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## **Ex situ environmental risk assessment of polluted soils using threshold guide values for the land snail *Cantareus aspersus***

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24 European contaminated soils based on global index calculations: i) The sum of the excess of  
25 transfers (SETs) and ii) the weighted SETs based on the general toxicity points of each  
26 contaminant used to evaluate the risk of transferred MEs, PAHs and PCBs (ERITMEs,  
27 ERITPAHs and ERITPCBs, respectively). In addition, the influence of soil physico-chemical  
28 properties on accumulation was modelled to better understand their roles in bioavailability.  
29 The presented *ex situ* TGV and the associated indicators (the global sum of the excess of  
30 transfers and global ecotoxicological risk) provide a basis by which stakeholders can  
31 prioritize the management of polluted soils depending on the risk they may represent. The  
32 determination of *ex situ* TGVs for organic and inorganic compounds provides new tools to  
33 characterize excess contaminant transfers, and it will also allow the use of snails for ERAs,  
34 notably for common pollutants, such as PAHs and PCBs for which guide values are not  
35 available.

36

## 37 **1. Introduction**

38 Anthropogenic activities are at the origin of 2.5 million polluted fields in the European  
39 Union (EEA, 2019). Contaminated soils are still a subject of concern, as they can cause health  
40 problems (Eeva et al., 2000), notably cancers for humans (Nawrot et al., 2006) and the  
41 collapse of animal populations (Laskowski and Hopkin, 1996) and disturb community  
42 structures (Miglioniri et al., 2004; Cébron et al., 2011). For the environmental risk assessment  
43 (ERA) of polluted soils and sites, a combination of chemical, biological and ecological  
44 measurements can be used *in situ* and *ex situ*, as described in the TRIAD methodology (ISO  
45 19204:2017). Understanding the relationship between soil pollution and environmental effects  
46 is essential for the establishment of management plans, and this relationship can only be  
47 assessed by measuring bioavailability through a combination of chemical and biological

48 methods (Lanno et al., 2004). Indeed, bioavailability is a dynamic three steps process  
49 (environmental availability, environmental bioavailability, and toxicological bioavailability,  
50 ISO 17402:2008). Thus, the measurement of bioavailability prevents the misinterpretation of  
51 the environmental risk level of contaminated soils. Therefore, the ERA of contaminated soils  
52 must take into account not only the total concentration of pollutants in soils but also the  
53 transfer of pollutants modulated by soil characteristics (e.g., pH, clay content, organic matter  
54 content) (Allen, 2002; Pauget et al., 2011; Pauget et al., 2016).

55 The *in situ* methodology developed with the land snail *Cantareus aspersus* by Pauget and  
56 de Vaufleury (2015) allows the assessment of environmental risk in the field of soil  
57 contaminated for 14 metallic elements (MEs). To accomplish this, the environmental  
58 bioavailability is estimated by the accumulation quotient (AQ) after 28 days of snail caging  
59 on a site and with a global index of the sum of excess transfers (SETs), which integrates the  
60 transfers of the 14 MEs to the snails. Based on the bioavailability assessment with the AQ, the  
61 toxicological risk can be estimated by weighting the AQ with the intrinsic toxicity of each  
62 transferred contaminant (toxicity point (TP) from ATSDR, 2015). This allows the evaluation  
63 of the environmental risks with the calculation of a risk coefficient (RC) for each  
64 contaminant. Then, the sum of each RC gives an index of the evaluation of the risk of  
65 transferred metallic elements (ERITME). The SET and ERITME index have been shown to  
66 be relevant for identifying the environmental risk of contaminated soils, e.g., resulting from  
67 past mining activities (Mariet et al., 2017). Until now, it has not been possible to apply this  
68 methodology to organic compounds due to the lack of basic data, such as the usual internal  
69 concentration of these molecules in snail tissues. A total of 55.3% of identified soil  
70 contaminations in France are caused by MEs (44.7%), but polycyclic aromatic hydrocarbons  
71 (PAHs) and polychlorinated biphenyls (PCBs) are also frequently found in polluted sites  
72 (8.1% and 2.6%, respectively) (MTES, 2019). The choice of *C. aspersus* was made for

73 several reasons, among which are its worldwide repartition, its particular position at the soil-  
74 air-plant interface (Dallinger et al., 2001), and its non-negligible ecological role (Iglesias and  
75 Castillejo, 1999). Indeed, *C. aspersus* is a primary consumer phyto- and saprophagous which  
76 also ingest soil) (Gomot et al., 1989; Barker, 2001; Chevalier et al., 2001; Dallinger et al.,  
77 2001). It is also usable as a bioindicator in ERAs of polluted soils (ISO 15952:2018; Larba  
78 and Soltani, 2014; Nica et al., 2013; de Vaufleury, 2015). Furthermore, due to the  
79 accumulation abilities of the land snail species, they can be involved in the trophic  
80 bioavailability of MEs, *i.e.*, transfer in food webs (Beeby et al., 1985; Laskowski and Hopkin,  
81 1996; Hispard et al., 2008). The *in situ* methodology enables snail exposure to various  
82 transfer sources, including not only the soil source but also, *e.g.*, plants, humus, and air.  
83 Currently, no *ex situ* assay with threshold guide values exists with the land snail to assess the  
84 environmental bioavailability of contaminants in soils. *Ex situ* exposure should facilitate the  
85 comparison of several soils by avoiding the influence of confounding factors, such as  
86 pedoclimatic conditions and site specific parameters, such as the diversity of plants. In the  
87 proposed *ex situ* bioassay, the soil constitutes the main source of exposure of snails, which is  
88 relevant, in the context of the ERA of excavated soils, *e.g.*, reused in embankments. Such *ex*  
89 *situ* approach will contribute to the ERA of destructured soils and guide management  
90 decisions for polluted soil stakeholders (*e.g.*, the rehabilitation of excavated polluted soils,  
91 considering the transfer of pollutants to biota). Therefore, the *in situ* approach characterizes  
92 the risk of transferring contaminants from structured sites to biota, and the *ex situ* approach  
93 characterizes the risk of transferring contaminants from destructured soil to biota.

94 The assessment of the environmental risk of contaminated soils has to help stakeholders of  
95 polluted sites to elaborate appropriate decisions for sustainable soil management. For this  
96 purpose, some methodologies including the ratio called risk quotient (predicted environmental  
97 concentrations/predicted no effect concentrations; PEC/PNEC) exist (Ahlers, 2001).

98 Calculation of PNEC require disposing of toxicity data (NOEC and ECx values) to which  
99 assessment factors are then applied. Among other risk assessment methods, the species  
100 sensitivity distribution (SSD) can be applied but it requires a lot of ecotoxicity data for  
101 numerous species (Jänsch et al., 2007). As for many soils bioindicators, ecotoxicity metrics  
102 for snails are not available for all the contaminants considered in the present study, thus  
103 neither the ratio risk nor the SSD approaches can be applied. Furthermore these methods are  
104 applied to each contaminant separately and here the aim was to provide global indices of the  
105 fate and potential effect of several contaminants at the same time. Other methodologies are  
106 proposed for the risk assessment of contaminated soils, such as the TRIAD method (ISO  
107 19204:2017). Among the parameters that are recommended in the TRIAD method, is the  
108 bioavailability that can be estimated notably with the snail, as bioindicator of the soil quality  
109 (ISO 17402:2008; ISO 19204:2017).

110 The aim of our study is to develop an *ex situ* approach for the main substances in  
111 contaminated soils, *i.e.*: 15 MEs (As, Cd, Co, Cr, Cu, Hg, Mo, Mn, Ni, Pb, Sb, Se, Sn, Sr and  
112 Zn), 16 PAHs and 7 PCBs. Almost all of these contaminants are ranked in the substance  
113 priority list (except for Mo and Sn; ATSDR, 2017). First, the present study determined the *ex*  
114 *situ* TGVs (threshold guide values) of the land snail for 15 MEs, 16 PAHs and 7 PCBs in a  
115 bio-assay with 68 soils from Europe. These soils have various physico-chemical properties  
116 that are representative of the natural soil variability. Then, this study presents the application  
117 of this *ex situ* assay to perform an ERA for various soils contaminated by MEs and PAHs, and  
118 one polluted soil by PCBs. Third, this study examines the relationships between the AQ of  
119 metal(loid)s and the influence of soil physico-chemical properties to better understand the role  
120 of soil parameters on contaminant transfers to snails.

