultimately, how these descriptors should be handled
326 in a view of environmental risk assessment. In this work, we studied the correlations between TE
327 extracted by standardized chemical methods and those actually accumulated in two model organisms
328 (plants and snails). We also investigated the influence of soil properties and contamination source on
329 TE bioaccumulation.

330 4.1. Standardized chemical methods for TE extraction from soils poorly predict TE accumulation
331 in plants and snails

332 Classically, two different points of view can be considered for TE analysis in soil, with the aim of
333 risk assessment for ecological receptors. The default assumption is that the totality of TE present in the
334 soil could pose a risk at a short-, medium- or long-term scale, *i.e.*, that availability could be 100%. A
335 less conservative assumption is that only part of total TE is actually bioavailable and thus poses a risk
336 to living organisms. From these points of view, a number of total and partial extraction schemes have
337 been designed for soil analysis in risk assessment procedures. Among the most commonly used
338 methods, are the standardized HF-HClO₄ mineralization for total TE (AFNOR, 1996), and the 0.01 M
339 CaCl₂ extraction, for “available” TE (NEN 5704, 1996). These are the methods we retained in this
340 work.

341 Our correlation studies showed that the total TE contents in soils (HF-HClO₄ extraction) were
342 poorly correlated to the concentrations of TE measured in plants. Indeed on average, only 18% of the
343 variability in plant TE contents was explained by the total contents in soils. There were however
344 noticeable differences between elements, with correlation coefficients ranging from 0 for Cd, Cu and

345 Zn, to > 0.3 for Cr and Pb. To consider “available” TE contents in soils (CaCl₂ extraction) was clearly
346 a better proxy for Cd ($r^2_{\text{adj}} = 0.3$) and, in a lesser extent, for Ni and Pb. This is in accordance with the
347 basic assumption that plants take up TE from the soil solution (Degryse *et al.*, 2009 and Smethurst,
348 2000), and that CaCl₂ extractions better reflect this soil compartment than do total extractions (Kabata-
349 Pendias, 2004, Meers *et al.*, 2007 and Römken *et al.*, 2009). However CaCl₂ extractions still did not
350 detect any correlation between soil and plant contents for Cu and Zn, while for As, plant contents
351 remained better explained by total than by “available” TE in soils.

352 For snails, about 29% of the variability of TE contents in viscera was explained by total
353 concentrations in soil; at the opposite, “available” TE explained only 11% of the variability of
354 viscera's contents. The greater influence of soil total TE content, as compared to “available”
355 concentrations, on snail's internal TE levels could be partly due to its foraging mode which multiplies
356 the sources of exposure. Indeed, in addition to their plant-based diet, snails also ingest significant
357 amounts of soil particles to satisfy their physiological needs (Gomot *et al.*, 1989). This could allow
358 snails to pick up quite strongly bounded TE on soil constituents (Pauget *et al.*, 2012). Thus, as
359 previously shown for earthworms (van Gestel, 2008), total soil concentrations are a better indicator of
360 the amounts of TE that are available for snails, than the CaCl₂ extractable pools. However, as observed
361 for plants, there were clear cut differences between elements, and Cu, Ni and Zn concentrations in
362 viscera were not, or slightly ($r^2 < 0.15$), correlated with soil contents.

363 It is interesting to note that, whatever the extraction method used and the organism considered, there
364 was no, or very low, correlations between the content in soils and the content in living organisms, for
365 those elements that are essential for nutrition (*i.e.*, Cu, Ni and Zn). This is likely due to the complex
366 biological processes regulating homeostasis of essential elements, allowing living organisms to
367 maintain almost stable internal levels under a certain range of external concentrations (Bargagli, 1998
368 and Menta and Parisi, 2001). Thus, it is only when homeostasis can no be longer controlled that the
369 internal levels of essential elements will rise significantly. It is obvious that chemical extraction
370 methods cannot take into account such biological response, making these methods poor proxies for

371 assessing environmental bioavailability of essential elements. At the opposite, for non essential TE
372 (As, Cd, Cr, Pb) whose concentrations in living organisms are not under strict homeostatic control,
373 correlations between contents in soil and in organisms were better, but were clearly dependent on the
374 fraction of TE (total vs “available” fraction) and the organism considered (plants vs snails).

375 Thus, as already emphasized by a number of authors for different types of soil, contamination levels
376 and living organisms (Lopes *et al.*, 2012, McLaughlin *et al.*, 2000, Mourier *et al.*, 2011, Murphy *et al.*,
377 2000, Remon *et al.*, 2005 and Van Gestel, 2008), these results confirm that neither total soil
378 concentrations nor “available” concentrations give enough information to foresee the actual
379 environmental bioavailability of TE on contaminated sites.

380 4.2. Factors modulating TE accumulation are different for plant and snails

381 It is well known that the speciation of TE in soils depends on the soil's characteristics such as pH,
382 redox potential, CEC or organic matter content and quality (Park *et al.*, 2011, Unamuno *et al.*, 2009
383 and Zeng *et al.*, 2011). Thus, we hypothesized that beside total or “available” TE contents in soils, as
384 determined by chemical extraction methods, the accumulation of TE in plants and snails could be
385 partly correlated to some soil parameters. Moreover, because snails are mostly herbivorous, we
386 postulated that TE accumulation in viscera could be closely linked to plant contamination.

387 When plants were considered, MLR models indeed improved the assessment of Cr and Ni
388 accumulation, and highlighted that soil factors such as pH, carbonates or total content of exchangeable
389 cations may play a significant role in the phytoavailability of TE. However for the other TE studied
390 (As, Cd, Cu, Pb and Zn) the MLR did not give better correlation coefficients than simpler models
391 using only “available” soil contents as explanatory variable. At the opposite for snails, considering the
392 soil parameters and/or plant contents in the regression models improved the assessment of all TE, but
393 As and Cu. As a rule, TE contents in snail viscera were mostly correlated to plant contents, except for
394 Cu. This is in agreement with the results of Scheifler *et al.* (2006) who showed that up to 90% of Cd
395 accumulated by snails came from plants. Internal TE contents in snails were also correlated to total
396 contents in soil (As, Cd, Cr) and/or to organic carbon (Cd), pH (Cr, Pb) and carbonates (Cr).

397 The aim of this work was not to elucidate the physicochemical drivers of TE accumulation in plant
398 and snails, but rather to compare the main parameters explaining variations in TE contents in both
399 organisms. Our results showed that TE contents in plants and snails were depending on very different
400 parameters. These differences are obviously due to the different exposition pathways, and finally to
401 the specific physiology of each type of organism. In fact, because of the development of their root
402 system, plants can accumulate TE from deeper soil layers than do snails, which are only exposed to
403 the topsoil (Berger and Dallinger, 1993). Moreover, the activity of plant roots and associated
404 microorganisms may trigger localized soil modifications, leading to a significant increase or decrease
405 of pH, redox potential and/or organic matter content (Ehrenfeld, 2013). Thus, these so-called
406 rhizosphere processes, can considerably modify the speciation of TE (Kabata-Pendias, 2004) and their
407 availability for plants; at the opposite, snails do not significantly modify soil properties and have very
408 likely no effect on TE speciation (Coourdassier *et al.*, 2007). Also, the fate of TE in plants and snails is
409 very different, in that snails are able to excrete some accumulated TE (Gimbert *et al.*, 2008b), while in
410 plants they are stored in the roots or above ground tissues but are rarely excreted (Weis and Weis,
411 2004). Lastly a major difference between these biological receptors lies in their source of
412 contamination. Indeed plants are mainly exposed to TE via the soil, whereas snails are exposed to both
413 soil and contaminated plants. Our results indeed confirmed that both sources of contamination had a
414 significant influence on snail's TE content.

