# Assessing the *in situ* bioavailability of trace elements to snails using accumulation kinetics

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## ARTICLE INFO

## ABSTRACT

The bioavailability of trace elements in soils is conditioned by both physico-chemical and biological parameters. In this study, the accumulation kinetics of cadmium (Cd), lead (Pb), arsenic (As) and antimony (Sb) were determined for 3 industrially impacted sites to assess the bioavailability of these contaminants to the garden snail (*Cantareus aspersus*). Mono and multivariate regressions allowed the identification of cation exchange capacity (CEC), silts and organic carbon content as the soil parameters modulating the *in situ* bioavailability of Cd and Pb. For all elements, the total concentrations in the soils were not good predictor (not significant correlation) of the bioavailability to snails. The Cd, As and Sb assimilation fluxes were correlated with the calcium chloride ($CaCl_2$) extract concentrations, but this correlation was not observed with Pb. The total soil concentration coupled with soil properties best explained the variation in Pb assimilation, whereas their influences on Cd bioavailability were lower, signifying that other parameters such as contamination sources may modulate Cd bioavailability. Here, the As and Sb *in situ* accumulation kinetics are described for the first time and highlighted a slight bioavailability to snails at the studied sites. The absence of a correlation between the As or Sb assimilation fluxes and total metals in the soil coupled with the absence of influence of soil properties on their bioavailability may result from the speciation of these metalloids, which are known to modulate their mobility in soils. This study highlights the need to consider both physico-chemical and biological aspects of metal and metalloid bioavailability to assess the risk of metal transfer from soil to organisms.

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## 1. Introduction

In France, many sites present soil contaminated by metals and metalloids due to anthropogenic activities such as soil mining (Gis, 2011; Matera et al., 2003). Currently, risk assessment procedures often ignore the soil characteristics, although these characteristics may influence both metal mobility in soil (Young et al., 2000) and the transfer of the contaminants to primary producers and primary consumers (Kabata-Pendias, 2004). This transfer corresponds to the flux of a pollutant from a compartment (biotic or abiotic) to organisms and is conditioned by trace element exposure and bioavailability. To analyze the risk of metal transfer, metal bioavailability must be estimated using biological measures (ISO, 2008).

For this purpose, the measurement of metal accumulation kinetics in bioindicators that consider the dynamic processes of bioavailability is an efficient method (Gimbert et al., 2006; Pauget et al., 2011; Van Straalen et al., 2008). Among the panel of bioindicators, the garden snail (*Cantareus aspersus*), already used in active biomonitoring (Fritsch et al., 2011) and for metal bioavailability assessment (Coeurdassier et al., 2010), presents numerous advantages: it lives at the soil–plant–air interface and then integrates the different sources (soil, plant, etc.) and routes (dermal and digestive) of contamination (Coeurdassier et al., 2002; de Vaufleury et al., 2006; Scheifler et al., 2006).

Currently, much work is being performed to find chemical extractants that allow the bioavailable pool of contaminants to

be mimicked (Caboche et al., 2010; Denys et al., 2009; ISO, 2008). Assessing metal bioavailability using accumulation kinetics requires the lethal measurement of metal concentration in tissues. To eliminate the sacrifice of organisms and to determine the effectiveness of the mimetic chemical method of metal bioavailability, an evaluation of the ability of chemical methods to assess metal bioavailability to snails must be conducted. Calcium chloride ($CaCl_2$) extraction has been determined as usable for the assessment of the phytoavailability and bioavailability of metals to soil organisms (ISO, 2008). However, the inability of $CaCl_2$ extract to assess the bioavailability of Cd and Pb to snails has been highlighted (Fritsch et al., 2011; Mourier et al., 2011; Pauget et al., 2011). The correlation between the metal extracted concentration and the *in situ* metal bioavailability to *C. aspersus* determined by active monitoring with consideration of the dynamic processes of bioavailability has never been studied. Accordingly, the objective of this study was first to evaluate the bioavailability of two metals (cadmium (Cd) and lead (Pb)) and, for the first time, two metalloids (arsenic (As) and antimony (Sb)) *in situ* at three polluted sites on the basis of their accumulation kinetics and the calculation of their assimilation flux (*a*). Another objective was to estimate the influence of *in situ* soil properties on metal bioavailability using multivariate equations. We also investigated whether the total soil concentration or $CaCl_2$ extract of metals could be used to assess the bioavailability of these 4 trace elements to snails.

## 2. Materials and methods

### 2.1. Site characteristics

Three industrial sites from among those of the Bioindicator 2 program (Pérès et al., 2011) were studied (the soil characteristics of each plot are presented in Table 1).

The Metaleurop site (a contaminated site) is located in northern France (50°29′ N, 2°59′ E) and extends around the former lead smelter of Noyelles–Godault, closed in 2003. Four areas were selected according to a metal pollution gradient in the vicinity (H, I and L for high, intermediate and low, respectively) and at 10 km (R for reference) from the former smelter, as well as two areas that had been used (woodland W and arable A) were studied. Due to French law, any arable plot must be located at least 0.5 km from the smelter leading to 7 plots (*i.e.* HW, IW, IA, LW, LA, RW and RA).

The Auzon site (45°23′12 N, 03°21′32 E) is an industrial waste site with trace element contamination, such as arsenic. Six plots (4 contaminated "Co", 2 controls "Ct") were selected according to a metal pollution gradient (from 62 to 3600 mg kg$^{-1}$ As) and vegetation cover (woodland "W", woodland on hydromorphic soil "WW", woodland hedge "WH" and wasteland "Wa").

