Assessing metals bioaccessibility to man in human health risk assessment of contaminated site

Sébastien Denys, Karine Tack, Julien Caboche, Patrice Delalain

Assessing metals bioaccessibility to Man in human health risk assessment of contaminated sites

S. DENYS, INERIS, Verneuil en Halatte, France, sebastien.denys@ineris.fr
K. TACK, INERIS, Verneuil en Halatte, France, karine.tack@ineris.fr
J. CABOCHE, INERIS, Verneuil en Halatte, France, julien.caboche@ineris.fr
P. DELALAIN, INERIS, Verneuil en Halatte, France, patrice.delalain@ineris.fr

1 INTRODUCTION

Soil ingestion is a significant exposure pathway to Man for sites contaminated with heavy metals. In France, risk assessments on polluted soils currently consider the total soil metal content for calculation of the dose exposure to Man. However several studies done on animals showed that this assumption was not accurate as the total soil metal content was not available for absorption though the gastro-intestinal system. The fraction that is actually entering the systemic circulation is the bioavailable fraction and was shown to be dependent on the solid metal distribution (Casteel et al., 2001; Henningsen et al., 1998). Measurement of metal bioavailability can be done using animals (pigs and monkeys) having similar digestive conditions than Humans. However these experiments are costly and can be ethically problematic. These last years, both North American and European scientists developed chemical tests to measure the bioaccessible fraction of the soil metals. These tests are based on two or three steps extraction method that simulates the extraction of the metal by the digestive fluxes (saliva, gastric and intestinal conditions). These fractions were shown to be correlated to the bioavailable fraction for some metalloids (arsenic) or metals (lead). The bioaccessible fraction of metal is then used to correct the soil metal total concentration. This data can be used to improve the estimation of the external fraction of the contaminant that is available for absorption through the digestive tract.

The objectives of this paper are to give a short overview of the methods available in the literature to assess the soil metal bioaccessibility, focusing on the tests developed by the European BARGE working group. One of these tests is then used on four soils sampled on a residential area on which soils are strongly contaminated with lead. Results of bioaccessibility are then discussed regarding the soil solid phase repartition of this metal using Tessier sequential extractions. Finally, a theoretical human health risk assessment is then carried out considering both the total and the bioaccessible lead fraction. Impact of bioaccessibility in terms of management of contaminated sites is then discussed.

2 Definition and Measurement of the bioaccessibility

2.1 Definition

Bioaccessibility is defined as the fraction of a pollutant that can be dissolved by the digestive fluids. This is the theoretical fraction available for adsorption through the gastrointestinal tract.

2.2 Overview of existing protocols to assess bioaccessible fraction of soil contaminant

Numerous tests are available in the literature to assess soil metal bioaccessibility. Most of these tests consist in a two steps extraction protocol: the intestinal and the stomachal step. A common characteristic among these tests is the difference in pH, ranging from low value in the intestinal step to close-to-neutral value in the stomachal step. Beyond this similarity, huge differences exist among these tests regarding experimental conditions or composition of the extractants used to simulate the digestive fluids. These differences might strongly influence measurement of bioaccessibility. Table 1 gives an overview of the chemical test available in the literature to estimate the bioaccessible fraction of a soil contaminant.

Table 1 Overview of the different methods available in the litterature to estimate a soil pollutant bioaccessibility to Man

Method	Type	Simulated digestive compartment	pH	T (°C)	Ratio L/S *	Residence Time	Tested metals
PBET	Batch	Stomach Small intestine	2.5 7	37 37	100/1 100/1	1 H 4H	As, Pb
SBET	Batch	Stomach	1.5	37	100/1	1H	As, Cd, Pb
IVG	Batch	Stomach Small intestine	1.8 5.5	37 37	150/1 150/1	1H 1H	As
US P	Batch	Stomach	1	37	1000/1	2H	Pb, Cr, As, Cd, Ni
MB & SR	Batch	Mouth Stomach Small intestine	6.4 2 7.5	37 37 37	160/1 2160/1 4770/1	5 s 2H 4H	Pb, Cr, As, Cd
DIN	Batch	Mouth Stomach Small intestine	6.4 2 7.5	37 37 37	15/1 50/1 100/1	0.5 H 2 H 6H	As, Cd, Pb, Cr, Hg
SHIME	Batch	Stomach Small intestine	5.2 6.5	37 37	2.5/1 4/1	3 H 5 H	As, Cd, Pb
RIVM	Batch	Mouth Stomach Small intestine	6.5 1.5 5.5	37 37 37	15/1 37.5/1 97.5/1	5 min 2 H 2H	As, Cd, Pb
TIM	Dyamic	Mouth Stomach Small intestine	5 2 7	37 37 37	5/1 30/1 51/1	5 min 1.5 H 6 H	As, Cd, Pb
AOAC	Batch	Stomach	1.12	37	150/1	16 H	Cu, Zn, Mn, Fe, Al

