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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Keywords: bioindicators, bioaccumulation, organic matter, integrative risk assessment

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.

Highlights:

- Influence of soil parameters (*e.g.,* organic matter) on human oral bioaccessibility
- Influence of soil parameters (*e.g.,* organic matter) on bioavailability to snails
- Strong relationships between oral bioaccessibility and bioavailability to snails
- Proposed models to link bioaccessibility to humans and bioavailability to snails

1. Introduction

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

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

2. Experimental

2.1. Soils

Twenty-nine soils were sampled from contaminated fields in France after humus removal (A horizon) (**Fig 1**), and the samples were dried (<40°C) and sieved at 250 µm. The physicochemical parameters were then measured with the appropriate ISO standard protocols (**Tab 1**). The pH_{water} ranged from 4.3 to 8.3, and the OM contents ranged from 15.4 to 360 mg kg^{-1}. The As, Cd and Pb concentrations were obtained by hot block system-assisted digestion (Environmental Express® SC100, Charleston, SC, USA) and determined by inductively coupled plasma mass spectrometry (ICP-MS, 7900, Agilent Technology, Les Ulis, France). More specifically, 300 mg of soil sample was digested in a mixture of 1.5 mL HNO_3 (70%) and 4.5 mL HCl (37%) at 95°C for 1 h and 30 min. After mineralization, digestion products were brought to 25 mL with ultrapure water (resistivity of 18.0 MΩ cm^{-1}) and stored at 4°C prior to analysis. The recovery rates as determined with a certified standard (ERM CC141, Loam soil, LGC Standards, Molsheim, France) for all metal(loids) were 115 ± 7%.

2.2. UBM test

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

2.3. Snail test

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

For each soil, six snails were exposed for four weeks (28 days) in triplicate in transparent polystyrene containers of 4032 cm^3 (24 x 21 x 8 cm). Exposure modalities are detailed in Pauget et al. [25]. One week before exposure, 100 g of soil (DW, <250 µm) was introduced to the containers and humidified (water holding capacity (WHC) adjusted to 50%) with demineralized water (with a pH of 6.5). Every two days, the containers were cleaned to remove faeces, and the lettuce was renewed. Snail food was offered *ad libitum* (corresponding to 1.5 g lettuce day^{-1} $snail^{-1}$) in a Petri dish left on the bottom of the container. At the end of exposure, snails were starved in clean containers (without soil) for two days (the faeces were removed every 12 h). Then, the snails were frozen (-80°C). After thawing, the visceral mass of each snail was dissected. Six visceral masses (2 per container x 3 replicates) per soil were freeze dried for two days before As, Cd and Pb analysis. Lyophilized viscera were digested between 47°C and 98°C for 265 min (DigiPREP MS, SCP Science, Courtaboeuf, France) in 7 mL of nitric acid (HNO_3 at 65%, Optima ultra trace purity, Fisher Scientific, Illkirch, France) that was diluted with MilliQ water (43 mL). Then, samples were filtered at 1 µm (Hydrophilic Teflon©, DigiFILTER 1.0 µm, SCP Science, Courtaboeuf, France) for ICP-MS analysis (Thermo Scientific X Series II, Courtaboeuf, France). Analyses were validated with a certified standard (TORT-2, lobster hepatopancreas, LGC Standards, Molsheim, France) with recovery rates of 114 ± 9% on average for all metal(loid)s. The bioaccumulated metal(loid) concentrations in the snails and the soil metal(loid) concentrations were used to calculate the bioaccumulation factors (BAF).