121

## 122 2. Material and Methods

## 123 2.1. Soils

124 The soils used in this study originate from various European locations (France, Germany  
125 and Czech Republic) (**Table S1**). The soils were sieved at 4 mm, and the physico-chemical  
126 parameters were measured with the appropriate ISO standard protocols (granulometry,  
127  $\text{pH}_{\text{water}}$ , etc.; **Figure 1**). To be applicable to various types of potentially contaminated soils  
128 from various soil locations, the TGV calculations are based on soil representing a wide range  
129 of physico-chemical properties (*e.g.*, forest, farming or grassland), as presented in **Table S1**.  
130 The min and max values for clay, silt, sand,  $\text{pH}_{\text{water}}$ , OM,  $\text{C}_{\text{org}}$ ,  $\text{CaCO}_3$  contents, CEC, Alox  
131 and Feox were 6.8 and 63.0%, 7.5 and 73.4%, 2.9 and 83.7%, 3.64 and 8.30, 12 and 360  $\text{g.kg}^{-1}$   
132 <sup>1</sup>, 4.1 and 207  $\text{g.kg}^{-1}$ , <0.5 and 277  $\text{g.kg}^{-1}$ , 4.30 and 1218  $\text{cmol.kg}^{-1}$ , 0.02 and 5.64  $\text{cmol.kg}^{-1}$   
133 and 0.005 and 5.14  $\text{cmol.kg}^{-1}$ , respectively.

134 For the analysis of MEs in the soils, 500 mg of the soils (dry weight (dw)) was sampled,  
135 and after *aqua regia* digestion ( $\text{HNO}_3\text{:HCl}$  2:5, v:v ultratracés, Optima®, Fisher Scientific,  
136 Illkirch, France), the concentrations of MEs were measured by ICP-MS (inductively coupled  
137 plasma mass spectrometry, Thermo Scientific X Series II, Courtaboeuf, France) (**Table S2**). A  
138 certified reference material (loamy clay 1, CRM052, LGC Standards, Molsheim, France) was  
139 employed and gave recovery rates of 80% to 120% for all the MEs. The concentrations of the  
140 PAHs and PCBs in the soils were measured by GC-MS/MS (**Tables S3 and S4**) according to  
141 [Morin-Crini et al. \(2014\)](#). Briefly, pressurized liquid extraction (PLE) was performed on 5 g  
142 dried samples with hexane using a Büchi (Rungis, France) SpeedExtractor E-914 system.  
143 After the concentration of the samples, sample extracts were analysed in the GC-MS/MS  
144 apparatus (Agilent, Massy, France), which included a 7890A GC system, a 7000 GC triple  
145 quadrupole mass spectrometer and an 80 GC Combipal autosampler and a (5% phenyl)-  
146 methylpolysiloxane (HP5MS) column (30 m x 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness, Agilent

19091J-433). The injection volume was 1  $\mu$ l, the injector temperature was 300°C, and injection was performed in splitless mode under a constant flow of He (purity of 99.9999%) at 1.5 mL.min<sup>-1</sup> followed by a purge flow to the split vent after 0.5 min. The temperature of the transfer line, ion source and quadrupole were 300°C, 300°C, and 150°C, respectively. An electron-impact ionization source at 70eV was utilized. Nitrogen with a purity of 99.9% was produced by a NiGen LC-MS 40-1 Claind generator (Gengaz, Wasquehal, France) and used as the collision gas at 1.5 mL.min<sup>-1</sup>. For PAH, the temperature gradient was from 45°C (held for 0.5 min) to 100°C at 30°C min<sup>-1</sup> and then from 100°C to 325°C at 10°C min<sup>-1</sup>. The total run time was 30 min. The abbreviations for the PAHs used in this study are as follows: NAP (naphthalene), ACY (acenaphthylene), ACE (acenaphthene), FLU (fluorene), PHE (phenanthrene), ANT (anthracene), FLT (fluoranthene), PYR (pyrene), BaANT (benzo(a)anthracene), CHY (chrysene), BbFLT (benzo(b)fluoranthene), BkFLT (benzo(k)fluoranthene), BaPYR (benzo(a)pyrene), dBahANT (dibenzo(a,h)anthracene), BghiPL (benzo(ghi)perylene), and IcdPYR (indeno[1,2,3-cd]pyrene). For PCB, the temperature gradient was from 120°C (held for 1 min) to 220°C at 3°C min<sup>-1</sup> and then from 220°C to 280°C at 6°C min<sup>-1</sup>. The total run time was 49 min. The PCBs analysed were indicators 28, 52, 101, 118, 138, 153 and 180. The soils were classified as contaminated on the basis of the soil guide values (SGVs) of MEs according to [Pauget and de Vaufleury \(2015\)](#) and [ADEME \(2018\)](#) for PAHs and PCBs (*Tables 1, S2, S3 and S4*).

## 166 **2.2. Snails: *Cantareus aspersus* (Müller, 1774)**

167 Snails were bred in our laboratory from individuals originating from a snail farm (CFPPA  
168 of Châteaufarine, 25000 Besançon, France). The species has been verified by DNA barcoding  
169 (Allgenetics, 15008 A Coruña, Spain). Juvenile land snails were reared in the laboratory  
170 under controlled conditions (20°C, 80% relative humidity, photoperiod of 18 h light and 6 h

171 dark) until the sub-adult stage (7-9 weeks), as described by [Gomot-de Vaufleury \(2000\)](#). The  
172 sub-adult stage was selected to avoid confounding factors related to growth and reproduction  
173 ([Gimbert et al., 2008](#); [Pauget et al., 2013](#)). At the beginning of exposure (t0), the sub-adult  
174 snails weighed  $4.98 \pm 0.76$  g and the internal concentrations of the MEs, PAHs and PCBs  
175 were measured in the visceral mass from twelve sub-adult snails (*Table 1*). The snails were  
176 fed during the growth period with uncontaminated commercial food, Helixal<sup>®</sup> (Berthon S.A.,  
177 France) and during the exposure period with uncontaminated fresh lettuce (from an organic  
178 farm, France) (*Table 1*).

### 179 **2.3. Exposure**

180 The snails were exposed to the soils, through digestive and cutaneous pathways for 28  
181 days. For each soil, six snails were exposed in triplicate in transparent polystyrene containers  
182 of 24 x 21 x 8 cm. The exposure modalities are detailed in [Pauget et al. \(2011\)](#). One week  
183 before exposure, 300 g of soil (dw) was introduced in containers and humidified (water  
184 holding capacity (WHC) adjusted to 50%) with osmotic water. The containers were cleaned  
185 every two days to remove faeces and renew the lettuce ( $1.5\text{g}\cdot\text{day}^{-1}\cdot\text{snail}^{-1}$ ). The lettuce was  
186 offered in a Petri dish left on the bottom of the container. At the end of the exposure, the  
187 snails were starved in clean containers (without soils) for two days (the faeces were removed  
188 every 12 h). Then, snails were frozen (-80°C).

### 189 **2.4. Chemical analysis**

190 After thawing, for the dissection of visceral mass from each snail, the whole soft body was  
191 removed from the shell, the foot and the anterior part of the body were separated to the  
192 visceral mass above the edge of the mantle ([Gomot-de Vaufleury and Pihan, 2002](#)). The  
193 visceral mass then includes the digestive gland (main storage for contaminants), the kidney,  
194 the lung and the main part of the genital tract. Two visceral masses per replicate (three

195 replicates per soil) were freeze dried for two days before the ME analysis. The lyophilized  
196 viscera were digested between 47°C and 98°C for 265 minutes (DigiPREP MS, SCP Science,  
197 Courtaboeuf, France) in 7 mL of nitric acid (HNO<sub>3</sub> at 65%, ultratracés Optima®, Fisher  
198 Scientific, Illkirch, France) and the volume was brought to 50 mL with milliQ water. Then,  
199 the samples were filtered to 1 µm (DigiFILTER, SCP Science, Courtaboeuf, France) and  
200 diluted 10x for ICP-MS analysis (Thermo Scientific X Series II, Courtaboeuf, France) as for  
201 soil samples. The analysis was validated with a certified reference material (TORT-2 and  
202 DOLT, LGC Standards, Molsheim, France), which had recovery rates of 107% ± 15. For the  
203 PAHs and PCBs, the fresh visceral mass was dissected and pooled (two snails) from each  
204 replicate. The PAHs and PCBs were extracted with the QuEACHERs (quick, easy, cheap,  
205 effective, rugged and safe) methodology (RESTEK, Lisse, France). Briefly, snail viscera  
206 samples (one pool of two animals per replicate) were weighted, mixed with equal parts of  
207 water and crushed with a ball mill until completely homogenized (25 ceramic sphere  
208 (FastPrep, MP Biomedicals, Illkirch, France) for two cycles (4.0 m/sec, 20 sec), with 180-  
209 second breaks). Samples were transferred into 50 mL Teflon centrifuge tubes, acetonitrile was  
210 added. After a 15min-extraction by vortex, salts were added and samples were further  
211 vortexed for 15 min. Extracts were centrifuged and an aliquot of supernatant was transferred  
212 to d-SPE polypropylene tubes for cleanup. Concentrations were measured by GC-MS/MS  
213 (7890A GC system, 7000 GC triple quadrupole mass spectrometer, HP5MS column, Agilent,  
214 Massy, France) in the same conditions than for soil samples. When the concentrations of the  
215 MEs, PAHs or PCBs in the snails were below the quantification limit (QL), the values of the  
216 concentrations used to calculate the AQ were replaced by QL/2 as recommended [Bergstrand](#)  
217 [and Karlsson \(2009\)](#).