415 4.3. Implications in risk assessment

416 It is widely admitted today that assessing and managing polluted soils on the sole basis of their total
417 contaminant concentrations (IEM, 2007, ISO 17402, 2008 and US EPA, 2007) may lead to inaccurate
418 conclusions and decisions (Brand *et al.*, 2013, Mourier *et al.*, 2011 and Van Gestel, 2008), resulting in
419 misestimating the actual risk for biological receptors. Thus considering the bioavailability of
420 contaminants could be a more relevant approach, as this integrates all aspects modulating their transfer
421 to living organisms (Fig. 4) (van Gestel, 2008). Given this, it is now argued that integrating the
422 concept of bioavailability in risk assessment methodologies is a necessity (ISO, 17402, 2008). In this

423 objective the use of CaCl₂ extraction is usually presented as a good chemical proxy for bioavailability.
424 However in this study we showed that such a chemical extraction poorly estimated TE accumulation
425 in plants and snails in large scale in situ experiments, *i.e.*, considering numerous elements,
426 contamination levels and soil types. This confirms the conclusions of several authors (Feng *et al.*,
427 2005 and Van Gestel, 2008) who emphasized the limitation of the CaCl₂ method for the prediction of
428 TE bioavailability in some field situations. To fill this gap a number of alternative weak extraction
429 procedures have been proposed so far, each focusing on particular elements, soil types or biological
430 receptors (Mourier *et al.*, 2011 and Smith *et al.*, 2010). This underlines the difficulty to find a
431 universal chemical extractant that could predict TE bioavailability. In fact, whatever the extractant
432 used, one of the major limitations of the “chemical extraction” approach to assess bioavailability, is
433 that it intrinsically considers the soil as the sole source of exposure. This may be a correct assumption
434 for plants, but this is obviously not true for numerous other organisms whose nutrition does not
435 exclusively depend on soil. For instance in snails, we showed that considering various exposition
436 pathways according to the trophic level of the target organism, strongly improved the prediction of
437 accumulation of several TE in viscera. This result highlights the importance of taking into account all
438 contamination sources, and not only the total or “available” TE contents in soil, when assessing the
439 bioavailability of contaminants on a given site. Consequently, although chemical extraction methods
440 are routinely used with the underlying idea that they can give a general insight into metal
441 bioavailability, they actually only inform about the easiness of metal leaching from soil by chemical
442 weak extractions, which is a measure of the environmental availability and not of the actual
443 bioavailability. So, owing that the accumulation of TE in living organisms depends on both the metal
444 speciation in soil and the physiological traits of the target organisms, the study of bioindicators could
445 be a relevant complement to chemical procedures, to get insights into TE bioavailability on
446 contaminated sites (Reeves and Chaney, 2008).

447 In this study we considered two types of bioindicators (plants and snails), representative of two
448 trophic levels. Indeed we assumed that coupling biological measures, by permitting the differences

449 between organisms to be taken into account, should allow a more accurate assessment of TE
450 bioavailability and a better site characterization. For bioindication using plants, the “composite
451 sample” strategy (Remon *et al.*, 2013) makes easy the prospection of large areas and allows to
452 overcome interspecific variations. Consequently, such a passive bioindication approach informs on the
453 global phytoavailability of TE and their potential transfer toward primary consumers. On the other
454 hand, bioindication using snails gives information about TE transfer to a specific link of the food chain
455 integrating multiple contamination sources (*i.e.*, soil and plants) and being a prey for a number of
456 carnivorous species (Barker, 2004 and Scheifler *et al.*, 2006). The snail-watch approach permits
457 accurate between-sites comparisons of TE bioavailability (Beeby and Richmond, 2003) as it uses
458 laboratory-born and calibrated animals. This active bioindication strategy informs on a species-
459 specific zooavailability of TE, allowing their potential transfer toward higher levels of the trophic
460 webs to be partly anticipated.

461 However, even though both bioindication strategies have many advantages, they also pose some
462 questions or present some limits (Varrault and Bermond, 2011). For instance, because of the potential
463 adaptation of plants to contaminated soils (Schipper *et al.*, 2011), TE phytoavailability on a given site
464 may change if plant communities are modified. Likewise, because snails only inform on the
465 bioavailability of TE inside the small area of the microcosms, the study of large and heterogeneous
466 sites may be relatively time consuming. Lastly it must be kept in mind that bioavailability of TE
467 cannot be quantified by a single value, as it closely depends on the organism of interest. Nevertheless,
468 accumulation bioindicators provide specific information about the extent of actual TE transfer toward
469 living organisms; consequently they allow a “physiologically defined” approach (Peijnenburg *et al.*,
470 2007) for measuring bioavailability. Thus, together with the classical chemical methods for assessing
471 environmental availability, actual uptake studies could be used as a guideline to assess the
472 environmental bioavailability of TE. These dual chemical and biological approaches could constitute a
473 more accurate way for raising (or decreasing) the alarm on soil quality for environmental risk
474 assessment.

475 ASSOCIATED CONTENT

476 **Supporting Information:**

477 S 1: Main pedological characteristics of the 25 plots from the 7 sites studied (median values, n=5)

478 S 2: Total (HF + HClO₄ extraction) and “available” (CaCl₂ extraction) TE contents at the 25 plots
479 from the 7 sites studied (median values, n=5). NA: no available data. *Italic values correspond to value*
480 *below the detection limit*

481 S 3: Principal plant species on the studied plots.

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489 ABBREVIATIONS

490 CEC, cation exchange capacity; MAD, median absolute deviation; MLR, multiple linear regression;
491 OM, organic matter; TE, trace element; SEC, sum of exchangeable cation

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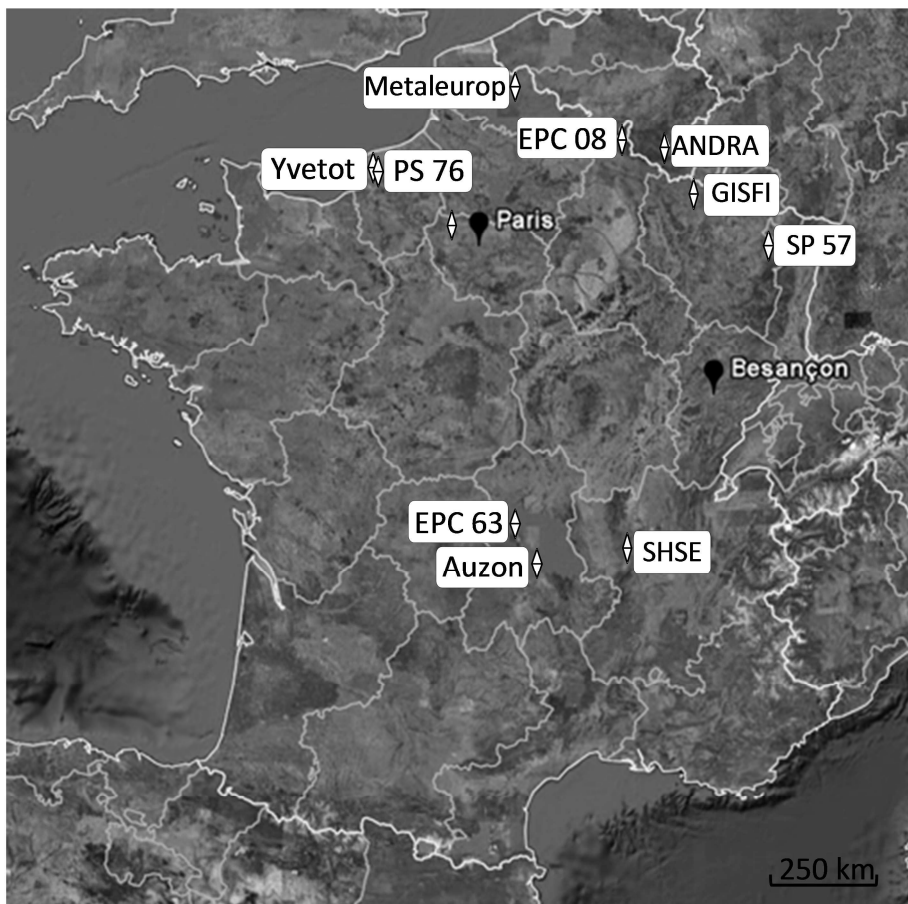
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- 670