*Liquid/Solid Ratio used in the extractions

2.3 The BARGE protocol

To homogenized protocols, a European working group (Bioaccessibility Research Group for Europe, BARGE) was created. This group aims to define and normalize one protocol for the measurement of the bioaccessibility for inorganic or organic compounds. Even if few adjustments of the protocol are still in progress, a general description of the method can be done. The protocol consists in a three steps extraction procedure conducted on a 0.6 g of a soil sample. A 9.0 mL simulated saliva fluid is added and thoroughly mixed to the soil sample. 5 to 15 min after addition of this saliva fluid, 13.5 mL of simulated gastric fluid is added to the suspension that is end-over rotated for 1 hour. After homogenization, pH of the suspension is adjusted to 1.2 to 1.7 using HCl. The stomach phase is then collected by centrifuging the soil suspension at 3000 g for 5 minutes. The supernatant is removed and metal content measured as the bioaccessible contaminant in the stomach phase. The gastric bioaccessible contaminant is measured after addition to the soil suspension of a simulated gastric fluid. pH of the suspension for the gastric step is adjusted to 6.3 using $NaHCO_3$ and the suspension is mixed for 4 hours. The soil suspension is then centrifuged at 3000 g for 5 minutes, the supernatant removed and metal content in this latter measured as the bioaccessible contaminant in the gastric phase. Simulated fluids are composed with both organic and inorganic reagents having specific composition. To these fluids, a simulated bile phase reagent is added to be as close as possible to the actual digestion conditions.

Assessment of lead bioaccessibility on a former mining extraction area

3.1 Material and methods

Lead bioaccessibility was determined on 4 soils sampled on 4 locations at a site contaminated with mining waste and located in a high carbonate soil area in the South of France. Each location corresponded to different soil use: garden (2 samples), waste material sample on a pedestrian pathway (1 sample) and a soil naturally developed on the former mined bedrock (1 sample). For each location, a representative sample was obtained by combining three point samples of the surface horizon (0-5 cm). After sampling, the soils were air-dried and sieved to < 250 µm particle size. This particle size is believed to be ingested or directly available for inhalation by children (Grön et Andersen, 2003; Oomen et al., 2003). Soil characteristics and lead content are given below (**Table 2**).

Table 2 : Soil characteristics and total lead content

	pH	Organic C g kg^{-1}	Total Carbonate g kg^{-1}	Pb content mg kg^{-1}
Garden 1	7.1	53.4	170.0	4 767
Garden 2	7.8	60.0	298.0	2 141
Waste material	7.9	9.4	346.0	77 007

Table 1). Temperature was maintained at 37°C throughout the extraction procedure. 0.6 g of soil was mixed to 9 mL of saliva (pH 6.5). This suspension was shaken for 5 minutes. Then 13.5 mL of gastric solution (pH 1.7) was added to the soil suspension. The pH of the solution was reduced to 1.2 using HCl. The suspension was end-over mixed for 2 hours and an aliquot of 0.25 mL was sampled for measurement of Pb in the solution (saliva-gastric phase). During the third step, bile and intestinal solution were added And the pH was increased to between 5.5 to 6. Solutions were mixed end over end for a further 2 hours and centrifuged at 3 000 g. The supernatant was then sampled to determine the Pb concentration in the solution (intestinal phase).Bioaccessibility tests were carried out on the four samples and the two reference materials. Results of bioaccessibility are expressed as the percentage of the total soil Pb content and for each sample, tests were carried out on three replicates.