## 218 **2.5. *Ex situ* threshold guide value calculation**

219 The *ex situ* TGVs were calculated on the basis of the internal concentrations of  
220 contaminants in the visceral mass of snails exposed *ex situ* to uncontaminated soils, according  
221 to the methodology published by [Pauget et al. \(2013\)](#) (Eq. 1). The outliers were removed  
222 before calculation.

$$223 \text{ } ex \text{ situ TGVs} = 75^{th} \text{ percentile} + 1.5 \times \text{interquatile range} \quad (1)$$

224 Uncontaminated soils were identified on the basis of soil guide values (SGVs, **Table 1**) which  
225 correspond to the concentrations of contaminants beyond which a soil can be considered as  
226 contaminated ([ADEME, 2018](#)). SGVs are guidance concentrations in soils to assess if a soil is  
227 contaminated and TGVs are guidance concentrations in snails viscera after 28 days of  
228 exposure to assess if an excess of transfer of contaminants exist from the soil to the snail.

229 For the *ex situ* TGVs calculation of the PAHs and PCBs, the concentrations accumulated in  
230 snails exposed to 26 and 7 uncontaminated soils were used, respectively (**Tables S1**). For the  
231 MEs, due to the strong differences between the concentrations of each ME in soils (**Table S1**),  
232 the *ex situ* TGVs were calculated with the concentration accumulated in the snail viscera over  
233 28 days from 44, 26, 66, 64, 37, 24, 51, 48, 66, 34, 37, 30, 40, 67, and 18 soils for As, Cd, Co,  
234 Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr and Zn, respectively (a soil can be considered  
235 uncontaminated for As but contaminated for Pb).

## 236 **2.6. Accumulation quotient, risk coefficient and index calculation**

237 For each snail and each category of contaminant (ME, PAH, and PCB), the accumulation  
238 quotient (AQ), SET index, risk coefficient (RC) and ERIT-ME, -PAH, -PCB indexes were  
239 calculated to assess the sum of excess transfers (exposure) and the toxicological risk,  
240 according to the methodology described by [Pauget and de Vaufleury \(2015\)](#). Briefly, the AQs  
241 for organic contaminants were calculated with the mean concentration in the snail tissue

242 instead of the median concentration (Eq. 2) due to the fact that three measurements (one per  
243 replicate) were performed per soil (pool of two individuals per replicate). The SET indexes  
244 were calculated based on the sum of each AQ (Eq. 3). According to [Pauget and de Vaufleury](#)  
245 [\(2015\)](#), the RCs (risk coefficient = exposure x hazard) were calculated based on the AQ (that  
246 reflects the exposure of the snails) and the toxicity points (TPs) from [ATSDR \(2015\)](#) as a  
247 surrogate of the hazard of each contaminant (**Table 1**; Eq. 4). Indeed, many biomarkers of  
248 ecotoxicity have been studied in land snails, such as growth and reproduction rates, the  
249 acetylcholinesterase activities, the oxidative stress management, the (epi-)genomic stability  
250 and/or the histopathologic disorders ([Regoli et al., 2006](#); [Radwan et al., 2010](#); [Itziou et al.,](#)  
251 [2011](#); [Angeletti et al., 2013](#); [Baurand et al., 2015](#); [Mleiki et al., 2015](#); [Leomanni et al., 2016](#);  
252 [Nica et al., 2017](#); [ISO 15952:2018](#); [Mleiki et al., 2018](#)). But these endpoints are not available  
253 for all the contaminants considered in the present study. Therefore, and according to [Pauget](#)  
254 [and de Vaufleury \(2015\)](#), the use of TP was applied here. For the PAHs and PCBs, the  
255 ERITME indexes are respectively reappointed ERITPAH and ERITPCB, respectively (Eq. 5).

$$256 \quad AQ = \frac{\text{Median Concentration}_{28 \text{ days}}}{TGV} \quad (2)$$

$$257 \quad SET = \sum(AQ - 1) \quad (3)$$

$$258 \quad RC = (AQ - 1) \times TP \quad (4)$$

$$259 \quad ERITME \text{ or } ERITPAH \text{ or } ERITPCB = \sum RC \quad (5)$$

260 The global SET index (GSET) and global evaluation of the risk of toxicological elements  
261 (GERITOXE) were integrative indexes of SET and ERIT- for all the families of compounds  
262 and were calculated with the sum of SET or ERITME, ERITPAH and ERITPCB (Eqs. 6 and  
263 7).

264  $GSET = \sum(SET\ ME + SET\ PAH + SET\ PCB)$  (6)

265  $GERITOXE = \sum(ERITME + ERITPAH + ERITPCB)$  (7)

266 **2.7. Statistical analysis**

267 The TGV calculation and all the statistical analysis were performed with R (version  
268 3.4.2.) (R Core Team, 2018). The relationships between the total concentrations of MEs in the  
269 soils, the SET or ERITME, ERITPAH and ERITPCB indexes, were assessed by univariate  
270 linear regressions, with data transformed by  $\log_{10}(x+1)$ . The assessment of the relationships  
271 between the total concentrations of the MEs in soil and the excess of transfers ( $AQ>1$ ) was  
272 performed by univariate linear regressions. The influence of soil physico-chemical parameters  
273 on  $AQ>1$  was modelled with multiple linear regressions. For each set of regressions, the best  
274 model (*i.e.*, the one providing the best adjusted coefficient of determination with the lowest  
275 number of independent variables) was chosen using the corrected Akaike criterion (AICc)  
276 (Burnham and Anderson, 2004). For statistical robustness, these assessments of the influence  
277 of soil physico-chemical parameters on anomalous transfers have been modelled only for  
278 compounds that have at least 10 soils for which  $AQ>1$ .

279

280 **3. Results and Discussion**

281 **3.1. *Ex situ* threshold guide values**

282 The *ex situ* TGV based on the concentrations in the snails viscera exposed to uncontaminated  
283 soils (**Figure 2**) are presented in **Table 1**. The *ex situ* TGVs are benchmarks for assessing the  
284 environmental bioavailability of contaminants in soils to snails. The *ex situ* TGVs for the MEs  
285 proposed in this study have the same order of magnitude as those of the CiRefs *in situ*  
286 developed by Pauget and de Vaufleury (2015) for 14 MEs. There are some differences for a

287 few MEs, such as Zn (*in situ* and *ex situ*: 1490 and 581 mg.kg<sup>-1</sup> dw, respectively), which may  
288 be the result of the influence of the vegetation or humus available for snails *in situ* and the  
289 relatively low content of this element in the commercial food (Helixal®) used for the growth  
290 of the snails (182 and 51.6 mg Zn.kg<sup>-1</sup> dw in 2013 and in 2017, respectively). These results  
291 underline the influence of ME content in the food used for snail rearing (Gomot and Pihan,  
292 1997) and the need to check the concentrations in the control snails before exposure (**Table 1**,  
293 Ct0). These new *ex situ* TGVs, developed with the same methodology as those used for the  
294 CiRefs *in situ* for land snails (Pauget and de Vaufleury, 2015), characterize two additional  
295 MEs, Se and Mn. Having information on these elements is essential, as Se can be responsible  
296 for soil pollution caused by the coal industry (Huang et al., 2009), and Mn pollution  
297 frequently occurs as a result of past and present activities at mining and smelting sites  
298 (Petitjean et al., 2016). This study provided also new data for organic compounds frequently  
299 found in contaminated soils, but for which no guidance values for gastropods were known due  
300 to the difficulty of extracting and analysing PAHs and PCBs with low QLs in such complex  
301 biological matrices. Concerning the PAHs (except for NAP) and PCBs, the uncontaminated  
302 soils contained only traces of these compounds, some of which were not quantifiable.  
303 Therefore, snails exposed to these soils did not accumulate enough contaminants to allow the  
304 calculation of the *ex situ* TGVs in the same way as for the MEs. Thus, the proposed *ex situ*  
305 TGVs for the PAHs (except for NAP) and PCBs were assimilated to the QL to avoid false  
306 positives, even though the concentrations of contaminants could potentially be weaker.  
307 Further technical improvements would allow a decrease in the QL and thus a more refined  
308 value of these TGVs.