672 FIGURES
673



674

675 Figure 1: Sites localization. <http://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicateur/>
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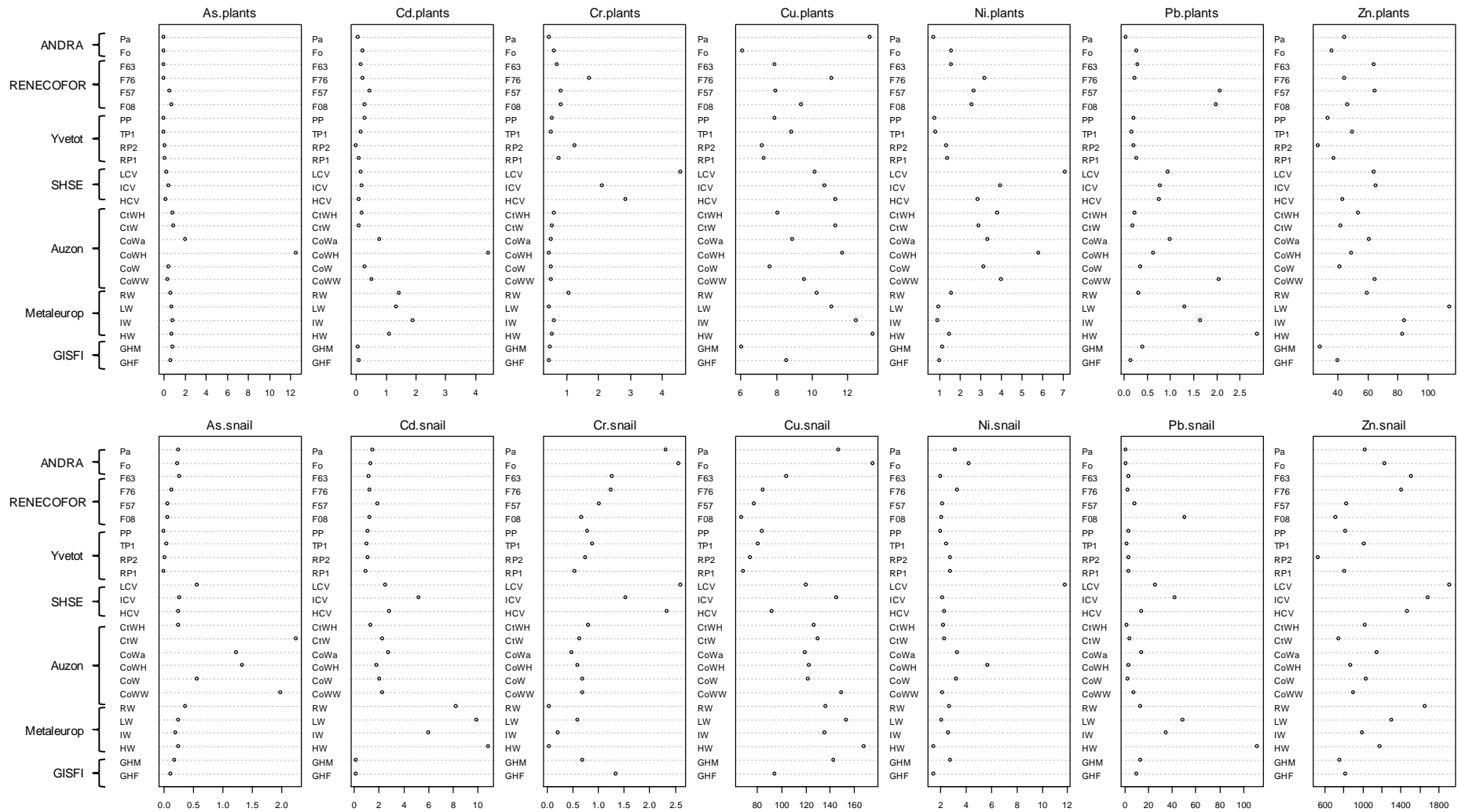


Figure 2: TE contents (mg kg⁻¹) in plants (up) and snails (down) sampled on 25 plots from 7 sites (median values, n= 5 for plants, n= 6 for snails).

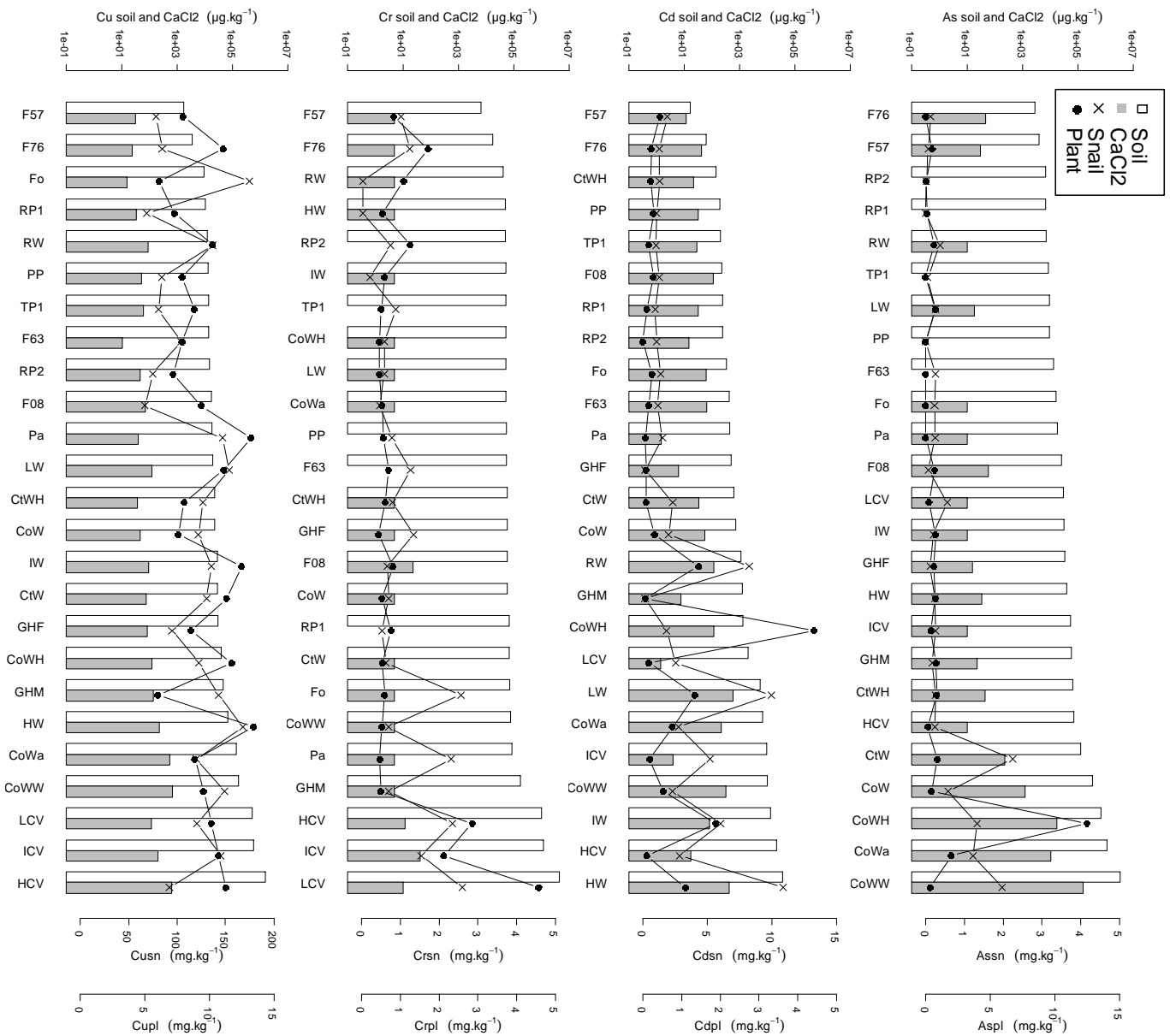


Figure 3 : TE contents in soil (total and CaCl₂ extractable fractions), in plants and in snails from 25 experimental plots (n= 12 for soils, n= 5 for plants, n= 6 for snails).

