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

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► To cite this version:

Brice Mourier, Clémentine Fritsch, Elie Dhivert, Frédéric Gimbert, Michael Coeurdassier, et al.. Chemical extractions and predicted free ion activities fail to estimate metal transfer from soil to field land snails.. Chemosphere, 2011, 85 (6), pp.1057-65. 10.1016/j.chemosphere.2011.07.035 . hal-00682800

HAL Id: hal-00682800

<https://hal.science/hal-00682800v1>

Submitted on 26 Mar 2012

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26 ABSTRACT

27 This study investigates the relevance of several soil chemical extractions (CaCl₂, acetic acid,
28 citric acid and a four-step sequential procedure) and of predicted free metal ion activities in
29 the soil solution (according to equations using total concentrations and pH) to characterize
30 the transfer of trace metals (Cd, Pb and Zn) from soil to snail soft tissues over a large
31 smelter-impacted area (Metaleurop Nord, Nord-Pas-de-Calais, France). The study was firstly
32 performed on 6 wild snail species together and then, specifically, on *Cepaea* sp and
33 *Oxychilus draparnaudi*, which were well distributed over the study area and have contrasted
34 diet (*Cepaea* snails are herbivorous while *O. draparnaudi* is carnivorous). When the 6
35 species were considered together, the accumulation depended most on the species, total or
36 extractable metal concentrations in soils, or predicted free ion activities, accounting for less
37 than 7% of the variation of metal concentrations in snail tissues. Species-specific analyses
38 showed that extractable concentrations explained around 25% of the variation of metal
39 concentrations in *O. draparnaudi* while it was up to 8% for *Cepaea* snails. When using total
40 soil concentrations and soil properties as explanatory variables, models were generally
41 slightly better, especially in species-specific analyses, explaining up to 42% of the variance.
42 These results show that the soil extraction procedures used in this study, and predicted free
43 ion activities of soil pore water, could not accurately estimate metal transfer from the
44 environment to snails, probably because they do not take into account other sources of
45 exposure than soil, and thus could not be used in risk assessment. Insight has to be gained
46 into the determination on food web structure and composition and subsequent contaminant
47 transfers, in order to improve risk assessment for such organisms.

48 *Keywords*

49 Biological monitoring, bioavailability, snails, toxic metals.

50

51 1. Introduction

52 Due to historical and modern industrial activities, soils can be severely contaminated with
53 metals (e.g. cadmium, Cd; lead, Pb; and zinc, Zn) and the release of such contaminants in
54 natural systems can consequently pose environmental and human health risks. In the case
55 of areas affected by metallurgical industries, the original load is primarily sourced from
56 smelter fallout and dusts from spoil heaps that have been transported across natural
57 systems. Exposure of organisms to trace metals depends on many non-biotic factors
58 (including, for instance, the spatial distribution of metals in soils, landscape or habitat
59 features, season etc) as well as the biological characteristics of the organism itself like its
60 diet and, more generally, its life history traits (Suter II, 1993). In line with that, Landrum et al.
61 (1994) developed the concept of environmental bioavailability as the fraction of the
62 environmental availability (modulated by physicochemically-driven processes) that is
63 available over the exposure period for uptake (physiologically-driven processes) by a given
64 species (Peijnenburg and Jager, 2003).

65 In the framework of ecotoxicology and ecological risk assessment, terrestrial invertebrates
66 have been extensively studied as biomonitors of environmental contamination by metals. The
67 relationships between concentrations of metals in snails and in soils have been well studied
68 in controlled conditions as well as on the field. Those studies generally have shown that
69 concentrations in snail tissues are related to the contamination of their environment (Berger
70 and Dallinger, 1993). The biota-to-soil concentrations ratios (BSAFs) that can be derived
71 from these studies may be useful in ecological risk assessment (Veltman et al., 2008).
72 However, relationships between total metal concentrations in soil and concentrations in snail
73 soft tissues are usually relatively low, hampering accurately predicting concentrations in
74 organisms from total soil concentrations. For instance, coefficient of determination (R^2) of the
75 relationship between *Cepaea nemoralis* snail and soil concentrations were of 0.33, 0.17 and
76 0.15 for Cd, Pb and Zn, respectively (Notten et al., 2005). Such low relationships may be due
77 to the fact that total metal concentrations are usually considered not to be the best estimate
78 of bioavailability for both plants and organisms (Harmsen, 2007).

79 In order to overcome this limitation, a variety of chemical methods has been developed to
80 estimate the available fraction of metals in soils, and then to correlate these available
81 concentrations with metal levels in tissues or with effects observed in organisms. Among
82 these methods, single or sequential extraction techniques have given satisfactory results for
83 certain pollutants, certain soils and/or certain organisms (see, for instance, Harmsen, 2007;
84 Meers et al., 2007; Hass and Fine, 2010), even if it is clear that no universal extractant
85 exists. Free metal ion activity in the soil solution has also been suggested as a pertinent
86 estimate of metal bioavailability and toxicity in aquatic organisms and higher plants
87 (Smolders et al., 2009) and may therefore be hypothesised as a good predictor of metal

88 concentrations in soil organisms (Sauvé et al., 2000). Though, studies examining
89 relationships between metal concentrations in soil organisms and free ion activities in pore
90 water or extractable metal concentrations in soil are scarce.

91 Within this context, we hypothesized that chemically extractable metal concentrations in
92 soils, or predicted free ion activity, would give better relationships with metal concentrations
93 in snail tissues, than total metal concentrations in soils. We thus aimed at comparing the
94 relationships between total or extractable (using three selective extractions and a four-step
95 sequential procedure) soil concentrations or predicted free ion activities and metal
96 concentrations in snail soft tissues. As an alternative to the use of extractable concentrations,
97 we also modelled metal concentrations in snail tissues using total metal concentrations in
98 soils and soil properties.

99

100 **2. Materials and methods**

101

102 *2.1. Study site and sampling strategy*

103 This study was carried out in the surroundings of the former “Metaleurop-Nord” smelter in
104 Northern France (Noyelles-Godault, Nord-Pas-de-Calais, 50°25'42 N 3°00'55 E). This area is
105 considered as highly polluted with Cd, Pb and Zn for both levels of contamination and
106 surfaces of concern (Sterckeman et al., 2002b; Douay et al., 2008; Douay et al., 2009;
107 Fritsch et al., 2010). A 40 km² (8 x 5 km) study area, centred on the former smelter, was
108 defined. This area was split into 160 squares (500 m-sided), which constituted our sampling
109 units. Because of logistical constraints, the present study was performed on 30 squares
110 located along a soil pollution gradient, chosen among the 160. In each of the 30 squares,
111 one to 10 composite soil samples (constituted each by 15 randomly placed sub-samples in a
112 homogeneous patch) were taken in woody patches (woodlots, hedgerows, tree plantations,
113 etc) during autumn 2006. Soil sampling was carried out on the first 25 cm and the litter layer
114 (OL layer constituted by the accumulation of no- or few decomposed leaves and woody
115 fragments on the soil surface) was removed. However, the humus layer (OF layer constituted
116 of fragmented residues) was sampled with the top mineral soil material, in accordance with
117 the most frequently recommended protocol in Europe. Detailed data about soil sampling, soil
118 physico-chemical characteristics and contamination are given in Douay et al (Douay et al.,
119 2009) and Fritsch et al (Fritsch et al., 2010). Snails were hand-searched during autumn 2006
120 in patches where soils were sampled. Snails were sampled in the morning, stored in plastic
121 bags without food and frozen at night. This allowed a depuration period of around 8 hours.

