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Bioaccessibility of metal(loid)s in soils to humans and their bioavailability to snails: a way to associate human health and ecotoxicological risk assessment?

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Abstract

Human health risk assessment (HHRA) and ecotoxicological risk assessment (ERA) of contaminated soils are frequently performed separately and based on total soil concentrations without considering the concepts of mobility, bioaccessibility and bioavailability. However, some **chemical and biological assays** rarely used in combination **can be applied** to more accurately assess the exposure of organisms to metal(loid)s and thus to better estimate the links between soil contamination and effects. For humans, the unified bioaccessibility method (UBM) assesses oral bioaccessibility, while for soil fauna such as land snails, the bioaccumulation test reflects the bioavailability of contaminants. **The aim of this study is to explore the relationship between oral bioaccessibility and the bioavailability of arsenic, cadmium and lead in twenty-nine contaminated soils. The results show a modulation of bioaccumulation and bioaccessibility of metal(loid)s by soil physicochemical parameters (organic matter especially). For the three metal(loid)s studied, strong relationships were modelled between the UBM and snail tests ($0.77 < r^2_{adj.} < 0.95$), depending on the parameters of the linear regressions (contaminant and phases of the UBM test). The original models proposed demonstrate the feasibility of linking bioaccessibility to humans and bioavailability to snails and the relevance of their association for an integrative risk assessment of contaminated soils.**

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Highlights:

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- Influence of soil parameters (*e.g.*, organic matter) on human oral bioaccessibility

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- Influence of soil parameters (*e.g.*, organic matter) on bioavailability to snails

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- Strong relationships between oral bioaccessibility and bioavailability to snails

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- Proposed models to link bioaccessibility to humans and bioavailability to snails

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57 1. Introduction

58 Human health risk assessment (HHRA) and ecotoxicological risk assessment (ERA) of
59 contaminated soils are challenging because they involve a mixture of scientific, regulatory and
60 budgetary requirements. In the current context of contaminated soils [1], HHRA and ERA are
61 often conducted independently and are mainly based on the measurement of total contaminant
62 concentrations [2,3]. Considering that the total amount of a contaminant in the soil is mobile
63 and available for organisms could lead to discrepancies between the real and estimated mobility
64 of contaminants. Indeed, contaminants could be highly mobile in slightly contaminated soil and
65 relatively low mobile in highly contaminated soil. To prevent misinterpretation, risk assessment
66 of contaminated soils should consider bioavailable concentrations, which may be modulated by
67 the physicochemical properties of soils (e.g., pH, clays content, and organic matter (OM)
68 content) [4-7]. In this context, bioavailability assessment based on oral bioaccessibility (i.e., the
69 fraction of an ingested contaminant that is soluble in digestive fluids) to humans and
70 bioaccumulation by soil organisms are relevant surrogates for evaluating the exposure of
71 organisms to contaminants in soils [8]. In addition, assessment of organism exposure can be
72 required for polluted soil management to determine the cause-and-effect relationships
73 (toxicological bioavailability) [7,8]. The oral bioaccessibility of contaminants in soils can be
74 assessed with *in vitro* assays [9,10], such as the unified bioaccessibility method (UBM) for
75 arsenic (As), cadmium (Cd) and lead (Pb) [11,12]. The principle of the UBM is to mimic the
76 human digestive process with chemical extractions simulating gastric and gastro-intestinal
77 phases [13]. The UBM has been shown to be relevant in estimating human exposure in various
78 contexts, such as mining soils [14], kitchen gardens and lawns [15], old town soils [16] and the
79 influence of earthworm bioturbation [17]. The bioavailability of contaminants in soils can also
80 be assessed using *ex situ* (e.g., in laboratory) bioaccumulation studies with bioindicators [8],
81 such as the terrestrial gastropod *Cantareus aspersus* [18-20]. This land snail integrates three
82 pathways of exposure: digestive (ingestion of soil particles, up to 40% to satisfy its
83 physiological needs), cutaneous (transfer from the soil to the snail foot) and pulmonary
84 (inhalation of soil particles) [20-24]. For this ubiquitous soil invertebrate, relationships between
85 total concentration, bioavailability, bioaccumulation, and the influence of various factors
86 (sources of exposure, physicochemical parameters of soils, etc.) have been investigated in
87 several studies, specifically for metallic elements in soils [22-25], making this organism a
88 relevant candidate to explore possible the convergence between HHRA and ERA.

89 Currently, HHRA and ERA approaches have rarely been conducted together on contaminated
90 soils to offer an integrative risk assessment despite the growing interest in associating them in
91 recent years [26,27]. Toxicological data for humans and ecotoxicological data are available but
92 seldom associated, except in safety data sheets for chemical compounds. Surprisingly, their
93 association for the evaluation of polycontaminated environmental matrices, *e.g.*, in an *a*
94 *posteriori* ERA approach, is infrequent [26]. However, such an association needs to be
95 developed for the preservation of both human and environmental health, *e.g.*, to promote the
96 One Health concept to assess the effects of multiple stressors at the human-animal-ecosystem
97 interface [28]. For the time being, integrative risk assessment has focused on a few
98 contaminants, such as nonylphenol [29], but rarely on polycontaminated matrices, as is often
99 the case with soils. However, some recent soil quality indices developed with land snails have
100 been shown to be relevant to highlighting the potential transfer of metals to living organisms,
101 including human populations [30]. The potential of such indices for the development of
102 methodologies for an integrative risk assessment of contaminated soils remains to be explored.
103 In this context, the aim of this study is to confront assessment methodologies for exposure to
104 metal(loid)s in soils for humans (UBM test) and for a soil fauna organism living at the air-soil-
105 plant interface (snail test). Using a wide range of soils, we also aim to estimate the influence of
106 the physicochemical parameters of soils (*e.g.*, texture, pH and OM content) on the metal(loid)s
107 in soils that can potentially be absorbed by humans (*i.e.*, bioaccessibility) and on the
108 metal(loid)s absorbed and accumulated in snails (*i.e.*, bioavailability).