### 309 **3.2. Application to contaminated soils**

310 The *ex situ* TGVs were used to assess the environmental risk of soils contaminated by  
311 MEs with the SET index and ERITME index (**Table 2**). The AQ calculated with the *ex situ*  
312 TGVs identifies the contaminants that are accumulated in excess in snails (Pauget et al.,  
313 2013). The highest AQs were found for As, Cd, Hg, Pb, Sb, and Sn, with maximal values of  
314 343, 59.5, 15.2, 360, 211 and 39.2, respectively (**Tables 2A and S5**). Consequently, these  
315 contaminants are the principal drivers of the SET index in this study, with a maximal value of  
316 608 (L3 soil). Associating the AQ with the TP of each ME allows the estimation of  
317 environmental risk (RC) according to the toxicity of the transferred ME. The highest RCs  
318 were again for As, Pb, Cd, Hg, Sb and Sn, with maximal values of 205200, 143600, 23400,  
319 8520, 2100, and 2025, respectively, and a maximal value of the ERITME index of 230664  
320 (L3 soil) (**Tables 2B and S5**). The relationships between the sum of the 15 MEs in the soils  
321 and the SET or ERITME indexes are presented in **figure 3** and have  $r^2$  adj. values for the  
322 linear regression models of 0.560 and 0.430, respectively. Half of the variance in the AQ is  
323 explained by the total concentration of MEs in the soils. The other part of the variance can be  
324 explained by the physicochemical properties of the soils and biotic factors (Pauget et al.,  
325 2013). The coefficient of determination ( $r^2$  adj.) better explains (+0.13) the relations of the  
326 sum of the 15 MEs in the soils with the SET index values than with the ERITME index  
327 values. This is in accordance with the low accumulation (high excretion) and the low toxicity  
328 (low TP) of essential elements and with the high accumulation (sequestration and  
329 detoxification) and the high toxicity (high TP) of non-essential elements, such as Cd (Betzer  
330 and Pilson, 1975; Berger and Dallinger, 1989; Gomot and Pihan, 1997; Pauget et al., 2012).  
331 These results highlighted that the commonly used rule based on “ the highest contaminated  
332 soil presents the highest risk” can lead to misinterpretation of risks, notably when considering  
333 the numerous SET/ERITME values that were null or close to 0 for effectively contaminated  
334 soils (**Figure 3**). For each soil, the values of SET and ERITME were not driven by the same

335 ME; for example, for O15, the SET is mainly influenced by Cr and the ERITME, mainly by  
336 Cd and Pb, due to the value of their TP. The AQ, SET index and risk index (ERITME)  
337 showed the environmental bioavailability to snails of the contaminants in the soils  
338 investigated.

339 The new *ex situ* TGVs for PAHs and PCBs have been used to assess *ex situ* the environmental  
340 risk of soils moderately contaminated by PAHs and PCBs with the SET PAH and ERITPAH  
341 indexes (**Table 3**, data presented only for soils causing a SET PAH and ERITPAH > 0) and  
342 SET PCB and ERITPCB indexes (**Table 4**). With the studied contaminated soils by PAHs, the  
343 results mainly evidenced a CHY bioavailability to snails. No PAH transfers have been  
344 detected for the G1 soil, which contains the highest PAH concentrations, possibly because of  
345 its high organic matter content (175 mg.kg<sup>-1</sup>) and the ageing of contamination, as described by  
346 [Vasseur et al. \(2008\)](#) and [Spasojevic et al. \(2017\)](#). This could explain the highest value  
347 reached by the SET-PAH index for the K1 soil (*i.e.*, 4.6) whose OM content is six times lower  
348 than the G1 soil. For the PCBs, the N2 soil is highly contaminated (the sum of the PCBs in  
349 the soil is 34.9 mg.kg<sup>-1</sup> and the SGV is 1 mg.kg<sup>-1</sup>), and the PCB with the highest transference  
350 is PCB153 (AQ = 5.05). This result reveals the bioavailability of PCBs in this soil, notably  
351 PCB153, which is often bioaccumulated in biota ([Atuma et al., 1996](#); [Danis et al., 2006](#)).  
352 These results also showed that the *ex situ* TGVs developed in this study are relevant for  
353 assessing the environmental bioavailability by bioaccumulation measurements of MEs, PAHs  
354 and PCBs over 28 days in low contaminated soils.

355 Contaminated soils are often polycontaminated; therefore, this study developed GSET and  
356 GERITOXE indexes for an integrated risk assessment. GSET and GERITOXE provide an  
357 overall picture of the risk resulting of the contamination of the environment. On the basis of  
358 the SET and ERIT- indexes calculated for each soil and each family of contaminants

359 analysed, GSET and GERITOXE were determined (*Table 5*). These calculations are  
360 presented for the soils for which data were available for ME and PAH (*Table 5*). The GSET  
361 and GERITOXE values vary according to the bioavailable compounds to snails as found for  
362 example for soils G2 and H2. For these soils the total concentrations of MEs (and PAHs)  
363 were 1007 – (3.56) and 985 – (0.204 mg.kg<sup>-1</sup>) respectively, whereas their GSET equal 2.1 vs  
364 14.6 and their GERITOXE equal 374 vs 4751 showing that similar ME-PAH soil  
365 contamination leads to contrasted level of risk. In term of the total contamination G2 soil was  
366 the most contaminated of the two soils, but in term of risk, according to the values of GSET  
367 and GERITOXE, H2 soils was the most risked soils (*Table 5*). These findings evidence  
368 reflects that the highest contaminated soils are not necessarily those showing the highest  
369 environmental risk.

370 Our original approach also provides global indexes useful for multicontaminated sites (SET  
371 and GERITOXE) which is not possible with the ratio risk (PEC/PNEC) or the SSD methods.  
372 Finally, these indexes are the same than those used in human health risk assessments and pave  
373 the way to develop integrative methodologies that conciliate ERA and human health risk  
374 assessment ([Louzon et al., 2020](#)), such as the ISS (interpretation of the state of soils or IEM  
375 for the French acronym), which combines the assessments of exposure and the toxicological  
376 risk ([Mossmann, 2010](#)). Results of the “TGV-AQ-GSET-GERITOXE-*ex situ* method” will be  
377 helpful to orientate the further use of the excavated soils (depending on the level of risk  
378 identified: eliminated as a waste, or need to be covered, or submitted to soil remediation  
379 techniques, or used for renaturation of sites...). In the latter case, this can, for example, limit  
380 the environmental disturbances and the cost associated to the excavation of soils.

### 381 **3.3. Influence of soil physico-chemical properties on the accumulation quotient**

382 The influence of soil physico-chemical properties on the accumulation quotient (AQ>1),  
383 which characterized the excess of transfer of contaminants to snails, was assessed by i)  
384 univariate linear regressions with concentrations of the compounds in the soils and by ii)  
385 multivariate linear regressions, by taking into consideration all of the physico-chemical  
386 properties that have been summarized in **Figure 1**. First, the results presented in **Table 6**  
387 showed that anomalous transfers were mainly related to the total concentrations of  
388 metal(loid)s in the soils, with  $r^2$  adj. for significant models of 0.56, 0.78, 0.63, 0.72 and 0.56  
389 for As, Cd, Cr, Pb and Sb, respectively. The regressions were improved by taking account the  
390 physico-chemical characteristics of soils, only for Cd and Zn with  $r^2$  adj. increased up to 0.88  
391 and 0.34, respectively. The models underline the modulating influence of Al/Fe oxides and  
392 clay in relation with their impact on ME mobility in soils (adsorptions/desorptions) ([Gadde](#)  
393 [and Laitinen, 1974](#); [Violante et al., 2003](#); [Hamid et al., 2019](#)) and transfers to snails ([Pauget et](#)  
394 [al., 2013](#)). When the concentrations of the exposure were quite low, *i.e.*, close to those of the  
395 pedogeochemical background, the influence of soil properties (pH, OM content, oxides) has  
396 been identified by multiple linear regressions for non-anomalous transfers of MEs (AQ<1)  
397 (**Table S5**). However, when the concentration of MEs increases in the soil, the influence of  
398 the soil physico-chemical properties is less significant. The anomalous transfers (AQ>1) were  
399 consequently driven by the total concentration of MEs in the soils (**Table 6**).