Pb distribution within the four soil samples was evaluated using a slightly modified sequential extraction according to the Tessier et al (1979) protocol.

Table 3 : Step of the sequential extraction procedure according to Tessier et al (1979) and slightly modified

Step	Fraction	pH	Reagents
I	Soluble	5.7	Ultrapure Water
II	Exchangeable	5.0	Magnesium nitrate 1M
III	Acid-soluble	4.5	Sodium acetate/ Acetic acid
IV a	Manganese oxydes	3.5	Hydroxylammonium chloride
IV b	Amorphous Iron Oxydes	3.0	Ammonium oxalate 0,2M/ oxalic acid 0,2M
IV c	Crystalline Iron Oxydes	2.25	Ammonium oxalate 0,2 M- oxalic acid 0,2 M-ascorbic acid 0,1 M
Va	Organic Matter		H_2O_2 35%
Vb	Sulfite		Nitric Acid 7N
VI	Residual		HF+HNO_3

For the waste material only, which was the most contaminated material, the solid residue was kept for further physical analysis. The high concentration of Pb in the waste material allowed analysis of this matrix by physical methods. X Ray Diffractometry (XRD), Scanning electron microscopy couple to EDX analysis and Infra-red spectrometry analysis were done both on the waste material and on the residues obtained after each step of the sequential extraction.

3.2 Results

3.2.1 Pb bioaccessibility

For all soil samples, Pb bioaccessibility was significantly higher in the saliva-gastric phase compared to the intestinal phase (α = 5%) (Table 5). Recovery of the total Pb content using the in vitro bioaccessibility test varied between 20% on the G2 soil and 65% on the waste material.

*

Table 4 : Bioaccessibility (% of total lead content) of lead in the four soil samples

		Garden (G1)	Garden (G2)	Waste material	Geochemical background
Bioaccessibility	Saliva - Gastric	56 ± 7 (c)	15 ± 3 (f)	50 ± 7 (d)	21 ± 4 (e)
	Intestinal	25 ± 3 (a)	5 ± 1 (d)	15 ± 2 (b)	9 ± 2 (c)
	Pb recovery	81	20	65	30

numbers followed by the same letter are not significantly different (Neuwmann-Keuls test, α = 5

Bioaccessibility values for both phases varied among samples. For the saliva-gastric phase, it ranged between 15% and 56% of the total Pb content; for the intestinal phase, it ranged from 5% (G2) to 25% (G1) of the total Pb content. For each phase, bioaccessibility values were significantly different between samples (α = 5%).

3.2.2 Pb distribution within the soil samples as determined by sequential extractions

For each French soil sample, results of sequential extractions are described. Whatever the sample, Pb was mostly extracted during the steps III (acid-soluble fraction),Vb (oxidable fraction). For step III the concentration of the extracted Pb ranged from 20% (waste) to 72% (G2) of the total soil Pb content. For step Vb, the concentration of the Pb in the extract varied between 31% (G1) and 62% (geochemical background) of the total soil Pb content. Pb content was always higher in extracts following step Vb except for the G2 sample in which Pb concentration was higher in the extract following step III.

Beyond steps III and Vb, Pb concentration measured after the other steps of the extraction were below 1% of the total soil Pb content, except for the step IVc (iron-oxide fraction) in which Pb concentration could reach 4% of the total soil Pb content. Non negligible Pb concentration were also obtained in the residual fraction (step VI).

3.2.3 Pb distribution within the initial waste material or its residues from each step of the sequential extraction

XRD showed the presence of cerrussite ($PbCO_3$) as the predominant Pb bearing mineral in the waste material. The same technique carried out on the residues showed that cerrussite was detected after only the first two steps of the extraction procedure. Scanning electron microscopy couple to EDX analysis showed that Pb was not detected after the step V of the sequential extraction. Morevoer, Pb in the residues from the first and second step was associated to carbon and oxyge wheras it was mostly associated to sulfur, iron and zinc in the residues obtained in the following steps of the extraction procedure.