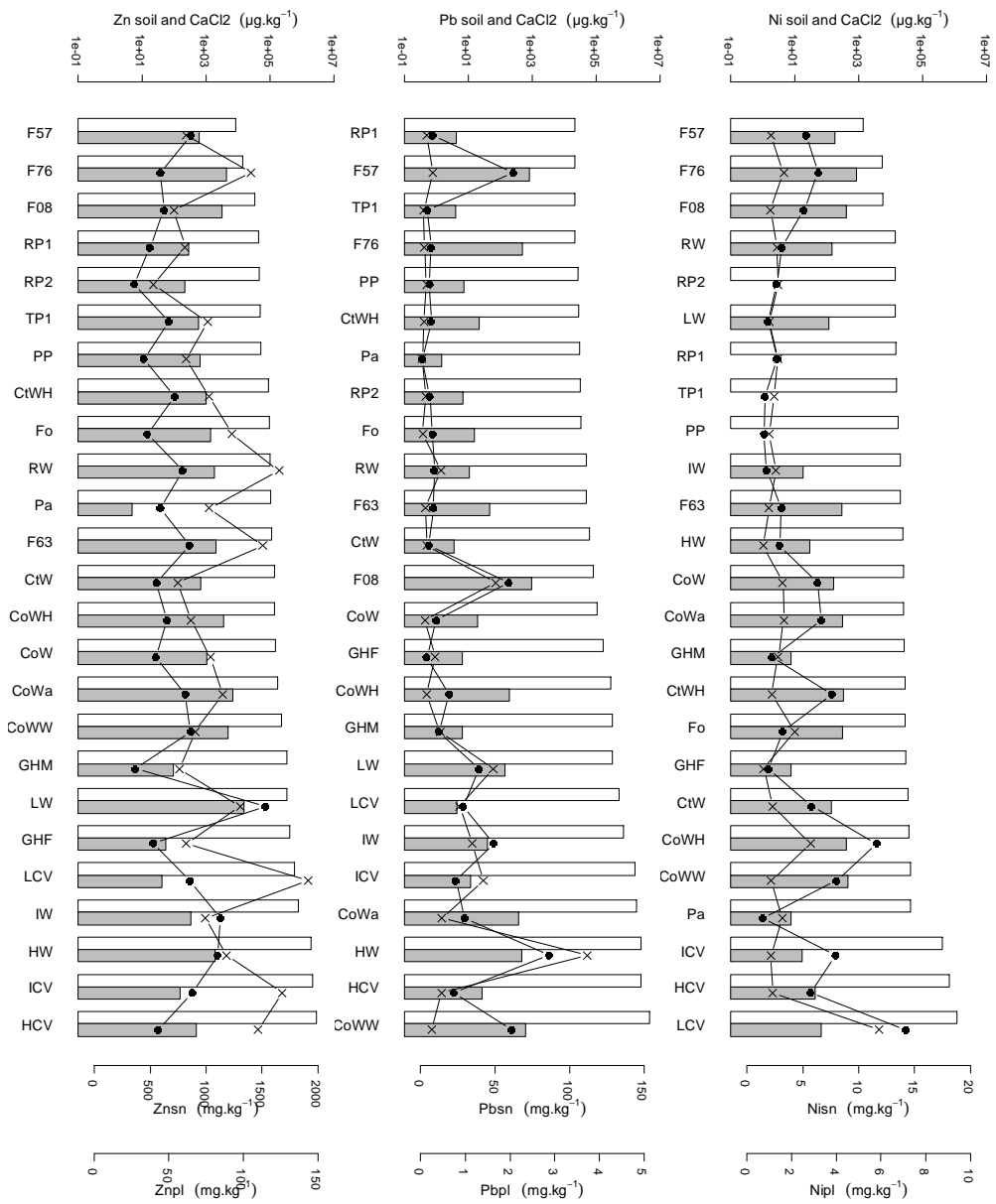
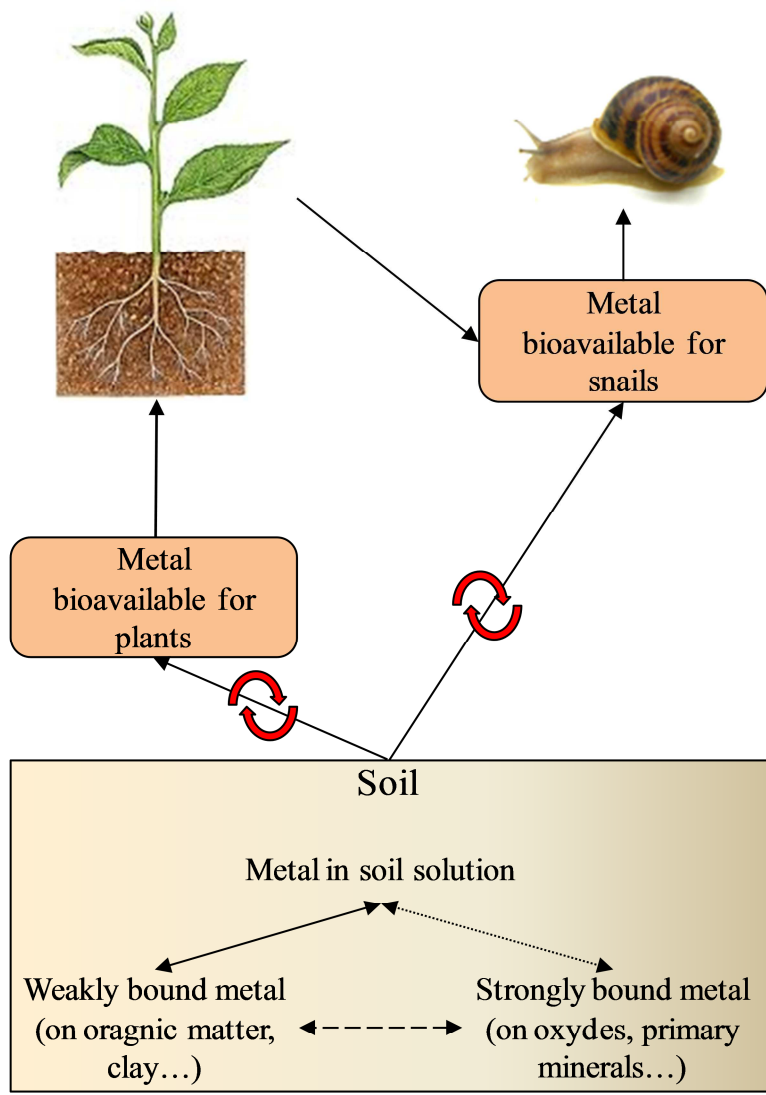


Figure 3: (continued)