122

123 *2.2. Determination of snail species and age and preparation of snail tissues*

124 Species were determined according to morphometric criteria (Kerney and Cameron, 2006).
125 Snails belonging to the *Cepaea* genus were classified according to two classes of relative
126 age (adults or juveniles), according to the presence or the absence, respectively, of a clear
127 white or brown-black lip at the mouth of their shell (Williamson, 1979). The presence of this
128 lip indicates that the snail has attained adulthood. In these *Cepaea* snails, the determination
129 at a species level is only possible on adults. In the case of non reproductive juveniles, the
130 snails were called “*Cepaea* juveniles”. Class age was not determined for the other snail
131 species due to the absence of published ageing method and/or to insufficient sample size to
132 take age into account in statistical treatments.

133 The soft body of the snails was separated from the shell and dried in an oven (60°C) to
134 constant dry weight before metals analysis. Snails were generally analysed individually, but
135 when their dry mass was lower than 0.1 g, two to 10 individuals (depending on the mass of
136 the individuals) were pooled to obtain a sufficient biomass for metals analysis. For both
137 individual and pooled samples, snail metal concentrations were normalized to soft tissues
138 weight.

139

140 *2.3. Analyses of metals concentrations in soils and animals*

141 Soils were analyzed for total and extractable metal concentrations and for soil properties.
142 The protocols are detailed in Douay et al. (2009). Briefly, samples were dried, disaggregated
143 and homogenized before sieving to 250 μm . The following soil characteristics were
144 measured: granulometry, pH (water suspension), organic carbon content (OC), organic
145 matter (OM), total carbonate content (CaCO_3) and cation exchange capacity (CEC). Total Cd
146 and Pb concentrations were measured by inductively-coupled argon plasma mass
147 spectrophotometry (ICP-MS) and Zn concentrations by inductively-coupled argon plasma
148 atomic emission spectrophotometry (ICP-AES) after a total digestion with a mixture of
149 hydrofluoric (HF) and perchloric (HClO_4) acids. Soil properties as well as metals
150 concentrations in soils were analyzed by the *Laboratoire d'Analyse des Sols* of the *Institut*
151 *National de la Recherche Agronomique* (INRA), of Arras (France), which is accredited by the
152 *French Accreditation Committee* (COFRAC, n°1-1380) for the analytical quality for soil
153 characteristics and metals concentration measurements.

154 Three selective extractions and one sequential four-step extraction were chosen. Calcium
155 chloride (CaCl_2 , 0.01M) was chosen to estimate cation exchange reactions and because it is
156 considered as relevant for the assessment of the bioavailable fraction of Cd, Pb and Zn to
157 plants and soil invertebrates (Harmsen, 2007; ISO, 2008). Acetic acid ($\text{C}_2\text{H}_4\text{O}_2$, 0.11M) and
158 citric acid ($\text{C}_6\text{H}_8\text{O}_7$, 0.11M) are two low molecular weight organic acids (LMWOA) mostly
159 present in the rhizosphere of many plants and used to simulate complexing behaviour of root
160 exudates (Meers et al., 2007). These two LMWOA increase the overall metal solubility by

161 dissolution of hydroxides and carbonates and by acting as complexing agents. Acetic acid
162 extractable fraction has been used as an estimate of the metal bioavailability to soil animals
163 (Morgan and Morgan, 1999; Lukkari et al., 2004). Citric acid has been used to simulate the
164 physicochemical characteristics of root environment of the plant (Ahumada et al., 2004). A
165 three-stage sequential extraction procedure was also performed, allowing the extraction of
166 four operationally defined fractions: acid-soluble extracted by 0.11M acetic acid
167 (exchangeable and carbonate bound), reducible extracted by 0.5 M of hydroxylammonium
168 chloride at pH 2 (Fe- and Mn-oxide bound), oxidizable extracted by 8.8 M of H₂O₂ followed
169 by 1 M of ammonium acetate at pH 2 (organic matter and sulfide bound) and residual
170 extracted by hydrofluoric (48 %) and perchloric (70 %) acids (included within silicate matrix).
171 The extractable concentrations of those four fractions will be called a, b, d and r,
172 respectively, later in this article. Extractable metal concentrations were quantified using
173 atomic absorption spectrometry (AAS, AA-6800, Shimadzu) by the *Laboratoire Sols et*
174 *Environnement* of the *Institut Supérieur d'Agriculture* (ISA), Lille, France. Three independent
175 replicates were performed for each sample and blanks were measured in parallel for each
176 set of analyses, measurements of certified reference materials (i.e. BCR-701 and BCR-483)
177 was were used to ensure the quality of the results obtained. For all metals average
178 recoveries of the CRMs were at XX% ± 10%.

179 Finally, predicted free ion activities in the soil solution (pPb²⁺, pCd²⁺, pZn²⁺) were calculated
180 from pH and total metal concentrations using the equations proposed by (Sauvé et al., 1997;
181 Sauvé et al., 2000; Stephan et al., 2008), respectively. As calculated by those equations, the
182 activity of metal ions corresponds to -log(free ion concentration in M).

183 Metal concentrations in snail soft bodies were measured by furnace (Cd, Pb) or flame (Zn)
184 atomic absorption spectrometry (VARIAN 220Z and 220FS, respectively) at the Chrono-
185 Environment Department of the University of Franche-Comté, Besançon, France. Digestion
186 of samples was performed using dissolution in nitric acid (HNO₃, 65%, Carlo-Erba analytical
187 quality) in a dry oven (65°C) during 72h. After digestion, samples were diluted adding ultra-
188 pure water (18.2 MΩ.cm⁻²). Blanks (acid + ultra-pure water) and Certified Reference
189 Materials (CRMs, TORT-2 and DOLT-3, National Research Council, Canada) were prepared
190 and analyzed using the same methods.

191 Average recoveries of the CRMs were at 95% ± 10% (n = 38) for Cd, 101% ± 17% (n = 42)
192 for Pb, 80% ± 2% (n = 22) for Zn. Detection limits in snail soft bodies were 0.14, 0.20 and 4.4
193 µg.g⁻¹ for Cd, Pb and Zn, respectively. For both soils and animals, metal concentrations were
194 expressed as micrograms per grams dry weight (µg.g⁻¹ dw).

195

196 *2.4. Statistical analyses*

197 For each sampled individual, the following data were available: snail species, metal
198 concentrations in soft tissues, and age for *Cepaea* snails. Environmental data available were
199 total and extractable metal concentrations and soil properties (clay, silt, sand, pH, CaCO₃,
200 CEC, OC and OM) in the soil of the patch where the individual was caught. The statistical
201 distribution of the data was checked with the test of Shapiro. Because metal concentrations
202 in soils and animals were skewed, variables were $\log_{10}(x + 1)$ transformed for statistical
203 analyses. These transformations allowed matching the assumptions of normality and linearity
204 which are required to perform General Linear Models (GLM).

205 First, principal component analyses were performed on soil properties to identify redundant
206 variables. According to those analyses, pH, OM, CEC, clay and silt were retained for further
207 analyses. Simple linear regressions were also performed to explore relationships between
208 snail and soil concentrations, and between biotic (species or age) and abiotic variables (soil
209 properties or metal concentrations).

210 Second, relationships between metal concentrations in snail tissues and in soils (total or
211 extractable concentrations, or predicted free ion activities in the soil pore water) were
212 determined. General Linear models (GLM) (Grafen and Hails, 2002) were performed using
213 metal concentration in snail tissues as dependant variable and species and metal
214 concentrations in soils as explanatory variables. Specific analyses were then made for *O.*
215 *draparnaudi* and *Cepaea* sp. because of their relative abundance and their homogeneous
216 spatial distribution over the study area. Moreover, these two species have clearly distinct
217 diet, *O. draparnaudi* being carnivorous while *Cepaea* snails are herbivorous (Kerney and
218 Cameron, 2006). Concentrations of metals in *O. draparnaudi* were modeled using soil
219 concentrations as only explanatory variable. For *Cepaea* snails, preliminary analyses (linear
220 models) showed no difference of concentration between adult *C. nemoralis* and *C. hortensis*,
221 whatever the metal. Metal concentrations in *Cepaea* sp individuals were thus modeled using
222 age and soil concentrations as explanatory variables, without further considering the species.