Infra red spectrometry conducted on the initial waste material was used to detect the two bands (678 cm^{-1} and 839 cm^{-1}) characterizing cerrussite. Quantification of this mineral using calibration curves showed that between 30% and 40% of Pb was occurring in the cerrussite form in the initial waste material. This technique allowed to detect occurrence of cerrussite in the residual fractions obtained from the steps I and II of the sequential extraction whereas $PbCO_3$ was not occurring anymore in the fraction obtained from the following steps of the extraction procedure. This latter technique was also conducted on the residual waste material after the in-vitro bioaccessibility measurement test and showed that cerrussite was not detected in this matrix.

3.3 Discussion

Bioavailability of Pb in human health risk assessment is supposed to be dependent on geochemical factors controlling Pb speciation in soil. From US EPA reports, it was underlined that cerrussite contains highly bioavailable Pb (US EPA, 1999). Bioaccessibility, which significantly controls bioavailability, should also be dependent on the soil Pb speciation. Consequently, risk management of soils contaminated by Pb might be based on the soil mineralogical composition. Particularly, special attention should be given to high carbonate areas in which Pb could occur in carbonates and therefore being strongly bioaccessible to humans.

The aims of this study were to verify this hypothesis on soil samples from a high carbonate dolomitic area in which Pb was supposed to occur mostly as carbonates by studying the solid phase distribution of Pb in these soils and the Pb bioaccessibility obtained by an in-vitro test.

Pb bioaccessibility results were significantly different between soil samples. Bioaccessible Pb measurements on the NIST reference material were similar to the results published in the literature using the same protocol, confirming the accuracy of our measurements (Oomen et al., 2002 ; Oomen et al., 2004).

Physical analysis of the contaminated waste material confirmed that 40% of Pb was present as cerrussite ($PbCO_3$). These analyses show that about 20% of this mineral is dissolved between the first and the third step of the sequential extraction. So, even if $PbCO_3$ was not occurring anymore in the fractions after step due a too high detection limit for IRS technique, it is clear that $PbCO_3$ is also dissolved during the following steps or the extraction procedure. This confirms that bioaccessibility of Pb is partly controlled by the presence of cerrussite. Moreover, this also confirms that cerrussite is a highly bioaccessible Pb-bearing mineral because of its entire dissolution during the bioaccessibility test.

Assuming that cerrussite strongly controls Pb bioaccessibility in high carbonate soils, then there should be a link between the bioaccessible Pb and the Pb concentration as measured in the third step of the sequential extraction.. However, the bioaccessibility tests shows that 65% of Pb was bioaccessible in the waste material. This means that a significant fraction (25%) of the Pb which is not occurring as cerrussite is also bioaccessible. In addition, for the three other soils, Pb bioaccessibility is significantly different among them whereas the Pb concentration in the extract following the step III is quite similar (c.1000 mg kg^{-1}). Moreover, bioaccessibility of Pb is higher for soil G1 compared to the waste material whereas the Pb concentration in the sequential extract of the third step of soil G1 is a factor of ten lower than the Pb concentration for the same extract from the waste material. Assuming that most of the carbonated forms were totally dissolved during step III of the sequential extraction procedure, this means that non-carbonate forms of Pb may be significantly bioaccessible. From physical analysis, Pb in the residues obtained after the last three steps of the extraction seems to be associated to sulphur and oxygen. The results of sequential extraction show that Pb is mostly extracted during the last step of the protocol (excepted for the G2 soil). These observations confirm a significant association of Pb to more stable mineralogical forms even if that have not been fully quantified in this present study. The bioaccessibility of Pb contained in these stable minerals can be explained by the chemical conditions of the digestion test. Indeed, the intestinal phase consists of an acid extraction (pH = 1.2) in which quite stable minerals are likely to be soluble. Another parameter to consider for bioaccessibility is the residence time of the matrix in the gastric compartment. The higher the residence time of the matrix in the gastric compartment, the higher the Pb extraction for even quite stable minerals. Therefore, even in carbonated areas, Pb bioaccessibility cannot be simply predicted from the carbonated minerals content of soil. The other Pb bearing mineral also play a significant role in overall Pb bioaccessibility to humans.