 Influence of soil properties

Figure 4: Synthetic schema of metal mobility and transfer from soil to organisms

TABLES

Table 1: Main distribution parameters of TE contents (mg kg⁻¹ DW) in plants, snails and soil (total and CaCl₂) sampled on 25 plots from 7 sites

Metal		As	Cd	Cr	Cu	Ni	Pb	Zn
Plants (n=125)	Median	0.519	0.233	0.644	9.20	1.75	0.395	50.4
	Min	0.000	0.000	0.250	4.98	0.359	0.000	17.9
	Max	28.6	7.42	165	25.6	10.4	6.24	193
	MAD	0.609	0.233	0.264	2.78	1.22	0.374	22.8
Snails (n=150)	Median	0.227	1.65	0.839	121	2.57	5.26	1072
	Min	0.001	0.142	0.030	22.9	0.033	0.689	391
	Max	11.1	33.0	20.0	222	33.4	313	2422
	MAD	0.237	1.01	0.532	44.4	1.11	4.91	381
Soil concentration (Total, n=25)	Median	30.35	0.612	56.8	22.8	25.7	78.9	138
	Min	2.905	0.0165	6.55	1.73	1.4	21.045	8.61
	Max	3285	34.4	4345	1555	1180	4575	2830
	MAD	34.7	0.709	12.2	15.6	15.8	85.8	135
Soil concentration (CaCl ₂ , n=25)	Median	26.925	33.6	5	76	150.5	19	689
	Min	10	1.415	5	10.65	7.5	1.5	5
	Max	155050	578	41.9	681	847	924	15100
	MAD	25.1	43.1	0	61.8	212	22.2	940

Table 2: Simple linear regression analysis and correlation study between TE contents in plants or in snails, and total TE in soil (extracted with HF + HClO₄). (n= 25)

Element	Organism	Regression equation	r ² _{adj}	p-value
As	Plants	As _{pl} = -0.081 ^{ns} + 0.170 As _{tot} **	0.27	0.005
	Snails	As _{sn} = -0.108* + 0.153 As _{tot} ***	0.63	<0.001
Cd	Plants	Cd _{pl} = ns	--	--
	Snails	Cd _{sn} = 0.324*** + 0.395 Cd _{tot} ***	0.45	<0.001
Cr	Plants	Cr _{pl} = -0.095 ^{ns} + 0.190 Cr _{tot} ***	0.49	<0.001
	Snails	Cr _{sn} = 0.019 ^{ns} + 0.135 Cr _{tot} **	0.23	0.009
Cu	Plants	Cu _{pl} = ns	--	--
	Snails	Cu _{sn} = ns	--	--
Ni	Plants	Ni _{pl} = 0.285** + 0.140 Ni _{tot} *	0.15	0.032
	Snails	Ni _{sn} = 0.417*** + 0.106 Ni _{tot} *	0.15	0.031
Pb	Plants	Pb _{pl} = -0.070 ^{ns} + 0.132 Pb _{tot} **	0.35	0.001
	Snails	Pb _{sn} = 0.104 ^{ns} + 0.408 Pb _{tot} ***	0.42	<0.001
Zn	Plants	Zn _{pl} = ns	--	--
	Snails	Zn _{sn} = 2.821*** + 0.091 Zn _{tot} *	0.14	0.037

Symbols for p-values in the regression equations are: ^o<0.1, *<0.05, **<0.01, ***<0.001

Table 3: Simple linear regression analysis and correlation study between TE contents in plants or in snails, and “available” TE in soil (extracted with 0.01 M CaCl₂). (n= 25)

Element	Organism	Regression equation	r ² _{adj}	p-value
As	Plants	As _{pl} = 0.034 ^{ns} + 0.103 As _{CaCl2}	0.24	0.016
	Snails	As _{sn} = -0.017 ^{ns} + 0.093 As _{CaCl2} ***	0.60	<0.001
Cd	Plants	Cd _{pl} = -0.097 ^{ns} + 0.160 Cd _{CaCl2}	0.32	0.001
	Snails	Cd _{sn} = 0.178 ^{ns} + 0.201 Cd _{CaCl2} *	0.20	0.013
Cr	Plants	Cr _{pl} = ns	--	--
	Snails	Cr _{sn} = ns	--	--
Cu	Plants	Cu _{pl} = ns	--	--
	Snails	Cu _{sn} = ns	--	--
Ni	Plants	Ni _{pl} = 0.220 ^o + 0.153 Ni _{CaCl2} *	0.23	0.017
	Snails	Ni _{sn} = ns	--	--
Pb	Plants	Pb _{pl} = -0.014 ^{ns} + 0.149 Pb _{CaCl2} ***	0.54	<0.001
	Snails	Pb _{sn} = ns	--	--
Zn	Plants	Zn _{pl} = ns	--	--
	Snails	Zn _{sn} = ns	--	--

Symbols for p-values in the regression equations are: ^o<0.1, *<0.05, **<0.01, ***<0.001

Table 4: Multiple linear regression analysis and correlation study between TE content in plants or in snails, and the main soil parameters as independent variables. Selected soil parameter were pH, cation exchange capacity (CEC), sum of exchangeable cations (SEC), organic carbon content (C_{org}), clay and silts contents and total soil concentration (extracted with HF + HClO₄) of each studied element. (n= 25)

Element	Organism	Regression equation	r^2_{adj}	<i>p-value</i>
As	Plants	$As_{pl} = -0.081^{ns} + 0.170 As_{tot}^{**}$	0.27	0.005
	Snails	$As_{sn} = -0.108^* + 0.153 As_{tot}^{***}$	0.63	<0.001
Cd	Plants	$Cd_{pl} = ns$	--	--
	Snails	$Cd_{sn} = 0.750^{***} + 0.403 Cd_{tot}^{***} - 0.271 C_{org}^*$	0.55	<0.001
Cr	Plants	$Cr_{pl} = 0.178^{\circ} + 0.205 Cr_{tot}^{***} - 0.277 SEC^{***}$	0.69	<0.001
	Snails	$Cr_{sn} = 0.464^{**} + 0.230 Cr_{tot}^{***} - 0.117 pH^{**} + 0.153 CaCO_3^*$	0.45	0.001
Cu	Plants	$Cu_{pl} = ns$	--	--
	Snails	$Cu_{sn} = ns$	--	--
Ni	Plants	$Ni_{pl} = 1.484^{***} + 0.226 Ni_{tot}^{**} - 0.089 pH^{***} - 0.310 Silts^{**}$	0.62	<0.001
	Snails	$Ni_{sn} = 0.641^{***} + 0.128 Ni_{tot}^{**} - 0.235 SEC^*$	0.27	0.012
Pb	Plants	$Pb_{pl} = 0.106^{ns} + 0.184 Pb_{tot}^{***} - 0.046 pH^*$	0.45	<0.001
	Snails	$Pb_{sn} = 0.104^{ns} + 0.408 Pb_{tot}^{***}$	0.42	<0.001
Zn	Plants	$Zn_{pl} = ns$	--	--
	Snails	$Zn_{sn} = 2.960^{***} + 0.150 Zn_{tot}^{**} - 0.245 SEC$	0.24	0.02

Symbols for *p-values* in the regression equations are: $^{\circ}$ <0.1, *<0.05, **<0.01, ***<0.001

Table 5: Regression analysis and correlation study between TE content in snails, and various sets of explanatory variables (n=25). PC: plant TE content; STC: soil TE total content; SSP: selected soil properties (pH, CEC, SEC, C_{org}, clay and silts contents).