109 2. Experimental

110 2.1. Soils

111 Twenty-nine soils were sampled from contaminated fields in France after humus
112 removal (A horizon) (Fig 1), and the samples were dried (<40°C) and sieved at 250 µm. The
113 physicochemical parameters were then measured with the appropriate ISO standard protocols
114 (Tab 1). The pH_{water} ranged from 4.3 to 8.3, and the OM contents ranged from 15.4 to 360 mg
115 kg⁻¹. The As, Cd and Pb concentrations were obtained by hot block system-assisted digestion
116 (Environmental Express® SC100, Charleston, SC, USA) and determined by inductively
117 coupled plasma mass spectrometry (ICP-MS, 7900, Agilent Technology, Les Ulis, France).
118 More specifically, 300 mg of soil sample was digested in a mixture of 1.5 mL HNO₃ (70%) and
119 4.5 mL HCl (37%) at 95°C for 1 h and 30 min. After mineralization, digestion products were
120 brought to 25 mL with ultrapure water (resistivity of 18.0 MΩ cm⁻¹) and stored at 4°C prior to

121 analysis. The recovery rates as determined with a certified standard (ERM CC141, Loam soil,
122 LGC Standards, Molsheim, France) for all metal(oids) were $115 \pm 7\%$.

123 2.2. UBM test

124 The bioaccessibility was measured in triplicate for each soil sample with an *in vitro*
125 validated extraction method: the UBM test [11-13] adapted by Pelfrêne and Douay [31] and
126 Pelfrêne et al. [32]. Briefly, 0.6 g of dry soil was placed in a centrifuge tube and mixed with 9
127 mL of simulated saliva fluid. After quick manual shaking (10 s), 13.5 mL of simulated gastric
128 fluid was added, and the pH of the solution was adjusted to 1.2 ± 0.05 with HCl (37%). The
129 tubes were shaken end-over-end at 37°C for 1 h and centrifuged at 4500 x g for 5 min. The
130 supernatant constitutes the gastric-only phase (UBM G). Then, the gastro-intestinal (GI) phase
131 was prepared from the gastric phase, by adding 27 mL of simulated duodenal fluid and 9 mL
132 of simulated bile solution. The final pH ranged from 5.8 to 6.8 by adjustment with NaOH (10
133 M). Tubes were shaken end-over-end at 37°C for 4 h and centrifuged at 4500 x g for 5 min
134 (UBM GI). Bioaccessible concentrations of As, Cd and Pb in the supernatants of the UBM G
135 and UBM GI phases were measured by inductively coupled plasma mass spectrometry (ICP-
136 MS, 7900, Agilent Technology, Les Ulis, France). To evaluate analytical recovery, a blank and
137 a NIST standard reference material (SRM2710a) were used. The recovery rates of SRM2710a
138 (n=3) were $93.2 \pm 4\%$ on average for all metal(loid)s and UBM phases. For each metal(loid)
139 and each phase of the UBM test, the percentage of the bioaccessible fraction was the ratio
140 between the bioaccessible and soil concentrations.

141 2.3. Snail test

142 Juvenile land snails (*Cantareus aspersus*, Müller, 1774) were reared in the laboratory
143 under controlled conditions (20°C, 80% relative humidity, and a photoperiod of 18 h of light
144 and 6 h of dark) until the sub-adult stage (7-9 weeks), as described by Gomot-de-Vaufleury
145 [33]. The sub-adult stage was selected to avoid marked mass changes related to growth and
146 reproduction [6,23]. At the beginning of exposure, sub-adult snails weighed 4.95 ± 0.66 g, and
147 the internal concentrations of As, Cd and Pb were measured in visceral mass from six sub-adult
148 snails (0.127 ± 0.001 , 1.53 ± 0.175 , 0.510 ± 0.332 mg kg⁻¹ dw, respectively). Snails were fed
149 during growth with uncontaminated (As, Cd and Pb contents: 0.718, 0.430 and 0.784 mg kg⁻¹
150 dw, respectively) commercial food (Helixal®, Berthon S.A., France) and were fed during the
151 exposure stage with uncontaminated (As, Cd and Pb contents: 0.127, 0.648 and 0.108 mg kg⁻¹
152 dw, respectively) fresh lettuce (organic farm, France).

153 For each soil, six snails were exposed for four weeks (28 days) in triplicate in transparent
154 polystyrene containers of 4032 cm³ (24 x 21 x 8 cm). Exposure modalities are detailed in Pauget
155 et al. [25]. One week before exposure, 100 g of soil (DW, <250 µm) was introduced to the
156 containers and humidified (water holding capacity (WHC) adjusted to 50%) with demineralized
157 water (with a pH of 6.5). Every two days, the containers were cleaned to remove faeces, and
158 the lettuce was renewed. Snail food was offered *ad libitum* (corresponding to 1.5 g lettuce day⁻¹
159 snail⁻¹) in a Petri dish left on the bottom of the container. At the end of exposure, snails were
160 starved in clean containers (without soil) for two days (the faeces were removed every 12 h).
161 Then, the snails were frozen (-80°C). After thawing, the visceral mass of each snail was
162 dissected. Six visceral masses (2 per container x 3 replicates) per soil were freeze dried for two
163 days before As, Cd and Pb analysis. Lyophilized viscera were digested between 47°C and 98°C
164 for 265 min (DigiPREP MS, SCP Science, Courtaboeuf, France) in 7 mL of nitric acid (HNO₃
165 at 65%, Optima ultra trace purity, Fisher Scientific, Illkirch, France) that was diluted with
166 MilliQ water (43 mL). Then, samples were filtered at 1 µm (Hydrophilic Teflon©, DigiFILTER
167 1.0 µm, SCP Science, Courtaboeuf, France) for ICP-MS analysis (Thermo Scientific X Series
168 II, Courtaboeuf, France). Analyses were validated with a certified standard (TORT-2, lobster
169 hepatopancreas, LGC Standards, Molsheim, France) with recovery rates of 114 ± 9% on
170 average for all metal(loid)s. The bioaccumulated metal(loid) concentrations in the snails and
171 the soil metal(loid) concentrations were used to calculate the bioaccumulation factors (BAF).

172 2.4. Statistical analysis

173 Statistical analysis was performed with R (version 3.4.2) [34]. The data (except pH) were
174 transformed by log₁₀ (x+1) to fulfil the residual normality and variance homogeneity
175 requirements (Kolmogorov-Smirnov and Bartlett tests). Assessment of the relationships
176 between metal(loid) concentrations in the soil and/or bioaccessible concentrations and/or
177 bioaccumulated concentrations in viscera was performed by simple linear regression. Then, the
178 influence of soil physicochemical parameters on bioaccessible concentrations and/or
179 bioaccumulated concentrations in viscera was modelled with multiple linear regression. Based
180 on the best models obtained, the bioaccessible concentrations were estimated from the
181 concentrations in snails and soil physicochemical parameters. It is possible to test the efficiency
182 of models on a subset of data when data from another experiment with other soils are not
183 available by performing an internal cross-validation test [35,36]. In this approach, the proposed
184 models were considered valid if the differences between adjusted q² and adjusted r² (r²_{adj.}) do
185 not exceed 0.3 [37]. All the models were validated with an internal cross-validation test [35,36].