400 Finally, the interest of the methodology developed in this paper with *ex situ*-TGV to assess  
401 the bioavailability is to consider the influence of physico-chemical properties of the soils and  
402 the specificity of each sites (*e.g.*, ageing of the contamination) on the exposure of organisms,  
403 to estimate real risks. To estimate the exposure of organisms and consequently to take in  
404 consideration the specificity of each site, bioavailability can be assessed with the method  
405 presented here and the determination of accumulation quotient (AQ). Then using toxicity

406 point (TP) allows to estimate the resulting risk coefficient (RC) for each compounds (Pauget  
407 and de Vaufleury, 2015).

408

#### 409 **4. Conclusion**

410 Studies based on threshold values and bioaccumulation measurements used to assess the  
411 fate and impact of contamination have been performed by *in situ* caging of invertebrates in  
412 terrestrial (*Cantareus aspersus*: Paugé and Vaufleury, 2015) and aquatic (*Gammarus*  
413 *fossarum*: Besse et al., 2013; Ciliberti et al., 2017) environments. Although these studies are  
414 relevant in biomonitoring, they are currently subjected to the influence of pedoclimatic  
415 conditions and specificity of sites (*e.g.*, plants and humus). Currently, the use of guide values  
416 and indexes of risk, such as TGV, SET and GERITOXE (ERITME, ERITPAH and  
417 ERITPCB), in ecotoxicology, initially developed *in situ* for snails, is of increasing interest, as  
418 evidenced by the recent application to earthworms (Coehlo et al., 2018; Coehlo, 2019). The  
419 interest of the present study is to propose guidance values for an *ex situ* bioassay (TGVs)  
420 using snails to assess the environmental risks, *e.g.*, of excavated contaminated soils. The *ex*  
421 *situ* TGVs determined in this study provide new and essential data (the GSET and  
422 GERITOXE indexes) for assessing the *ex situ* environmental risks of soil contamination by 15  
423 MEs, 16 PAHs and 7 PCBs and preventing misinterpretation during ERAs according to the  
424 bioavailability concept. Considering AQ instead of raw/absolute internal concentrations in  
425 organisms account for the specific ability of an organism to concentrate some compounds.  
426 Thus, misinterpretation of the transfer of the compounds from soil to biota is limited. This  
427 study provides original knowledge on the influence of the physico-chemical properties of the  
428 soil on contamination transfer to biota which was found to be variable depending on the level  
429 of the bioavailable concentration of metal(loid)s in soils. Indeed, when considering  $AQ > 1$ , a

430 low incidence of physico-chemical properties of soils has been demonstrated on the excess of  
431 transfers to organisms. Our objective was to propose the first TGVs for organic pollutants in a  
432 terrestrial organism that is currently used mainly as a bioindicator of ME accumulation. These  
433 TGVs showed bioavailability of PAHs and PCBs (mainly CHY and PCB153 in the panel of  
434 soils in this study) in contaminated soils. To help the stakeholders of polluted soils in their  
435 make management, *ex situ* TGVs for snails are a basis for interpreting contamination levels:  
436 when *ex situ* TGVs are exceeded, the contaminant is bioavailable, and therefore, soil  
437 contamination should be managed. The presented results also provide a basis for prioritizing  
438 the management of contaminated soils for limiting the impact on the environment and by  
439 considering the risk of subsequent management measures (*e.g.*, soil excavation and  
440 embankment use).

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450

## 451 **7. Conflict of interest**

452 The authors declare no conflict of interest.

453

## 454 **8. Supplementary data**

455 The supplementary data associated with this article can be found in the online version.

456

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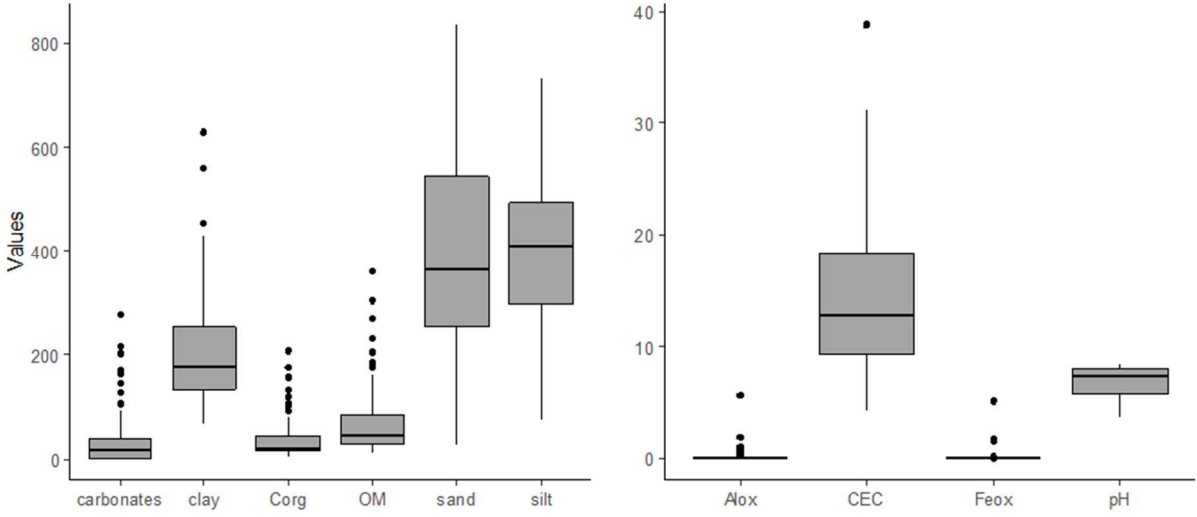
**Figures:**

**Figure 1: Diversity of the physico-chemical parameters of the soils** (clay, silt, sand, OM, Corg, and CaCO<sub>3</sub> are in g.kg<sup>-1</sup>; CEC, Alox and Feox are in cmol.kg<sup>-1</sup>).

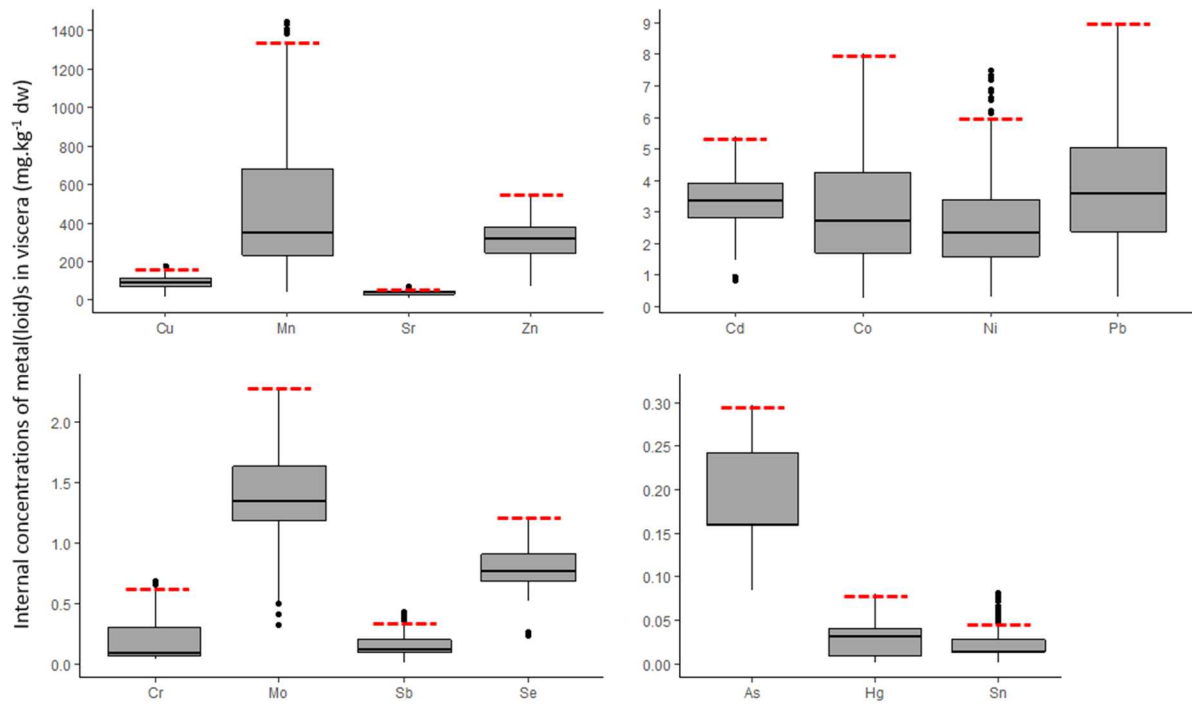
**Figure 2: Concentrations of the MEs in the viscera of snails exposed to uncontaminated soils for the determination of the ex situ TGVs** (in mg.kg<sup>-1</sup> dw; TGVs are shown by the dotted red lines).