Element	Explanatory variables	Regression equation	r ² _{adj}	p-value
As	PC	As _{sn} = 0.075* + 0.310 As _{pl} **	0.22	0.01
	STC + PC	As _{sn} = -0.108* + 0.153 As _{tot} ***	0.63	<0.001
	STC + PC + SSP	As _{sn} = -0.108* + 0.153 As _{tot} ***	0.63	<0.001
Cd	PC	Cd _{sn} = 0.365*** + 0.838 Cd _{pl} **	0.27	0.004
	STC + PC	Cd _{sn} = 0.272*** + 0.324 Cd _{tot} *** + 0.530 Cd _{pl} *	0.54	<0.001
	STC + PC + SSP	Cd _{sn} = 0.660*** + 0.339 Cd _{tot} *** + 0.468 Cd _{pl} * - 0.241 C _{org} *	0.62	<0.001
Cr	PC	Cr _{sn} = 0.146* + 0.496 Cr _{pl} *	0.21	0.012
	STC + PC	Cr _{sn} = 0.019 ^{ns} + 0.135 Cr _{tot} **	0.23	0.009
	STC + PC + SSP	Cr _{sn} = 0.464** + 0.230 Cr _{tot} *** - 0.117 pH** + 0.153 CaCO ₃ *	0.45	0.001
Cu	PC	Cu _{sn} = ns	--	--
	STC + PC	Cu _{sn} = ns	--	--
	STC + PC + SSP	Cu _{sn} = ns	--	--
Ni	PC	Ni _{sn} = 0.376*** + 0.402 Ni _{pl} **	0.25	0.006
	STC + PC	Ni _{sn} = 0.376*** + 0.402 Ni _{pl} **	0.25	0.006
	STC + PC + SSP	Ni _{sn} = 0.376*** + 0.402 Ni _{pl} **	0.25	0.006
Pb	PC	Pb _{sn} = 0.519*** + 2.171 Pb _{pl} ***	0.56	<0.001
	STC + PC	Pb _{sn} = 0.519*** + 2.171 Pb _{pl} ***	0.56	<0.002
	STC + PC + SSP	Pb _{sn} = -0.333 ^{ns} + 2.085 Pb _{pl} *** + 0.139 pH***	0.75	<0.001
Zn	PC	Zn _{sn} = 2.196*** + 0.481 Zn _{pl} **	0.24	0.007
	STC + PC	Zn _{sn} = 2.196*** + 0.481 Zn _{pl} **	0.24	0.007
	STC + PC + SSP	Zn _{sn} = 2.196*** + 0.481 Zn _{pl} **	0.24	0.007

Symbols for p-values in the regression equations are: °<0.1, *<0.05, **<0.01, ***<0.001

ASSOCIATED CONTENT : **Supporting Information**

S 1: Studied sites: land use, soil occupation, constrains and number of plots.

Site	Land uses	Soil occupation	constraints	No. of plots
GISFI (French Scientific Interest Group - Industrial wasteland)	Contaminated	Wastelands	Contamination in PAH	2
Metaleurop	Contaminated	Arable, woodlands	Multi-metals contamination (Cd, Pb, As)	7
Auzon	Contaminated	Arable, woodlands	Located contamination (As)	6
SHSE (Slag Heap of Saint-Etienne)	Contaminated	Wastelands	Metal diffused contamination (Pb, Cd, Zn...)	3
Yvetot	Cultivated	Arable, pastures	Age of pastures	6
RENECOFOR (national Network of long-term follow-up of the forest ecosystems)	Forest	Forest	Scots pines, spruces, fir tree	4
Andra (French national radioactive waste management agency)	Cultivated / Forest	Arable, forest	Different soil occupations	2

S 2: Main pedological characteristics of the 25 plots from the 7 sites studied (median values, n=5)

Site	Plot	Clay (g kg ⁻¹)	Silts (g kg ⁻¹)	Sands (g kg ⁻¹)	C _{org} (g kg ⁻¹)	pH _w	CEC (cmol kg ⁻¹)	SEC cmol+.kg ⁻¹	CaCO ₃ (g kg ⁻¹)	N _{tot} (g kg ⁻¹)
GISFI	GHF	143	225	611	121	8.3	15.0	17.8	249	2.99
	GHM	94.0	172	736	210	8.3	11.0	14.6	137	4.17
Metaleurop	HW	298	601	101	48.3	8.0	29.3	31.4	50.6	3.40
	IW	294	609	96.0	26.6	8.2	24.3	27.0	66.4	2.05
	LW	203	283	517	31.8	6.5	17.2	17.1	0.500	2.27
	RW	163	525	313	20.2	6.5	12.2	12.4	0.500	1.58
Auzon	CoWW	238	427	346	59.6	5.4	19.1	19.5	0.500	3.96
	CoW	148	279	574	40.8	5.7	13.0	13.6	0.500	2.90
	CoWH	89.5	167	757	44.2	5.2	11.0	11.5	0.500	3.33
	CoWa	98.5	167	713	34.9	5.8	9.46	9.90	0.500	2.48
	CtW	138	299	556	40.8	6.2	14.9	15.6	0.500	2.90
	CtWH	148	225	628	26.2	5.1	8.90	9.29	0.500	2.03
SHSE	HCV	48.5	142	803	46.9	8.1	10.5	13.0	48.6	2.26
	ICV	44.5	131	820	30.4	8.7	6.60	10.8	74.5	1.36
	LCV	33.5	77.0	886	14.8	8.6	3.47	5.72	39.9	0.905
Yvetot	RP1	140	665	196	11.1	5.6	5.43	6.40	0.500	1.14
	RP2	128	665	210	14.4	6.0	6.97	7.71	0.500	1.41
	TP1	149	652	202	18.9	5.5	7.25	8.09	0.500	1.83
	PP	163	634	203	25.8	5.5	8.06	8.59	0.500	2.45
RENECOFOR	F08	262	630	106	87.4	4.0	10.4	9.76	0.500	4.72
	F57	44.5	73.0	886	13.0	4.1	2.14	1.82	0.500	0.689
	F76	78.0	354	566	104	3.8	7.10	4.55	0.500	3.07
	F63	257	411	326	156	4.9	9.42	8.94	1.40	9.17
Andra	Fo	255	425	299	27.5	4.9	6.81	6.86	0.500	1.88
	Pa	499	360	134	59.1	7.3	33.5	34.7	21.3	5.61

SEC : sum of exchangeable cations (Al, Ca, Fe, Mg, Mn, Na,)

S 3: Total (HF + HClO₄ extraction) and “available” (CaCl₂ extraction) TE contents at the 25 plots from the 7 sites studied (median values, n=5). NA: no available data. *italic values correspond to value below the detection limit*