223 Third, GLM involving concentrations in snails tissues as a dependent variable and total metal
224 concentrations in soils and soil properties as explanatory variables were performed.
225 Preliminary monivariate analyses having shown that biotic variables (e.g. species when
226 analyses were performed on the six snail species and age for *Cepaea* snails) were of
227 primary importance in explaining metal concentrations in tissues, they were placed first in the
228 models. Then, to assess the influence of soil properties conditionally to total soil
229 concentrations, this last variable was placed in second in the models. These two variables in
230 the model being fixed (biotic variables and total concentration), soil properties were added.
231 Precisely, the order of soil properties in the models was chosen according to preliminary
232 monivariate analyses linking concentrations in snails to soil properties. All soil variables
233 were placed in the model in the decreasing order of their coefficient of determination (R^2)

234 obtained in monovariate analyses. The complete model was then analysed by ANOVA and
235 non-significant variables were excluded.

236 Post-hoc multiple comparisons were performed using Tukey's Honest Significant Difference
237 test ($p < 0.05$) to test significant differences of metal concentrations between species.

238 Biota-to-Soil Accumulation Factors (BSAFs) were calculated as the ratio of metal concentration in
239 the soft tissues on total metal concentration in the soil. The relationship between BSAF and
240 total soil concentration (conditionally to species) was assessed using a linear models.

241 When models had two variables, interactions between the variables were studied when
242 relevant. For all tests, significance was chosen at $p < 0.05$. All statistical analyses were
243 performed using the software R 2.10.1 with *pgirmess* and *ade4* packages
244 (RDevelopmentCoreTeam, 2010).

245

246 3. Results

247

248 3.1. Snail species, metal concentrations in soils and in snail and BSAF

249 In the present paper, analyses were conducted on six out of the 19 species sampled. These
250 six species were selected because they were relatively abundant and well spatially
251 distributed over the study area. A total of 264 snail samples were found in 48 habitats within
252 the 30 squares where they were sampled. The 264 snails include 20 copse snails (*Arianta*
253 *arbustorum*), 47 white-lipped banded snails (*Cepaea hortensis*), 54 brown-lipped grove snails
254 (*Cepaea nemoralis*) and 30 juvenile *Cepaea sp* snails, 19 garden snails (*Cantareus*
255 *aspersus*, ex-*Helix aspersa*), 78 Draparnaudi's glass snails (*Oxychilus draparnaudi*) and 16
256 strawberry snails (*Trichia striolata*).

257 Soils from the study site were heavily polluted with metals (Table 1). Median total
258 concentrations of Cd, Pb and Zn were much higher than the background values of woody
259 topsoils of the region which are 0.2, 53.3 and 53.4 $\mu\text{g g}^{-1}$, respectively (Sterckeman et al.,
260 2002a), except six topsoils that exhibited background concentrations. Soil properties varied
261 greatly according to the different sampling sites (Table 1). Topsoil texture was mainly loamy
262 to silty-loamy. All other soil properties exhibited large variations, up to one order of
263 magnitude for organic matter content, showing a great variability of types of soils within the
264 study area.

265 Metal concentrations in snail soft bodies were characterized by distinct patterns, which varied
266 between species and as a function of the element considered (Table 1, Figure1). Median Cd
267 concentrations in snails ($45.1 \mu\text{g.g}^{-1}$) were higher than those measured in soils ($p < 0.001$).
268 Only *Arianta arbustorum* and juvenile *Cepaea sp* snails exhibited significant lower Cd
269 concentration compared to other species (Figure 1). Inversely, Pb concentrations in snail
270 tissues (median Pb concentration of $33.2 \mu\text{g.g}^{-1}$) were lower than those measured in soils (p
271 < 0.001). No significant intra-species differences were detected for Pb concentrations.
272 Finally, Zn concentrations in snails were of the same order of magnitude than those found in
273 soils ($p = 0.58$). Higher Pb concentrations were measured for *Oxychilus draparnaudi* and
274 *Trichia striolata* compared to other species (Figure 1).

275

276 Cd biota-to-soil accumulation factors (BSAF) were consistently higher than 1 for all species
277 (Figure 2). The transfer of this metal was the lowest for *A. arbustorum* (median BSAF of 1.2)
278 and juvenile *Cepaea* snails (median BSAF of 1.7), and the highest for *T. striolata* (median
279 BSAF of 50.6) (data not shown). The four other species exhibited similar intermediate
280 median BSAFs ranging from 3.5 to 7.4. For Pb, median values of BSAF between species
281 ranged from 0.031 to 0.13 and did not show inter-species differences, except for *T. striolata*,
282 for which BSAFs reached 0.53. The inter-species pattern was similar for Zn, with all species

283 exhibiting median BSAFs ranging from 0.49 to 1.23, while it was 15.0 for *T. striolata*.
284 When considering all species together, the relationship of BSAF to exposure was significant
285 and negative for all the metals considered (Figure 2, left). Comparing the carnivorous *O.*
286 *draparnaudi* to the herbivorous *Cepaea sp.* (Figure 2, right), the decrease of BSAF values for
287 Cd and Pb along the pollution gradient were significantly higher for *Cepaea* snails (slopes = -
288 0.93 and -0.12, respectively) than for *O. draparnaudi* (slopes = -0.41 and -0.05, respectively).
289 For Zn, BASF decreased similarly for *Oxychilus* (slope = -0.35) and *Cepaea* snails (slope = -
290 0.37).

291

292 *3.2 Influence of total or extractable concentrations, and of free metal ion activities in the soil* 293 *solution on concentrations in snail tissues*

294 Whatever the extraction, Cd concentrations in the 6 species of snails were poorly (at best
295 6.8%) explained by total or extractable soil concentrations (Table 2). Three of the four steps
296 of the sequential procedure even led to non significant relationships. No relationship (except
297 for Pb_b concentrations) was evidenced for Pb. Zn concentrations in snail tissues were very
298 poorly explained by soil concentrations. Whatever the metal, the influence of the species was
299 always higher than that of soil concentrations (total or extractable) in explaining metal
300 concentrations in snail soft tissues. Species and Pb_b extracted concentrations accounted for
301 11% of the variation of Pb concentrations in snail tissues. Depending on the considered
302 extraction, species and metal concentrations explained 18.3 to 21.7% of the variance for Cd,
303 and from 35.4 to 38.5% for Zn. For both metals, however, some extractable concentrations
304 were not significantly related to snail concentrations. The use of predicted free activities led
305 to similar low correlations for each metal (Table 2).

306 Considering all extractions together, the relationships between concentrations in *O.*
307 *draparnaudi* snails and soil concentrations were much better than when all species were
308 considered together and also better than for *Cepaea* snails (Table 2). Metal concentrations in
309 *O. draparnaudi* tissues were never significantly correlated with free ion activities in the soil
310 solution. Cadmium levels in *O. draparnaudi* increased significantly with total as well as
311 extractable (all extractants) Cd concentrations in soils (Table 2). The various extractions
312 explained from 5.5 (Cd_d) to 24.3% (acetic acid) of the variations of Cd concentrations in
313 snails. Total as well as Pb_d concentrations were poorly related to Pb concentrations in snail
314 tissues. Three other extractable concentrations (citric and acetic acids, and Pb_b) explained
315 from 19.1 to 25.3% of the variations of Pb concentrations. CaCl₂-extractable concentrations
316 were the only non-significantly related concentrations for Zn. Zinc concentrations in snail
317 tissues were best related (25.2% of variance explained) to Zn_b concentrations. Total
318 concentrations and the other extractants explained 13.7 and from 6.4 to 10.9% of the
319 variations of Zn concentrations in snails, respectively.