**Figure 3: Relations between the sum of the concentration of the fifteen MEs in the soils (mg.kg<sup>-1</sup> dw) and SET or ERITME index values** (data are transformed by log<sub>10</sub>(x+1)).

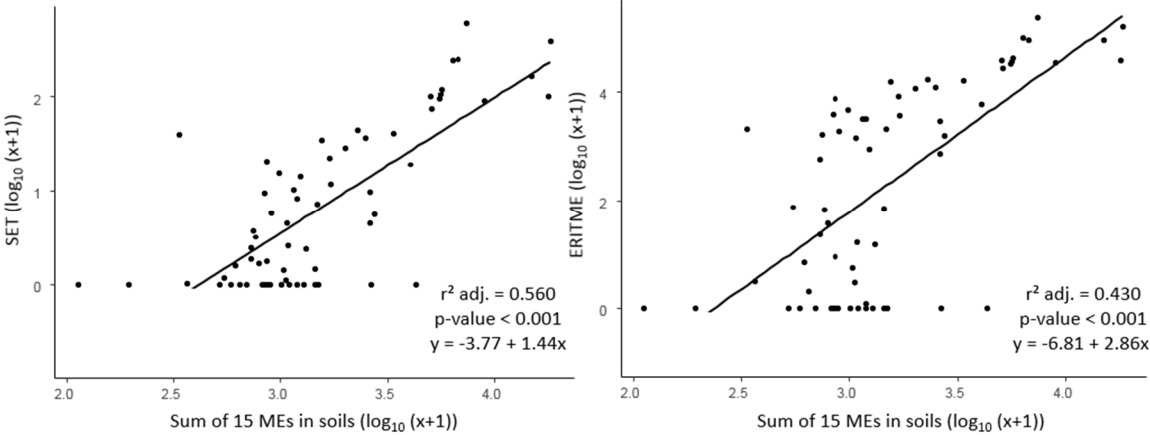
**Figure 1: Diversity of the physico-chemical parameters of the soils** (clay, silt, sand, OM, Corg, and  $\text{CaCO}_3$  are in  $\text{g.kg}^{-1}$ ; CEC, Alox and Feox are in  $\text{cmol.kg}^{-1}$ ).



**Figure 2: Concentrations of the MEs in the viscera of snails exposed to uncontaminated soils for the determination of the ex situ TGVs (in  $\text{mg}\cdot\text{kg}^{-1}\text{ dw}$ ; TGVs are shown by the dotted red lines).**



**Figure 3: Relations between the sum of the concentration of the fifteen MEs in the soils (mg.kg<sup>-1</sup> dw) and SET or ERITME index values (data are transformed by log<sub>10</sub>(x+1)).**



## Tables

**Table 1: Soil guide values (SGVs) in the soils; toxicity points (TPs) for each contaminant; concentrations of the contaminants in the visceral mass before exposure (Ct0, n=12), in lettuce, and in Helixal®; and transfer guide values (TGVs) calculated from the visceral mass of *Cantareus aspersus* sub-adults after 28 days of exposure to uncontaminated soils** (Sources for SGVs are 1: [Pauget and de Vaufleury \(2015\)](#), 2: [Baize \(2000\)](#), 3: [Villanneau et al. \(2008\)](#), 4: [ADEME \(2018\)](#)). The toxicity points used are from [ATSDR \(2015\)](#). The Ct0 are median (min|max) for MEs and mean  $\pm$  standard deviation pour PAHs and PCBs. The concentrations and TGVs for the ME are in  $\text{mg.kg}^{-1}$  dry weight (dw) and those for the PAHs and the PCBs are in  $\mu\text{g.kg}^{-1}$  fresh mass (fm) pooled from two individuals).

**Table 2: (A)-Accumulation quotient (AQ) and SET index and (B)-Risk coefficient (RC) and ERITME index based on the concentration of metallic elements (MEs) in the visceral mass of snails after 28 days of exposure to soils. Color gradient from green to red as function of the levels of the transfers and risks.** Detailed results for all soils are presented in table S5.

**Table 3: Accumulation quotient (AQ) | risk coefficient (RC) and SET | ERITPAH indexes based on the visceral mass of snails after 28 days of exposure to polycyclic aromatic hydrocarbons (PAHs)** (the bold values are  $>1$  for AQ,  $>5$  for SET,  $>0$  for RC and  $> 1000$  for ERITPAH, only data for soils for which a SET PAH and ERITPAH  $> 0$  are presented in this table ).

**Table 4: Accumulation quotient (AQ) | risk coefficient (RC) and SET | ERITPCB indexes based on the visceral mass of snails after 28 days of exposure to polychlorinated biphenyl indicators (PCBs)** (the bold values are  $>1$  for AQ,  $>5$  for SET,  $>0$  for RC and  $> 1000$  for ERITPCB).

**Table 5: Total concentrations of the contaminants in the soils ( $\text{mg.kg}^{-1}$  dw), global sum of the excess of transfer (GSET) and global evaluation of the risk of the toxicological elements (GERITOXE) in soils** (na: data not available. The color gradient varies from green to red according to the value of the ME and GERITOXE indices).

**Table 6: Influence of total metal(loid) concentrations and soil parameters on the accumulation quotient (AQ $>1$ )** (significant variables are ranked with stars as a function of the p-value obtained: \*  $< 0.05$ , \*\*  $< 0.01$ , \*\*\*  $< 0.001$ ; the data are transformed by  $\log_{10}(x + 1)$ ; N=number of soils assessed in this study with AQ $>1$ ).

**Table 1: Soil guide values (SGVs) in the soils; toxicity points (TPs) for each contaminant; concentrations of the contaminants in the visceral mass before exposure (Ct0, n=12), in lettuce, and in Helixal®; and transfer guide values (TGVs) calculated from the visceral mass of *Cantareus aspersus* sub-adults after 28 days of exposure to uncontaminated soils** (Sources for SGVs are 1: [Pauget and de Vaufleury \(2015\)](#), 2: [Baize \(2000\)](#), 3: [Villanneau et al. \(2008\)](#), 4: [ADEME \(2018\)](#)). The toxicity points used are from [ATSDR \(2015\)](#). The Ct0 are median (min|max) for MEs and mean ± standard deviation pour PAHs and PCBs. The concentrations and TGVs for the ME are in mg.kg<sup>-1</sup> dry weight (dw) and those for the PAHs and the PCBs are in µg.kg<sup>-1</sup> fresh mass (fm) pooled from two individuals).

Contaminants		SGV	TP	Concentrations	Concentrations	Concentrations	TGV
		(mg.kg <sup>-1</sup> dw)		in snails (Ct0)	in lettuce	in Helixal®	
ME	As	25 <sup>2</sup>	600	0.11 (0.09 0.13)	0.13	0.72	0.364
	Cd	0.45 <sup>2</sup>	400	1.49 (1.08 1.62)	0.65	0.43	5.60
	Co	23 <sup>2</sup>	400	1.22 (0.90 1.76)	0.07	0.66	8.12
	Cr	90 <sup>2</sup>	10	0.93 (0.66 3.66)	0.05	7.77	0.653
	Cu	20 <sup>2</sup>	10	55.9 (34.9 84.4)	10.1	7.99	173
	Hg	0.1 <sup>2</sup>	600	0.02 (0.01 0.03)	0.01	0.49	0.089
	Mn	754 <sup>2</sup>	10	208 (80.7 348)	46.4	69.5	1355
	Mo	1.59 <sup>3</sup>	53	2.76 (1.85 3.38)	0.21	1.01	2.31
	Ni	60 <sup>2</sup>	178	5.11 (3.63 7.42)	0.37	3.10	6.10
	Pb	62.3 <sup>3</sup>	400	0.42 (0.26 1.21)	0.11	0.78	9.06