2.4. Statistical analysis

Statistical analysis was performed with R (version 3.4.2) [34]. The data (except pH) were transformed by $\log_{10}(x+1)$ to fulfil the residual normality and variance homogeneity requirements (Kolmogorov-Smirnov and Bartlett tests). Assessment of the relationships between metal(loid) concentrations in the soil and/or bioaccessible concentrations and/or bioaccumulated concentrations in viscera was performed by simple linear regression. Then, the influence of soil physicochemical parameters on bioaccessible concentrations and/or bioaccumulated concentrations in viscera was modelled with multiple linear regression. Based on the best models obtained, the bioaccessible concentrations were estimated from the concentrations in snails and soil physicochemical parameters. It is possible to test the efficiency of models on a subset of data when data from another experiment with other soils are not available by performing an internal cross-validation test [35,36]. In this approach, the proposed models were considered valid if the differences between adjusted q^2 and adjusted r^2 ($r^2_{adj.}$) do not exceed 0.3 [37]. All the models were validated with an internal cross-validation test [35,36].

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

3. Results and discussion

3.1. Oral bioaccessible fractions for humans and bioavailability to snails

The bioaccessible fractions for humans and bioaccumulated concentrations for snails are presented in **Tab 2**. The *in vitro* bioaccessibility of As, Cd and Pb measured in the twenty-nine 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} 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 mg kg^{-1} of soil for the GI phase, respectively (**Tab 2**). The mean ratios/percentages of the bioaccessible fractions of As, Cd and Pb in the G phase were 37 ± 23%, 84 ± 15% and 78 ± 23%, respectively, of the pseudo-total concentrations in the soils. In the GI phase, the bioaccessible fractions of As, Cd and Pb decreased to 30 ± 16%, 38 ± 13% and 11 ± 14%, respectively. Overall, for the twenty-nine soils studied, bioaccessible fractions of Cd and Pb were higher (2.3 and 4.6 times, respectively) in the G phase than in the GI phase. These results are explained by the pH of the simulated fluids, which are more acidic in the G phase and lead to higher solubilization of metals [9,11]. However, this is much less marked for As, which showed similar bioaccessible fractions, in most cases, in both phases with average values of 37% *vs* 30% (*i.e.*, 1.2-fold less), for G and GI phases, respectively (**Tab 2**). This difference could be related to the particular geochemical behaviour of As in the simulated fluid conditions [11,26], which may lead to reduced adsorption and precipitation reactions at the neutral pH in the GI phase for As than for Cd and Pb [9,11]. These results for the three studied metal(loid)s are in accordance with those in other studies [9,11,39,40], especially those of Denys et al. [12], in an *in vivo* validation of the UBM test on juvenile swine.

The *in vivo* bioavailability of As, Cd and Pb measured with the bioaccumulation test after *C. 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 mg kg^{-1} of dry weight (dw) viscera, respectively (**Tab 2**). The comparison between bioaccessible and bioaccumulated concentrations of As and Pb for each soil showed that the concentrations in snails were lower than the bioaccessible phase concentrations (*e.g.*, the As and Pb concentrations in snails were approximately 9 and 6 times lower than those in the G phase, respectively). In contrast, for Cd, the concentrations were 2 times higher in snails than in the G phase of the UBM test due to the snail physiology and internal management of metal(loid)s; specifically, Pb is highly excreted with cell debris, while Cd is stored and associated with metallothionein in the cytosolic fraction [24,41]. Hence, elevated BAFs were recorded for Cd (ranging from 0.859 and 25.3), and low BAFs were recorded for As and Pb (ranging from 0.004 to 0.169 or 0.055 to 0.769, respectively) (**Tab 2**). These BAFs are in accordance with those reported in various studies in which *C. aspersus* was classified as a deconcentrator of As and Pb and a macroconcentrator of Cd [22,23,25,42,43].

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

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

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

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

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

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

The robustness of the improved models was checked using a cross-validation approach. The small differences (< 0.17) between the $r^2_{adj.}$ (**Tab 4**) and $q^2_{adj.}$ (**Tab 5**) values testify to the efficiency of the proposed models for assessing metal(loid) bioaccessibility to humans using a bioavailability assessment with snails. Finally, we verified the estimation potential of these validated models by comparing measured and estimated values of bioaccessible concentrations (**Fig 3**). For each digestive phase and metal(loid) studied, the slopes were relatively close to 1 (ranging from 0.78 to 0.95), demonstrating that bioavailability to snails may be an efficient way to estimate oral bioaccessibility for humans.