320 For *Cepaea* snails, two extractable Cd concentrations (CaCl_2 and Cd_b) and pCd^{2+} were
321 significantly related to concentrations in soft tissues. For Pb, only acetic acid extractable
322 concentrations and pPb^{2+} were significantly related to *Cepaea* tissue concentrations, the
323 relationship being negative for acetic acid. CaCl_2 -extractable and Zn_r concentrations, and
324 pZn^{2+} were significantly correlated with Zn concentrations in *Cepaea* tissues. Inversely to *O.*
325 *draparnaudi*, predicted free ion activities in the soil pore water were significantly correlated to
326 concentrations in *Cepaea* for all metals. Although Cd concentrations in *Cepaea* tissues were
327 age-dependant, this was not the case for Pb and Zn (Table 2).

328 Generally speaking, for all metals, extractable concentrations were weakly correlated with
329 snail concentrations when the six species were considered together. A specific analysis
330 shows that few extractable concentrations were significantly although weakly related to
331 *Cepaea* tissues. In *O. draparnaudi*, extractable concentrations better correlated with
332 concentrations in snail tissues but the best extractant explained 24.3, 25.3 and 25.2% of the
333 variance for Cd, Pb and Zn, respectively.

334

335 3.3. Influence of soil properties on concentrations in snail tissues

336 Models using soil properties (conditionally to species or age and total soil concentrations)
337 were not more efficient (adjusted R^2 of the models of 13.4, 23.0 and 41.6% for Pb, Cd and
338 Zn, respectively) than extraction-based models to explain concentrations in soft tissues for
339 the six species considered together (Table 3). The species still was the most important
340 explaining variable. Considering the influence of total concentrations and soil properties
341 separately (partial R^2), conditionally to species, total soil concentration was shown to be
342 weakly related to concentrations in snails and was even non significant for Pb. No clear trend
343 concerning the influence of soil properties could be evidenced: one to three soil properties
344 appeared to be significant depending on the metal of concern and, whatever the metal,
345 explained no more than 4.6% of the variance of snail concentrations.

346 As observed for extraction-based models, Pb and Zn concentrations in snail soft tissues
347 were better explained for *O. draparnaudi* than for *Cepaea sp* (Table 3). For Cd, adjusted R^2
348 models were similar for both species with 30.0% for *O. draparnaudi* and 29.0% for *Cepaea*.

349 Metal concentrations in *O. draparnaudi* were positively related with total metal concentrations
350 in soils and negatively with organic matter contents. For Cd, the influence of soil
351 concentration was higher than that of organic matter whereas it was the contrary for Pb and
352 Zn.

353 For *Cepaea*, models involved more soil variables than for *O. draparnaudi*. As for extraction-
354 based models, the age was an important variable to explain Cd concentrations in soft
355 tissues. Its importance was lower for Pb and not significant for Zn. Total metal concentrations
356 in soils were never significant for this species. Silt and pH influenced negatively snail

357 concentrations for all metals. Organic matter was negatively correlated with snail
358 concentrations for Cd, whereas it was positively correlated for Pb and Zn.

359

360

361

362 4. Discussion

363 4.1. BSAFs are influenced by both the species and the total soil concentration

364 For most species, median values of BSAF were around 5 for Cd, which is in accordance with
365 Martin and Coughtrey who showed Cd bioaccumulation factors of 1.37–7.57 in various
366 species of snails (Martin and Coughtrey, 1982). Few inter-species differences were noted for
367 Pb, median BSAFs being lower than 0.5 for all species, indicating either an important
368 excretion and/or a low assimilation of this metal. Except for *Trichia striolata*, median BSAFs
369 for Zn values generally ranged from 0.5 to 1. Our data showed a negative relationship
370 between BSAFs and total soil concentrations for all investigated metals. The highest BCF
371 values for all metals were at low exposure concentrations, while the lowest BCF values were
372 at elevated metal exposure levels. These results are consistent with those of Spurgeon and
373 Hopkin (Spurgeon and Hopkin, 1996) and Veltman et al (Veltman et al., 2008), who found
374 inverse relationships between BASFs in animals from all trophic levels and metal
375 concentrations in soils. Similarly, this trend has also been described in aquatic environments,
376 with various aquatic species (McGeer et al., 2003). The fact that the BSAF decreases linearly
377 with increasing soil concentration, almost with a slope of 1.0, indicates a significant degree of
378 control over metal accumulation, probably regulation. A constant BSAF would in fact suggest
379 the contrary, namely that metals level in the snails does increase with increasing soil
380 concentrations. Consequently, our results suggest that the degree of control over metal
381 accumulation was stronger for Cd than for Zn. The relatively gentle slopes observed for Pb
382 indicate that BSAF appear to be independent of the soil concentration. Moreover, slope
383 differences observed for the carnivorous *O. draparnaudi* with the herbivorous *Cepaea sp.*
384 suggest the influence of species for metal accumulation and regulation.

385 The physiological basis for the inverse relationship of BSAF to metal exposure concentration
386 arises from metal uptake and control mechanisms (McGeer et al., 2003). Lock and Janssen
387 (2001) concluded that uptake rate constants for cadmium decrease with increasing metal
388 concentration, based on experimental results on two terrestrial oligochaetes, *Eisena fetida*
389 and *Enchytraeus albidus*. At low environmental Zn levels, snails are able to sequester and
390 retain Zn in tissues for essential functions. When Zn exposure levels are elevated, snails are
391 able to control bioaccumulation. Despite it is known that *Cantareus aspersus* snails are able
392 to regulate Pb concentrations more closely than other metals (Beeby and Richmond, 2010),
393 our results indicate no-regulation controls on Pb bioaccumulation. These authors suggest
394 that BSAF are unreliable indicators of an evolved regulatory mechanism for Pb.

395

396 4.2. Total and extractable metal concentrations in soils are poor predictors of metal
397 concentrations in snail tissues

398 While selective and sequential extractions are frequently considered as a promising tool to
399 measure environmental availability of trace metals in soils (Harmsen, 2007; ISO, 2008),
400 relatively few data are available on relationships between extractable concentrations from
401 field soils and concentrations in tissues of wild animals. Our data show that extractable
402 concentrations from three selective and one sequential four-step extractions, as well as
403 predicted free ions activities in the soil solution, explain up to 42% of the variations of the
404 concentrations of Cd, Pb and Zn in the tissues of snails sampled in the surroundings of a
405 former Pb-smelter and belonging to six species.

406 Among the data relating metal concentrations in wild animals with various soil extracts,
407 earthworms are probably the most studied taxa. In three species of earthworms
408 (*Allolobophora chlorotica*, *Apporectodea caliginosa*, and *Lumbricus rubellus*) from a
409 moderately contaminated Dutch floodplain, HNO₃⁻, CaCl₂-extractable and pore water Cd, Cu,
410 Pb and Zn concentrations in soils were non significantly correlated with concentrations in the
411 worm tissues (Van Vliet and Van der Zee, 2005). Total soil concentrations were weakly ($R^2 <$
412 0.10) correlated with worms for Cd, Cu and Zn but not for Pb. Adding flooding, a frequent
413 environmental stress in those habitats, as a new variable in the models allowed improving R^2
414 to 0.67 for Cd, 0.10 for Cu and 0.33 for Zn. The relationship remained non significant for Pb.
415 Modeling each species separately, only total soil concentrations were significantly related to
416 metal concentrations in worm tissues (not for all species or metals) with R^2 ranging from 0.14
417 to 0.80. As in the Dutch case on earthworms, our data showed that Pb concentrations in
418 soils, whatever the extractant used, generally do not correlate with Pb concentrations in
419 snails when all species are considered together. The influence of species is the most
420 important variable explaining snail concentrations. In a study of another Dutch floodplain, Cd
421 and Cu concentrations in *A. caliginosa* and *L. rubellus* have also been found to correlate best
422 with total rather than to pore-water and CaCl₂-extractable concentrations (Hobbelen et al.,
423 2006).

