



In situ assessment of phyto and zooavailability of trace elements: A complementary approach to chemical extraction procedures



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HIGHLIGHTS

- Bioavailability of As, Cd, Cr, Cu, Ni, Pb and Zn to plants and snails is assessed.
- Modulating parameters of metals bioavailability are estimated for plant and snails.
- Coupling bioindicators improves the assessment of trace elements bioavailability.
- No unique chemical method allows the assessment of bioavailability of metals.
- Contamination sources are important to consider during metal transfer assessment.

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ABSTRACT

For an accurate risk assessment of sites contaminated by trace elements (TE), measurements of bioavailability must be performed. This is routinely achieved using the standardized 0.01 M CaCl₂ method. However, the suitability of chemical extractions as proxies of bioavailability is questionable. We analyzed the correlations between chemically estimated TE bioavailability and TE actually accumulated by coupling plant and snails bioindicators. Results showed a better correlation between plant TE contents and CaCl₂ fraction while total soil concentration better explained snail TE contents. However in both cases chemical measures were not suitable to predict TE accumulation and bioavailability. Considering the soil properties only improve the estimation of Cr, Ni and Pb accumulation by plants while for snails, TE contents in viscera were dependent both on soil and plant contents and soil properties. It highlights the complementarities of biomonitoring methods to assess bioavailability. This dual approach allows a “physiologically defined” evaluation of bioavailability.

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1. Introduction

Since a few decades, soil degradation (e.g., erosion, loss of organic matter, sealing, pollution, ...) is an increasing problem worldwide. Contamination by trace elements (TE) is considered as one of the main threats (Jeffery et al., 2010) because of human health issues (Qingdong et al., 2007; Science Communication Unit, 2013), and also as they may cause severe ecological disturbances to both organisms and their habitats (Moriarty, 1999). For these reasons, various protective thresholds for total contents in soils have been proposed for different TE (Carlou, 2007). However it is largely acknowledged today that the toxicity of TE rather depends on their bioavailability than on their total contents in soils (Van Gestel et al., 2009). Consequently, protective

thresholds based on total TE contents in soils are only coarse indications of the potential hazard, and should be completed by methods allowing to assess TE's bioavailability (ISO, 17402, 2008).

However, although the term “bioavailability” can be easily understood as how much of a contaminant is available for living organisms, the underlying concept is much more complex and a great number of definitions, and assessment methods, have been proposed so far (Harmsen, 2007; Naidu et al., 2008; Semple et al., 2004). In an effort to offer a clear working definition of this concept, Semple et al (2004) proposed to distinguish the part of the contaminant which is “bioaccessible” (i.e., “which is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical”), from that which is actually “bioavailable” (i.e., “which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time”). However this distinction has not been retained in the definition adopted in ISO 17402 (ISO, 17402, 2008) which simply

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states that “bioavailability is the degree to which chemicals present in the soil may be absorbed or metabolized by human or ecological receptors or are available for interaction with biological systems”. Whatever the terms and definitions considered, there is however a consensus today to regard bioavailability as a multi-level concept involving three distinct notions: the environmental availability, the environmental bioavailability and the toxicological bioavailability (Gimbert et al., 2006; ISO, 17402, 2008; Lanno et al., 2004; Peijnenburg et al., 1997).

The environmental availability depends on multiple physico-chemical processes governing metal partition between the solid and liquid phases of the soil. Environmental availability is generally assessed as the so-called “available” or “easily exchangeable” TE fractions, using more or less weak chemical extractants, such as neutral salt solutions at low concentration or diluted weak acids. Because these chemical extraction methods are easy to use, reproducible and based on an easily understandable concept (the more the TE are easy to extract, the more they can interfere with living organisms), they are routinely used for risk assessment of contaminated sites. However numerous studies (Meers et al., 2007; Pauget et al., 2012; Peakall and Burger, 2003; van Gestel, 2008) have shown that the level of “available” elements, as determined by chemical extraction methods, is often a poor proxy of the fraction of TE that actually interacts with living organisms. By contrast, the environmental bioavailability refers to the fraction of contaminant that is actually taken up by biological receptors. It depends on complex species-specific physiological processes, controlling desorption of contaminants from the solid matrix and their assimilation (absorption and excretion) by the organism. To assess the environmental bioavailability of TE in soils, the use of accumulation bioindicators is obviously highly relevant (Peakall and Burger, 2003). Indeed, during the last two decades, plants (Le Guédard et al., 2012; Remon et al., 2013; Vergé et al., 2002) or soil organisms, such as snails or earthworms (Dallinger and Berger, 1992; Gimbert et al., 2008a; Pauget et al., 2013; Rabitsch, 1996; Scheiffler et al., 2003), have been proposed as accumulation bioindicators. However, the transfer of TE and their accumulation by living organisms and in food webs depends both on the species, its trophic level and exposure pathways. It is thus questionable to use a single species or to consider a single trophic level for assessing bioavailability, as this may lead to misinterpretations.