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

4. Conclusions

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

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

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

5. Acknowledgements

The authors would like to thank ADEME for funding the study programme COMBINE: Coupler des indicateurs chiMiques et Biologiques pour une évaluation Intégrative des risques sanitaires et Environnementaux (contract n°1572C0310) and the programme ODESSA: Optimisation de la gestion DEs Sites et Sols pollués par une mesure simple de bioAccessibilité (contract n°1472C0046). The authors thank Dominique Rieffel and Caroline Amiot for their technical assistance. The authors warmly thank the reviewers for their constructive remarks.

6. Conflict of interest

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 mg* kg^{-1} *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	$[As]_{UBM\text{-}G} = -0.422^{*} + 0.952[As]_{soil}^{***}$	0.650	<0.001
	$[As]_{UBM\text{-}G} = -2.09^{**} + 1.00[As]_{soil}^{***} + 0.623[silt]^{*}$	0.714	<0.001
	$[As]_{UBM\text{-}GI} = -0.337 + 0.851[As]_{soil}^{***}$	0.631	<0.001
	$[As]_{UBM\text{-}GI} = -2.06^{**} + 0.905[As]_{soil}^{***} + 0.644[silt]^{**}$	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	$[Cd]_{UBM\text{-}G} = -0.054^{*} + 0.996[Cd]_{soil}^{***}$	0.987	<0.001
	$[Cd]_{UBM\text{-}G}$ = no influence of soil parameters	-	-
	$[Cd]_{UBM\text{-}GI} = -0.144^{***} + 0.833[Cd]_{soil}^{***}$	0.947	<0.001
	$[Cd]_{UBM\text{-}GI} = 0.160 + 0.893[Cd]_{soil}^{***} - 0.193[OM]^{**}$	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	$[Pb]_{UBM\text{-}G} = -0.350^{*} + 1.07[Pb]_{soil}^{***}$	0.945	<0.001
	$[Pb]_{UBM\text{-}G}$ = no influence of soil parameters	-	-
	$[Pb]_{UBM\text{-}GI} = -1.68^{***} + 1.18[Pb]_{soil}^{***}$	0.748	<0.001
	$[Pb]_{UBM\text{-}GI}$ = no influence of soil parameters	-	-
	$[Pb]_{snail} = -0.540^{*} + 0.928[Pb]_{soil}^{***}$	0.846	<0.001
	$[Pb]_{snail}$ = no influence of soil parameters	-	-