Site	Plot	Total fraction (mg kg ⁻¹)							Available fraction (µg kg ⁻¹)						
		[As] _{tot}	[Cd] _{tot}	[Cr] _{tot}	[Cu] _{tot}	[Ni] _{tot}	[Pb] _{tot}	[Zn] _{tot}	[As] _{CaCl2}	[Cd] _{CaCl2}	[Cr] _{CaCl2}	[Cu] _{CaCl2}	[Ni] _{CaCl2}	[Pb] _{CaCl2}	[Zn] _{CaCl2}
GISFI	GHF	32.8	0.499	57.1	30.3	29.0	165	408	15.5	6.12	<i>5.00</i>	84.6	<i>7.50</i>	6.39	55.1
	GHM	58.5	1.23	172	45.3	26.9	309	323	23.4	7.56	<i>5.00</i>	143	<i>7.50</i>	6.52	94.8
Metaleurop	HW	39.3	34.4	48.6	68.4	23.5	2485	1885	34.2	415	<i>5.00</i>	234	30.4	465	1965
	IW	30.9	13.3	50.3	27.6	19.8	731	745	<i>10.0</i>	79.3	<i>5.00</i>	93.3	18.3	39.1	340
	LW	9.42	5.44	52.0	19.4	14.2	319	332	18.6	578	<i>5.00</i>	123	120	142	15100
	RW	7.11	1.09	41.5	12.3	13.6	48.8	102	<i>10.0</i>	122	<i>5.00</i>	87.8	151	10.7	1835
Auzon	CoWW	3285	9.97	76.5	159	41.4	4575	225	155050	326	<i>5.00</i>	681	466	617	5020
	CoW	339	0.722	57.9	22.9	25.7	104	148	1238	53.8	<i>5.00</i>	47.7	163	19.0	1080
	CoWH	661	1.32	51.8	38.6	36.3	282	140	16895	120	<i>5.00</i>	126	418	194	3500
	CoWa	1087	6.73	52.3	140	25.7	1834	173	10735	219	<i>5.00</i>	538	318	377	6660
	CtW	123	0.612	67.3	27.7	35.0	60.1	138	233	33.6	<i>5.00</i>	76.0	136	3.54	689
	CtWH	62.5	0.143	56.8	22.8	27.6	28.1	88.1	44.6	21.2	<i>5.00</i>	38.0	335	21.4	1006
SHSE	HCV	73.3	21.0	982	1555	685	2525	2830	<i>10.0</i>	17.6	11.6	653	43.5	26.5	492
	ICV	54.8	9.37	1158	570	405	1616	2180	<i>10.0</i>	3.98	41.9	200	17.4	11.8	153
	LCV	30.3	1.99	4345	525	1180	513	577	<i>10.0</i>	1.42	10.2	118	69.5	4.35	42.2
Yvetot	RP1	6.93	0.236	66.3	10.3	14.6	21.0	42.7	NA	32.6	NA	34.3	NA	4.21	284
	RP2	6.76	0.243	49.0	14.4	13.9	30.6	47.0	NA	14.7	NA	46.1	NA	6.80	215
	TP1	8.52	0.205	51.2	13.7	15.6	21.4	48.4	NA	29.3	NA	61.4	NA	4.04	587
	PP	9.48	0.198	53.8	13.4	16.9	26.3	51.5	NA	32.3	NA	51.2	NA	7.24	640
RENECOFOR	F08	25.4	0.235	57.9	17.1	5.68	78.9	34.0	58.9	111	22.5	71.5	427	924	3175
	F57	3.83	0.017	6.55	1.73	1.40	21.1	8.61	30.5	11.7	<i>5.00</i>	32.5	179	826	604
	F76	2.91	0.063	17.7	3.48	5.53	21.9	13.5	46.4	41.5	<i>5.00</i>	24.0	847	485	4290
	F63	12.8	0.414	55.0	14.1	20.2	49.7	113	NA	64.0	NA	10.7	300	46.7	2035
Andra	Fo	16.6	0.328	71.1	9.57	27.9	32.5	93.3	<i>10.0</i>	62.7	<i>5.00</i>	15.9	316	16.0	1410
	Pa	18.4	0.447	82.7	18.5	42.3	29.9	104	<i>10.0</i>	1.45	<i>5.00</i>	38.9	<i>7.50</i>	<i>1.50</i>	<i>5.00</i>

S 3: Principal plant species on the studied plots.