	<b>Sb</b>	<b>2.01<sup>1</sup></b>	<b>10</b>	0.05 (0.04 0.08)	0.12	0.07	<b>0.360</b>
	<b>Se</b>	<b>0.70<sup>2</sup></b>	<b>178</b>	1.51 (1.18 1.80)	0.05	0.11	<b>1.25</b>
	<b>Sn</b>	<b>4.31<sup>1</sup></b>	<b>53</b>	0.04 (0.01 0.07)	0.04	0.02	<b>0.048</b>
	<b>Sr</b>	<b>224<sup>1</sup></b>	<b>1</b>	28.0 (17.8 39.5)	27.0	223	<b>71.0</b>
	<b>Zn</b>	<b>100<sup>2</sup></b>	<b>53</b>	459 (298 634)	37.0	51.6	<b>581</b>
<b>PAH</b>	<b>NAP</b>	<b>0.11<sup>4</sup></b>	<b>178</b>	21.0 ± 6.10	<1.00	<1.00	<b>26.1</b>
	<b>ACY</b>	<b>0.23<sup>4</sup></b>	<b>10</b>	<16.9	<1.00	<1.00	<b>16.9</b>
	<b>ACE</b>	<b>0.11<sup>4</sup></b>	<b>178</b>	<13.7	<1.00	<1.00	<b>13.7</b>
	<b>FLU</b>	<b>0.11<sup>4</sup></b>	<b>10</b>	<6.45	<1.00	<1.00	<b>6.45</b>
	<b>PHE</b>	<b>0.40<sup>4</sup></b>	<b>10</b>	6.88 ± 5.95	<1.00	<1.00	<b>4.20</b>
	<b>ANT</b>	<b>0.60<sup>4</sup></b>	<b>10</b>	<9.63	<1.00	<1.00	<b>9.63</b>
	<b>FLT</b>	<b>1.22<sup>4</sup></b>	<b>178</b>	6.27 ± 2.96	<1.00	<1.00	<b>1.92</b>
	<b>PYR</b>	<b>1.02<sup>4</sup></b>	<b>10</b>	8.72 ± 5.03	<1.00	<1.00	<b>1.83</b>
	<b>BaANT</b>	<b>0.63<sup>4</sup></b>	<b>400</b>	<1.44	<1.00	<1.00	<b>1.44</b>
	<b>CHY</b>	<b>0.71<sup>4</sup></b>	<b>178</b>	3.99 ± 1.18	<1.00	<1.00	<b>1.47</b>

	<b>BbFLT</b>	<b>0.91<sup>4</sup></b>	<b>600</b>	<2.28	<1.00	<1.00	<b>2.28</b>
	<b>BkFLT</b>	<b>0.40<sup>4</sup></b>	<b>400</b>	<2.01	<1.00	<1.00	<b>2.01</b>
	<b>BaPYR</b>	<b>0.75<sup>4</sup></b>	<b>600</b>	<6.45	<1.00	<1.00	<b>6.45</b>
	<b>dBahANT</b>	<b>0.34<sup>4</sup></b>	<b>600</b>	<4.08	<1.00	<1.00	<b>4.08</b>
	<b>BghiPL</b>	<b>0.56<sup>4</sup></b>	<b>10</b>	<5.31	<1.00	<1.00	<b>5.31</b>
	<b>IcdPYR</b>	<b>0.58<sup>4</sup></b>	<b>178</b>	<5.25	<1.00	<1.00	<b>5.25</b>
	<b>PCB28</b>	<b>0.024<sup>4</sup></b>		<0.16	<0.16	<0.16	<b>0.12</b>
	<b>PCB52</b>	<b>0.024<sup>4</sup></b>		<0.26	<0.26	<0.26	<b>0.18</b>
	<b>PCB101</b>	<b>0.022<sup>4</sup></b>		<0.27	<0.27	<0.27	<b>0.18</b>
<b>PCB</b>	<b>PCB118</b>	<b>0.022<sup>4</sup></b>	<b>10</b>	<0.28	<0.28	<0.28	<b>0.24</b>
	<b>PCB138</b>	<b>0.026<sup>4</sup></b>		<0.57	<0.57	<0.57	<b>0.51</b>
	<b>PCB153</b>	<b>0.026<sup>4</sup></b>		<0.42	<0.42	<0.42	<b>0.36</b>
	<b>PCB180</b>	<b>0.022<sup>4</sup></b>		<0.58	<0.58	<0.58	<b>0.23</b>

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38 **Table 2: (A)-Accumulation quotient (AQ) and SET index and (B)-Risk coefficient (RC) and ERITME index based on the concentration of metallic**  
 39 **elements (MEs) in the visceral mass of snails after 28 days of exposure to soils. Color gradient from green to red as function of the levels of the**  
 40 **transfers and risks. Detailed results for all soils are presented in table S5.**

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(A)	AQ															
Soils	As	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	Zn	SET
A1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
B2	1	1	1	2.60	1	1	1	1	1	1	1	1	1	1	1	1.60
C3	1	1	1.16	1	1	1	1.30	1	1	1	1	1	1	1	1	0.46
H2	7.99	1.26	1	2.24	1.02	1.16	1	1	1.25	1	1	1	6.65	1	1	14.6
L2	9.67	9.86	1	1	1	2.25	1	1	1	74.8	6.33	1.13	2.10	1	1.60	99.8
L3	343	2.05	1	1	1	9.39	1	1	1	45.4	211	1	4.13	1	1	608
M2	22.7	1	1	1	1	1.2	2.06	1	1	360	1.57	1	1	1	1	382
O1	1	1	1	1.16	1	1.62	1	1	1	9.34	1.32	1	1.90	1	1.44	10.8
O2	1	1.01	1	1	1	1	1	1.10	1	4.78	1	1	1.46	1	1.37	4.72
O4	3.54	10.7	1	1	1	6.26	1	1	1	72.0	13.4	1.15	1	1	2.67	102
O15	1.32	1.08	1	11.7	1	1	1	1	1	2.32	1.43	1	1.08	1	1.26	13.2
O18	3.82	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2.82
O24	2.28	2.21	1	1.07	1	1.75	1	1	1.05	3.93	1.70	1	1.33	1	2.51	8.84
O25	1.53	1	1	1	1	1	1	1	1	1.62	1	1	1.29	1	1	1.44
O30	3.19	3.64	1	1	1	1	1	1	1	80.6	1.17	1	3.48	1	3.61	89.7

(B)	RC																
	Soils	As	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	Zn	ERITME
A1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B2	0	0	0	16.0	0	0	0	0	0	0	0	0	0	0	0	0	16.0
C3	0	0	64.0	0	0	0	3.00	0	0	0	0	0	0	0	0	0	67.0
H2	4194	104	0	12.4	0.200	96.0	0	0	44.5	0	0	0	300	0	0	0	4751
L2	5202	3544	0	0	0	750	0	0	0	29520	53.3	23.1	58.3	0	31.8	0	39183
L3	205200	420	0	0	0	5034	0	0	0	17744	2100	0	166	0	0	0	230664
M2	13020	0	0	0	0	120	10.6	0	0	143600	5.70	0	0	0	0	0	156757
O1	0	0	0	1.60	0	372	0	0	0	3336	3.20	0	47.7	0	23.3	0	3784
O2	0	4.00	0	0	0	0	0	5.30	0	1512	0	0	24.4	0	19.6	0	1565
O4	1524	3880	0	0	0	3156	0	0	0	28400	124	26.7	0	0	88.5	0	37200
O15	192	32.0	0	107	0	0	0	0	0	528	4.30	0	4.24	0	13.8	0	881
O18	1692	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1692
O24	768	484	0	0.700	0	450	0	0	8.90	1172	7.00	0	17.5	0	80.0	0	2988
O25	318	0	0	0	0	0	0	0	0	248	0	0	15.4	0	0	0	581
O30	1314	1056	0	0	0	0	0	0	0	31840	1.70	0	131	0	138	0	34482

44 **Table 3: Accumulation quotient (AQ) | risk coefficient (RC) and SET | ERITPAH indexes based on the visceral mass of snails after 28 days of**  
 45 **exposure to polycyclic aromatic hydrocarbons (PAHs)** (the bold values are >1 for AQ, >5 for SET, >0 for RC and > 1000 for ERITPAH, only data for soils  
 46 for which a SET PAH and ERITPAH > 0 are presented in this table ).