186 For this internal verification step, one-third (n=10) of samples were randomly chosen, and
187 multiple regressions were re-modelled. Comparisons between q^2 (determination coefficient of
188 the cross-validation test) and r^2 (models proposed) allowed for model validation. For each set
189 of regressions, the best model (*i.e.*, the one providing the best adjusted coefficient of
190 determination with the lowest number of independent variables) was chosen using the corrected
191 Akaike criterion (AICc) [38].

192 3. Results and discussion

193 3.1. Oral bioaccessible fractions for humans and bioavailability to snails

194 The bioaccessible fractions for humans and bioaccumulated concentrations for snails are
195 presented in **Tab 2**. The *in vitro* bioaccessibility of As, Cd and Pb measured in the twenty-nine
196 soil samples ranged from 0.508 to 245 mg kg⁻¹, 0.082 to 321 mg kg⁻¹ and 10.7 to 10730 mg kg⁻¹
197 of soil for the G phase and from 0.583 to 89.1 mg kg⁻¹, 0.035 to 142 mg kg⁻¹ and 0 to 2460
198 mg kg⁻¹ of soil for the GI phase, respectively (**Tab 2**). The mean ratios/percentages of the
199 bioaccessible fractions of As, Cd and Pb in the G phase were 37 ± 23%, 84 ± 15% and 78 ±
200 23%, respectively, of the pseudo-total concentrations in the soils. In the GI phase, the
201 bioaccessible fractions of As, Cd and Pb decreased to 30 ± 16%, 38 ± 13% and 11 ± 14%,
202 respectively. Overall, for the twenty-nine soils studied, bioaccessible fractions of Cd and Pb
203 were higher (2.3 and 4.6 times, respectively) in the G phase than in the GI phase. These results
204 are explained by the pH of the simulated fluids, which are more acidic in the G phase and lead
205 to higher solubilization of metals [9,11]. However, this is much less marked for As, which
206 showed similar bioaccessible fractions, in most cases, in both phases with average values of
207 37% vs 30% (*i.e.*, 1.2-fold less), for G and GI phases, respectively (**Tab 2**). This difference
208 could be related to the particular geochemical behaviour of As in the simulated fluid conditions
209 [11,26], which may lead to reduced adsorption and precipitation reactions at the neutral pH in
210 the GI phase for As than for Cd and Pb [9,11]. These results for the three studied metal(loid)s
211 are in accordance with those in other studies [9,11,39,40], especially those of Denys et al. [12],
212 in an *in vivo* validation of the UBM test on juvenile swine.

213 The *in vivo* bioavailability of As, Cd and Pb measured with the bioaccumulation test after *C.*
214 *aspersus* in 28 days ranged from 0.098 to 21.9 mg kg⁻¹, 2.53 to 333 mg kg⁻¹ and 1.42 to 856
215 mg kg⁻¹ of dry weight (dw) viscera, respectively (**Tab 2**). The comparison between
216 bioaccessible and bioaccumulated concentrations of As and Pb for each soil showed that the
217 concentrations in snails were lower than the bioaccessible phase concentrations (*e.g.*, the As
218 and Pb concentrations in snails were approximately 9 and 6 times lower than those in the G

219 phase, respectively). In contrast, for Cd, the concentrations were 2 times higher in snails than
220 in the G phase of the UBM test due to the snail physiology and internal management of
221 metal(loid)s; specifically, Pb is highly excreted with cell debris, while Cd is stored and
222 associated with metallothionein in the cytosolic fraction [24,41]. Hence, elevated BAFs were
223 recorded for Cd (ranging from 0.859 and 25.3), and low BAFs were recorded for As and Pb
224 (ranging from 0.004 to 0.169 or 0.055 to 0.769, respectively) (**Tab 2**). These BAFs are in
225 accordance with those reported in various studies in which *C. aspersus* was classified as a
226 deconcentrator of As and Pb and a macroconcentrator of Cd [22,23,25,42,43].

227 We observed substantial inter-soil variability in bioaccessible fractions and bioaccumulated
228 concentrations in snails (**Tab 2**). This variability highlights the influence not only of soil
229 contamination (concentration of metal(loid)s and mixture) but also of the physicochemical
230 parameters of soils (*e.g.*, pH and OM content) on metal(loid) mobility and transfer.

231 **3.2. Influence of soil parameters on oral bioaccessible concentrations for humans** 232 **and bioavailability to snails**

233 The relationships between the bioaccessibility of As, Cd and Pb and their total
234 concentrations in soils were investigated. The modulating influence of the physicochemical
235 parameters of soils was also examined because soil parameters can influence metal(loid)
236 availability and bioavailability [44-48]. Strong relationships between total concentrations of
237 contaminants in soil and their extractable concentrations observed in the UBM test were
238 evidenced by r^2_{adj} values ranging from 0.63 to 0.99, according to the contaminant and
239 bioaccessible phase (G or GI) considered (**Tab 3**). For As, very close relationships were found
240 for the G and GI phases, while the r^2_{adj} values in the G phase were always higher for Cd and Pb.
241 These results are related to the differential solubilization, precipitation and therefore absorption
242 potential of the stomach and intestine, as discussed above. The consideration of soil parameters
243 rather than just the total concentrations of metal(loid)s (**Tab 1**) can improve the regression
244 models (**Tab 3**). For As in G and GI phases, the content of silt increased the r^2_{adj} values up to
245 0.71 and 0.72, respectively, and for Cd in the GI phase, the OM content increased the r^2_{adj} up
246 to 0.96. No influence of soil physicochemical parameters was observed for Cd in the G phase
247 or Pb in either phase. It is likely that the strong acidic pH of the first gastric solution of the
248 UBM test prevented the influence of the soil physicochemical parameters on Cd and Pb
249 bioaccessibility. For the GI phase, OM content negatively influenced the Cd bioaccessibility.
250 The neutral pH of the intestinal phase might have led to readsorption of Cd to OM particles of
251 soil [49,50]. For both phases, As bioaccessibility was positively modulated by the silt content

252 of the soils. Hence, the As of this granulometric fraction seemed to be transferred to the
253 simulated solutions of UBM tests.