424 In the present work, the use of free metal ion activities in the soil pore water to model metal
425 concentrations in snails gave no better results than chemical extractions. This is in
426 agreement with the results of a study aiming at using *Helix aspersa* snail as quantitative
427 sentinels of total and predicted free Pb concentrations in soils (Beeby and Richmond, 2003).
428 In this work where adult snails were sampled from 23 locations in England and Wales,
429 correlations between free Pb activity calculated using the same equation (Sauvé et al., 1997)
430 and Pb concentrations in snail tissues were low ($R^2 = 0.16$, $p < 0.001$). In a study on *Eisenia*
431 *fetida* exposed to nine Cd-spiked soils, similar low relationships were found between free Cd
432 activity and Cd accumulation in the worm tissues (Li et al., 2009). However, the introduction
433 of pore water pH as an explanatory variable increased R^2 to 0.45, indicating that Cd uptake

434 by *E. fetida* may be moderated by the presence of other cations in the soil pore water,
435 especially H⁺ ions.

436 In a synthesis of the researches undertaken on Dutch floodplains, van Gestel concluded that
437 bioavailability could not be predicted from available concentrations in pore water or 0.01 M
438 CaCl₂ soil extracts (Van Gestel, 2008). Our results on several species of gastropod mollusks
439 reinforce the conclusion that extractable concentrations might not provide predictive models
440 accurate enough to be used in routine risk assessment.

441 Another key factor in understanding the transfer of metals from the environment to organisms
442 relies on food chain transfers. Indeed, in a study of metal transfers in a soil - plant (*Urtica*
443 *dioica*) - snail (*Cepaea nemoralis*), relationships between metal concentrations in plants and
444 snails were stronger than between soils and snails (Notten et al., 2005). The authors
445 interpreted this phenomenon as a major contribution of plant materials to the transfers of
446 metals to snails, compared to the contribution of the soil, which was, though, considered
447 significant. In a study aiming at evaluating *H. aspersa* snails as sentinels for mapping
448 pollution, however, correlations between soil and snail Cd concentrations were slightly higher
449 than correlations between *Taraxacum* leaves and snails (Beeby and Richmond, 2002),
450 suggesting a rather equal contribution of soil and plant in the accumulation of metals by the
451 snails. Moreover, the relative contribution of soil and plants in the accumulation of metals by
452 snails has been suggested to vary greatly depending on the metal considered (Scheifler et
453 al., 2006). Therefore, depending on the metal and on the actual diet of the different snail
454 species, relationships between metal concentrations in snail tissues and soil concentrations
455 are likely to drastically vary.

456 Snails have various diets depending on the considered species (Speiser, 2001) and
457 obviously do not only depend on the soil when considering their exposure and subsequent
458 contamination. Plants of all developmental stages (from seedlings to senescing plants), leaf
459 litter, wood, dead and even alive animals have been recorded as gastropod food (Speiser,
460 2001). Therefore, when the transfer of metals from the environment to the snail has to be
461 modelled, one should not only consider the soil on which snails are crawling and feeding but
462 also the rest of their diet. However, even in this case, one would obtain metal concentrations
463 in the soils and in various supposed food items, which would not allow modelling the
464 transfers without knowing the proportion of each item is actually ingested.

465

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467

468 *4.3. Modeling metal concentrations in snail tissues using soil total metal concentrations and*
469 *properties slightly increases predictions*

470 When data are handled with all species together, models associating total concentrations
471 and soil properties do not explain metal concentrations in snail tissues better than extraction-
472 based ones, the influence of the species remaining the most important variable. Looking at
473 species separately, however, the models using soil properties are generally slightly better
474 than extraction-based. To our knowledge, only few studies investigated the role of soil
475 properties in explaining metal accumulation in wild snail tissues. In the present study, soil pH
476 was found to be inversely related to the accumulation of metals in snails. Soil pH affects the
477 partitioning of metals in the different soil compartments and, therefore, the metal
478 concentration available for exposure from soil solution. Several studies performed on *in situ*
479 contaminated soils with different species of worms found that total metal concentrations in
480 soils was a poor predictor of earthworm metal accumulation due to a number of modifying
481 factors such as pH, organic matter content, and clay particle content (Nahmani et al., 2007).
482 In the present study, metal concentrations in *O. draparnaudi* snails increased with total
483 concentrations and decreased with organic matter values, as also observed on *Lumbricus*
484 *rubellus* (Corp and Morgan, 1991). This suggests that metal adsorption on organic matter
485 occurred, reducing the bioavailability of metals at high organic matter contents. Clay content
486 has also been found to negatively influence metal availability for animals at high contents
487 (Janssen et al., 1997; Owojori et al., 2010). In our study, clay content was positively
488 correlated with Pb concentrations in the six species considered together and in *Cepaea*
489 snails, but not in *Oxychilus* individuals. Our results also showed a negative relationship
490 between tissues Cd and Pb concentrations in the six species and in *Cepaea* snails and silt
491 content, but this was not observed in *Oxychilus* snails. Taken together, our results do not
492 show a straightforward influence of soil properties, whose influence was species- and metal-
493 specific.

494

495

496 **5. Conclusion**

497 In this study, metal concentrations in snails were poorly explained by total and extractable
498 soil concentrations, whatever the chemical extractant, and by predicted free ion activities in
499 the soil solution. When total metal concentrations and soil characteristics were used as
500 explanatory variables, the explanation of the variations of metal concentrations in snail
501 tissues slightly increased, particularly when data were handled species-specifically, but
502 remained lower than 42%. Biotic parameters like species when a multi-species dataset is
503 studied, or age when it can be determined (i.e. for certain species for which a method exists),
504 were of primary importance in explaining snail tissue concentrations. Taken together, those
505 results suggest that correlations between snail tissues and external (total, or extractable
506 concentrations, predicted free ion activities) are not high enough to be used for predictive

507 purposes. Data coming from laboratory or microcosm studies, particularly those using freshly
508 spiked soils, sometimes show better or even good relationships between invertebrate tissue
509 and external concentrations, and this has lead to consider chemical extractions as a
510 promising tool to predict metal bioavailability. The few field data (mainly on worms and, in the
511 present work, on snails) reinforce the well known feeling that extrapolation from laboratory to
512 field conditions is not straightforward and that both approaches should be combined to
513 understand and to predict bioavailability. Moreover, important insights have to be gained in
514 food web approaches allowing determining food web structure and composition, and hence,
515 a better understanding of the transfer (and subsequent effects) of contaminants in
516 ecosystems.

517

518 **Acknowledgements**

519 Francis Douay and his colleagues from the *Laboratoire Sols et Environnement* are gratefully
520 acknowledged for having performed soil samples and analyses. We warmly thank Cécile
521 Grand from the *Agence De l'Environnement et de la Maîtrise de l'Energie* (ADEME) for many
522 fruitful scientific discussions. The STARTT programme is financially supported by the *Agence*
523 *Nationale de la Recherche* (ANR, contract n°ANR-05-ECCO-004) and the ADEME (contract
524 n°0572C0058). Patrick Giraudoux, Francis Raoul and Dominique Rieffel are acknowledged
525 for their help in field work. Brice Mourier is supported by a grant from the *Centre National de*
526 *la Recherche Scientifique* (CNRS).

527

528 **Table 1.** Descriptive statistics of total concentrations of Cd, Pb and Zn in the 25 cm soil
 529 layer ($n = 48$) and in snail soft bodies ($n = 264$), and physico-chemical parameters of soils,
 530 from the former “Metaleurop-Nord” smelter (Noyelles-Godault, France). Different letters
 531 represent significant differences of metal concentrations between soils and snails according
 532 to linear models.