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AQ   RC																	
Soil	NA	AC	AC	FL	PH	AN		PY	BaAN		BbFL		BaPY	dBahAN	BghiP	IcdPY	SET   ERITPAH
s	P	Y	E	U	E	T	FLT	R	T	CHY	T	BkFLT	R	T	L	R	H
<b>G2</b>	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	<b>3.1 37</b>	1 0	1 0	1 0	1 0	1 0	1 0	<b>2.1 374</b>
										<b>4</b>							
<b>K1</b>	1 0	1 0	1 0	1 0	1 0	1 0	<b>1.2 35.</b>	1 0	<b>1.3 12</b>	<b>4.8 67</b>	1 0	<b>1.3 12</b>	1 0	1 0	1 0	1 0	<b>4.6 952</b>
							<b>6</b>		<b>0</b>	<b>6</b>		<b>0</b>					
<b>L2</b>	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	<b>2.6 28</b>	1 0	1 0	1 0	1 0	1 0	1 0	<b>1.6 285</b>
										<b>5</b>							
<b>L4</b>	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	<b>1.1 17.</b>	1 0	1 0	1 0	1 0	1 0	1 0	0.1 17.8
										<b>8</b>							
<b>O1</b>	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	<b>2.2 21</b>	1 0	1 0	1 0	1 0	1 0	1 0	<b>1.2 214</b>
										<b>4</b>							

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52 **Table 4: Accumulation quotient (AQ) | risk coefficient (RC) and SET | ERITPCB indexes based on the visceral mass of snails after 28 days of exposure**  
 53 **to polychlorinated biphenyl indicators (PCBs)** (the bold values are >1 for AQ, >5 for SET, >0 for RC and > 1000 for ERITPCB).

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AQ   RC								
Soils	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	SET   ERITPCB
<b>C2</b>	1   0	1   0	1   0	1   0	1   0	1   0	1   0	0   0
<b>C3</b>	1   0	1   0	1   0	1   0	1   0	1   0	1   0	0   0
<b>C4</b>	1   0	1   0	1   0	1   0	1   0	1   0	1   0	0   0
<b>C5</b>	1   0	1   0	1   0	1   0	1   0	1   0	1   0	0   0
<b>C6</b>	1   0	1   0	1   0	1   0	1   0	1   0	1   0	0   0
<b>C7</b>	1   0	1   0	1   0	1   0	1   0	1   0	1   0	0   0
<b>N2</b>	1   0	1   0	1   0	<b>4.96   49.6</b>	1   0	<b>5.05   50.5</b>	1   0	<b>8.01   80.1</b>

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**Table 5: Total concentrations of the contaminants in the soils (mg.kg<sup>-1</sup> dw), global sum of the excess of transfer (GSET) and global evaluation of the risk of the toxicological elements (GERITOXE) in soils (na: data not available. The color gradient varies from green to red according to the value of the ME and GERITOXE indices).**

Soils	[ME]soils	[PAH]soils	[PCB]soils	SET ME	SET PAH	SET PCB	GSET	ERITME	ERITPAH	ERITPCB	GERITOXE
J1	112	0.044	na	0	0	na	0	0	0	na	0
I1	192	0.427	na	0	0	na	0	0	0	na	0
E2	586	0	na	0	0	na	0	0	0	na	0
F1	688	0.745	na	0	0	na	0	0	0	na	0
A1	810	0.205	na	0	0	na	0	0	0	na	0
A2	844	0.270	na	0	0	na	0	0	0	na	0
A3	871	0.502	na	0	0	na	0	0	0	na	0
A4	880	0.572	na	0	0	na	0	0	0	na	0
C5	1003	0.059	0.001	0	0	0	0	0	0	0	0
C7	1089	0.315	0.001	0	0	0	0	0	0	0	0
C6	1184	2.54	0.001	0	0	0	0	0	0	0	0
D2	1280	0.622	na	0	0	na	0	0	0	na	0
C4	1432	0.099	0.002	0	0	0	0	0	0	0	0
C2	1493	0.063	0.001	0	0	0	0	0	0	0	0
C1	2640	0.190	0.001	0	0	0	0	0	0	0	0
B3	1194	0.081	na	0.02	0	na	0.02	0.20	0	na	0.20
H1	643	0.121	na	0.02	0	na	0.02	1.06	0	na	1.06
F3	1036	0.275	na	0.45	0	na	0.45	4.50	0	na	4.50
E1	614	0.049	na	0.61	0	na	0.61	6.10	0	na	6.10
B1	854	1.43	na	0.79	0	na	0.79	7.90	0	na	7.90
D1	1302	0.518	na	1.38	0	na	1.38	13.8	0	na	13.8
B2	1078	0.058	na	1.60	0	na	1.60	16.0	0	na	16.0
I2	718	0.154	na	0.89	0	na	0.89	22.2	0	na	22.2
F2	753	1.30	na	2.24	0	na	2.24	66.0	0	na	66.0

C3	1445	0.083	0.001	0.46	0	0	0.46	67.0	0	0	67.0
G1	544	11.3	na	0.19	0	na	0.19	76.0	0	na	76.0
G2	1007	3.56	na	0	2.1	na	2.1	0	374	na	374
K1	4295	4.00	na	0	4.6	na	4.6	0	952	na	952
L1	1141	1.03	na	9.36	0	na	9.36	3232	0	na	3232
O1	1698	7.16	na	10.8	1.2	na	12	3784	214	na	3998
H2	985	0.204	na	14.6	0	na	14.6	4751	0	na	4751
L2	4989	1.96	na	99.8	1.6	na	101	39183	285	na	39468
L4	14916	9.97	na	166	0.1	na	166	89384	17.8	na	89402
L3	7389	2.86	na	608	0	na	608	230664	0	na	230664

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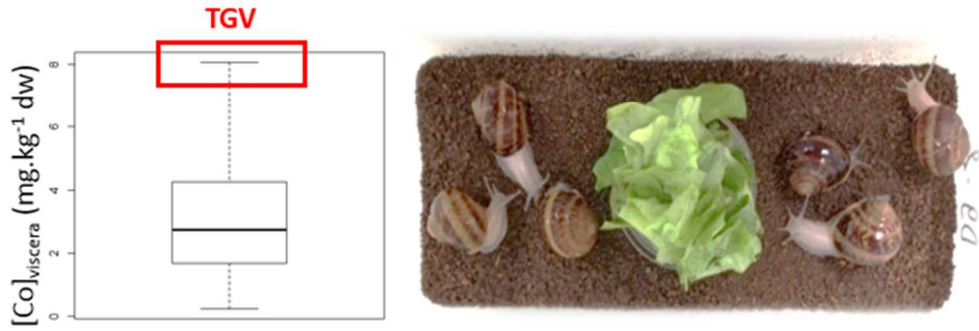
79 **Table 6: Influence of total metal(loid) concentrations and soil parameters on the accumulation quotient (AQ>1)** (significant variables are ranked with  
80 stars as a function of the p-value obtained: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001; the data are transformed by  $\log_{10}(x + 1)$ ; N=number of soils assessed in this study  
81 with AQ>1).

Elements	Equations	N	adj. r <sup>2</sup>	p-value
<b>As</b>	$AQ = -0.333 + 0.700[As]_{soil}^{***}$	23	0.564	< 0.001
	AQ = no influence of soil parameters			
<b>Cd</b>	$AQ = 0.034 + 0.678[Cd]_{soil}^{***}$	24	0.782	< 0.001
	$AQ = -0.563^* + 0.626[Cd]_{soil}^{***} - 2.15[Alox]^{**} + 0.567[clay]^{**}$			
<b>Cr</b>	$AQ = -1.03^* + 0.895[Cr]_{soil}^*$	11	0.631	0.002
	AQ = no influence of soil parameters			
<b>Hg</b>	$AQ = 0.458^{***} + 0.266[Hg]_{soil}$	19	0.083	0.123
	AQ = no influence of soil parameters			
<b>Pb</b>	$AQ = -0.922^{***} + 0.746[Pb]_{soil}^{***}$	34	0.715	< 0.001
	AQ = no influence of soil parameters			
<b>Sb</b>	$AQ = 0.243^* + 0.393[Sb]_{soil}^{***}$	26	0.558	< 0.001
	AQ = no influence of soil parameters			
<b>Sn</b>	$AQ = 0.590^{***} + 0.393[Sn]_{soil}$	25	0.022	0.498
	AQ = no influence of soil parameters			
<b>Zn</b>	$AQ = 0.034 + 0.151[Zn]_{soil}$	21	0.133	0.058
	$AQ = -0.166 + 0.214[Zn]_{soil}^{**} + 18.6[Alox]^* - 72.6[Feox]^*$			

1 **Graphical abstract**

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***Ex situ* assessment of environmental risk of contaminated soils with land snails based on threshold guide values (TGV)**



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