The SHSE site is a metallurgical landfill near Saint-Etienne (45°43′ N, 4°39′ E), with high metal contamination combined with herbicide contamination. Three plots were selected according to their level of plant cover, with a gradient from the "high level of cover of vegetation" plot (HCV) to the "low level of cover of vegetation" plot (LCV). Briefly, all the plots were sampled on a grid (10 m × 10 m) subdivided into 4 sampling-zones (5 m × 5 m). In each 25 m$^2$ sampling zone, 12 randomized soil samples were taken (0–15 cm depth, after removal of vegetation residues) and pooled to characterize soil physico-chemical parameters. More details on sampling strategy and analysis are presented in Pérès et al. (Pérès et al., 2011).

### 2.2. Animals

Juvenile brown garden snails (*Cantareus aspersus aspersus* Müller, 1774, syn. *Helix aspersa aspersa*) were reared as described

**Table 1**
Medians (n = 4) of the physico-chemical characteristics of the three studied sites of the Bioindicator program. Control plots are in italics.

| Site | Plot | pH$_w$ | Silts (g kg$^{-1}$) | Sands (g kg$^{-1}$) | OM (g kg$^{-1}$) | Clay (g kg$^{-1}$) | C$_{org}$ (g kg$^{-1}$) | CEC (cmol kg$^{-1}$) | [Al]$_{ox}$ (cmol kg$^{-1}$) | [Fe]$_{ox}$ (cmol kg$^{-1}$) | [Cd]$_{tot}$ (mg kg$^{-1}$) | [Pb]$_{tot}$ (mg kg$^{-1}$) | [As]$_{tot}$ (mg kg$^{-1}$) | [Sb]$_{tot}$ (mg kg$^{-1}$) | [Cd]$_{CaCl_2}$ (mg kg$^{-1}$) | [Pb]$_{CaCl_2}$ (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Metaleurop | HW | 7.99 | 601 | 101 | 83.5 | 298 | 48.3 | 29.3 | 0.038 | 0.014 | 34.4 | 2485 | 39.3 | | 0.415 | 0.465 |
| | IW | 8.19 | 609 | 96.0 | 46.0 | 294 | 26.6 | 24.3 | 0.040 | 0.020 | 13.3 | 731 | 30.9 | | 0.079 | 0.039 |
| | IA | 8.21 | 474 | 211 | 45.1 | 318 | 26.1 | 29.3 | 0.054 | 0.014 | 8.54 | 482 | 18.1 | | 0.066 | 0.031 |
| | LW | 6.45 | 283 | 517 | 55.0 | 203 | 31.8 | 17.2 | 0.059 | 0.015 | 5.44 | 319 | 9.42 | | 0.578 | 0.142 |
| | LA | 7.90 | 366 | 452 | 29.3 | 181 | 16.9 | 13.2 | 0.078 | 0.020 | 3.12 | 143 | 8.67 | | 0.047 | 0.009 |
| | *RW* | *6.55* | *525* | *313* | *34.9* | *163* | *20.2* | *12.2* | *0.053* | *0.011* | *1.09* | *48.8* | *7.11* | | *0.122* | *0.011* |
| | *RA* | *6.96* | *531* | *290* | *25.5* | *181* | *14.7* | *13.0* | *0.048* | *0.010* | *0.968* | *41.7* | *8.60* | | *0.045* | *0.005* |
| Auzon | CoWW | 5.41 | 427 | 346 | 103 | 238 | 59.6 | 19.1 | 0.425 | 0.015 | 9.97 | 4575 | 3285 | 3930 | 0.326 | 0.617 |
| | CoW | 5.73 | 279 | 574 | 70.6 | 148 | 40.8 | 13.0 | 0.176 | 0.016 | 0.722 | 104 | 339 | 37.8 | 0.054 | 0.019 |
| | CoWH | 5.23 | 167 | 757 | 76.5 | 89.5 | 44.2 | 11.0 | 0.410 | 0.017 | 1.32 | 282 | 661 | 176 | 0.120 | 0.194 |
| | CoWa | 5.81 | 167 | 713 | 60.3 | 98.5 | 34.9 | 9.46 | 0.111 | 0.009 | 6.74 | 1834 | 1087 | 2221 | 0.219 | 0.377 |
| | *CtW* | *6.22* | *299* | *556* | *70.7* | *138* | *40.9* | *14.9* | *0.069* | *0.012* | *0.612* | *60.1* | *123* | *11.9* | *0.034* | *0.004* |
| | *CtWH* | *5.10* | *225* | *628* | *45.2* | *148* | *26.2* | *8.90* | *0.814* | *0.016* | *0.143* | *28.1* | *62.5* | *9.93* | *0.021* | *0.021* |
| SHSE | HCV | 8.10 | 142 | 803 | 81.1 | 48.5 | 46.9 | 10.5 | 0.081 | 0.030 | 21.0 | 2525 | 73.3 | | 0.018 | 0.027 |
| | ICV | 8.74 | 131 | 820 | 52.5 | 44.5 | 30.4 | 6.60 | 0.065 | 0.026 | 9.37 | 1616 | 54.8 | | 0.004 | 0.012 |
| | LCV | 8.57 | 77 | 886 | 25.6 | 33.5 | 14.8 | 3.47 | 0.070 | 0.031 | 1.99 | 513 | 30.4 | | 0.001 | 0.004 |

pH$_w$: pH$_{water}$; OM: organic matter; CEC: cation exchange capacity; ox: oxide; [Metal]$_{tot}$: total metal concentration in soil; [Metal]$_{CaCl_2}$: concentration of metal extracted by 0.01 M calcium chloride.