254 A similar approach was carried out with As, Cd and Pb concentrations accumulated in snails as
255 an indicator of their bioavailability (Tab 3). For each element, the total concentration in soils
256 appeared as an important explanatory variable ($r^2_{adj.} = 0.51, 0.92$ and 0.85 for As, Cd and Pb,
257 respectively); although physicochemical parameters of soils did not improve the regression
258 model for Pb, some parameters improved the regression models for As and Cd (Tab 3). Hence,
259 for As, the content of OM and silts increased the $r^2_{adj.}$ up to 0.65, and for Cd, the OM content,
260 pH and cation exchange capacity (CEC) increased the $r^2_{adj.}$ up to 0.96. These results are in
261 accordance with previous laboratory experiments with snails and are related to the low lability
262 of Pb in soils and the reactivity of As and Cd to soil pH and components such as OM [25].

263 3.3. Relationships between oral bioaccessibility and bioavailability of metal(loid)s

264 We first looked for relationships between extractible concentrations in the UBM test
265 and accumulated concentrations in the snail tissues of As, Cd and Pb (Fig 2). The related
266 regressions revealed, regardless of the digestive compartment considered (G or GI), strong and
267 positive relationships, particularly for Cd and Pb and to a lesser extent for As (Tab 4). As
268 previously described, the potential influence of soil parameters was also investigated in a new
269 modelling step to better connect the oral bioaccessible concentrations to humans with the
270 bioaccumulated concentrations for snails. The obtained regressions were then strongly
271 improved, especially when considering the soil OM content, with a rise of 17% and 15% of
272 variance explained ($r^2_{adj.}$) for As in the G and GI phases, respectively and to a lesser extent for
273 Pb (+4% and +6%) and Cd (+2%) (Tab 4). This improvement in estimating the bioaccessible
274 concentrations can be explained by the limited ability of snails to access the metal(loid) fraction
275 associated with organic particles in soils. Indeed, the pH in the snail digestive tract ranges
276 between 6.1 and 7.5 [51], which is probably not sufficient to reach the desorption capacities
277 achieved by the very acidic pH in the human stomach [52].

278 The robustness of the improved models was checked using a cross-validation approach. The
279 small differences (< 0.17) between the $r^2_{adj.}$ (Tab 4) and $q^2_{adj.}$ (Tab 5) values testify to the
280 efficiency of the proposed models for assessing metal(loid) bioaccessibility to humans using a
281 bioavailability assessment with snails. Finally, we verified the estimation potential of these
282 validated models by comparing measured and estimated values of bioaccessible concentrations
283 (Fig 3). For each digestive phase and metal(loid) studied, the slopes were relatively close to 1

284 (ranging from 0.78 to 0.95), demonstrating that bioavailability to snails may be an efficient way
285 to estimate oral bioaccessibility for humans.

286 Our objective was not to oppose or to prioritize the UBM test and the bioassay with snails but
287 to demonstrate their complementarity, both in terms of response and use. Indeed, to our
288 knowledge, studies that try to link chemical approaches for bioaccessibility assessment and
289 biological tests to estimate bioavailability with soil invertebrates are rare. The majority of the
290 studies concerning the discovered relationships with mammals, such as mice, have shown that
291 the UBM assay can estimate the bioavailability of As, Cd and Pb in contaminated soils [53-56].
292 To date, only the study of Rahman et al. [26] has reported correlations ($r^2=0.95$) between
293 bioaccumulation factors in earthworms (*Lumbricus rubellus*) and the human bioaccessible
294 fraction (gastric phase) of As in six aged pesticide-contaminated soils. Thus, for these two
295 bioindicators of soil quality (*L. rubellus* and *C. aspersus*), strong relationships between
296 bioaccumulation and oral bioaccessibility to humans exist for As. Similarity between these
297 results and ours was not necessarily expected because of the differences between the considered
298 organisms (*i.e.*, humans and soil invertebrates) in terms of sources, routes of exposure and
299 physiology.

300 4. Conclusions

301 This study provides effective methods to establish links between the bioaccessibility to
302 humans and the bioavailability to snails, and new evidence confirming that HHRA and ERA
303 methodologies may converge to reinforce risk assessment procedures for both sanitary and
304 environmental perspectives. Hence, in the case where only physicochemical properties of soils
305 and environmental data for ERA are available, we propose a way to approach, in a first attempt,
306 HHRA at least for As, Cd and Pb; similarly, HHRA data could be used to approach ERA.

307 In HHRA procedures, daily doses of exposure based on total concentrations of contaminants in
308 soils are used to estimate a hazard quotient (HQ) [2]. As discussed in the study of Jia et al. [57]
309 on a rapidly urbanization area of Yangtze (China), the measurements of oral bioaccessible and
310 bioaccumulated concentrations can be used to refine HQs to provide a more accurate estimation
311 of the exposure and consequently a better interpretation of the environmental state. However,
312 these data and models are available for a limited number of contaminants and soil quality
313 bioindicators.

314 **The original findings we provide in this study rely** on the establishment of relationships
315 connecting the oral bioaccessibility **to** humans and bioavailability to snails **for** As, Cd and Pb.
316 In the current context of increasing sanitary and environmental crises, these results constitute a
317 useful contribution to coordinated risk assessment strategies. As exposure assessment is a key
318 factor in assessing the risk of contaminated soil, this contribution joins the current trend of the
319 One Health concept, associating humans and other organisms in an integrative risk assessment.

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327 **6. Conflict of interest**

328 The authors declare no conflicts of interest.

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TABLES AND FIGURES

Bioaccessibility of metal(loid)s in soils to humans and their bioavailability for snails: a way to associate human health and ecotoxicological risk assessment?

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Figures:

Figure 1: Map of soil locations (from map background of OpenStreetMap©)

Figure 2: Relationship between bioaccessible concentrations obtained with the UBM test and bioaccumulated concentrations for snails (data are transformed by $\log_{10}(x+1)$; grey bands are 95% confidence intervals of the linear regression models that are represented by black lines).

Figure 3: Relationship between bioaccessible concentrations measured with the UBM test and estimated concentrations from snails (with equations in **Tab 4**, data are transformed by $\log_{10}(x+1)$; grey bands are 95% confidence intervals of the linear regression models that are represented by black lines).