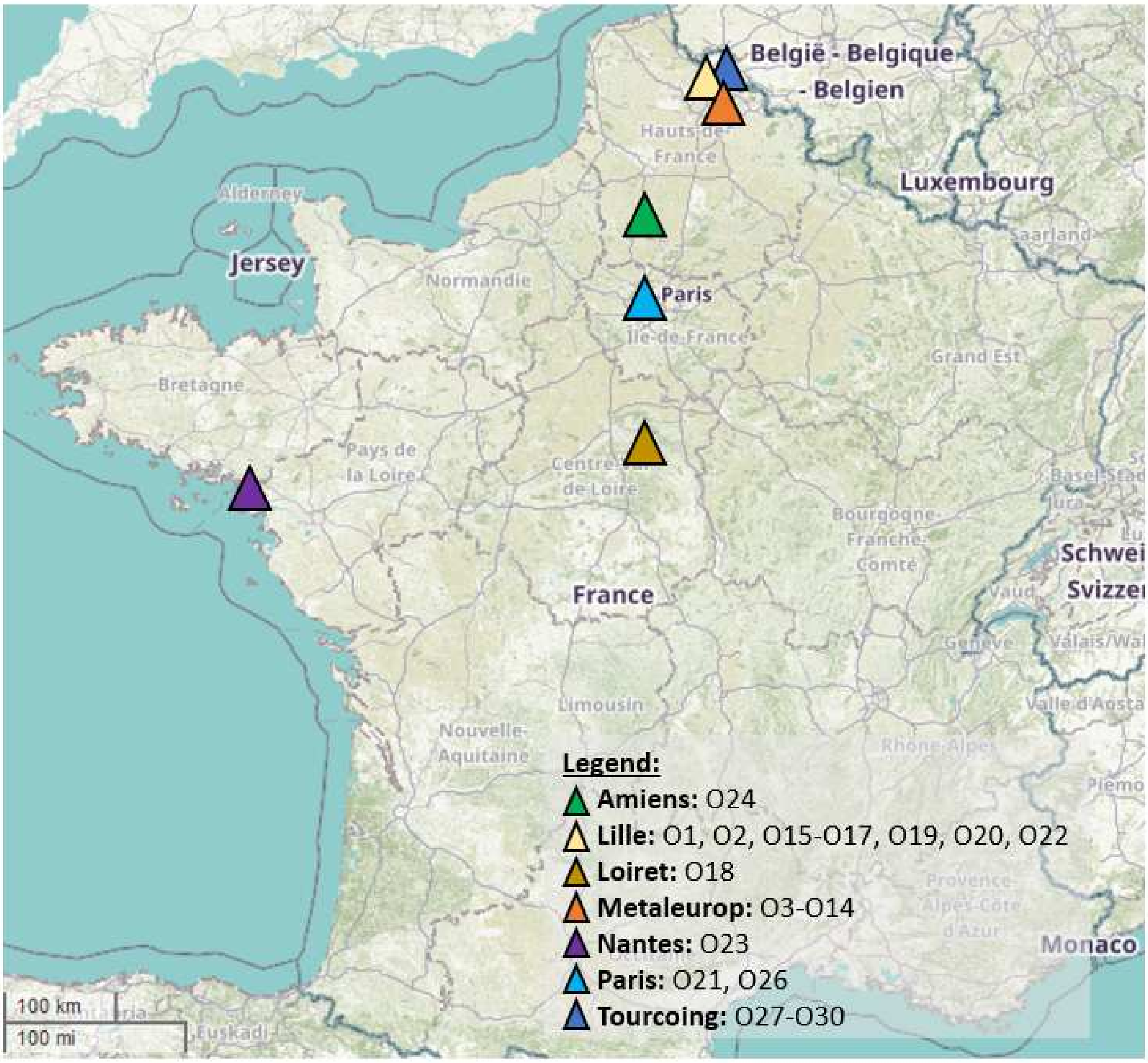
Table 4:

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

Table 5:

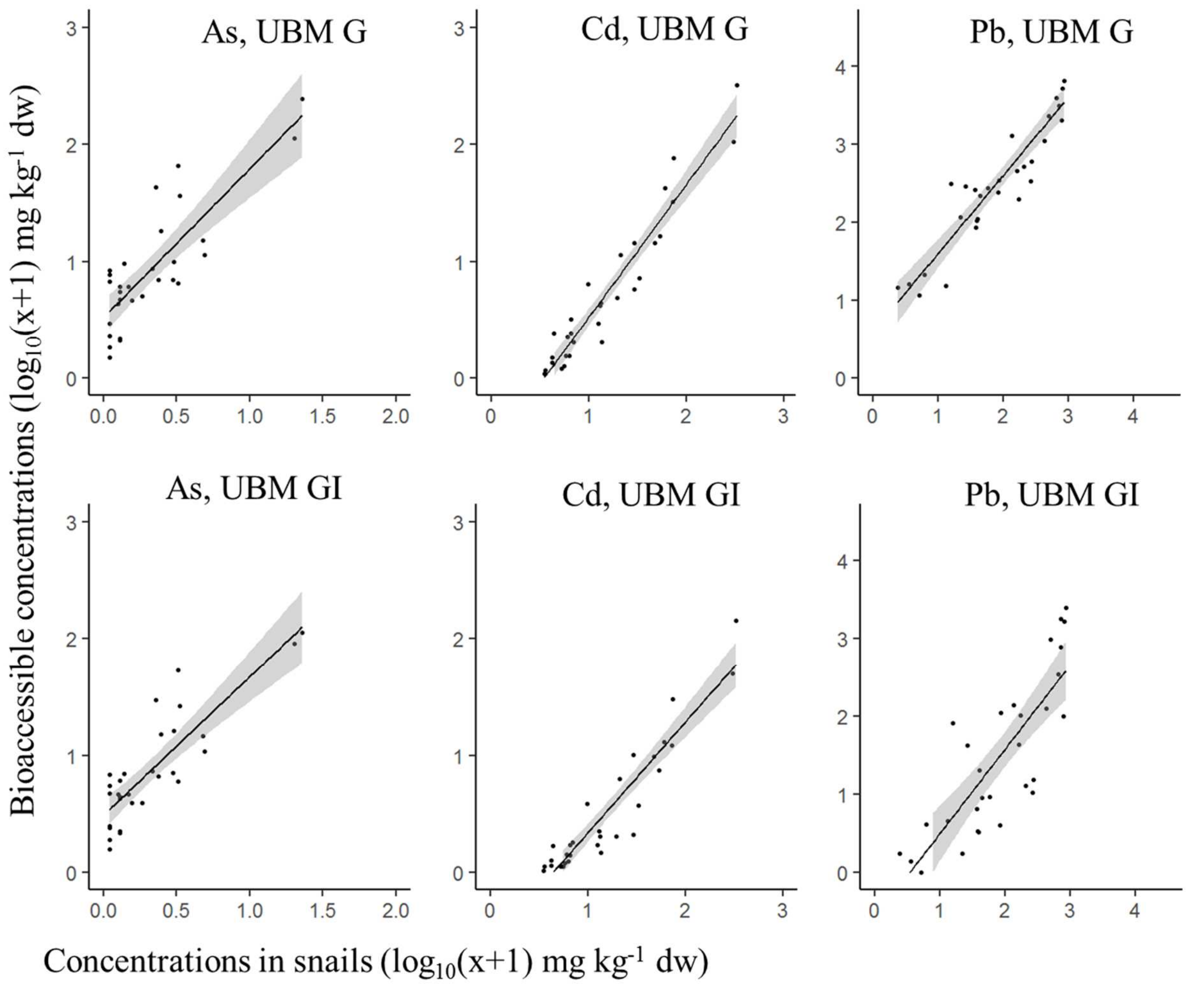
Elements	Internal cross-validation equations	$q^2_{adj.}$	p-value
As	$[As]_{UBM\ G} = -0.973 + 1.24[As]_{snail}{}^{**} + 0.800[OM]^{*}$	0.800	<0.01
	$[As]_{UBM\ GI} = -0.815 + 1.03[As]_{snail}{}^{***} + 0.742[OM]^{**}$	0.843	<0.001
Cd	$[Cd]_{UBM\ G} = -1.61^{**} + 1.37[Cd]_{snail}{}^{***} + 0.286[OM]$	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:



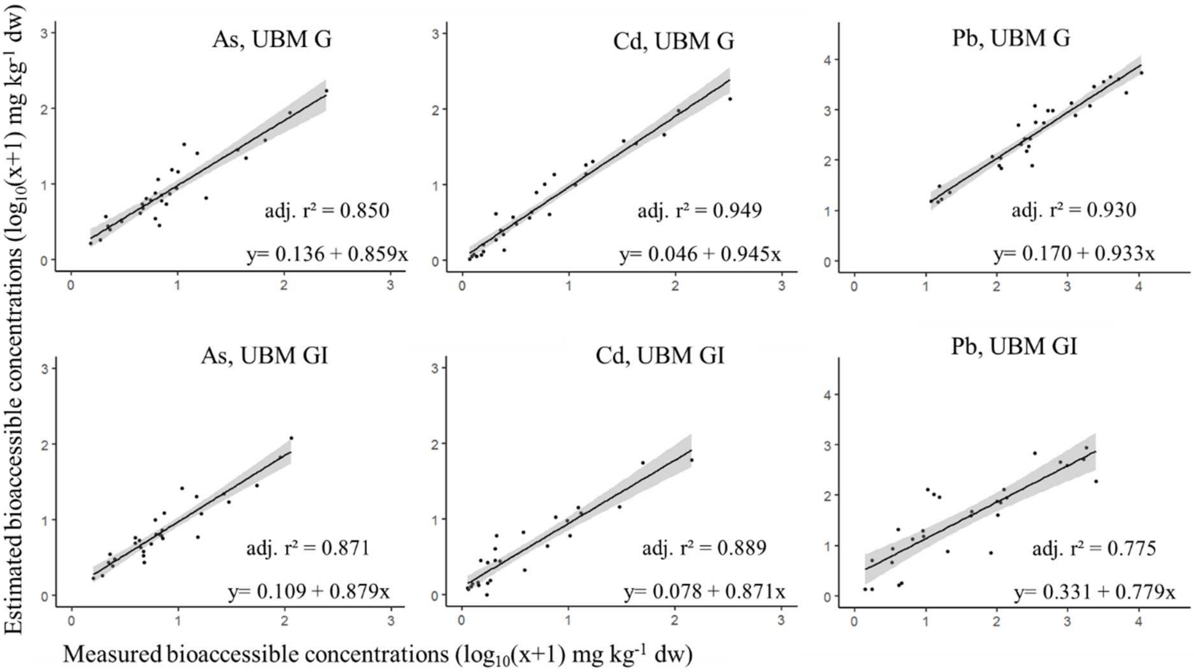
België - Belgique - Belgien
Hauts-de-France
Luxembourg
Saarland
Alderney
Jersey
Normandie
Paris
Île-de-France
Grand Est
Bretagne
Pays de la Loire
Centre-Val de Loire
Bourgogne-Franche-Comté
Schwei
Svizzer
Vaud
France
Genève
Valais/Wal
Limousin
Valle d'Aosta
Nouvelle-Aquitaine
Rhône-Alpes
Piemo
Provence-Alpes-Côte d'Azur
Monaco
Euskadi
100 km
100 mi
Legend:
Amiens: O24
Lille: O1, O2, O15-O17, O19, O20, O22
Loiret: O18
Metaleurop: O3-O14
Nantes: O23
Paris: O21, O26
Tourcoing: O27-O30

Fig 2:



As, UBM G
Cd, UBM G
Pb, UBM G
As, UBM GI
Cd, UBM GI
Pb, UBM GI
Bioaccessible concentrations ($\log_{10}$(x+1) mg kg^{-1} dw)
Concentrations in snails ($\log_{10}$(x+1) mg kg^{-1} dw)

Fig 3:



As, UBM G
adj. r² = 0.850
y= 0.136 + 0.859x
Cd, UBM G
adj. r² = 0.949
y= 0.046 + 0.945x
Pb, UBM G
adj. r² = 0.930
y= 0.170 + 0.933x
As, UBM GI
adj. r² = 0.871
y= 0.109 + 0.879x
Cd, UBM GI
adj. r² = 0.889
y= 0.078 + 0.871x
Pb, UBM GI
adj. r² = 0.775
y= 0.331 + 0.779x
Estimated bioaccessible concentrations (log10(x+1) mg kg-1 dw)
Measured bioaccessible concentrations (log10(x+1) mg kg-1 dw)