Integration of bioaccessibility in human health risk assessment and in the calculation of rehabilitation level

Bioaccessibility can simply be integrated to human health risk assessment to adjust the dose exposure to Man by soil ingestion. Equation 1 shows how the bioaccessibility fraction has to be integrated to the calculation of the dose exposure.

Equation 1 :

*Dose Exposure $_{adujusted}$ = Soil Total Content (mg kg^{-1}) * Bioaccessibility (%) * Amount of ingested soil (mg day^{-1})*

Impact concerning the integration of this value in human health risk assessment is shown here, based on a theoretical residential scenario. A hazard quotient can be calculated for the soil ingestion pathway, considering a child chronic exposure to the garden soil 2 (total lead content = 2142 mg kg^{-1}; lead bioaccessibility = 20%; yearly exposure time = 350 days; toxicological reference value = 3.5 µg kg^{-1} day^{-1}). The hazard quotient varies from 5.8 when bioaccessibility is not considered to 1.1 when bioaccessibility is considered.

A rehabilitation level might also be calculated for this soil considering or not bioaccessibility. To reach a hazard quotient of 1, a lead concentration of 380 mg kg^{-1} is needed without considering bioaccessibility. When bioaccessibility of lead in the soil garden 2, the rehabilitation level is around 1 800 mg kg^{-1}.

5 Conclusion

The bioavailability of a soil contaminant to Man can be approached by the measurement of the fraction of this metal that is dissolved in the gastro-intestinal tract (bioaccessible fraction). This fraction can be measured by chemical extraction tests based on two or three steps simulating the different steps of the digestion process. Numerous tests can be found in the literature. The European Barge Group aims to currently develop and normalize one homogeneous test involving a three steps extraction procedure (saliva, gastric and intestinal extraction steps). Here one test selected among the available tests in the litterature and defined by the Dutch RIVM was chosen to characterized lead bioaccessibility in four soils sampled around a former mining extraction area. Lead bioaccessibility in these samples ranged from 20 to 80% of the soil total lead content. Discussion of these values regarding lead distribution within the soil solid phase was done using both chemical and physical methods. Even if carbonate lead (cerrussite) was identified as a highly bioaccessible form of lead, dissolution of lead associate to quite stable mineral (lead associated to sulfur) also occured during the chemical tests showing that these forms might contribute sensitively to overall lead bioaccessibility.

Implication of the bioaccessibility concept in human health risk assessment allows for an improved estimate of the contaminant which is actually available for adsorption through the gastro-intestinal tract. However, some studies are still needed even if bioaccessibility is ever currently used in European countries. Particularly further researchs are needed on the relationships between bioaccessibility values and bioavailability values as measured using animal models on the same soil samples.

6 References

Casteel, S. , Evans, T., Turk, J., Basta, N., Weis, C., Henningsen, G., Hoffman, E. (2001) Refining the risk assessment of metal-contaminated soils. Short Communication. Int. J. Hyg. Environ. Health 203, 473-474.

Henningsen G., Weis C., Casteel S, Brown L, Hoffman, E, Brattin, W, Drexler J., Christensen, S. (1998) Differential bioavailability of Pb mixtures from twenty different soil matrices at Superfund mining sites. Toxicological Sciences. 42.

Oomen, A.G.(2000). Determination of oral bioavailability of soil-borne contaminants. PhD, University of Utrecht.

Oomen, A.G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Scoeters, G., Verstraete, W., Van de Wiele, T., Wragg, J., Rompelberg, C.J.M., Sips,A., Van Wijnen, J.H.(2002). Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. Environmental Sciences and Technology, 36, 3326-3334.

Oomen, A.G., Rompelberg, C.J.M., Van de Kamp, E., Pereboom, D.P.K.H., De Zwart, L.L., Sips, A.J.A.M.(2004). Effect of bile type on the bioaccessibility of soil contaminants in an in vitro digestion model. Archives of Environmental Contamination and Toxicology, 46, 183-188.

Tessier , A., Campbell, P.G.C. and Bisson, M.; 1979. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry, 51, 844-850.

US EPA (1999) Short Sheet : IEUBK Model Bioavailability variable. Office of Solid Waste and Emergency Response. EPA#540-F-00-006.