	Cd _{soil}	Pb _{soil}	Zn _{soil}	Cd _{snail}	Pb _{snail}	Zn _{snail}	OM	Clay	Silt	CEC	pH
	μg.g ⁻¹						g.kg ⁻¹			cmol ⁺ .kg ⁻¹	
Minimum	0.7	74.7	87.5	4.3	4.2	164	24	29	90	7.4	4.0
1 st quartile	5.3	266	405	24.6	16.9	381	55	146	328	14.0	6.2
Median	10.7	402	672	45.1	33.2	606	78	197	414	19.2	7.8
Mean	11.9 ^a	733 ^a	882 ^a	52.9 ^b	63.0 ^b	836 ^a	107	212	431	18.3	7.1
3 rd quartile	14.1	1144	1304	66.7	76.2	1029	150	243	520	21.0	7.9
Maximum	104	2360	3790	258	825	6619	243	391	719	29.2	8.3

533

534

535 **Table 2.** Partial R² of explanatory variables (when significant) and adjusted-R² of the models
 536 (in brackets, when significant) linking snail metal concentrations to soil concentrations (total
 537 or extractable) and free metal ion activities in the soil solution (pMetal²⁺). Best R² are
 538 indicated in bold. Negative relationships between metal concentrations in snail tissues and
 539 explanatory variables are indicated in between bracket (-). Partial R² of biotic variables
 540 (species or age) can be deduced by subtracting values of partial R² of soil concentrations
 541 from the R² of the model.

TM	[TM] _{total}	[TM] _{CaCl2}	[TM] _{citric acid}	[TM] _{acetic acid}	[TM] _a	[TM] _b	[TM] _d	[TM] _r	pTM ²⁺
6 snail species (<i>n</i> = 264, model: log ₁₀ [TM+1] _{snail} ~ species + log ₁₀ [total or extractable TM + 1] _{soil})									
Cd	3.2 (20.2)	5.7 (18.3)	4.6 (20.5)	6.0 (21.7)	ns ^a (17.0)	6.8 (20.2)	ns (14.8)	ns (16.9)	2.3 (-) (15.5)
Pb	ns (6.4)	Ns (5.6)	ns (8.4)	ns (14.1)	ns (3.7)	2.4 (-) (11.0)	ns (5.8)	ns (5.9)	3.5 (-) (8.6)
Zn	2.9 (39.5)	Ns (36.1)	2.0 (-) (38.5)	ns (37.3)	1.1 (36.5)	3.6 (42.1)	1.7 (37.4)	3.1 (38.7)	2.2 (-) (37.8)
<i>Oxychilus draparnaudi</i> (<i>n</i> = 78, model: log ₁₀ [TM+1] _{snail} ~ log ₁₀ [total or extractable TM + 1] _{soil})									
Cd	21.9	8.6	23.1	24.3	11.5	11.8	5.5	7.3	ns
Pb	7.0	Ns	19.1	23.0	ns	25.3	7.4	ns	ns
Zn	13.7	Ns	10.9	6.4	4.4	25.2	7.2	7.6	ns
<i>Cepaea sp</i> (<i>n</i> = 131, model: log ₁₀ [TM+1] _{snail} ~ age + log ₁₀ [total or extractable TM + 1] _{soil})									
Cd	ns (16.5)	5.1 (21.7)	ns (16.8)	ns (18.3)	ns (17.8)	4.9 (21.5)	ns (20.1)	ns (20.6)	4.3 (-) (20.9)
Pb	ns	Ns	ns	8.1 (-) (9.6)	ns	ns	ns	ns	6.2 (-) (7.6)
Zn	ns	3.2 (3.2)	ns	ns	ns	ns	ns	3.9 (3.9)	6.4 (-) (6.4)

542 ^ans: non significant

543

544 **Table 3.** Partial R² of each variable, models and their adjusted-R² (Adj-R²) for relationships
 545 linking snail metal concentrations to biotic variables (species and age), external total metal
 546 concentrations and soil properties in 6 snail species, in *Oxychilus draparnaudi* and in *Cepaea*
 547 *sp.* The negative or positive relationship between metal concentration in snail tissues and
 548 each quantitative explanatory variable is indicated by a sign minus or plus in bracket.

	Species	Age	[TM] _{total}	OM	pH	Clay	Silt	CEC	Adj-R ²
6 snail species (n = 264)									
Cd	14.9	nc ^a	3.1 (+)	4.6 (-)	ns ^b	ns	2.6 (-)	ns	
	Log(cd _{int} +1) ~ species + log(cd _{tot} +1) + log(OM+1) + log10(silt+1)								23.0
Pb	7.0	nc	ns	ns	4.0 (-)	2.5 (+)	1.8 (-)	ns	
	Log(pb _{int} +1) ~ species + log(pb _{tot} +1) + log10(pH+1) + log10(clay+1) + log(silt+1)								13.4
Zn	36.8	nc	3.2 (+)	ns	3.0 (-)	ns	ns	ns	
	Log(zn _{int} +1) ~ species + log(zn _{tot} +1) + log10(pH+1)								41.6
<i>Oxychilus draparnaudi</i> (n = 78)									
Cd	nc	nc	22.9 (+)	8.9 (-)	ns	ns	ns	ns	
	Log(cd _{int} +1) ~ log(cd _{tot} +1) + log(OM+1)								30.0
Pb	nc	nc	8.3 (+)	22.4 (-)	ns	ns	ns	ns	
	Log(pb _{int} +1) ~ log(pb _{tot} +1) + log(OM+1)								29.0
Zn	nc	nc	15.2 (+)	22.0 (-)	ns	ns	ns	ns	
	Log(zn _{int} +1) ~ log(zn _{tot} +1) + log(OM+1)								34.7
<i>Cepaea sp</i> (n = 131)									
Cd	nc	18.2	ns	4.5 (-)	3.9 (-)	ns	5.2 (-)	ns	
	Log(cd _{int} +1) ~ age + log(cd _{tot} +1) + log10(silt+1) + log(pH+1) + log(OM+1)								29.0
Pb	nc	3.0	ns	4.2 (+)	5.7 (-)	4.2 (+)	6.7 (-)	ns	
	Log(pb _{int} +1) ~ age + log(pb _{tot} +1) + log10(silt+1) + log(pH+1) + log(OM+1) + log(clay+1)								20.0
Zn	nc	ns	ns	3.7 (+)	3.5(-)	ns	8.6 (-)	ns	
	Log(zn _{int} +1) ~ age + log(zn _{tot} +1) + log10(silt+1) + log(OM+1) + log10(pH+1)								15.1

549 ^a: not concerned

550 ^b: not significant

551

552 **Figure captions**

553

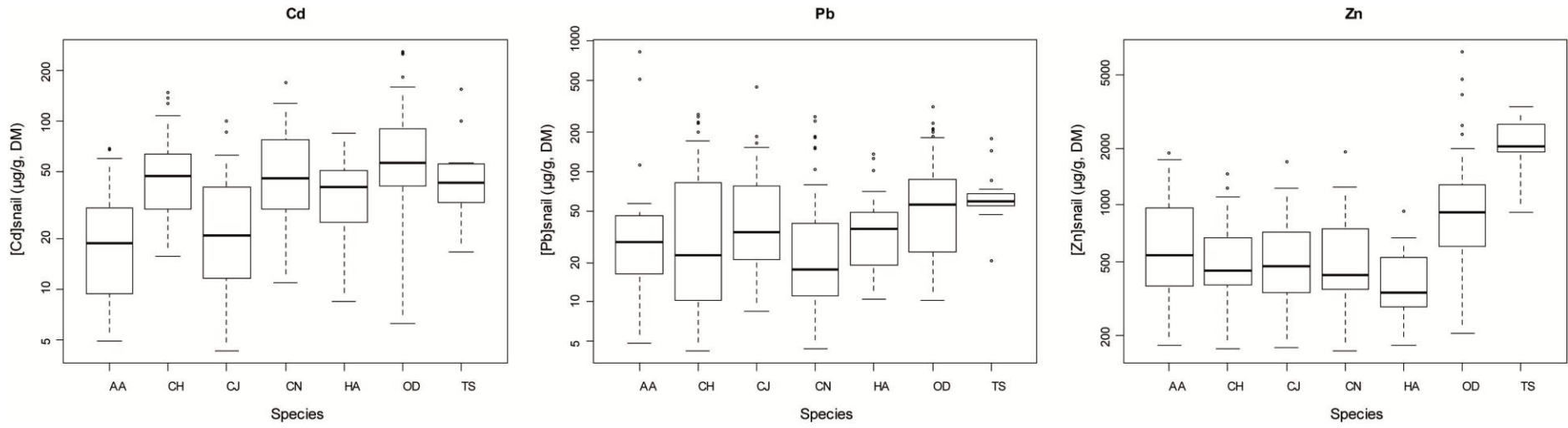
554 **Figure 1.** Cd, Pb and Zn concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dw) measured in soft tissues of each snail
555 species sampled in the area of the former “Metaleurop-Nord” smelter (Noyelles-Godault,
556 France). AA: *Arianta arbustorum*, CH: *Cepaea hortensis* (adults), CJ: juvenile of *Cepaea sp.*,
557 CN: *Cepaea nemoralis* (adults), HA: *Helix aspersa* (syn. *Cantareus aspersus*), OD:
558 *Oxychilus draparnaudi*, TS: *Trichia striolata*.

559

560 **Figure 2.** Cd, Pb and Zn biota-to-soil accumulation factors (BSAF) for snails as a function of
561 soil total metal concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dw) for 6 snail species (left) and for *Oxychilus*
562 *draparnaudi* (OD) and *Cepaea sp.* (Csp) individuals (right) sampled in the area of the former
563 “Metaleurop-Nord” smelter (Noyelles-Godault, France). Lines represent model predictions
564 with associated coefficients (slope and intercept). For the purpose of readability, metal
565 concentration values are given in $\mu\text{g}\cdot\text{g}^{-1}$ dw in both x- and y-axis of the figure. The equations
566 exhibit log-transformed values.

567

568 **Figure 1.**



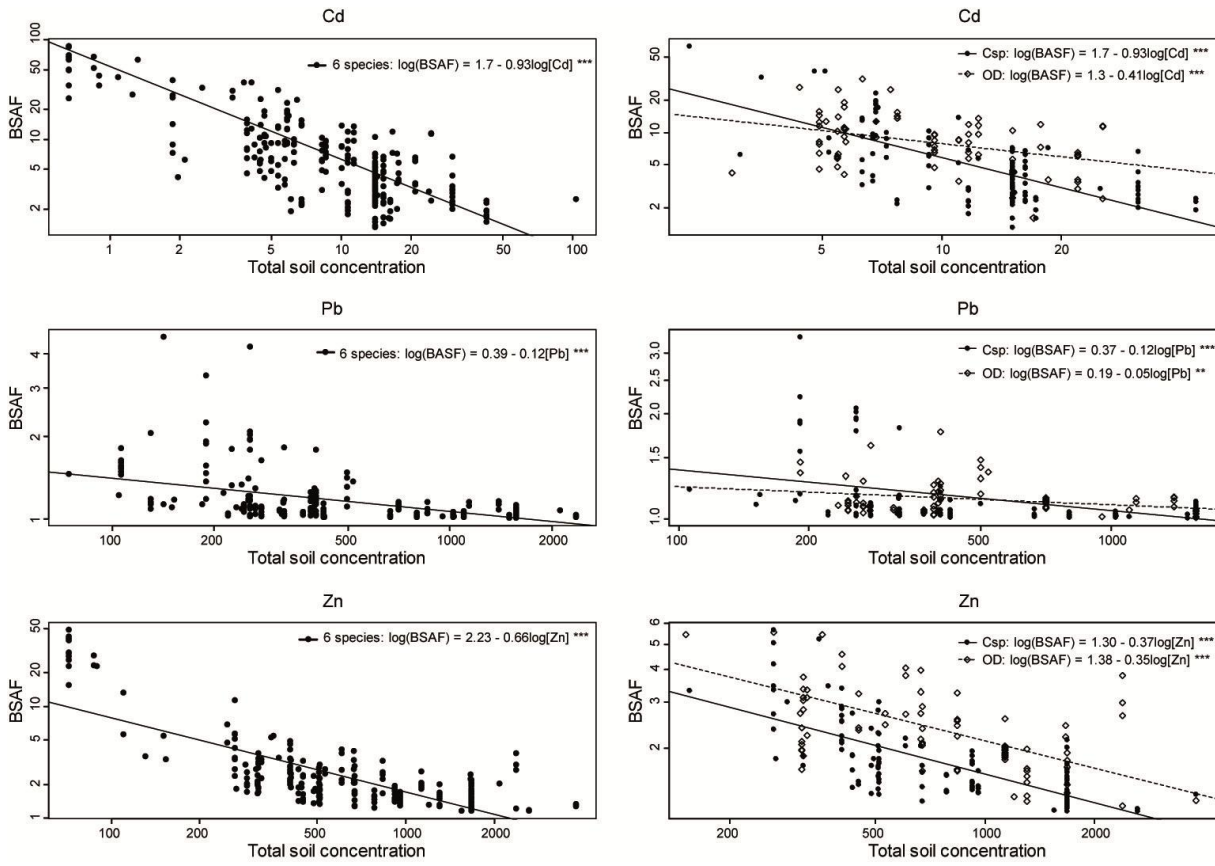
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571

