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Chemical extractions and predicted free ion activities fail to estimate metal transfer from soil to field land snails

Brice Mourier, Clémentine Fritsch, Elie Dhivert, Frédéric Gimbert, Michaël Cœurdassier, Benjamin Pauget, Annette de Vaufleury, Renaud Scheifler *

Chrono-Environment, UMR 6249 University of Franche-Comté/CNRS UsC INRA, Place Leclerc, F-25030 Besançon Cedex, France

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ABSTRACT

This study investigates the relevance of several soil chemical extractions (calcium chloride, acetic acid, citric acid and a four-step sequential procedure) and predicted free metal ion activities in the soil solution to characterise the transfer of trace metals (Cd, Pb, and Zn) from soil to snail soft tissues over a large smelter-impacted area (Metaleurop Nord, Nord-Pas-de-Calais, France). The study was first performed on six snail species together and then specifically on *Cepaea* sp. and *Oxychilus draparnaudi*. When the six species were considered together, the accumulation of metals depended mostly on the species. When significant, total or extractable metal concentrations, or the predicted free ion activities, accounted for less than 7% of the variation of the metal concentrations in the snail tissues. Species-specific analyses showed that extractable concentrations explained approximately 25% of the variation of the metal concentrations in *O. draparnaudi*, and up to 8% in *Cepaea* snails. When using total soil concentrations and soil properties as explanatory variables, the models were generally slightly better, explaining up to 42% of the variance. The soil extraction procedures and predicted free ion activities used in this study did not accurately estimate the metal transfer from soil to snails and could not be used in risk assessment.

1. Introduction

Because of historical and modern industrial activities, soils can be severely contaminated with metals (e.g., cadmium, Cd; lead, Pb; and zinc, Zn). The release of such contaminants in natural systems can consequently pose environmental and human health risks. In the case of areas affected by metallurgical industries, most of the metal pollution primarily comes from smelter fallout and dusts from spoil heaps that have been transported across natural systems. The exposure of organisms to trace metals depends on many non-biotic factors, such as the spatial distribution of metals in soils, landscape or habitat features, season, and the biological characteristics of the organism, such as diet and, more generally, life history traits (Suter, 1993). When the sources (air, water, soil, food) and routes (respiratory, cutaneous, digestive) of exposure are quantified, pollutant bioavailability is another key parameter that affects the transfer of the pollutant from the environment to the organism. The concept of bioavailability has long been debated (Harmsen, 2007), but it can be defined as the fraction of the total concentration of a pollutant that is available or that can be made available over the exposure period, for uptake (Peijnenburg and Jager, 2003). Generally, bioavailability is handled as a dynamic species-, soil- and pollutant-specific process that can be described by three parameters: (i) the availability of the contaminant in the soil (i.e., environmental availability), (ii) the uptake of the contaminant by the organism (i.e., environmental bioavailability), and (iii) the accumulation and/or effect of the contaminant within the organisms (i.e., toxicologic bioavailability) (Peijnenburg et al., 1997; Lanno et al., 2004). One of the major challenges of ecotoxicology is producing reliable methods to quantitatively measure each of these parameters and to find relationships among them. Even more challenging, but very important for predictive and applied purposes, is the quest for tools that can estimate bioavailable fractions for several organisms, soils, and/or pollutants.

A variety of chemical methods have been developed to estimate the available fraction of metals in soils and then to correlate these available concentrations with metal levels in tissues or with the observed effects in organisms. Among these methods, single or sequential extraction techniques have produced satisfactory results for certain pollutants, soils and/or organisms (see, for instance, Harmsen, 2007; Meers et al., 2007; Hass and Fine, 2010), even though it is clear that no universal extractant exists. Free metal ion activity in the soil solution has also been suggested as a

* Corresponding author. Tel.: +33 (0) 381 665 740; fax: +33 (0) 381 665 797.
E-mail addresses: brice.mourier@entpe.fr (B. Mourier), clementine.fritsch@univ-fcomte.fr (C. Fritsch), elie.dhivert@orange.fr (E. Dhivert), frederic.gimbert@univ-fcomte.fr (F. Gimbert), michael.coeurdassier@univ-fcomte.fr (M. Cœurdassier), benjamin.pauget@univ-fcomte.fr (B. Pauget), annette.devaufleury@univ-fcomte.fr (A.de Vaufleury), renaud.scheifler@univ-fcomte.fr (R. Scheifler).

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pertinent estimate of metal bioavailability and toxicity in aquatic organisms and higher plants (Smolders et al., 2009) and may be a good predictor of metal concentrations in soil organisms (Sauvé et al., 2000). However, studies examining relationships between either extractable metal concentrations in soil or free ion activities in the soil solution and organisms sampled in the field (and not exposed under controlled conditions) are scarce.

In the framework of ecotoxicology and ecological risk assessment, terrestrial snails have been extensively studied as biomonitors of environmental contamination by metals. The relationships between concentrations of metals in snails and in soils have been well studied in controlled conditions as well as in the field. Studies have generally shown that concentrations in snail tissues are related to the contamination of their environment (Berger and Dallinger, 1993). The biota-to-soil concentration ratios (BSAF) that can be derived from these studies may be useful in ecological risk assessment (Veltman et al., 2008). However, relationships between total metal concentrations in soils and concentrations in snail soft tissues are usually relatively low, which diminishes the accuracy of predicting concentrations in organisms with total soil concentrations. For instance, the coefficients of determination (R^2) for the relationship between *Cepaea nemoralis* snail and total soil concentrations were 0.33, 0.17, and 0.15 for Cd, Pb and Zn, respectively (Notten et al., 2005). Such low relationships may be because total metal concentrations are usually not considered to be the best estimate of bioavailability for both plants and organisms (Harmsen, 2007). Moreover, aboveground soil organisms, like snails, are obviously exposed to not only soil contamination but also food web transfer. Chemical extractants could therefore provide a synthetic measurement of bioavailable amounts of pollutants in the environment (soil) and in food webs (plants and soil invertebrates), possibly providing better relationships than total concentrations in studied organisms.