In this work we hypothesized that considering different organisms, representative of different trophic levels (primary producers and primary consumers), could be a relevant approach to get information on the environmental bioavailability of TE in soils. Transfer to primary producers was estimated by analyzing TE content in composite plant samples, as recently proposed by Remon et al. (2013). This passive biomonitoring approach informs on the phytoavailability of TE for a plant community, considered as a whole. Transfer to primary consumers was assessed by active biomonitoring with garden snails, informing on the zooavailability of TE (Fritsch et al., 2011; Gimbert et al., 2008a). We also assumed that the transfer of TE from soil to organisms at various spatial scales, could be partly conditioned by their environmental availability or/and by the soil properties. To address these issues, we performed an extensive study on 25 experimental plots (7 geographical sites) exemplifying different land use and contamination levels. We analyzed (i) the correlations between environmental availability and environmental bioavailability, as determined by chemical and biological methods, (ii) the influence of soil properties on TE accumulation by snails and plants and (iii) the relationship between TE concentrations in plants and TE accumulation by snails.

2. Materials and methods

2.1. Selected sites and studied soils

Seven sites (hereafter named Andra, Auzon, GISFI, Metaleurop, RENECOFOR, SHSE and Yvetot, S1) were selected throughout France (Fig. 1), among those studied in the French national research

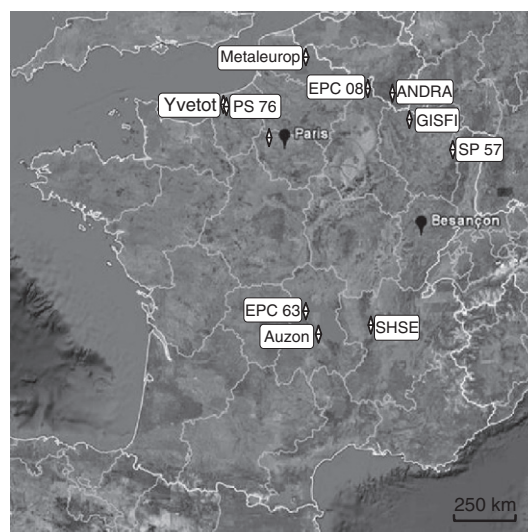


Fig. 1. Sites localization. <http://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicateur/>.

program “Bioindicators 2”; <http://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicateur>. Each site was subdivided into two to six 100 m² (10 m x 10 m) experimental plots, according to the local typology and/or land use. The Auzon (6 plots), Metaleurop (4 plots), SHSE (3 plots) and GISFI (2 plots) sites were industrial landfills or peri-industrial woodlands and grasslands more or less impacted by industrial activities; the RENECOFOR site (4 plots) belonged to a network of French forests (<http://www.onf.fr/renecofor>); the Yvetot site (4 plots) was a cultivated grassland (Plassart et al., 2008) and the Andra site presented two plots, one in a forest and one in a grassland. All these sites and experimental plots have been previously described and located in Pérès et al. (2011) and in Pauget et al. (2013).

Samples of each plot were taken on a grid (10 m x 10 m) subdivided into 4 sampling-zones (5 m x 5 m). In each 25 m² sampling zone, 12 randomized soil samples were taken (0–15 cm depth after removal of the humus) and pooled to characterize soil physico-chemical parameters.

Soils from the 25 plots studied were analyzed for their pedological characteristics, as well as for their total and “available” TE contents. All analyses were performed by the Laboratory for Soil Analyses of the National Institute for Agronomic Research (INRA Arras, France), which benefits from the COFRAC (French Accreditation Committee) accreditation n°1-1380 (available at www.cofrac.fr) for its analytical insurance in soil metal(loid) measurements. Briefly, total metals (Cd, Cr, Cu, Ni, Pb and Zn) in soil samples (routinely 250 mg dry soil, ground at <250 μm) were extracted using hydrofluoric (HF) and perchloric (HClO₄) acids, according to the NF X 31-147 procedure (AFNOR, 1996). For the determination of total As, soil samples were extracted with a mixture of sulfuric acid (H₂SO₄)/nitric acid (HNO₃) (2/1, V/V) in the presence of vanadium pentoxide (V₂O₅) at 100 °C for 3 h. For the determination of “available” metal(loids), extractions were performed with 0.01 M calcium chloride (CaCl₂), according to the NEN 5704 procedure (NEN 5704, 1996). Metal concentrations in the various extracts were measured using inductively coupled plasma atomic emission spectrometry (ICP-OES). Selected pedological characteristics of the 25 plots studied and their total and CaCl₂ extractable TE contents are given in Supplementary Material S2 and S3.

2.2. Biological material and sampling protocol

2.2.1. Plants

To get a general insight into TE transfer toward plants, and to avoid species-specific responses, metal analyses were performed at a plant community level, i.e., using composite plant samples (Remon et al.,

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