572 **Figure 2.**

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576 **5. References**

- 577 Ahumada, I., Escudero, P., Ascar, L., Mendoza, J., Richter, P., 2004. Extractability of arsenic, copper,
578 and lead in soils of a mining and agricultural zone in central Chile. *Commun Soil Sci Plan.* 35,
579 1615-1634.
- 580 Beeby, A., Richmond, L., 2002. Evaluating *Helix aspersa* as a sentinel for mapping metal pollution.
581 *Ecol Indic.* 1, 261-270.
- 582 Beeby, A., Richmond, L., 2003. Do the soft tissues of *Helix aspersa* serve as a quantitative sentinel of
583 predicted free lead concentrations in soils? *Appl Soil Ecol.* 22, 159-165.
- 584 Beeby, A. & Richmond, L. 2010. Magnesium and the regulation of lead in three populations of the
585 garden snail *Cantareus aspersus*. *Environmental Pollution.* 158(6): 2288-2293.
- 586 Berger, B., Dallinger, R., 1993. Terrestrial snails as quantitative indicators of environmental metal
587 pollution. *Environ Monit Assess.* 25, 65-84.
- 588 Corp, N., Morgan, A.J., 1991. Accumulation of heavy metals from polluted soils by the earthworm,
589 *Lumbricus rubellus*: can laboratory exposure of 'control' worms reduce biomonitoring problems?
590 *Environ Pollut.* 74, 39-52.
- 591 Douay, F., Pruvot, C., Roussel, H., Ciesielski, H., Fourrier, H., Proix, N., Waterlot, C., 2008.
592 Contamination of urban soils in an area of Northern France polluted by dust emissions of two
593 smelters. *Water Air Soil Poll.* 188, 247-260.
- 594 Douay, F., Pruvot, C., Waterlot, C., Fritsch, C., Fourrier, H., Lorette, A., Bidar, G., Grand, C., de
595 Vaufléury, A., Scheifler, R., 2009. Contamination of woody habitat soils around a former lead
596 smelter in the North of France. *Sci Total Environ.* 407, 5564-5577.
- 597 Fritsch, C., 2010. Utilisation intégrée de bioindicateurs pour la surveillance des sols et des
598 écosystèmes terrestres. UMR 6249 UFC/CNRS Chrono-Environnement UsC INRA. PhD Thesis,
599 Université de Franche-Comté, Besançon, France.
- 600 Fritsch, C., Giraudoux, P., Coeurdassier, M., Douay, F., Raoul, F., Pruvot, C., Waterlot, C., de
601 Vaufléury, A., Scheifler, R., 2010. Spatial distribution of metals in smelter-impacted soils of woody
602 habitats: influence of landscape and soil properties, and risk for wildlife. *Chemosphere.* 81, 141-
603 155.
- 604 Grafen, A., Hails, R., 2002. *Modern statistics for the life sciences.* Oxford University Press, Oxford,
605 UK.
- 606 Harmsen, J., 2007. Measuring bioavailability: from a scientific approach to standard methods. *J*
607 *Environ Qual.* 36, 1420-1428.
- 608 Hass, A., Fine, P., 2010. Sequential selective extraction procedures for the study of heavy metals in
609 soils, sediments, and waste materials: a critical review. *Crit Rev Env Sci Tec.* 40, 365-399.
- 610 Hobbelen, P.H.F., Koolhaas, J.E., van Gestel, C.A.M., 2006. Bioaccumulation of heavy metals in the
611 earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in relation to total and available metal
612 concentrations in field soils. *Environ Pollut.* 144, 639-646.
- 613 ISO, 2008. Soil quality - Requirements and guidance for the selection and application of methods for
614 the assessment of bioavailability of contaminants in soil and soil materials. ISO 17402:2008.
615 International Organization for Standardization, Geneva, Switzerland.
- 616 Janssen, R.P.T., Posthuma, L., Baerselman, R., Den Hollander, H.A., Van Veen, R.P.M., Peijnenburg,
617 W.J.G.M., 1997. Equilibrium partitioning of heavy metals in Dutch field soils. II. Prediction of metal
618 accumulation in earthworms. *Environ Toxicol Chem.* 16, 2479-2488.
- 619 Kerney, M.P., Cameron, R.A.D., 2006. *Guide des escargots et limaces d'Europe.* Delachaux et
620 Niestlé, Paris, France.
- 621 Li, L.Z., Zhou, D.M., Wang, P., Allen, H.E., Sauve, S., 2009. Predicting Cd partitioning in spiked soils
622 and bioaccumulation in the earthworm *Eisenia fetida*. *Appl Soil Ecol.* 42, 118-123.
- 623 Lock K, Janssen CR. Zinc and cadmium body burdens in terrestrial oligochaetes: use and significance
624 in environmental risk assessment. *Environ Toxicol Chem* 2001;20:2067-72.
- 625 Lukkari, T., Taavitsainen, M., Väisänen, A., Haimi, J., 2004. Effects of heavy metals on earthworms
626 along contamination gradients in organic rich soils. *Ecotox Environ Safe.* 59, 340-348.
- 627 McGeer, J. C., Brix, K. V., Skeaff, J. M., DeForest, D. K., Brigham, S. I., Adams, W. J. & Green, A.
628 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals:
629 Implications for hazard assessment of metals in the aquatic environment. *Environmental*
630 *Toxicology and Chemistry.* 22(5): 1017-1037.
- 631 Martin, M.H., Coughtrey, P.J., 1982. Biological monitoring of heavy metal pollution: land and air. in:
632 Mellanby, K. (Ed.). *Applied Science Publishers, London, UK,* pp. 253-271.
- 633 Meers, E., Du Laing, G., Unamuno, V., Ruttens, A., Vangronsveld, J., Tack, F.M.G., Verloo, M.G.,
634 2007. Comparison of cadmium extractability from soils by commonly used single extraction
635 protocols. *Geoderma.* 141, 247-259.

- 636 Morgan, J.E., Morgan, A.J., 1999. The accumulation of metals (Cd, Cu, Pb, Zn and Ca) by two
637 ecologically contrasting earthworm species (*Lumbricus rubellus* and *Aporrectodea caliginosa*):
638 implications for ecotoxicological testing. *Appl Soil Ecol.* 13, 9-20.
- 639 Nahmani, J., Hodson, M.E., Black, S., 2007. A review of studies performed to assess metal uptake by
640 earthworms. *Environ Pollut.* 145, 402-424.
- 641 Notten, M.J.M., Oosthoek, A.J.P., Rozema, J., Aerts, R., 2005. Heavy metal concentrations in a soil-
642 plant-snail food chain along a terrestrial soil pollution gradient. *Environ Pollut.* 138, 178-190.
- 643 Owojori, O.J., Reinecke, A.J., Rozanov, A.B., 2010. Influence of clay content on bioavailability of
644 copper in the earthworm *Eisenia fetida*. *Ecotox Environ Safe.* 73, 407-414.
- 645 Peijnenburg, W.J.G.M., Jager, T., 2003. Monitoring approaches to assess bioaccessibility and
646 bioavailability of metals: matrix issues. *Ecotox Environ Safe.* 56, 63-77.
- 647 R Development Core Team, 2010. R: a language and environment for statistical computing. R
648 Foundation for Statistical Computing, Vienna, Austria.
- 649 Sauvé, S., McBride, M.B., Hendershot, W.H., 1997. Speciation of lead in contaminated soils. *Environ*
650 *Pollut.* 98, 149-155.
- 651 Sauvé, S., Norvell, W.A., McBride, M., Hendershot, W., 2000. Speciation and complexation of
652 cadmium in extracted soil solutions. *Environ Sci Technol.* 34, 291-296.
- 653 Scheifler, R., De Vaufleury, A., Coeurdassier, M., Crini, N., Badot, P.M., 2006. Transfer of Cd, Cu, Ni,
654 Pb, and Zn in a soil-plant-invertebrate food chain: A microcosm study. *Environ Toxicol Chem.* 25,
655 815-822.
- 656 Smolders, E., Oorts, K., van Sprang, P., Schoeters, I., Janssen, C.R., McGrath, S.P., McLaughlin,
657 M.J., 2009. Toxicity of trace metals in soil as affected by soil type and aging after contamination:
658 using calibrated bioavailability models to set ecological soil standards. *Environ Toxicol Chem.* 28,
659 1633-1642.
- 660 Speiser, B., 2001. Food and Feeding Behaviour. in: Barker, G.M. (Ed.). *The Biology of Terrestrial*
661 *Molluscs.* CABI Publishing, Oxon, UK, pp. 259-288.
- 662 Spurgeon, D.J., Hopkin, S.P., 1996. Risk assessment of the threat of secondary poisoning by metals
663 to predators of earthworms in the vicinity of a primary smelting works. *Sci Tot Environ.* 187, 167-
664 183.
- 665 Stephan, C.H., Courchesne, F., Hendershot, W.H., McGrath, S.P., Chaudri, A.M., Sappin-Didier, V.,
666 Sauvé, S., 2008. Speciation of zinc in contaminated soils. *Environ Pollut.* 155, 208-216.
- 667 Sterckeman, T., Douay, F., Fourrier, H., Proix, N., 2002a. Référentiel pédo-géochimique du Nord-Pas
668 de Calais. Conseil Régional du Nord-Pas de Calais, Ministère de l'Aménagement du Territoire et
669 de l'Environnement, Lille, France, p. 130.
- 670 Sterckeman, T., Douay, F., Proix, N., Fourrier, H., Perdrix, E., 2002b. Assessment of the
671 contamination of cultivated soils by eighteen trace elements around smelters in the North of
672 France. *Water Air Soil Pollut.* 135, 173-194.
- 673 Suter II, G.W., 1993. Exposure. in: Suter II, G.W. (Ed.). *Ecological Risk Assessment.* Lewis
674 Publishers, Boca Raton, FL, pp. 153-172.
- 675 Van Gestel, C.A.M., 2008. Physico-chemical and biological parameters determine metal bioavailability
676 in soils. *Sci Tot Environ.* 406, 385-395.
- 677 Van Vliet, P.C.J., Van der Zee, S.E.A.T.M., 2005. Heavy metal concentrations in soil and earthworms
678 in a floodplain grassland. *Environ Pollut.* 138, 505-516.
- 679 Veltman, K., Huijbregts, M.A.J., Hendriks, A.J., 2008. Cadmium bioaccumulation factors for terrestrial
680 species: Application of the mechanistic bioaccumulation model OMEGA to explain field data. *Sci*
681 *Tot Environ.* 406, 413-418.
- 682 Williamson, P., 1979. Age determination of juvenile and adult *Cepaea*. *J Mollus Stud.* 45, 52-60.