Site	Plot	Principal species on the plots
GISFI	GHF	<i>Achillea millefolium</i> , <i>Agrostis stolonifera</i> , <i>Arrhenatherum elatius</i> , <i>Artemisia vulgaris</i> , <i>Artemisia campestris</i> , <i>Bromus hordeaceus</i> , <i>Bromus sterilis</i> , <i>Cerastium</i> sp., <i>Chenopodium album</i> , <i>Dactylis glomerata</i> , <i>Echium ulgare</i> , <i>Epilobium parviflorum</i> , <i>Erigeron annuus</i> , <i>Euphorbia cyparissias</i> , <i>Hypericum perforatum</i> , <i>Medicago lupulina</i> , <i>Melilotus albus</i> , <i>Malva moschata</i> , <i>Myosotis</i> sp., <i>Oenothera biennis</i> , <i>Petrorhagia rotifera</i> , <i>Picris hieracioides</i> , <i>Plantago lanceolata</i> , <i>Prunus spinosa</i> , <i>Robinia pseudoacacia</i> , <i>Rubus</i> sp., <i>Scrophularia</i> sp., <i>Sedum acre</i> , <i>Senecio jacobaea</i> , <i>Silene latifolia</i> , <i>Tanacetum vulgare</i> , <i>Tragopogon ubius</i> , <i>Veronica chamaedrys</i> , <i>Vicia hirsuta</i> , <i>Vulpia ciliata</i>
	GHM	<i>Acer pseudoplatanus</i> , <i>Arrhenatherum elatius</i> , <i>Artemisia vulgaris</i> , <i>Astragalus glycyphyllos</i> , <i>Centaurea jacea</i> , <i>Chenopodium album</i> , <i>Cirsium arvense</i> , <i>Clematis vitalba</i> , <i>Dactylis glomerata</i> , <i>Dipsacus fullonum</i> , <i>Epilobium</i> sp., <i>Erigeron annuus</i> , <i>Euphorbia cyparissias</i> , <i>Geranium robertianum</i> , <i>Hypericum perforatum</i> , <i>Medicago sativa</i> , <i>Oenothera biennis</i> , <i>Papaver rhoeas</i> , <i>Plantago lanceolata</i> , <i>Reseda lutea</i> , <i>Rosa</i> sp., <i>Rubus</i> sp., <i>Rumex acetosella</i> , <i>Sanguisorba minor</i> , <i>Silene latifolia</i> , <i>Verbascum</i> sp., <i>Vulpia ciliata</i>
Metaleurop	HW	<i>Acer pseudoplatanus</i> , <i>Alnus glutinosa</i> , <i>Arrhenatherum elatius</i> , <i>Betula pubescens</i> , <i>Calystegia sepium</i> , <i>Carex palustris</i> , <i>Cornus sanguinea</i> , <i>Crataegus monogyna</i> , <i>Elytrigia repens</i> , <i>Epilobium</i> sp., <i>Fraxinus excelsior</i> , <i>Galium aparine</i> , <i>Glechoma hederacea</i> , <i>Myosotis arvensis</i> , <i>Quercus</i> sp., <i>Ranunculus repens</i> , <i>Robinia pseudoacacia</i> , <i>Rubus</i> sp., <i>Salix</i> sp., <i>Sambucus nigra</i> , <i>Symphytum officinale</i> , <i>Urtica dioica</i>
	IW	<i>Acer pseudoplatanus</i> , <i>Arrhenatherum elatius</i> , <i>Cornus sanguinea</i> , <i>Crataegus monogyna</i> , <i>Dipsacus fullonum</i> , <i>Elytrigia repens</i> , <i>Epilobium</i> sp., <i>Fragaria vesca</i> , <i>Galium aparine</i> , <i>Glechoma hederacea</i> , <i>Myosotis arvensis</i> , <i>Populus</i> sp., <i>Ranunculus repens</i> , <i>Rubus</i> sp., <i>Sambucus nigra</i> , <i>Symphytum officinale</i> , <i>Urtica dioica</i>
	LW	<i>Acer pseudoplatanus</i> , <i>Alnus glutinosa</i> , <i>Betula pubescens</i> , <i>Carpinus betulus</i> , <i>Castanea sativa</i> , <i>Corylus avellana</i> , <i>Crataegus monogyna</i> , <i>Epilobium</i> sp., <i>Fraxinus excelsior</i> , <i>Galium aparine</i> , <i>Geranium robertianum</i> , <i>Glechoma hederacea</i> , <i>Hedera helix</i> , <i>Ilex aquifolium</i> , <i>Lamium album</i> , <i>Milium effusum</i> , <i>Myosotis arvensis</i> , <i>Parthenocissus inserta</i> , <i>Poa</i> sp., <i>Populus</i> sp., <i>Prunus avium</i> , <i>Quercus</i> sp., <i>Rubus</i> sp., <i>Sambucus nigra</i> , <i>Stellaria holostea</i> , <i>Symphytum officinale</i> , <i>Urtica dioica</i>
	RW	<i>Acer pseudoplatanus</i> , <i>Clematis vitalba</i> , <i>Crataegus monogyna</i> , <i>Epilobium</i> sp., <i>Equisetum arvense</i> , <i>Euonymus europaeus</i> , <i>Fraxinus excelsior</i> , <i>Galium aparine</i> , <i>Geranium robertianum</i> , <i>Glechoma hederacea</i> , <i>Hedera helix</i> , <i>Lamium album</i> , <i>Myosotis arvensis</i> , <i>Populus</i> sp., <i>Prunus avium</i> , <i>Quercus</i> sp., <i>Ranunculus repens</i> , <i>Rubus</i> sp., <i>Salix</i> sp., <i>Urtica dioica</i> , <i>Vicia hirsuta</i>
Auzon	CoWW	<i>Agrostis capillaris</i> , <i>Arum maculatum</i> , <i>Cornus sanguinea</i> , <i>Crataegus monogyna</i> , <i>Equisetum arvense</i> , <i>Euphorbia dulcis</i> subsp <i>incompta</i> , <i>Hedera helix</i> , <i>Lonicera periclymenum</i> , <i>Quercus</i> sp., <i>Rubus</i> sp., <i>Sambucus nigra</i> , <i>Urtica dioica</i> ,
	CoW	<i>Apiaceae</i> sp., <i>Cornus sanguinea</i> , <i>Corylus avellana</i> , <i>Crataegus monogyna</i> , <i>Euonymus europaeus</i> , <i>Hedera helix</i> , <i>Ligustrum vulgare</i> , <i>Lonicera periclymenum</i> , <i>Quercus</i> sp., <i>Rubus</i> sp.
	CoWH	<i>Agrostis capillaris</i> , <i>Alliaria petiolata</i> , <i>Apiaceae</i> sp., <i>Cirsium arvense</i> , <i>Cornus sanguinea</i> , <i>Crataegus monogyna</i> , <i>Epilobium</i> sp.1, <i>Epilobium</i> sp. 2, <i>Equisetum arvense</i> , <i>Euonymus europaeus</i> , <i>Humulus lupulus</i> , <i>Rubus</i> sp., <i>Urtica dioica</i> ,
	CoWa	<i>Achillea millefolium</i> , <i>Agrostis capillaris</i> , <i>Apiaceae</i> sp., <i>Cytisus scoparius</i> , <i>Epilobium</i> sp., <i>Euphorbia cyparissias</i> , <i>Galium aparine</i> , <i>Populus tremula</i> , <i>Quercus</i> sp., <i>Rubus</i> sp., <i>Rumex acetosella</i> , <i>Urtica dioica</i> ,
	CtW	<i>Alliaria petiolata</i> , <i>Anthriscus sylvestris</i> , <i>Arum maculatum</i> , <i>Chelidonium majus</i> , <i>Crataegus monogyna</i> , <i>Euonymus europaeus</i> , <i>Galium aparine</i> , <i>Hedera helix</i> , <i>Lamium galeobdolon</i> , <i>Ligustrum vulgare</i> , <i>Lunaria annua</i> , <i>Prunus spinosa</i> , <i>Sambucus nigra</i> , <i>Tillia platyphyllos</i> , <i>Urtica dioica</i> ,
	CtWH	<i>Euonymus europaeus</i> , <i>Galium aparine</i> , <i>Glechoma hederacea</i> , <i>Hedera helix</i> , <i>Lamiaceae</i> sp., <i>Prunus spinosa</i> , <i>Quercus</i> sp., <i>Rubus</i> sp., <i>Tillia platyphyllos</i>
	HCV	<i>Ailanthus altissima</i> , <i>Calamintha nepeta</i> , <i>Clematis vitalba</i> , <i>Cornus sanguinea</i> , <i>Echium vulgare</i> , <i>Euonymus europaeus</i> , <i>Hypericum perforatum</i> , <i>Lactuca serriola</i> , <i>Melilotus albus</i> , <i>Oenothera biennis</i> , <i>Urtica dioica</i> , <i>Verbascum pulverulentum</i>
SHSE	ICV	<i>Ailanthus altissima</i> , <i>Calamintha nepeta</i> , <i>Fraxinus excelsior</i> , <i>Hypericum perforatum</i> , <i>Melilotus albus</i> , <i>Oenothera biennis</i> , <i>Plantago sacbra</i> subsp <i>scabra</i> , <i>Populus nigra</i> , <i>Reseda lutea</i> , <i>Rosa</i> sp., <i>Sambucus nigra</i> , <i>Urtica dioica</i> , <i>Verbascum pulverulentum</i>
	LCV	<i>Echium vulgare</i> , <i>Lactuca serriola</i> , <i>Melilotus albus</i> , <i>Hypericum perforatum</i> , <i>Oenothera biennis</i> , <i>Plantago sacbra</i> Moench subsp <i>scabra</i> , <i>Populus nigra</i> , <i>Reseda lutea</i> , <i>Scrophularia canina</i> , <i>Verbascum pulverulentum</i>
Yvetot	RP1	<i>Carpinus betulus</i> , <i>Fumaria officinalis</i> , <i>Lamium purpureum</i> , <i>Lolium perenne</i> , <i>Poa annua</i> , <i>Rumex acetosa</i> , <i>Stellaria media</i> , <i>Taraxacum</i> sp., <i>Trifolium repens</i> , <i>Urtica dioica</i> , <i>Veronica chamaedrys</i> ,

	RP2	<i>Lolium perenne, Poa annua, Stellaria media, Trifolium repens,</i>
	TP1	<i>Cirsium sp., Holcus lanatus, Lamium album, Lamium purpureum, Lolium perenne, Ranunculus repens, Rumex acetosa, Rumex acetosella, Stellaria holostea, Stellaria media, Taraxacum sp., Trifolium repens, Urtica dioica</i>
	PP	<i>Cirsium sp., Crataegus monogyna, Fagus sylvatica, Lamium album, Lamium purpureum, Plantago major, Ranunculus repens, Rumex acetosella, Stellaria holostea, Symphytum officinale, Taraxacum sp., Urtica dioica</i>
RENECOFOR	F08	<i>Picea abies, Calluna vulgaris, Carex pilulifera, Deschampsia flexuosa, Galium mollugo, Rubus fruticosus, Sorbus aucuparia, Vaccinium myrtillus</i>
	F57	<i>Abies alba, Deschampsia flexuosa, Fagus sylvatica, Festuca altissima, Luzula luzuloides, Oxalis acetosella, Picea abies, Rubus fruticosus, Rubus idaeus, Sorbus aucuparia, Vaccinium myrtillus</i>
	F76	<i>Pinus sylvestris, Calluna vulgaris, Deschampsia flexuosa, Fagus sylvatica, Molinia caerulea, Rubus fruticosus, Vaccinium myrtillus</i>
	F63	<i>Abies alba, Agrostis capillaries, Campanula rotundifolia, Carex pilulifera, Cytisus scoparius, Digitalis purpurea, Epilobium montanum, Fagus sylvatica, Gallium rotundifolium, Hypericum humifusum, Picea abies, Rubus idaeus, Rumex acetosella</i>
ANDRA	Fo	<i>Fagus sylvatica, Galium aparine, Glechoma hederacea, Hedera helix, Lamium album, Lamium galeobdolon, Rubus fruticosus</i>
	Pa	<i>Lolium perenne, Ranunculus repens, Rumex acetosella, Taraxacum sp., Trifolium repens</i>