Tables:

Table 1: Soil types and physicochemical parameters (OM: organic matter, Corg: organic carbon, CEC: cation exchange capacity, Alox and Feox are exchangeable cations in soils, Emb.: embankment, Farm.: farming, Gras.: grassland, Indus.: industrial, U: unknown, Urb.: urban).

Table 2: Bioaccessible concentrations and fractions for humans and bioaccumulated concentrations and bioaccumulation factors (BAFs) for snails for each soil (results are presented in $\text{mg kg}^{-1} \text{ dw}$ for concentrations (mean for bioaccessibility and median for bioaccumulated concentrations for snails) and in % for bioaccessible fractions and BAF is the ratio between concentrations in snails and concentrations in soils. G and GI: gastric and gastro-intestinal phases of the UBM test, respectively).

Table 3: Influence of total metal(loid) concentrations and soil parameters on oral bioaccessibility and bioaccumulation (significant variables are ranked with stars as a function of p-value obtained: * < 0.05, ** < 0.01, *** < 0.001, data are transformed by $\log_{10}(x+1)$).

Table 4: Relationship between concentrations accumulated in visceral mass of snails and in the UBM test phases G and GI for gastric and gastro-intestinal phases, respectively (significant variables are ranked with stars as a function of the p-value obtained: * < 0.05, **

< 0.01 , *** < 0.001 , data are transformed by $\log_{10}(x + 1)$. Models in bold are used in **Tab 5** to perform an internal cross-validation).

Table 5: Equations of the internal cross-validated models assessing the bioaccessible concentrations according to the accumulated concentrations in snails and physicochemical parameters of soils (data are transformed by $\log_{10}(x+1)$; significant variables are ranked with stars as a function of p-value obtained: * < 0.05 , ** < 0.01 , *** < 0.001).

Table 1:

Soils	Site use	Clay (%)	Silt (%)	Sand (%)	pH _{water}	OM (g kg ⁻¹)	C _{org} (g kg ⁻¹)	CaCO ₃ (g kg ⁻¹)	CEC (cmol kg ⁻¹)	Alox (cmol kg ⁻¹)	Feox (cmol kg ⁻¹)	As (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Pb (mg kg ⁻¹)
O1	Urb.	15.2	41.4	41.9	7.7	161	92.7	15.0	20.9	0.023	0.005	13.4	1.32	430
O2	Ind.	16.1	47.4	36.5	7.4	71.1	40.9	1.3	17.5	0.035	0.006	11.5	1.91	320
O3	Ind.	23.1	47.8	29.1	8.0	61.1	35.1	84.0	18.0	0.020	0.005	228	257	6256
O4	Ind.	16.6	41.0	42.4	7.8	304	175	22.3	12.3	0.020	0.005	74.4	48.0	5224
O5	Ind.	12.3	35.0	52.7	6.8	360	207	3.6	11.7	0.054	0.007	108	85.7	8971
O6	Ind.	29.1	45.9	25.0	8.1	24.3	14.0	91.2	24.6	0.020	0.005	139	109	2184
O7	Ind.	21.5	44.0	34.5	8.3	112	64.1	145	22.3	0.020	0.005	12.8	4.2	385
O8	Ind.	22.8	70.6	6.6	8.3	27.1	15.6	162	16.5	0.020	0.005	5.64	0.640	30.7
O9	Ind.	20.0	56.9	23.1	8.0	111	63.9	38.6	17.3	0.020	0.005	23.3	7.34	534
O10	Ind.	23.7	48.2	28.1	5.7	205	118	1.6	31.2	0.020	0.005	48.6	27.8	2233
O11	Ind.	16.2	55.1	28.7	6.8	72.9	41.9	<1.0	18.2	0.020	0.005	27.9	18.2	1260
O12	Ind.	15.7	52.1	32.2	4.3	46.8	26.9	<1.0	10.2	0.028	0.005	7.74	1.42	222
O13	Ind.	16.8	46.9	36.3	5.2	59.3	34.1	<1.0	18.4	0.020	0.005	12.6	6.50	484
O14	Ind.	33.3	59.3	7.4	8.0	75.2	43.2	40.7	38.8	0.030	0.008	23.4	15.4	719
O15	Ind.	13.5	42.6	43.9	7.9	30.6	17.6	37.5	10.9	0.020	0.005	13.5	1.30	177
O16	U	16.7	73.4	9.6	7.6	18.2	10.5	3.0	12.4	0.020	0.005	5.52	0.640	17.8
O17	U	30.2	34.9	34.2	5.7	51.4	29.6	1.0	16.2	0.027	0.005	17.8	0.200	33.7
O18	Farm.	41.6	21.5	36.3	8.0	35.8	20.6	27.0	28.4	0.020	0.005	74.4	0.520	46.6
O19	Ind.	16.9	54.8	28.5	7.2	33.5	19.3	3.6	12.3	0.020	0.005	10.1	4.49	117
O20	Ind.	22.0	29.5	48.5	8.1	15.9	9.1	17.9	10.2	0.035	0.005	26.0	0.370	150
O21	Gras.	8.0	10.0	82.0	7.6	43.5	25.0	31	11.1	0.020	0.005	11.3	2.32	288
O22	U	8.8	7.5	83.7	8.2	15.4	8.9	18	6.5	0.020	0.005	3.28	0.235	19.7
O23	Farm.	16.3	38.1	45.6	6.8	28.3	16.3	3.2	7.2	0.020	0.007	57.0	0.102	660
O24	Farm.	18.3	42.0	39.7	7.6	55.9	32.2	106	18.1	0.020	0.005	12.0	3.57	303
O26	Ind.	11.3	16.9	71.8	7.5	41.7	24.0	169	4.3	0.020	0.005	13.3	0.450	312
O27	Emb.	6.8	20.2	73.0	7.9	134	77.0	51.5	8.6	0.020	0.007	28.5	7.43	1882
O28	Emb.	9.1	30.3	60.6	7.8	230	132	16.9	12.1	0.020	0.007	25.8	2.60	467
O29	Emb.	7.6	30.8	61.6	7.9	185	106	29.9	9.0	0.020	0.005	36.1	27.2	5258
O30	Emb.	9.0	22.0	69.0	8.1	179	103	27.2	10.1	0.042	0.005	28.3	12.8	3241
Min.		6.8	7.5	6.6	4.3	15.4	8.9	<1.0	4.30	0.020	0.005	3.28	0.102	17.8
Max.		41.6	73.4	83.7	8.3	360	207	169	38.8	0.054	0.008	228	257	8971