Within this context, we hypothesised that the chemically extractable metal concentrations in soils or the predicted free ion activity in the soil solution would provide better relationships with metal concentrations in snail tissues than total metal concentrations in soils. Thus, we compared the relationships between (i) total or (ii) extractable (using three selective extractions and a four-step sequential procedure) soil concentrations or (iii) predicted free ion activities and metal concentrations in snail soft tissues. As an alternative to the use of extractable (non-total) concentrations, we also modelled metal concentrations in snail tissues using total metal concentrations in soils and soil properties.

2. Materials and methods

2.1. Study site and sampling strategy

This study was carried out in the surroundings of the former "Metaleurop-Nord" smelter in Northern France (Noyelles-Godault, Nord-Pas-de-Calais, 50°25′42N 3°00′55E). This area is considered to be highly polluted with Cd, Pb, and Zn for both levels of contamination and surfaces of concern (Sterckeman et al., 2002b; Douay et al., 2008, 2009; Fritsch et al., 2010). A 40 km^2 (8 × 5 km) study area, centred on the former smelter, was defined. This area was divided into 160 squares (500 m × 500 m) that constituted our sampling units. Because of logistical constraints, the study was performed on 30 of those 160 squares. In each of the 30 squares, one to 10 composite soil samples (each constituted by 15 randomly placed subsamples in a homogeneous patch) were taken in woody patches (woodlots, hedgerows, tree plantations, etc.) during the autumn of 2006. Soil sampling was carried out on the first 25 cm, and the litter layer (OL) was removed (the OL layer constituted no or some accumulation of decomposed leaves and woody fragments on the soil surface). However, the humus layer (OF, the OF layer constituted fragmented residues) was sampled with the top mineral soil material in accordance with the most frequently recommended protocols in Europe (Theocharopoulos et al., 2001). Detailed data about soil sampling, soil physico-chemical characteristics and contamination are provided in Douay et al. (2009) and Fritsch et al. (2010). Snails were hand-searched in patches where soils were sampled. Because of logistical constraints, complete depuration of the snail gut content was not performed. Snails were sampled in the morning and were stored in plastic bags without food for the rest of the day. After returning from the field at night, the snail samples were checked, annotated and frozen. This procedure provided a period of depuration of approximately 8 h. Unpublished data from our department showed that *C. aspersus* snails may contain from 1% to 10% of their mass in soil and food in their gut. Using these data, the mean dry mass of the different species and the mean metal concentrations in soils and snail tissues, we calculated the bias from an absence of depuration (in the worst case, snails would not have excreted at all during the depuration period). Depending on the species, the bias ranged from 0.2% to 4.8% for Cd, 7.8% to 161.4% for Pb, and 0.4% to 21.4% for Zn. Therefore, if the bias was acceptable for Cd and Zn, it might have been significant for Pb.

2.2. Determination of snail species and age, and preparation of snail tissues

Species were determined according to morphometric criteria (Kerney and Cameron, 2006). Snails belonging to the *Cepaea* genus were classified as adults or juveniles according to the presence or the absence, respectively, of a clear white or brown-black lip at the mouth of their shell (Williamson, 1979). In these *Cepaea* snails, the determination at the species level is possible only on adults. Hereafter, juvenile individuals will be called "*Cepaea* juveniles". Class age was not determined for the other snail species because of the absence of published ageing methods and the insufficient sample size for considering age in statistical treatments.

Before performing the metal analyses, the soft bodies of the snails were separated from the shell and dried in an oven (60 °C) to a constant dry mass. Snails were generally analysed individually, but when their dry mass was lower than 0.1 g, two to 10 individuals were pooled to obtain a sufficient biomass for metal analysis.

2.3. Analyses of metal concentrations in soils and animals

The soils were analysed for total and extractable metal concentrations and for soil properties. The protocols are detailed in Douay et al. (2009). Briefly, the samples were dried, disaggregated and homogenised before sieving to 250 μm. The following soil characteristics were measured: granulometry, pH (water suspension), organic carbon content (OC), organic matter (OM) content, total carbonate content ($CaCO_3$) and cation exchange capacity (CEC). After a total digestion with a mixture of hydrofluoric (HF, 48%) and perchloric ($HClO_4$, 70%) acids, total Cd and Pb concentrations were measured by inductively coupled argon plasma mass spectrophotometry (ICP-MS), and Zn concentrations were measured by inductively coupled argon plasma atomic emission spectrophotometry (ICP-AES). Soil properties and metal concentrations in soils were analysed by the *Laboratoire d'Analyse des Sols* of the *Institut National de la Recherche Agronomique* (INRA) of Arras, France, which is accredited by the *French Accreditation Committee* (COFRAC, no. 1-1380) for the analytical quality of soil characteristics and metal concentration measurements.

Three selective extractions and one sequential four-step extraction were chosen. Calcium chloride ($CaCl_2$, 0.01 M) was chosen to estimate cation exchange reactions, as it is considered to be relevant for the assessment of the bioavailable fraction of Cd, Pb, and