Table 2:

Soils	As				Cd				Pb			
	G Phase	GI Phase	Snail	BAF	G Phase	GI Phase	Snail	BAF	G Phase	GI Phase	Snail	BAF
O1	5.05 (38%)	5.08 (38%)	0.298	0.022	1.25 (95%)	0.413 (31%)	5.02	3.80	349 (81%)	111 (26%)	84.6	0.197
O2	3.69 (32%)	3.39 (29%)	0.298	0.026	1.40 (73%)	0.407 (21%)	5.63	2.95	217 (68%)	7.93 (2%)	43.3	0.135
O3	245 (108%)	112 (49%)	21.9	0.096	321 (125%)	142 (55%)	333	1.29	6542 (105%)	2460 (39%)	856	0.137
O4	42.4 (57%)	28.5 (38%)	1.29	0.017	41.2 (86%)	11.9 (25%)	60.0	1.25	3964 (76%)	342 (7%)	652	0.125
O5	64.5 (60%)	52.7 (49%)	2.25	0.021	75.9 (89%)	29.0 (34%)	73.6	0.859	10730 (120%)	1801 (20%)	731	0.081
O6	111 (80%)	89.1 (64%)	19.2	0.138	104 (96%)	48.8 (45%)	307	2.83	2034 (93%)	100 (5%)	783	0.359
O7	4.49 (35%)	3.25 (25%)	0.298	0.023	3.85 (92%)	1.04 (25%)	18.6	4.43	337 (88%)	9.52 (2%)	267	0.694
O8	1.19 (21%)	1.19 (21%)	0.298	0.053	0.541 (85%)	0.250 (39%)	5.33	8.33	20.4 (66%)	3.11 (10%)	5.21	0.169
O9	10.4 (45%)	9.72 (42%)	3.93	0.169	6.15 (84%)	2.75 (37%)	32.0	4.36	514 (96%)	11.8 (2%)	208	0.389
O10	35.3 (73%)	25.4 (52%)	2.31	0.048	31.1 (112%)	11.2 (40%)	71.9	2.58	2330 (104%)	973 (44%)	503	0.225
O11	14.0 (50%)	13.6 (49%)	3.78	0.135	15.4 (85%)	6.48 (36%)	52.5	2.91	1104 (88%)	124 (10%)	431	0.342
O12	1.09 (14%)	1.25 (16%)	0.298	0.039	1.04 (73%)	0.478 (34%)	12.5	8.80	198 (89%)	101 (45%)	171	0.769
O13	3.36 (27%)	3.67 (29%)	0.262	0.021	4.82 (74%)	1.10 (17%)	28.3	4.35	457 (94%)	42.8 (9%)	162	0.335
O14	9.00 (38%)	15.2 (65%)	2.02	0.086	13.3 (87%)	8.72 (57%)	46.7	3.04	609 (85%)	14.4 (2%)	271	0.377
O15	5.05 (38%)	3.64 (27%)	0.482	0.036	1.02 (78%)	0.814 (63%)	6.04	4.65	114 (64%)	0.745 (0%)	21.0	0.119
O16	0.841 (15%)	0.910 (16%)	0.098	0.018	0.544 (85%)	0.203 (32%)	4.80	7.50	10.7 (60%)	0.000 (0%)	4.12	0.232
O17	1.95 (11%)	1.50 (8%)	0.098	0.006	0.154 (77%)	0.120 (60%)	2.56	12.8	13.7 (41%)	0.733 (2%)	1.42	0.042
O18	5.97 (8%)	5.58 (8%)	1.39	0.019	0.344 (66%)	0.135 (26%)	3.20	6.15	15.3 (33%)	0.391 (1%)	2.56	0.055
O19	1.30 (13%)	1.39 (14%)	0.098	0.010	3.20 (71%)	1.26 (28%)	12.1	2.70	84.8 (72%)	2.36 (2%)	37.4	0.320
O20	5.94 (23%)	6.08 (23%)	1.98	0.076	0.261 (71%)	0.126 (34%)	4.59	12.4	105 (70%)	2.30 (2%)	38.9	0.259
O21	5.50 (49%)	4.96 (44%)	2.23	0.008	1.93 (83%)	0.721 (31%)	11.5	4.96	243 (84%)	3.03 (1%)	83.7	0.291
O22	0.508 (16%)	0.583 (18%)	0.098	0.030	0.199 (87%)	0.132 (57%)	4.24	18.4	14.2 (72%)	3.54 (18%)	12.1	0.615
O23	17.3 (30%)	14.1 (25%)	1.48	0.026	0.082 (82%)	0.035 (35%)	2.53	25.3	111 (17%)	19.2 (3%)	39.1	0.059
O24	4.02 (34%)	2.90 (24%)	0.829	0.069	3.41 (96%)	1.03 (29%)	12.4	3.47	259 (86%)	5.47 (2%)	35.6	0.118
O26	5.68 (43%)	3.70 (28%)	0.098	0.007	0.490 (109%)	0.275 (61%)	3.20	7.11	275 (88%)	8.19 (3%)	58.0	0.186
O27	6.73 (24%)	4.48 (16%)	0.098	0.003	5.43 (73%)	2.86 (38%)	8.81	1.19	1281 (68%)	137 (7%)	137	0.073
O28	7.36 (29%)	5.84 (23%)	0.098	0.004	2.18 (84%)	0.716 (28%)	5.52	2.12	292 (63%)	41.6 (9%)	25.5	0.055
O29	8.58 (24%)	5.92 (16%)	0.379	0.011	13.3 (49%)	9.17 (34%)	28.3	1.04	5196 (99%)	1667 (32%)	820	0.156
O30	7.69 (27%)	6.29 (22%)	1.16	0.041	10.3 (81%)	5.28 (41%)	20.4	1.60	3136 (97%)	783 (24%)	730	0.225
Mean	21.9 (37%)	14.9 (30%)	2.38	0.043	22.9 (84%)	9.91 (38%)	40.8	5.63	1399 (78%)	303 (11%)	249	0.246
Standard-deviation	48.9 (22%)	26.3 (16%)	5.16	0.044	62.1 (15%)	27.4 (13%)	80.3	5.55	2447 (22%)	632 (14%)	295	0.188

Table 3:

Elements	Equations	r ² _{adj.}	p-value
As	$[As]_{UBM-G} = -0.422* + 0.952[As]_{soil}^{***}$	0.650	<0.001
	$[As]_{UBM-G} = -2.09** + 1.00[As]_{soil}^{***} + 0.623[silt]^*$	0.714	<0.001
	$[As]_{UBM-GI} = -0.337 + 0.851[As]_{soil}^{***}$	0.631	<0.001
	$[As]_{UBM-GI} = -2.06** + 0.905[As]_{soil}^{***} + 0.644[silt]**$	0.717	<0.001
	$[As]_{snail} = -0.463** + 0.553[As]_{soil}^{***}$	0.505	<0.001
	$[As]_{snail} = -1.38** + 0.665[As]_{soil}^{***} + 0.492[silt]** - 0.276[OM]^*$	0.646	<0.001
Cd	$[Cd]_{UBM-G} = -0.054* + 0.996[Cd]_{soil}^{***}$	0.987	<0.001
	$[Cd]_{UBM-G} = \text{no influence of soil parameters}$	-	-
	$[Cd]_{UBM-GI} = -0.144*** + 0.833[Cd]_{soil}^{***}$	0.947	<0.001
	$[Cd]_{UBM-GI} = 0.160 + 0.893[Cd]_{soil}^{***} - 0.193[OM]**$	0.959	<0.001
	$[Cd]_{snail} = 0.563*** + 0.802[Cd]_{soil}^{***}$	0.917	<0.001
	$[Cd]_{snail} = 1.08*** + 0.869[Cd]_{soil}^{***} - 0.052pH^* + 0.300[CEC]^* - 0.293[OM]^{***}$	0.961	<0.001
Pb	$[Pb]_{UBM-G} = -0.350* + 1.07[Pb]_{soil}^{***}$	0.945	<0.001
	$[Pb]_{UBM-G} = \text{no influence of soil parameters}$	-	-
	$[Pb]_{UBM-GI} = -1.68*** + 1.18[Pb]_{soil}^{***}$	0.748	<0.001
	$[Pb]_{UBM-GI} = \text{no influence of soil parameters}$	-	-
	$[Pb]_{snail} = -0.540* + 0.928[Pb]_{soil}^{***}$	0.846	<0.001
	$[Pb]_{snail} = \text{no influence of soil parameters}$	-	-

Table 4:

Elements	Equations	r^2_{adj}	p-value
As	$[As]_{UBM\ G} = 0.523^{***} + 1.27[As]_{snail}^{***}$	0.678	<0.001
	$[As]_{UBM\ G} = -0.513^* + 1.28[As]_{snail}^{***} + 0.567[OM]^{***}$	0.845	<0.001
	$[As]_{UBM\ GI} = 0.493^{***} + 1.18[As]_{snail}^{***}$	0.715	<0.001
	$[As]_{UBM\ GI} = -0.403^* + 1.19[As]_{snail}^{***} + 0.490[OM]^{***}$	0.867	<0.001
Cd	$[Cd]_{UBM\ G} = -0.647^{***} + 1.15[Cd]_{snail}^{***}$	0.924	<0.001
	$[Cd]_{UBM\ G} = -1.07^{***} + 1.08[Cd]_{snail}^{***} + 0.282[OM]^{**}$	0.946	<0.001
	$[Cd]_{UBM\ GI} = -0.637^{***} + 0.963[Cd]_{snail}^{***}$	0.882	<0.001
	$[Cd]_{UBM\ GI} = \text{no influence of soil parameters}$	-	-
Pb	$[Pb]_{UBM\ G} = 0.510^{**} + 1.04[Pb]_{snail}^{***}$	0.887	<0.001
	$[Pb]_{UBM\ G} = -0.183 + 0.872[Pb]_{snail}^{***} + 0.553[OM]^{***}$	0.928	<0.001
	$[Pb]_{UBM\ GI} = -0.735^* + 1.14[Pb]_{snail}^{***}$	0.704	<0.001
	$[Pb]_{UBM\ GI} = -1.83^{***} + 0.879[Pb]_{snail}^{***} + 0.875[OM]^{**}$	0.766	<0.001

Table 5:

Elements	Internal cross-validation equations	q^2_{adj}	p-value
As	$[As]_{UBM\ G} = -0.973 + 1.24[As]_{snail}^{**} + 0.800[OM]^*$	0.800	<0.01
	$[As]_{UBM\ GI} = -0.815 + 1.03[As]_{snail}^{***} + 0.742[OM]^{**}$	0.843	<0.001
Cd	$[Cd]_{UBM\ G} = -1.61^{**} + 1.37[Cd]_{snail}^{***} + 0.286[OM]$	0.921	<0.001
	$[Cd]_{UBM\ GI} = -1.18^{**} + 1.27[Cd]_{snail}^{***}$	0.859	<0.001
Pb	$[Pb]_{UBM\ G} = -0.374 + 0.959[Pb]_{snail}^* + 0.534[OM]$	0.789	<0.01
	$[Pb]_{UBM\ GI} = 2.66^* + 1.93[Pb]_{snail}^{**} - 0.012[OM]$	0.714	<0.01

Fig 1:

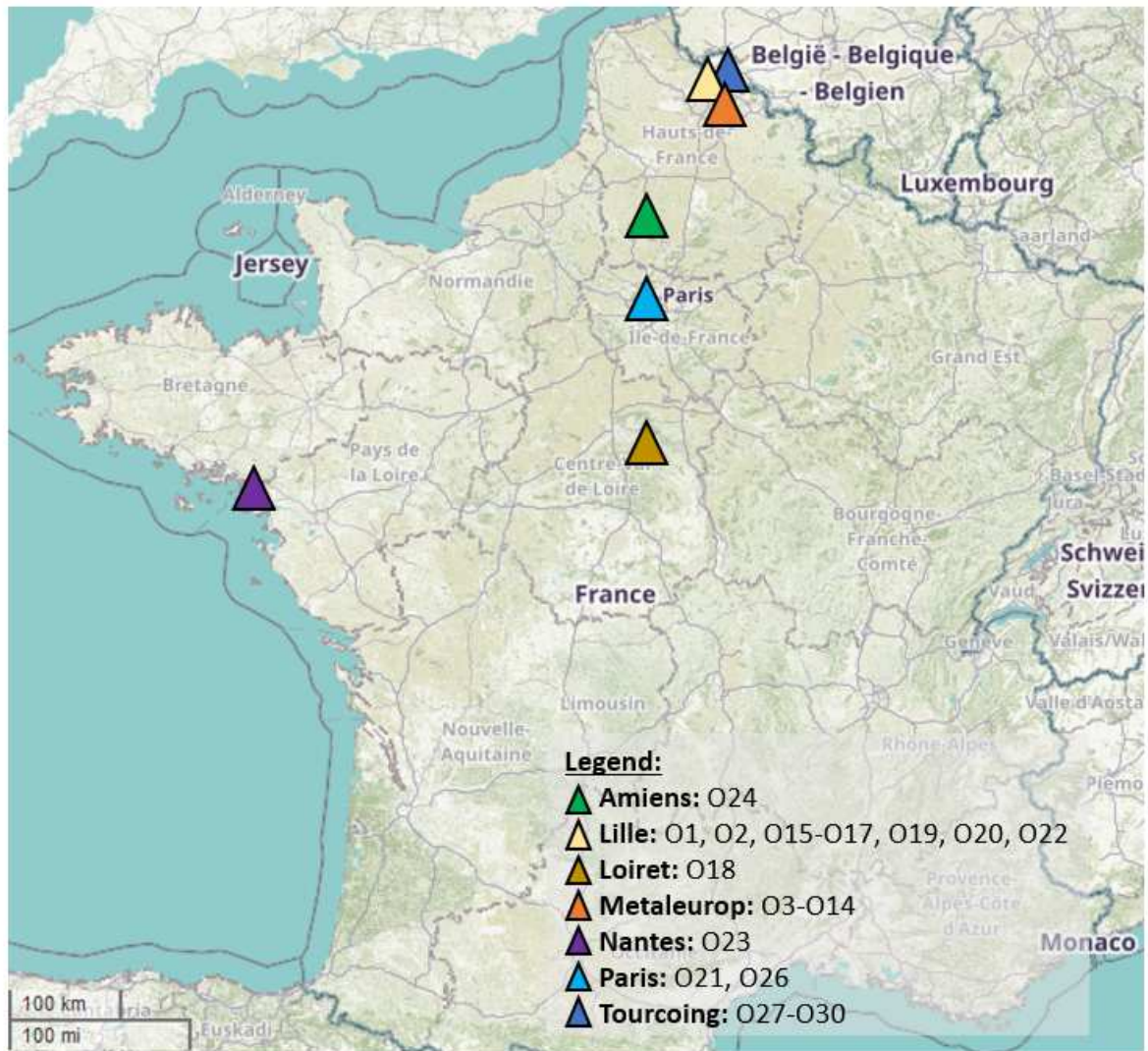


Fig 2:

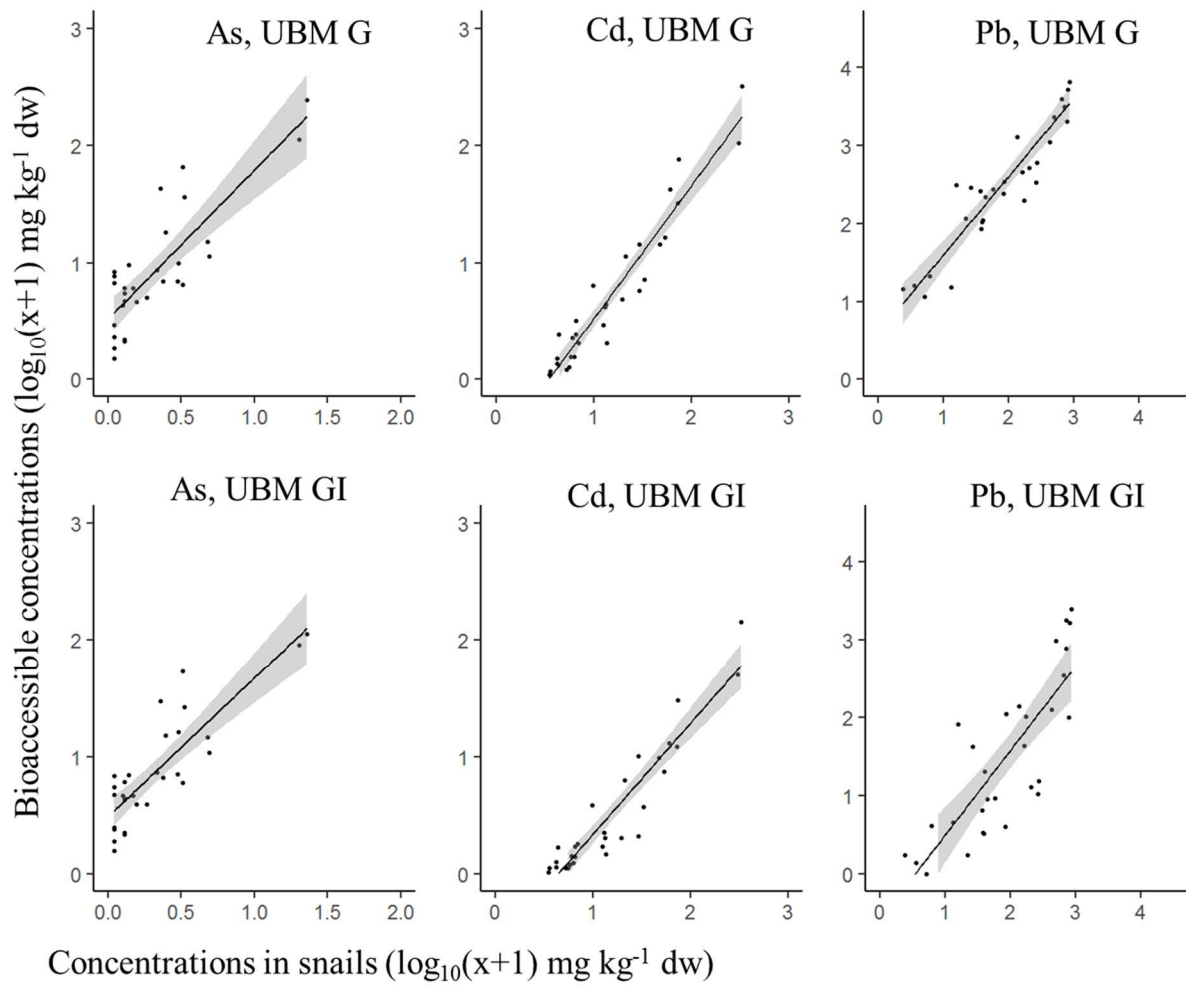


Fig 3:

