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### ► To cite this version:

Thibaut Powolny, Renaud Scheifler, Francis Raoul, Clémentine Fritsch. Is blood a reliable indicator of trace metal concentrations in organs of small mammals?. *Chemosphere*, 2019, 217, pp.320-328. 10.1016/j.chemosphere.2018.10.215 . hal-02124731

**HAL Id: hal-02124731**

**<https://hal.science/hal-02124731>**

Submitted on 7 Feb 2022

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1 Is blood a reliable indicator of trace metals concentrations in  
2 organs of small mammals?

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8 Keywords: Non-invasive methods, biomonitoring, wildlife, inorganic pollutants, toxic elements

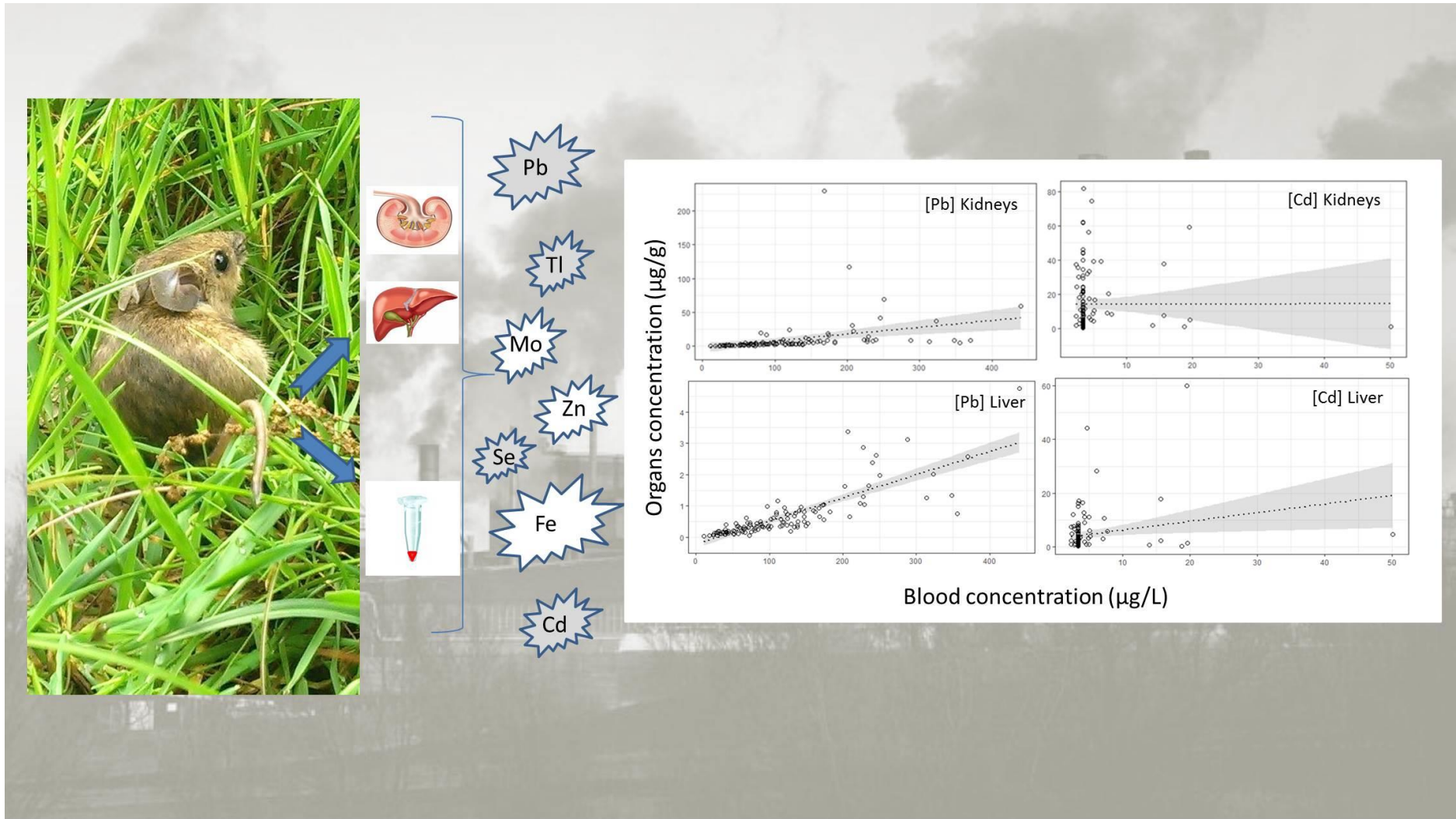
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10 **Highlights**

- 11 • Non-destructive biomonitoring approach is required for scientific and ethical reasons.
- 12 • We tested whether blood metal levels can serve as indicator of organ concentrations.
- 13 • This hypothesis was confirmed for Pb, Se, and Tl, with accurate modelling.
- 14 • For the other 8 investigated metals above the limit of detection, relationships were weak or non-
- 15 significant.
- 16 • More toxicokinetic research is needed to develop the use of blood in ecotoxicology.

17

18 Graphical abstract



20 **Abstract**

21 In wildlife ecotoxicology, the rationale for using blood rather than other body fluids or tissues is that  
22 sampling blood is a minimally invasive technique, without animal's sacrifices, providing both ethical and  
23 scientific benefits. To date, only few data on the relationships between blood and organ metal  
24 concentrations are available for small mammals living in contaminated sites. The present work aims at  
25 studying the relationships between the concentrations of 18 essential and non-essential metals in blood  
26 and their concentrations in the liver and kidneys, two accumulation and target organs, in wood mice from  
27 a former lead and zinc smelter named Metaleurop Nord, Northern France. Results from Se, Pb and Tl  
28 indicate that blood levels may be used to predict concentrations in organs of small mammals. Conversely,  
29 for Cd, Cu, Fe, Mo, Ti and Zn, blood concentrations were poorly or not related to liver and kidney  
30 concentrations. On top of accurately predicting concentrations of some metals in target organs, blood can  
31 provide important information about physiological and biochemical status of organisms, but further  
32 toxicokinetic research would be required to develop the use of blood as low-invasive biomonitoring and  
33 ecotoxicological method in wildlife.

34

## 35           **1. Introduction**

36   In human, blood tests are often used in health care to determine physiological and biochemical states,  
37   giving information about organ function or diseases. In wildlife ecotoxicology, blood has also widely been  
38   used to evaluate various parameters of individuals' health (Gorriz et al., 1996; Nunes et al., 2001; Topashka-  
39   Ancheva et al., 2003 ; Rogival et al., 2006; Sánchez-Chardi et al., 2008 ; Adham et al., 2011). The rationale  
40   for using blood rather than other body fluids or tissues is that sampling blood is a minimally invasive  
41   technique that does not necessitate sacrificing individuals, which has obviously both ethical and scientific  
42   benefits. There is indeed an increasing will from the large public to perform scientific studies with minimal  
43   impacts on the species being studied. Many countries have therefore developed regulation (see e.g.  
44   Directive 2010/63/UE) that frame the use of lab and free-ranging animals (mostly vertebrates) used for  
45   scientific purposes. Accordingly, current scientific guidelines such as the one from the *American Society of*  
46   *Mammalogists* fully acknowledge those ethical issues and propose appropriate procedures minimizing  
47   stress, pain, and, generally speaking, impact on animals (Sikes and the Animal Care and Use Committee of  
48   the American Society of Mammalogists, 2016). From a scientific point of view, minimizing impact on studied  
49   animal models, particularly avoiding sacrificing individuals, is also crucial to limit disturbance of scientific  
50   investigations on the dynamics of local populations. This is a key issue for long-term monitoring and surveys,  
51   whose importance is increasingly recognized in ecotoxicology (Kendall, 2016). Moreover, lethal, or at least  
52   invasive methods inducing a risk for either survival or reproduction of captured individuals, cannot be  
53   applied on many rare and/or endangered species for conservation issues. In addition to important  
54   physiological parameters informing about the health status of individuals, blood, as integuments like  
55   feathers or hairs that are also widely used in ecotoxicology, provides information about the level of  
56   exposure to contaminants.

57   Free-ranging terrestrial vertebrates are considered to be exposed to metals mainly through ingestion of  
58   food and water, and secondarily through inhalation of aerosols and, in some instances, vapors (Shore and  
59   Rattner, 2001; Smith et al., 2007). Whatever the exposure route, metals, after uptake, are transported and  
60   distributed to different organs within the body through blood, the main transporting medium in the body

(Fig. 1). Excretion or clearance of metals from an organ or from the body as a whole can occur through various routes, including or not a transport through blood (Fig. 1), and depends on a number of metal-specific processes and their role (e.g. essential or non-essential metals) in organisms (Elder et al., 2015). Epidemiological studies on human and laboratory experiments on model organisms have allowed the development of toxicokinetic studies for several metals. Toxicokinetics refer to the processes of absorption, distribution, biotransformation (i.e. formation or breakdown of metal-carbon bonds, or change of oxidation state of a metal in the biological system) and excretion, quantitatively studied as a function of time (Elder et al., 2015). According to general concepts in toxicokinetics, a constant exposure, associated to constant absorption and elimination rates, would lead, over time, to a steady-state equilibrium, where metal concentrations in blood could be used as a reliable estimate of concentrations in the organs. In the wild, however, the exposure of small mammals in a contaminated site is likely to be far from constant. Exposure of free-ranging mammals to metals, indeed, has been shown to greatly vary over time and space, with e.g. heterogeneity of soil contamination, habitat use, diet and foraging behaviour of organisms, or variations of food resources in the field (Smith et al. 2007, van den Brink et al., 2011). Bioavailability (i.e. the fraction of the total concentration of a metal in a given compartment that can actually be transferred into an organism) of metals in soils can also dramatically vary with both characteristics of the considered metal and the properties of the contaminated medium (Harmsen, 2007). This is likely to be the case for metals in plant and animal tissues small mammals feed on but that issue has been less studied for terrestrial organisms than in the aquatic environment (see e.g. Rainbow et al., 2011) or for human foodstuffs (Elder et al., 2015). The rate of clearance of metals from organs (into blood or through excretion, Fig. 1), usually measured by the biological half-time (i.e. the time it takes to the concentration to be reduced to half of its initial value), is another process that influences the metal concentration in blood, and may therefore influence the relationship of metal concentrations between blood and organs.

In the line of some previous developments of minimally invasive ecotoxicological methods on small mammals (Tête et al., 2013; 2014; 2015), the present work aims at studying the relationships between the concentrations of 18 essential and non-essential trace elements in blood and their concentrations in the

87 liver and the kidneys, two accumulation and target organs, in small mammals sampled along a pollution  
88 gradient in Northern France. We hypothesise that blood concentrations of elements with high internal turn-  
89 over will show a strong relationship between blood and internal tissues, allowing to develop simple  
90 modelling to be further used in ecotoxicology of free-ranging small mammals. Weak or no relationships will  
91 probably be found for elements whose blood concentration is more linked to daily exposure and/or for  
92 other toxicokinetic reasons.

## 93 **2. Materials and Methods**

### 94 *2.1. Study site*

95 We conducted fieldwork in an area surrounding the former lead (Pb) and zinc (Zn) smelter named  
96 Metaleurop Nord (Hauts-de-France Region, northern France, 5025042N, 300055E). The smelter was  
97 established by the end of the 20<sup>th</sup> century, until its closure in 2003. Major emissions leading up to the  
98 smelter's closure were cadmium (Cd), lead (Pb), zinc (Zn). Soil contamination levels on this area are well  
99 documented and reported high Cd, Pb and Zn levels of contamination, ranging from 0.1 to 2,402 mg/kg for  
100 Cd, from 16 to 41,960 mg/kg for Pb and from 44 to 38,760 mg/kg for Zn (*per dry weight*, Douay et al., 2009;  
101 Fritsch et al., 2010a). Cadmium, Pb and Zn concentrations in soils from woody habitats are highly correlated,  
102 with coefficients of determination ranging from 0.6 to 0.8. Concentrations decrease with the distance to  
103 the smelter and are related to the prevailing SW–NE winds (Fritsch et al., 2010b) but anthropogenic  
104 activities disturb this global pattern (Douay et al., 2009). A 40 km<sup>2</sup> (8×5 km) study area around the former  
105 smelter was divided into 160 squares (500×500 m). Four sampling squares across an environmental metal  
106 contamination gradient were studied (from control to highly contaminated squares) for the present study.  
107 Based on the Cd, Pb and Zn concentrations, sampling squares were classified as: control (“C”), low (“L”),  
108 medium (“M”) and high (“H”) pollution level (Table 1).

### 109 *2.2. Data collection*

110 From the 30<sup>th</sup> of September to the 10<sup>th</sup> of October 2014, 10 lines of 10 non-lethal INRA traps spaced 3 m  
111 apart were placed on each site for 3 consecutive trap nights. A total of 120 wood mice (*Apodemus*  
112 *sylvaticus*) were trapped along the gradient of pollution, according to the following distribution: 27



113 individuals at site “control”, 33 at site “L”, 34 at site “M” and 26 at site “H” (Table 1). No difference in age  
114 using crystalline lens mass (ANOVA,  $F=1,83$ ;  $Df=3$ ;  $p=0,146$ ) nor in sex-ratio ( $F=1,56$ ;  $Df=1$ ;  $p=0,196$ ) were  
115 observed between sites. Before animal sacrifice by cervical dislocation (following the American Veterinary  
116 Medical Association ethical guidelines (Sikes and the Animal Care and Use Committee of the American  
117 Society of Mammalogists, 2016), blood was collected from the orbital sinus using a Pasteur pipette,  
118 immediately transferred into heparinised microtubes. Blood samples were then stored at 4-8°C before  
119 freezing at -20°C within the following next 10 hours. Individuals were weighed, measured and sexed during  
120 dissection at the laboratory. The crystalline lenses were sampled and kept in a 3% formalin solution. All  
121 procedures involving the handling of animals were conducted by certified persons. During dissections, livers  
122 and kidneys were collected (mean liver mass:  $1.010g \pm 0.261g$ ; mean kidney mass:  $0.222g \pm 0.045g$ ), dried  
123 (65°C, drying oven) and stored until analysis. Sampling authorization was given by the Direction Régionale  
124 de l’Environnement, de l’Aménagement et du Logement (DREAL) of the Hauts-de-France Region.

### 125 2.3. *Metal analysis*

126 The concentrations of 18 metals (aluminum (Al), antimony (Sb), arsenic (As), Cd, chromium (Cr), cobalt (Co),  
127 copper (Cu), iron (Fe), Pb, manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), strontium (Sr),  
128 tin (Sn), titanium (Ti), thallium (Tl), and Zn) were determined in blood samples and organs. Blood samples  
129 were acid digested in 700  $\mu$ l of nitric acid ( $HNO_3$ , 65%, analytical quality, Optima) for half a day in open  
130 tubes under fume hood, and then for 60 h in closed tubes at 60°C in an oven. After digestion, samples were  
131 diluted to 30 ml by the addition of ultra-pure water ( $18.2 MV.cm^{-2}$ ). Liver and kidney dried samples were  
132 digested with nitric acid ( $HNO_3$  at 69%) and then diluted by adding ultra-pure water (Elga,  $18.2 MV.cm^{-2}$ )  
133 prior to analyses. Blood and organs metals were analysed by Inductively Coupled Plasma—Mass  
134 Spectrometry (ICP-MS, ThermoFischer Scientific XSeries 2). Over the 18 metals measured, only 11 (Cd; Cu;  
135 Fe; Mn; Mo; Pb; Se; Sn; Ti, Tl, Zn) had a satisfactory proportion of values above quantification limits (Table  
136 S1), allowing further statistical analyses. For the values under quantification limits for these elements,  
137 values were replaced by the quantification limit divided by square root of two for statistical analysis (Helsel,  
138 2010). Repeatability (within-run precision) and reproducibility of the measurements were surveyed

139 (checking of RSD values and use of internal standards), and quality control (control solutions and blanks to  
140 check for the absence of drift) was conducted during measurements. Analysis accuracy was checked using  
141 certified reference materials (National Research Council Canada TORT-2 Lobster hepatopancreas,  
142 eEuropean Reference Material ERM<sup>®</sup>-CE196 Bovine blood). Blanks (acid + ultra-pure water) and certified  
143 reference materials were prepared and analysed using the same methods as the blood samples. Average  
144 recoveries of the certified reference materials are given Table S1. The concentrations of metals in the blood  
145 are expressed as  $\mu\text{g/L}$  and as  $\mu\text{g/g}$  dry mass for the liver and kidneys.

#### 146 *2.4. Statistical analyses*

147 General Linear Models were used to investigate the relationship between blood and organs (liver and  
148 kidney) metal concentrations. A backward selection procedure starting from a full model that included all  
149 factors and their two-way interactions was performed. Quasi likelihood estimations were used to estimate  
150 the scale parameter, and the significance of terms in the model was tested using F-tests. Starting by  
151 continuous covariates, non-significant terms at  $p \leq 0.05$  were sequentially removed to achieve a minimal  
152 adequate model (Crawley, 1993). Wherever an interaction term was left in a model, the two corresponding  
153 factors were also included as main effects. All tissue concentrations (from blood, liver and kidneys) were  
154 log-transformed ( $\log_{10}(x+1)$ ) and normality and homoscedasticity assumptions were checked graphically.  
155 Full models tested were: [Organ]  $\sim$  [Blood] + Sex + Crystalline mass + Sex\*[Blood] + Crystalline  
156 mass\*[Blood]. To reduce the effect of outliers, we removed points with a Cook's distance (Cook, 1977) of  
157  $\geq 0.95$ , and reran the linear regression. Tissue concentrations of metals were compared along the  
158 environmental contamination gradient of Cd, Pb and Zn using ANOVA test. When significant differences  
159 were detected among study squares, multiple comparisons were performed using Tukey HSD test. Each  
160 analysis was performed with the software R v. 3.15.1 (R Development Core Team).

### 161 **3. Results**

#### 162 *3.1. Metal concentrations around the former smelter*

163 Among the 18 elements measured, Cu, Fe, Pb, Se, and Zn were the only elements to exhibit concentrations  
164 above the quantification limits of the analytical instruments, whatever the fluid (blood) or tissue (kidney

165 and liver). Cd, Mn, Mo, Ti, and Tl were not always detectable in blood, while Sn was hardly detectable in  
166 organs. Over the 18 metals measured, only 11 (Cd; Cu; Fe; Mn; Mo; Pb; Se; Sn; Ti, Tl, Zn) had a satisfactory  
167 proportion of values above quantification limits and were included in further statistical analyses. Complete  
168 trace metal concentrations measured in blood, liver and kidneys are presented in Table S1.

169 The concentrations of Cd, Pb and Zn in blood, in the liver and in kidneys (Fig. 2) of wood mice showed a  
170 high variability, depending on squares. Lead concentrations in kidneys were significantly higher only in  
171 mouse coming from the most contaminated site ( $F=38.2$ ;  $Df=3$ ;  $p<0.001$ , Fig. 2A). In the liver, Pb  
172 concentrations also varied along the gradient ( $F=12.0$ ;  $Df=3$ ;  $p<0.001$ ; Fig. 2B), with concentrations from the  
173 lowly and the highly contaminated squares not differing among them, but being significantly higher than  
174 those in the control and the medium squares ( $p<0.001$ ). Concentrations from the highest contaminated  
175 square were also higher than in the medium level, and concentrations from the lowest contaminated  
176 square were higher than in the intermediate level of environmental pollution. The pattern was quite similar  
177 for (F=16.34; Df=3;  $p<0.001$ , Fig. 2H). For Cd, contrary to blood for which no differences were observed  
178 along the pollution gradient ( $F=0.29$ ;  $Df=3$ ;  $p=0.83$ , data not shown), liver ( $F=14.45$ ;  $Df=3$ ;  $p<0.001$ , Fig. 2C)  
179 and kidneys ( $F=16.89$ ;  $Df=3$ ;  $p<0.001$ , Fig. 2D) concentrations increased along the pollution gradient, except  
180 in the highest contaminated square where concentrations were not statistically higher than in the medium  
181 level of pollution. Zinc concentrations in blood (Fig. 2G), kidneys (Fig. 2E) and the liver (Fig. 2F) did not show  
182 any significant increase along the contamination gradient, even if, for blood and the liver, concentrations  
183 at the low level of contamination square were sometimes higher than in some other squares. Comparisons  
184 of concentrations in blood and the organs for the others metals (Cu, Fe, Mn, Mo, Se, Sn, Ti and Tl) along the  
185 contaminated gradient are presented in supplementary materials (Table S1 and Fig. S1), and are not  
186 presented here and will not be discussed extensively because the concentrations in the soils for those  
187 metals are not available. Only the relationships between blood and organs will be discussed for Cu, Fe, Mn,  
188 Mo, Se, Sn, Ti and Tl.

189 *3.2. Relationships between trace element concentrations in different tissues*

190 The relationships between blood and tissue concentrations varied considerably between elements and  
191 tissues. Copper (Cu) and Ti concentrations in tissues could not be modelled by any of our study variables  
192 (Table 2, Fig. S2 and S3). When examining Pb concentrations in liver and kidneys (Fig. 3A and 3B), increases  
193 in blood Pb predicted increases in organs ( $R^2=0.64$ ,  $p < 0.001$  for liver and  $R^2=0.56$ ,  $p < 0.001$  for kidneys,  
194 Table 2). Molybdenum (Mo) was the only other element for which blood concentration was the only  
195 variable retained in models to predict organ concentrations. Contrary to Pb, however, the coefficients of  
196 determination of the models for Mo were low ( $R^2= 0.26$  for the liver,  $R^2= 0.13$  for kidneys, Table 2). Selenium  
197 (Fig. 4) and Tl (Fig. S2 and S3) provided good models with coefficients of determination ranging from 0.69  
198 to 0.89, but sex was a variable to consider in the modelling of liver concentrations for both elements.  
199 Cadmium concentrations in kidneys (Fig. 3D) were mainly predicted by age as assessed by crystalline masses  
200 ( $R^2=0.43$ ;  $p = 0.01$ ; Table 2) and by the age (partial  $R^2= 0.04$ ;  $p < 0.001$ ) and blood concentration with a weak  
201 explanation power (partial  $R^2= 0.30$ ;  $p = 0.01$ ) in liver ( $R^2= 0.34$ ;  $p < 0.001$ ). Iron (Fe) concentrations in organs  
202 were poorly modelled ( $R^2<0.11$ ) by blood concentrations for kidneys and by blood concentrations and sex  
203 for the liver (Table 2, Fig. 4). The liver concentrations for Zn, as for Tl, were significantly related to blood  
204 concentrations ( $p = 0.01$  for Zn and  $p = 0.0001$  for Tl) and to the interaction term between blood  
205 concentration and crystalline mass or sex, respectively (blood concentration\*sex:  $p = 0.01$  for Zn and blood  
206 concentration\* crystalline mass:  $p = 0.02$  for Tl, Table 2). Models for Zn exhibited relatively low coefficients  
207 of determination ( $R^2= 0.28$  for the liver,  $R^2= 0.14$  for kidneys).

#### 208 4. Discussion

209 Measuring pollutants using internal tissue samples from small mammals is the current reference method  
210 to estimate the degree of exposure to, and the subsequent accumulation of, cumulative compounds.  
211 However, in recent years, for both scientific and ethical reasons, there is a strong and ongoing tendency to  
212 use non-destructive samples in biomonitoring studies. The main objective of the present study was  
213 therefore to evaluate whether blood concentration values can be indicative of liver and kidney trace metals  
214 concentration in wood mice (*Apodemus sylvaticus*) under field conditions. Studies of metal accumulation  
215 in small mammals have included many taxa, such as wood, field and yellow-necked mice (Damek-Poprawa

216 and Sawicka-Kapusta, 2003; van den Brink et al., 2010; Tête et al., 2014), voles (Appleton et al., 2000), moles  
217 (Komarnicki, 2000), or shrews (Hamers et al., 2006; Sánchez-Chardi and Nadal, 2007; Moriarty et al., 2012;  
218 Drouhot et al., 2014). However, most of these studies focused on organs (mainly liver and kidney) using  
219 lethal methods. Contrary to bird species (Dauwe et al., 2005; Scheifler et al., 2006; Espín et al., 2016), scarce  
220 information on blood metal concentrations are available in literature for small mammals in their natural  
221 environment (Rogival et al., 2006; Vermeulen et al., 2009).

222 Here, the significant correlations observed between blood and tissue concentrations for Se, Pb, and Tl  
223 indicates that blood samples are suitable to estimate concentrations of these metals in liver and kidneys in  
224 the wood mice. For both Se and Tl, sex or its interaction with blood concentration is a variable retained in  
225 the final models but, according to the partial  $R^2$  of the variables (Table 2), its weight is low, and sex can  
226 relatively easily determined on alive animals using anogenital distance. Furthermore, these correlations  
227 have been observed over large concentration ranges, and occurred despite the varying relative age (using  
228 the crystalline mass as a proxy) or sex. Blood sampling could therefore be an adequate non-lethal method  
229 for estimating these metal concentrations in internal tissues over a wide range of biological and  
230 environmental conditions. For some other metals, however, the low (Fe, Mo and Zn), or even absent (Cu,  
231 Ti) relationship between blood and organ concentrations indicates that blood samples could hardly or not  
232 be used to predict tissue contamination in wild wood mice, at least for the metal concentrations recorded  
233 on our study site. Cd concentrations in organs were shown to be much more dependent of the age (as  
234 estimated here by the crystalline lenses mass) than by the concentrations in the blood. Similar pattern was  
235 noted on the same study site using hair (Tête et al., 2014) and the influence of age on Cd accumulation in  
236 various tissues is a well-known issue. In the present study, investigations for this compound were however  
237 limited by the relatively low number of samples above detection limits in blood. It is worth to note that, in  
238 the present study, the essentiality of metals is not a key factor in explaining high or weak relationships  
239 between blood and organs. Rather, a better knowledge of toxicokinetics of the different metals wild animals  
240 may be exposed to would allow gaining insight in the understanding of internal exchanges between blood  
241 and organs.

242 Toxicokinetics of Cd, one of the main metals found in mining and smelting-impacted sites (including our  
243 study site of Metaleurop Nord), have been relatively extensively studied, mainly in birds. Cd accumulation  
244 occurs primarily in kidneys and in the liver (Scheuhammer, 1987) and the release from kidneys has been  
245 proved to be very slow (Mayack et al., 1981). Thus, under conditions of chronic dietary exposure, Cd  
246 concentrations in kidneys are thought to represent long-term accumulation (Scheuhammer, 1987). In  
247 contrast, Cd in the blood equilibrates with exposure over a much shorter period of time (Hammond and  
248 Beliles, 1980), suggesting that concentrations in blood represent short term exposure. In addition, relative  
249 Cd accumulation by kidneys and the liver is dependent on the magnitude of the exposure; with increasing  
250 exposure, a higher proportion is deposited in the liver (Hammond and Beliles, 1980; Scheuhammer, 1987).  
251 Since blood concentrations of Cd under steady-state conditions are expected to equilibrate with  
252 concentrations in both the liver and kidneys (García-Fernández et al., 1997), changes in liver-kidney ratios  
253 of Cd will likely also influence blood-kidney ratios. For example, changes in body and organ masses may  
254 alter blood-organ ratios of metals. Moreover, and contrary to the liver or kidneys, blood does not constitute  
255 a tissue of storage. In the present study, the lowest concentrations of most elements were indeed found in  
256 the blood. In wild birds, Garcia-Fernandez et al. (1997) showed that Cd levels in blood represent a very small  
257 part of only 0.5% of Cd body burden, while liver and kidneys together contain 92% of the total (of which  
258 61% for kidneys only). Moreover, it is possible that concentrations of elements in blood did not change in  
259 the same manner as those in the liver or kidneys because metal sequestration features and speciation  
260 (differed between the tissues, see Vijver et al., (2004) for details). For instance, it has been shown in the  
261 rainbow trout (*Oncorhynchus mykiss*) that the patterns of subcellular partitioning of Cd varied according to  
262 target organs, with a different pattern of Cd accumulation in subcellular fractions in the gill and in the liver  
263 (Kamunde, 2009). Thus, changes in body and organ masses associated to blood and organs differences in  
264 their proportions of free/bound metals may stress different levels of total metals between blood and  
265 organs.

266 The imprecise relationship between blood and hepatic concentrations of metals may also be attributed  
267 partially to their different elimination rates in tissues. The elimination rate is initially faster from liver than

268 from blood (Heinz et al., 1990). Additionally, binding of metals to inorganic molecules in the liver (as well  
269 as in other organs) may result in the formation of inert complexes with a long biological half-life  
270 (Scheuhammer et al., 1998) and the proportion of the total metal pool that is bound may affect its  
271 distribution among different tissues (Cuvin-Aralar and Furness, 1991). Taken together, those data indicate  
272 that internal exchanges and rates of turn-over among tissues are key parameters in explaining blood-organ  
273 relationships, an area that would need further research.

274 General increase in concentrations of essential (Fe, Mg, Mn, Cu, and Cr) and non-essential elements (Pb)  
275 with age have been reported in organs in the wood mice (Lopes et al., 2002; Beernaert et al., 2007) and in  
276 other mammals (Scheirs et al., 2006; Outridge and Scheuhammer, 1993; Sánchez-Chardi and Nadal, 2007).  
277 In our study, except for Cd, we did not detect age-related effects in the relationship between blood and  
278 organs concentrations. Age-related increases in organs have been reported for Cd (Sheffield et al., 2001)  
279 and translate a Cd accumulation in storage organs along small mammals life.

280 The present study provides original results on the relationships between blood and organs concentrations  
281 for several metals. Our study provides simple and relatively accurate models (with  $R^2$  ranging from 0.56 to  
282 0.91) for Se, Pb and Tl, suggesting that blood sampling may be a useful tool in order to study these metals  
283 in wild, free-ranging small mammals. Conversely, for other metals such as Cd, Cu, Fe, Mo, Ti, and Zn, and in  
284 our levels of metals concentrations, blood concentrations were not a good indicator of internal  
285 concentrations, suggesting that blood sampling as a non-lethal methodology to predict organ  
286 concentrations may not be relevant for those metals. Further studies are clearly needed on other sites, on  
287 several years or at different seasons, and on different (small) mammal species to test (and modify if needed)  
288 the models developed here. The method would particularly be applicable where destructive sampling  
289 cannot be allowed, in protected areas and/or on endangered species.

## 290 **Acknowledgements**

291 The authors gratefully thank Nadia Crini and Caroline Amiot for their technical help in metal analyses, and  
292 Virgile Baudrot, Javier Fernandez de Simon, Julie Montaz, Aurore Niechajowicz, Shinji Ozaki, Nicolas Tête,  
293 and Núria Vallverdu Coll, for their help in the field. The ADEME (Agence De l'Environnement et de la Maîtrise

294 de l'Energie, <https://www.ademe.fr/>) financially supported the present work (programmes BIOTROPH  
295 N°1172C0030 and DYSPAT N°1572C0309).

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422 **Tables**

423 Table 1. Summary of study squares characteristics. Median [minimum - maximum] Cd, Pb and ZN concentrations ( $\mu\text{g/g}$ , dry weight) in soils collected in the  
 424 vicinity of the former Metaleurop Nord smelter. Results are presented for each sampling unit (square) corresponding to each pollution level.

Sampling squares	Control	Low	Medium	High
Dominant habitat	Forest	Forest	Forest	Forest
Distance from Metaleurop (median, km)	11.2	3.0	1.4	0.6
Soil contamination levels (from Fritsch et al. 2010)				
<i>Pb</i>	110 [73 - 182]	261.5 [149 - 333]	526 [245 - 1260]	1357 [755 - 6809]
<i>Cd</i>	1.6 [1.3 - 2.0]	4.0 [1.5 - 6.0]	9.7 [3.6 - 19.5]	59.3 [15.3 - 236.5]
<i>Zn</i>	183 [150 - 267]	322.5 [114 - 407]	586 [303 - 1044]	1802 [1069 - 6035]
Number of wood mouse ( $\text{♂}$ ; $\text{♀}$ )	27 (11 ; 16)	33 (18 ; 15)	34 (23 ; 11)	26 (14 ; 12)

425

426

427 Table 2. Results from general linear models linking metal blood concentrations to liver and kidneys concentrations in wood mice. A backward selection  
428 procedure was used, with least significant variables being removed sequentially, until a minimum adequate model (presented). Bold indicates significant  $p$   
429 values.

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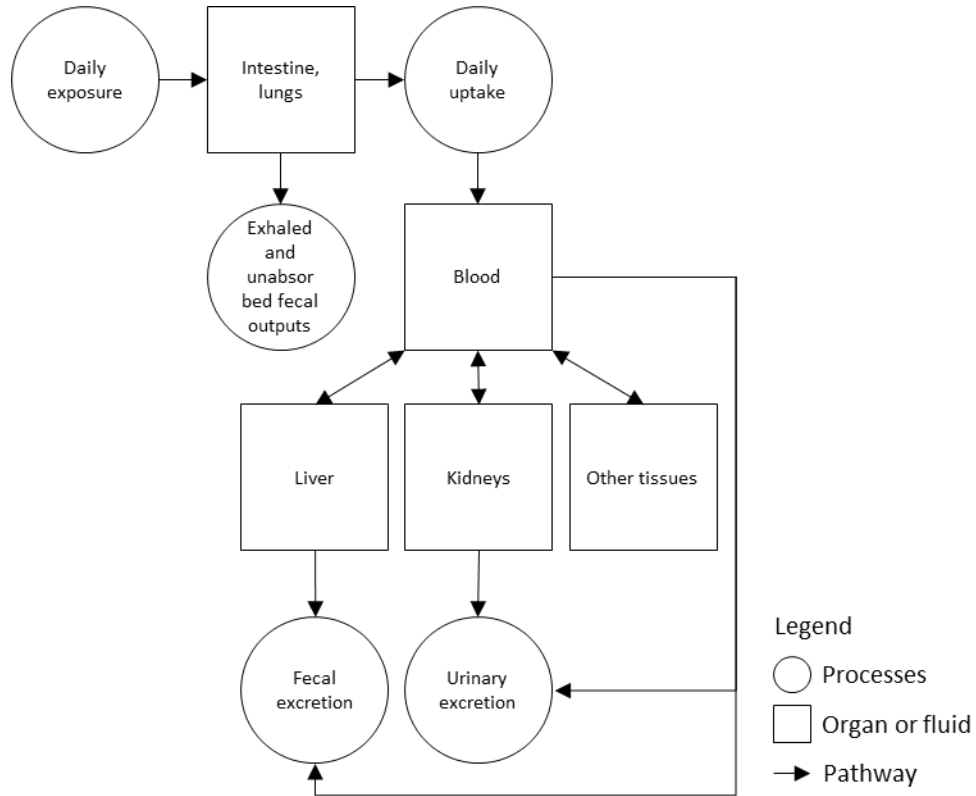
Elements	Organ	Variable	Estimates	Partial $r^2$	Df/DfDen*	F values	P	$r^2$
Cd	Liver	blood concentration	0.47	0.04	1, 116	6.89	<b>0.01</b>	0.34
		crystalline mass	57.56	0.30	1, 116	53.46	<b>&lt; 0.001</b>	
	Kidney	crystalline mass	82.38		1, 117	88.15	<b>&lt; 0.001</b>	0.43
Cu	Liver	blood concentration	0.05		1, 118	1.64	0.20	0.014
	Kidney	crystalline mass	-2.65		1, 117	1.36	0.24	0.01
Fe	Liver	blood concentration	0.52	0.05	1, 117	6.91	<b>0.01</b>	0.11
		sex	<i>female</i> :- 0.07	0.06	1, 117	7.75	<b>0.01</b>	
	Kidney	blood concentration	0.27		1, 117	4.44	<b>0.04</b>	0.04
Mo	Liver	blood concentration	0.21		1, 118	41.10	<b>&lt; 0.001</b>	0.26
	Kidney	blood concentration	0.11		1, 118	18.00	<b>&lt; 0.001</b>	0.13
Pb	Liver	blood concentration	0.37		1, 117	210.96	<b>&lt; 0.001</b>	0.64
	Kidney	blood concentration	0.98		1, 117	148.96	<b>&lt; 0.001</b>	0.56
Se	Liver	blood concentration	0.75	0.89	1, 117	996.40	<b>&lt; 0.001</b>	0.89
		sex	<i>female</i> : 0.03	0.004	1, 117	5.27	<b>0.02</b>	
	Kidney	blood concentration	0.04		1, 118	272.67	<b>&lt; 0.001</b>	0.69
Ti	Liver	blood concentration	0.01		1, 118	0.03	0.86	< 0.001
	Kidney	blood concentration	-0.0003		1, 118	0.001	0.99	< 0.001
Tl	Liver	blood concentration	0.12	0.89	1, 116	439.34	<b>&lt; 0.001</b>	0.91
		sex	0.004		1, 116	0.0128	0.91	
		blood concentration X sex	-0.04	0.02	1, 116	16.04	<b>0.0001</b>	
	Kidney	blood concentration	0.81		1, 116	886.36	<b>&lt; 0.001</b>	0.88
Zn	Liver	blood concentration	0.65	0.24	1, 116	38.97	<b>&lt; 0.001</b>	0.28
		sex	1.31		1, 116	0.41	0.52	
		blood concentration X sex	-0.36	0.04	1, 116	6.13	<b>0.01</b>	
	Kidney	blood concentration	0.23		1, 116	19.97	<b>&lt; 0.001</b>	0.14

\* Df = variable Df ; DfDen = error Df

431

432 **Figures**

433 Fig. 1. Flow scheme describing the movement of metals among various compartments in a terrestrial  
 434 vertebrate (modified from Elder et al. 2015) and its environment. Dermal contact and other minor pathways  
 435 (perspiration, exfoliation of skin, loss of hair and nails, lactation...) are not presented.



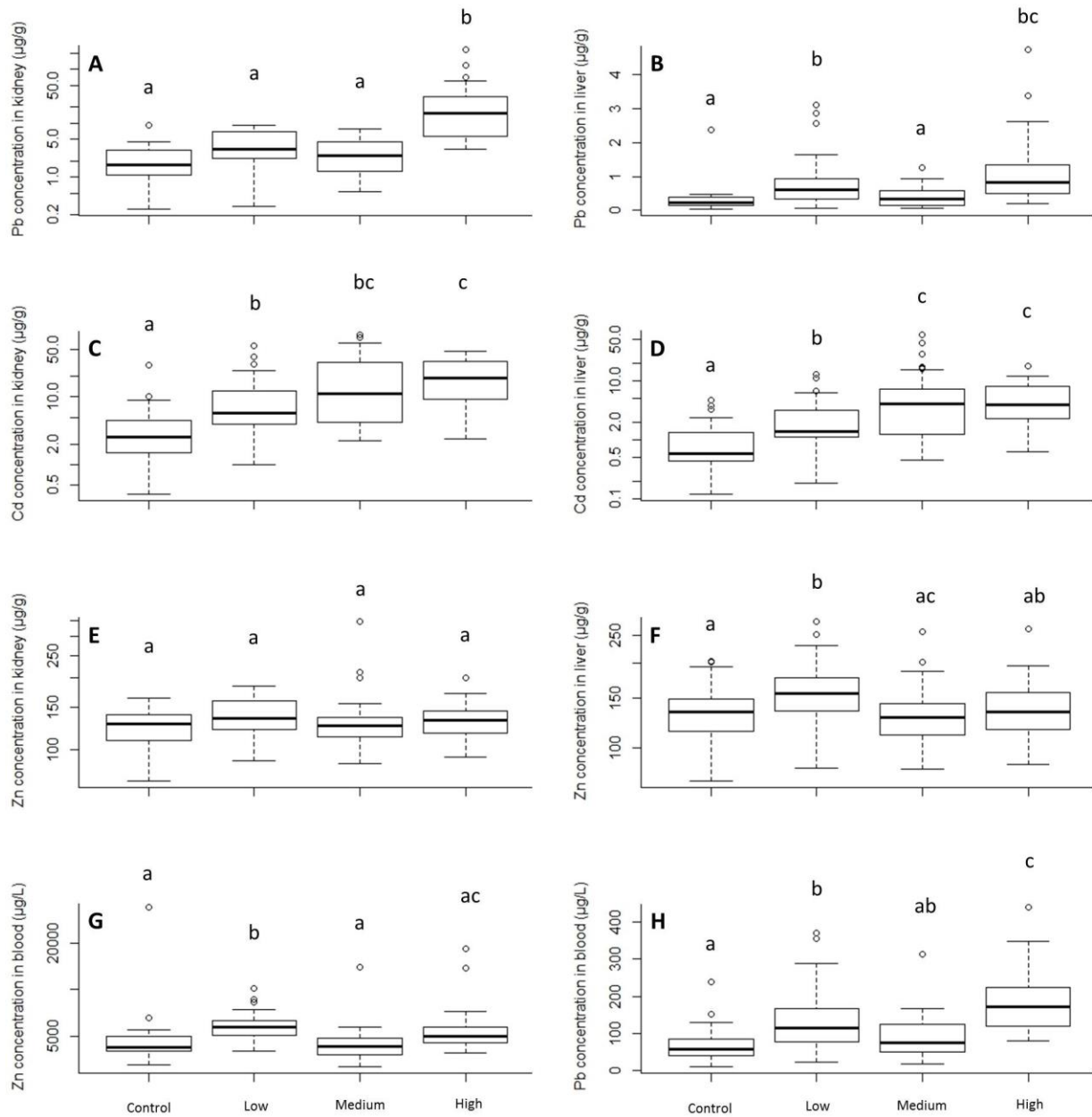
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440 Fig. 2. Lead concentrations in kidneys (A), liver (B) and blood (H), Cd concentrations in kidney (C) and liver (D), and Zn concentrations in kidney (E), liver (F) and blood (G) along the soil metal contamination gradient  
 441 (see Fritsch et al., 2010, for further details about soil contamination). Different letters correspond to  
 442 significantly different means (Tukey's HSD,  $p < 0.05$ ).  
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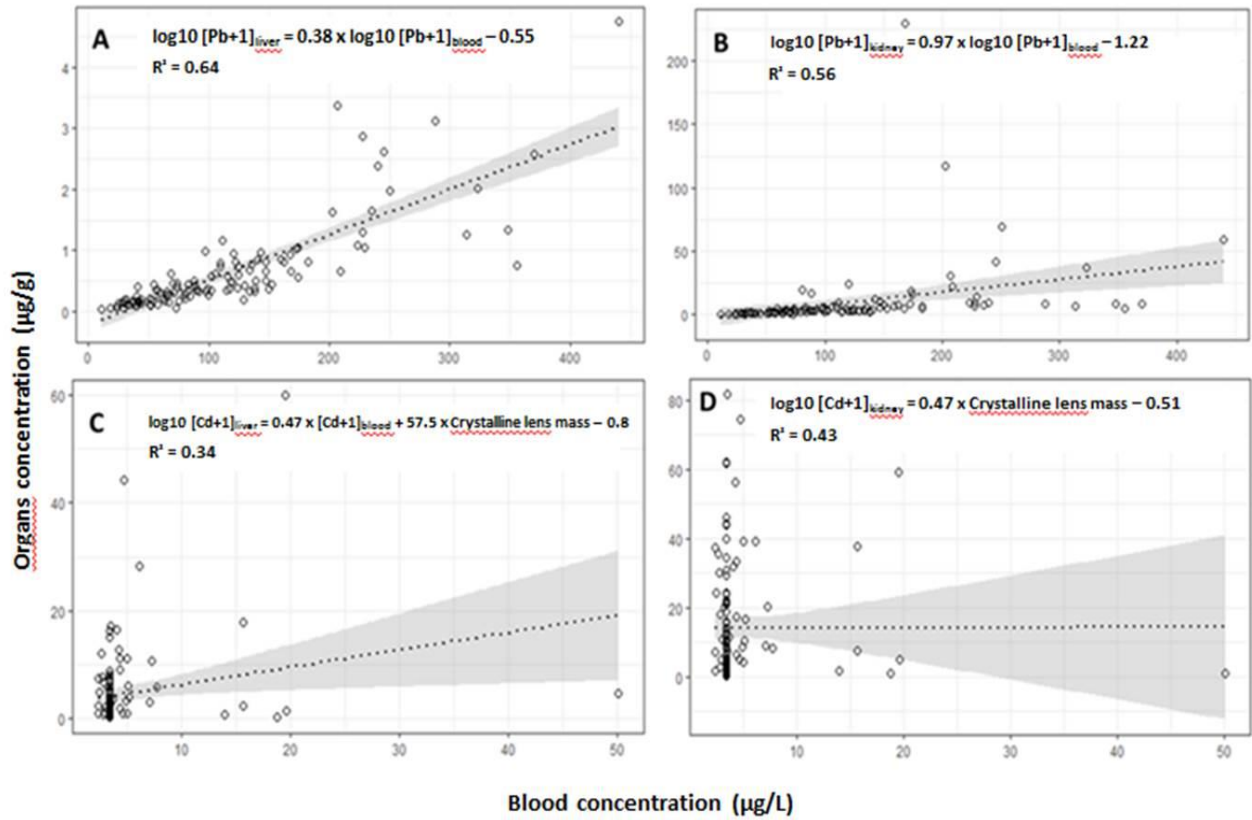


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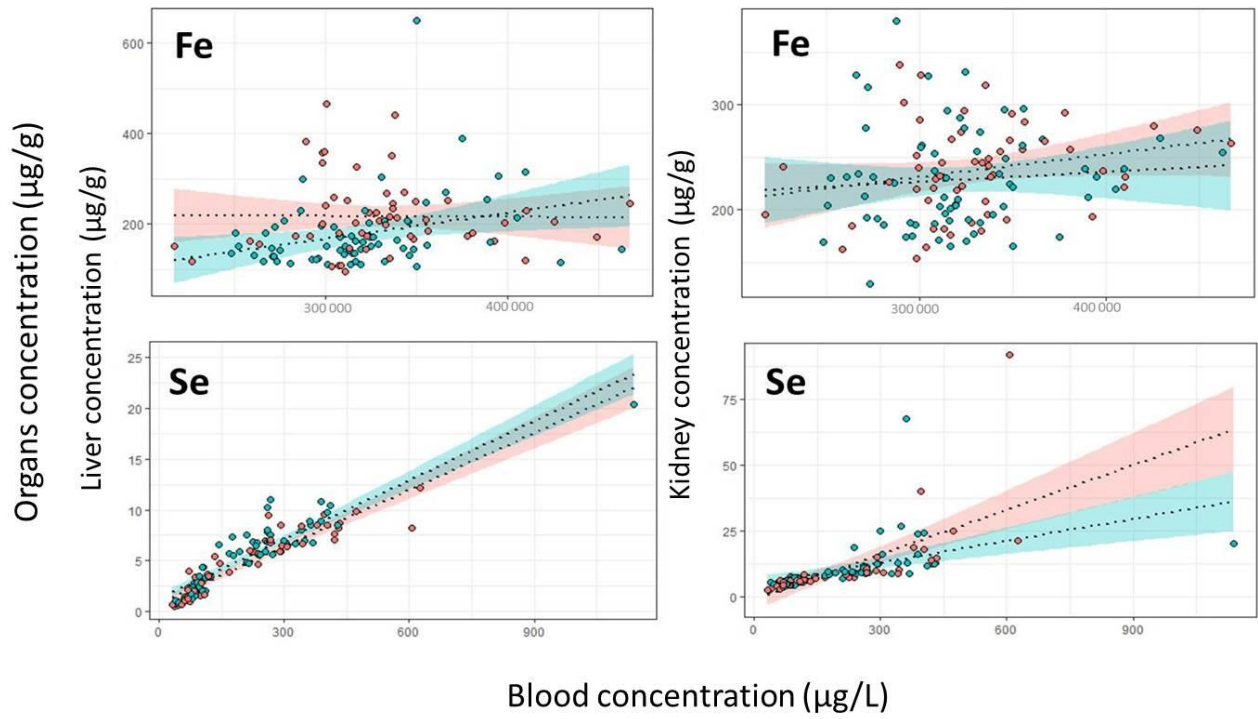
446 Fig. 3. Relationship between blood and organs concentrations in the wood mouse *Apodemus sylvaticus*. (A)  
 447 Blood/liver Pb concentrations, (B) Blood/kidney Pb concentrations, (C) Blood/liver Cd concentrations, (D)  
 448 Blood/kidney Cd concentrations. General Linear Models were used and all tissue concentrations were log  
 449 ( $\log_{10}(x+1)$ ) transformed (grey represents IC 95%).



450

451

452 Fig. 4. Relationship between blood and organs concentrations (liver and kidney) in male (blue) and female  
453 (red) wood mice *Apodemus sylvaticus* for iron (Fe) and selenium (Se). General Linear Models were used and  
454 all tissue concentrations were log (log 10(x+1)) transformed (blue and red represent IC 95% for each sex).



455

456 **Supplementary Data**

457 Table S1. Certified values and average recoveries of the Certified Reference Materials used in this study (the number of reference samples analyzed for each  
 458 certified reference materials is 9).

Element	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	Ti	Tl	Zn	
TORT-2	Certified																		
	values	NS <sup>a</sup>	21.6	26.7	0.51	0.77	106	105	13.6	0.95	2.5	0.35	NS	5.63	0.04 <sup>c</sup>	45.2	NS	NS	180
	( $\mu\text{g/g}$ )																		
ERM <sup>®</sup> - CE196	Certified																		
	Recovery	ND <sup>b</sup>	99	98	89	72	80	75	77	100	78	120	ND	109	171	101	ND	ND	107
	(%)																		
ERM <sup>®</sup> - CE196	Certified																		
	values	NS	NS	12.33	NS	NS	NS	NS	NS	NS	NS	772	NS	NS	NS	NS	NS	NS	NS
	( $\mu\text{g/L}$ )																		
ERM <sup>®</sup> - CE196	Certified																		
	Recovery	ND	ND	77	ND	ND	ND	ND	ND	ND	ND	79	ND	ND	ND	ND	ND	ND	ND
	(%)																		

459 <sup>a</sup>Not specified460 <sup>b</sup>Not determined461 <sup>c</sup>Indicative (not certified) value

462

463 Table S2. Trace metal (TM) concentrations (median, mean, minimum and maximum) in blood (expressed  
464 in  $\mu\text{g/L}$ ) and organs (liver and kidney expressed in  $\mu\text{g/g}$ ) for the 18 tested TM. Total number of samples  
465 and the percentage of data under the quantification limit are given.

Element	Sex	Soil contamination level	Blood concentration (µg/L)				Liver concentration (µg/g)				Kidney concentration (µg/g)				N	% < LQ in blood	% < LQ in liver	% < LQ in kidney
			Median	Mean	Min	Max	Median	Mean	Min	Max	Median	Mean	Min	Max				
Al	F	Control	1036	1036	1036	1036	2,75	8,48	0,263	36,6	0,316	2,89	0,316	18,0	16	100	31	69
		Low	1036	1166	1036	2980	3,27	3,43	0,263	8,88	0,316	3,75	0,316	12,9	15	93	20	53
		Medium	1036	1036	1036	1036	3,88	4,23	0,263	9,38	2,03	4,55	0,316	17,2	11	100	9	45
		High	1036	1036	1036	1036	1,08	4,61	0,263	22,4	3,67	4,06	0,316	13,6	12	100	50	33
	M	Control	1036	1137	1036	2143	4,47	6,17	0,263	23,0	0,316	4,08	0,316	14,3	11	91	9	64
		Low	1036	1036	1036	1036	0,263	1,60	0,263	10,1	3,43	6,79	0,316	35,6	18	100	78	39
		Medium	1036	1036	1036	1036	3,51	4,56	0,263	10,1	0,316	4,95	0,316	37,2	23	100	4	52
		High	1036	1036	1036	1036	4,04	5,61	0,263	25,7	0,316	5,03	0,316	25,4	14	100	7	57
As	F	Control	8,90	8,90	8,90	8,90	0,008	0,017	0,008	0,089	0,010	0,010	0,010	0,010	16	100	88	100
		Low	8,90	8,90	8,90	8,90	0,008	0,013	0,008	0,084	0,010	0,010	0,010	0,010	15	100	7	100
		Medium	8,90	8,90	8,90	8,90	0,008	0,008	0,008	0,008	0,010	0,010	0,010	0,010	11	100	100	100
		High	8,90	8,90	8,90	8,90	0,008	0,008	0,008	0,008	0,010	0,010	0,010	0,010	12	100	100	100
	M	Control	8,90	8,90	8,90	8,90	0,008	0,013	0,008	0,063	0,010	0,010	0,010	0,010	11	100	91	100
		Low	8,90	8,90	8,90	8,90	0,008	0,008	0,008	0,008	0,010	0,010	0,010	0,010	18	94	100	100
		Medium	8,90	8,90	8,90	8,90	0,008	0,014	0,008	0,144	0,010	0,017	0,010	0,173	23	100	4	96
		High	8,90	9,30	8,90	14,6	0,008	0,027	0,008	0,104	0,010	0,010	0,010	0,010	14	7	79	100
Cd	F	Control	3,46	3,46	3,46	3,46	0,731	1,13	0,261	3,79	3,40	5,27	0,741	29,2	16	100	0	0
		Low	3,46	5,56	2,40	19,6	2,27	3,97	0,186	12,8	6,87	15,9	1,02	56,4	15	40	0	0
		Medium	3,46	5,45	3,46	19,6	5,99	14,9	0,460	59,9	13,4	27,4	2,77	74,4	11	64	0	0
		High	3,46	4,46	2,72	15,6	3,38	5,62	0,635	17,9	19,4	21,1	2,40	46,1	12	58	0	0
	M	Control	3,46	9,24	3,46	50,0	0,523	0,945	0,121	4,70	1,74	2,70	0,376	7,56	11	82	0	0
		Low	3,46	3,49	2,40	4,68	1,36	1,72	0,740	3,59	5,23	6,31	2,23	12,2	18	67	0	0
		Medium	3,46	3,82	3,40	7,80	3,51	5,37	0,713	17,1	9,61	17,7	2,26	81,5	23	70	0	0
		High	3,46	3,99	2,40	7,28	3,96	5,14	1,13	11,0	18,5	19,8	4,48	37,3	14	50	0	0
Co	F	Control	6,01	6,14	5,64	7,64	0,085	0,076	0,002	0,189	0,149	0,182	0,055	0,870	16	81	25	0
		Low	6,01	6,01	6,01	6,01	0,055	0,063	0,002	0,195	0,099	0,117	0,050	0,362	15	100	33	0
		Medium	6,01	6,01	6,01	6,01	0,076	0,070	0,002	0,096	0,106	0,105	0,060	0,152	11	100	91	0
		High	6,01	6,01	6,01	6,01	0,073	0,077	0,002	0,151	0,098	0,110	0,002	0,196	12	100	17	8
	M	Control	6,01	6,10	6,01	7,04	0,12	0,13	0,002	0,260	0,111	0,125	0,002	0,224	11	91	9	91
		Low	6,01	6,36	6,01	11,96	0,067	0,067	0,002	0,173	0,096	0,145	0,002	1,02	18	94	28	11
		Medium	6,01	6,01	6,01	6,01	0,087	0,074	0,002	0,130	0,097	0,103	0,045	0,196	23	100	13	0
		High	6,01	6,01	6,01	6,01	0,086	0,084	0,002	0,171	0,105	0,119	0,065	0,218	14	100	7	0
Cr	F	Control	10,7	10,7	10,7	10,7	0,005	0,015	0,005	0,110	0,006	0,043	0,006	0,236	16	100	81	81
		Low	10,7	10,7	10,7	10,7	0,005	0,015	0,005	0,134	0,006	0,071	0,006	0,712	15	100	87	80
		Medium	10,7	10,7	10,7	10,7	0,005	0,009	0,005	0,049	0,006	0,047	0,006	0,189	11	100	91	27
		High	10,7	10,7	10,7	10,7	0,005	0,005	0,005	0,005	0,036	0,074	0,006	0,377	12	100	100	50
	M	Control	10,7	19,1	10,7	112	0,005	0,018	0,005	0,088	0,006	0,006	0,006	0,006	11	100	82	100
		Low	10,7	10,7	10,7	10,7	0,005	0,007	0,005	0,039	0,006	0,078	0,006	0,305	18	94	94	61
		Medium	10,7	10,7	10,7	10,7	0,005	0,008	0,005	0,036	0,006	0,037	0,006	0,263	23	100	91	78
		High	10,7	13,0	10,7	43,4	0,005	0,009	0,005	0,039	0,006	0,091	0,006	0,738	14	93	86	57
Cu	F	Control	496	503	377	674	10,4	10,6	7,72	13,9	18,8	18,1	10,2	21,7	16	0	0	0
		Low	883	941	624	1380	10,2	10,6	7,60	15,6	17,3	17,4	11,5	24,4	15	0	0	0
		Medium	736	847	502	1499	11,4	11,2	8,58	15,1	18,3	18,4	13,6	24,9	11	0	0	0
		High	820	962	725	2532	9,61	9,85	7,76	12,0	15,8	16,9	13,2	22,2	12	0	0	0
	M	Control	525	578	365	1232	11,2	10,9	8,25	14,0	18,3	18,4	12,4	23,9	11	0	0	0
		Low	975	995	761	1472	10,1	10,4	8,01	12,8	17,2	17,2	11,3	21,7	18	0	0	0
		Medium	749	809	563	1856	10,9	11,1	7,50	14,8	17,6	18,3	11,9	27,7	23	0	0	0
		High	876	917	726	1480	10,6	10,5	7,60	13,0	18,0	18,0	11,8	27,7	14	0	0	0
Fe	F	Control	322820	351815	283240	467200	187	196	95	384	232	238	154	338	16	0	0	0
		Low	339400	343781	298440	425600	210	239	167	441	251	252	190	294	15	0	0	0
		Medium	307600	299731	216720	342920	259	253	118	467	241	233	181	328	11	0	0	0
		High	322280	315720	258280	380640	178	186	124	245	215	224	163	302	12	0	0	0
	M	Control	348120	348317	266760	429200	161	222	114	649	220	218	174	268	11	0	0	0
		Low	316200	311915	248240	355880	151	157	112	248	224	226	129	331	18	0	0	0
		Medium	307640	311092	251880	462400	152	187	117	390	212	229	170	379	23	0	0	0
		High	326320	322020	250200	366000	154	162	107	270	247	238	165	327	14	0	0	0
Mn	F	Control	25,3	25,3	25,3	25,3	5,57	5,90	3,93	8,16	6,71	6,37	3,61	8,82	16	100	0	0
		Low	26,4	34,4	20,2	91,1	4,74	4,78	3,56	6,28	6,08	6,01	3,95	7,63	15	73	0	0
		Medium	25,3	29,1	21,5	62,2	6,54	6,29	4,29	8,42	6,16	6,12	4,77	8,08	11	64	0	0
		High	25,3	31,1	18,2	86,2	5,08	4,98	3,64	7,13	5,03	5,12	3,89	6,30	12	25	0	0
	M	Control	25,3	25,3	25,3	25,3	7,23	7,08	3,69	12,76	6,42	6,45	3,34	9,09	11	100	0	0
		Low	25,3	26,9	16,6	48,5	4,54	4,87	3,26	7,61	5,62	5,35	3,03	8,79	18	44	0	0
		Medium	25,3	27,3	20,9	59,4	5,10	5,18	3,16	7,81	5,51	5,77	2,94	9,97	23	78	0	0
		High	24,6	24,8	16,0	36,7	4,42	4,50	2,18	5,98	5,13	5,24	3,49	8,00	14	7	0	0
Mo	F	Control	6,80	7,45	3,80	19,5	2,76	2,96	1,77	4,67	1,73	1,77	0,994	2,40	16	0	0	0
		Low	6,52	6,57	2,77	16,4	2,59	2,54	1,01	4,71	1,64	1,60	0,834	2,20	15	33	0	0
		Medium	8,48	8,09	2,77	13,3	3,46	3,38	2,41	4,42	2,24	2,19	1,48	2,80	11	9	0	0
		High	3,69	6,47	2,77	16,5	2,89	2,92	2,05	4,70	2,01	1,87	1,12	2,33	12	60	0	0
	M	Control	5,94	6,95	3,52	15,4	3,07	3,15	1,90	5,05	1,58	1,73	1,35	2,41	11	0	0	0
		Low	6,32	7,09	2,77	13,6	2,84	2,90	2,03	4,15	1,77	1,77	0,927	2,32	18	22	0	0
		Medium	6,44	7,80	2,77	35,												

Element	Sex	Soil contamination level	Blood concentration (µg/L)				Liver concentration (µg/g)				Kidney concentration (µg/g)				N	% < LQ in blood	% < LQ in liver	% < LQ in kidney
			Median	Mean	Min	Max	Median	Mean	Min	Max	Median	Mean	Min	Max				
Ni	F	Control	101	191	56	1089	0,009	0,040	0,009	0,308	0,011	0,082	0,011	0,427	16	0	87	75
		Low	44	90	22	396	0,009	0,032	0,009	0,274	0,011	0,055	0,011	0,281	15	47	9	80
		Medium	117	157	22	365	0,009	0,294	0,009	3,15	0,011	0,134	0,011	1,21	11	82	83	18
		High	99	579	22	3116	0,009	0,024	0,009	0,117	0,011	0,079	0,011	0,390	12	50	64	67
	M	Control	71,0	771	21,9	8164	0,009	0,070	0,009	0,259	0,102	0,248	0,011	0,936	11	9	89	45
		Low	62,3	132	21,9	440	0,009	0,104	0,009	1,36	0,116	0,303	0,011	1,46	18	39	96	50
		Medium	72,4	163	21,9	890	0,009	0,012	0,009	0,072	0,011	0,095	0,011	0,480	23	83	86	70
		High	88,3	641	21,9	2124	0,009	0,061	0,009	0,633	0,011	0,027	0,011	0,161	14	36	0	86
Pb	F	Control	53,1	62,5	24,0	129	0,237	0,270	0,072	0,481	1,65	2,09	0,427	4,42	16	0	0	0
		Low	141	285	72,6	1901	0,816	0,922	0,055	2,88	3,97	4,59	0,286	9,17	15	0	0	0
		Medium	75,0	98,0	33,7	314	0,272	0,433	0,126	1,27	2,34	3,02	1,00	7,15	11	0	0	0
		High	145	156	80,3	250	0,698	0,774	0,207	1,97	12,8	34,9	4,64	229	12	0	0	0
	M	Control	64,7	75,8	10,8	240	0,257	0,433	0,047	2,38	1,96	2,66	0,259	9,10	11	0	0	0
		Low	91,4	107	23,0	288	0,346	0,643	0,082	3,11	3,16	3,89	0,451	9,14	18	0	0	0
		Medium	73,2	85,7	17,9	168	0,345	0,378	0,055	0,926	2,95	3,06	0,54	7,74	23	0	0	0
		High	178	207	87,6	440	1,00	1,48	0,196	4,74	17,5	27,1	3,26	117	14	0	0	0
Sb	F	Control	3,95	3,95	3,95	3,95	0,002	0,002	0,002	0,002	0,003	0,003	0,003	0,003	16	100	100	100
		Low	3,95	3,95	3,95	3,95	0,002	0,002	0,002	0,002	0,003	0,003	0,003	0,003	15	100	100	100
		Medium	3,95	3,95	3,95	3,95	0,002	0,002	0,002	0,002	0,003	0,003	0,003	0,003	11	100	92	100
		High	3,95	3,95	3,95	3,95	0,002	0,003	0,002	0,010	0,003	0,006	0,003	0,040	12	100	92	83
	M	Control	3,95	3,95	3,95	3,95	0,002	0,002	0,002	0,002	0,003	0,003	0,003	0,003	11	100	89	100
		Low	3,95	3,95	3,95	3,95	0,002	0,003	0,002	0,016	0,003	0,003	0,003	0,003	18	100	96	100
		Medium	3,95	3,95	3,95	3,95	0,002	0,003	0,002	0,008	0,003	0,003	0,003	0,003	23	100	93	100
		High	3,95	3,95	3,95	3,95	0,002	0,004	0,002	0,022	0,003	0,005	0,003	0,032	14	100	0	93
Se	F	Control	92,5	95,4	62,5	146	2,34	2,64	1,01	5,44	5,52	5,38	2,85	8,46	16	0	0	0
		Low	70,5	81,5	32,2	239	1,43	1,78	0,462	4,68	5,69	5,17	2,65	7,45	15	0	0	0
		Medium	267	277	218	345	6,63	6,98	5,76	9,45	9,21	9,89	7,16	15,1	11	0	0	0
		High	421	428	256	626	8,36	8,48	6,49	12,1	16,3	24,2	9,46	91,7	12	0	0	0
	M	Control	91,2	90,1	73,3	112	2,04	2,19	1,27	3,51	5,20	5,43	4,02	7,39	11	0	0	0
		Low	91,0	84,8	39,7	124	2,51	2,42	0,85	4,39	6,00	5,79	4,14	7,68	18	0	0	0
		Medium	242	239	145	369	6,73	6,85	4,75	11,0	9,18	9,43	7,09	12,5	23	0	0	0
		High	363	406	238	1137	8,50	9,01	5,61	20,3	17,3	21,2	9,19	67,5	14	0	0	0
Sn	F	Control	21,4	21,7	12,9	30,1	0,004	0,024	0,004	0,283	0,005	0,007	0,005	0,037	16	0	87	94
		Low	18,6	20,0	13,0	29,4	0,004	0,006	0,004	0,023	0,005	0,009	0,005	0,062	15	0	9	93
		Medium	13,2	31,2	8,36	208	0,004	0,008	0,004	0,050	0,005	0,005	0,005	0,005	11	0	92	100
		High	15,0	15,9	11,6	27,4	0,004	0,005	0,004	0,021	0,005	0,009	0,005	0,033	12	0	91	83
	M	Control	23,1	227	17,8	2341	0,004	0,006	0,004	0,028	0,005	0,005	0,005	0,005	11	0	89	100
		Low	14,1	15,7	9,04	32,0	0,004	0,011	0,004	0,084	0,005	0,005	0,005	0,005	18	0	89	100
		Medium	17,2	30,3	9,52	283	0,004	0,004	0,004	0,004	0,005	0,010	0,005	0,051	23	0	93	87
		High	16,8	31,8	11,2	202	0,004	0,005	0,004	0,019	0,005	0,008	0,005	0,051	14	0	69	93
Sr	F	Control	63,5	63,5	63,5	63,5	0,030	0,115	0,030	0,700	0,036	0,196	0,036	0,701	16	100	80	69
		Low	63,5	63,5	63,5	63,5	0,200	0,246	0,030	0,531	0,036	0,340	0,036	0,921	15	100	36	53
		Medium	63,5	63,5	63,5	63,5	0,209	0,182	0,030	0,346	0,036	0,371	0,036	0,988	11	100	50	55
		High	63,5	63,5	63,5	63,5	0,085	0,152	0,030	0,403	0,036	0,122	0,036	0,581	12	100	45	83
	M	Control	63,5	63,5	63,5	63,5	0,152	0,179	0,030	0,404	0,036	0,277	0,036	0,918	11	100	11	73
		Low	63,5	63,5	63,5	63,5	0,258	0,268	0,030	0,582	0,036	0,356	0,036	1,22	18	100	52	56
		Medium	63,5	63,5	63,5	63,5	0,030	0,135	0,030	0,561	0,036	0,224	0,036	1,13	23	100	86	65
		High	63,5	63,5	63,5	63,5	0,030	0,051	0,030	0,186	0,036	0,248	0,036	1,23	14	100	0	64
Tl	F	Control	59,1	63,2	59,1	124	1,86	1,99	1,57	2,83	2,30	2,27	1,10	3,68	16	94	0	0
		Low	111	114	59,1	219	1,88	1,95	1,54	2,69	1,91	2,21	1,49	3,41	15	87	0	0
		Medium	85,7	84,2	59,1	145	1,83	2,10	1,68	2,95	2,14	2,11	1,48	2,99	11	45	0	0
		High	59,1	78,6	59,1	151	1,76	2,02	1,42	3,45	2,46	2,43	1,29	3,43	12	58	0	0
	M	Control	59,1	59,1	59,1	59,1	2,26	2,33	1,56	3,27	2,08	2,26	0,024	4,36	11	100	0	91
		Low	98,0	95,5	59,1	172	1,77	1,83	1,45	2,43	2,58	2,62	1,59	4,13	18	17	0	0
		Medium	59,1	72,0	59,1	109	1,91	2,01	1,49	2,78	2,02	2,11	1,39	3,99	23	61	0	0
		High	60,6	76,2	59,1	128	1,93	1,90	1,31	2,28	1,77	1,96	1,17	2,84	14	50	0	0
Tl	F	Control	0,198	0,201	0,198	0,240	0,002	0,002	0,001	0,005	0,024	0,026	0,000	0,060	16	94	0	6
		Low	0,198	0,198	0,198	0,198	0,003	0,005	0,001	0,014	0,047	0,048	0,006	0,136	15	100	0	0
		Medium	0,198	0,205	0,198	0,280	0,004	0,005	0,001	0,015	0,026	0,040	0,013	0,132	11	91	0	0
		High	0,520	0,666	0,198	2,08	0,031	0,040	0,007	0,135	0,260	0,459	0,125	1,65	12	25	0	0
	M	Control	0,198	0,198	0,198	0,198	0,003	0,003	0,001	0,009	0,036	0,037	0,010	0,078	11	100	0	0
		Low	0,198	0,210	0,198	0,400	0,004	0,004	0,002	0,006	0,034	0,035	0,007	0,077	18	89	0	0
		Medium	0,198	0,198	0,198	0,198	0,004	0,007	0,002	0,023	0,039	0,074	0,009	0,286	23	100	0	0
		High	0,660	1,98	0,198	13,2	0,043	0,135	0,012	0,867	0,499	1,21	0,149	7,06	14	7	0	0
Zn	F	Control	4224	4411	3377	5300	124	134	76,4	203	134	129	73,7	165	16	0	0	0
		Low	6100	6290	4040	10192	147	162	84,9	280	136	138	89,4	178	15	0	0	0
		Medium	4356	4420	3208	5760	138	142	110	187	134	132	114	156	11	0	0	0
		High	4926	4994	3921	6144	137	139	97,5	196	134	136	111	172	12	0	0	0
	M	Control	4300	6848	3264	33888	135	140	105	201	124	126	105	163	11	0	0	0
		Low	5404	5673	4012	8700	159	162	125	253	134	143	103					

469 Table S3. Tukey's HSD tests summary. Comparisons between squares of increasing level of soil Cd, Pb and Zn  
 470 contamination are presented for blood, liver and kidneys.

### Blood

		<b>Fe</b>	Control	Low	Medium	High
F=4.99 <b>p&lt;0.003</b> Df=3	Control					
	Low		0.27			
	Medium		<b>0.001</b>	0.17		
	High		0.09	0.91	0.60	

		<b>Cu</b>	Control	Low	Medium	High
F=33.51 <b>p&lt;0.001</b> Df=3	Control					
	Low		<b>p&lt;0.001</b>			
	Medium		<b>p&lt;0.001</b>	<b>0.02</b>		
	High		<b>p&lt;0.001</b>	0.85	0.19	

		<b>Mo</b>	Control	Low	Medium	High
F=0.66 p=0.57 Df=3	Control					
	Low		0.92			
	Medium		0.10	0.82		
	High		0.73	0.97	0.59	

		<b>Se</b>	Control	Low	Medium	High
F=185.7 <b>p&lt;0.001</b> Df=3	Control					
	Low		0.11			
	Medium		<b>p&lt;0.001</b>	<b>p&lt;0.001</b>		
	High		<b>p&lt;0.001</b>	<b>p&lt;0.001</b>	<b>p&lt;0.001</b>	

		<b>Ti</b>	Control	Low	Medium	High
F=15.43 <b>p&lt;0.001</b> Df=3	Control					
	Low		<b>p&lt;0.001</b>			
	Medium		0.07	<b>p&lt;0.001</b>		
	High		0.09	<b>p&lt;0.001</b>	0.99	

		<b>Zn</b>	Control	Low	Medium	High
F=5.61 <b>p&lt;0.001</b> Df=3	Control					
	Low		<b>0.04</b>			
	Medium		0.85	<b>0.002</b>		
	High		0.24	0.90	<b>0.03</b>	

		<b>Cd</b>	Control	Low	Medium	High
F=0.30 p=0.82 Df=3	Control					
	Low		0.84			
	Medium		0.94	0.99		
	High		0.84	0.99	0.99	

		<b>Mn</b>	Control	Low	Medium	High
F=0.47 p=0.70 Df=3	Control					
	Low		0.98			
	Medium		0.99	0.91		
	High		0.85	0.65	0.94	

		<b>Pb</b>	Control	Low	Medium	High
F=16.34 <b>p&lt;0.001</b> Df=3	Control					
	Low		<b>p&lt;0.001</b>			
	Medium		0.28	0.07		
	High		<b>p&lt;0.001</b>	<b>0.04</b>	<b>p&lt;0.001</b>	

		<b>Sn</b>	Control	Low	Medium	High
F=2.88 <b>p=0.04</b> Df=3	Control					
	Low		<b>0.04</b>			
	Medium		0.13	0.94		
	High		0.11	0.99	0.99	

		<b>Tl</b>	Control	Low	Medium	High
F=19.08 <b>p&lt;0.001</b> Df=3	Control					
	Low		0.99			
	Medium		0.99	0.99		
	High		<b>p&lt;0.001</b>	<b>p&lt;0.001</b>	<b>p&lt;0.001</b>	

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## Kidney

		Control	Low	Medium	High
F=0.21 p=0.89 Df=3	Fe				
	Control				
	Low	0.92			
	Medium	0.99	0.90		
	High	0.99	0.95	0.99	

		Control	Low	Medium	High
F=0.56 p=0.64 Df=3	Cu				
	Control				
	Low	0.76			
	Medium	0.99	0.74		
	High	0.86	0.99	0.84	

		Control	Low	Medium	High
F=4.9 p=0.003 Df=3	Mo				
	Control				
	Low	0.91			
	Medium	0.03	0.002		
	High	0.96	0.67	0.11	

		Control	Low	Medium	High
F=92.1 p<0.001 Df=3	Se				
	Control				
	Low	0.99			
	Medium	p<0.001	p<0.001		
	High	p<0.001	p<0.001	p<0.001	

		Control	Low	Medium	High
F=1.09 p=0.36 Df=3	Ti				
	Control				
	Low	0.69			
	Medium	0.95	0.32		
	High	0.99	0.54	0.99	

		Control	Low	Medium	High
F=1.127 p=0.34 Df=3	Zn				
	Control				
	Low	0.30			
	Medium	0.95	0.57		
	High	0.81	0.86	0.97	

		Control	Low	Medium	High
F=17.02 p<0.001 Df=3	Cd				
	Control				
	Low	0.004			
	Medium	p<0.001	0.08		
	High	p<0.001	0.006	0.69	

		Control	Low	Medium	High
F=3.621 p=0.02 Df=3	Mn				
	Control				
	Low	0.19			
	Medium	0.44	0.95		
	High	0.008	0.50	0.22	

		Control	Low	Medium	High
F=38.2 p<0.001 Df=3	Pb				
	Control				
	Low	0.07			
	Medium	0.67	0.47		
	High	p<0.001	p<0.001	p<0.001	

		Control	Low	Medium	High
F=0.452 p=0.71 Df=3	Sn				
	Control				
	Low	0.99			
	Medium	0.83	0.92		
	High	0.76	0.86	0.99	

		Control	Low	Medium	High
F=19.08 p<0.001 Df=3	Tl				
	Control				
	Low	0.99			
	Medium	0.99	0.99		
	High	p<0.001	p<0.001	p<0.001	



## Liver

		Control	Low	Medium	High
F=0.76 p=0.52 Df=3	Fe				
	Control				
	Low	0.99			
	Medium	0.91	0.98		
	High	0.87	0.69	0.46	

		Control	Low	Medium	High
F=1.49 p=0.22 Df=3	Cu				
	Control				
	Low	0.79			
	Medium	0.93	0.39		
	High	0.61	0.98	0.25	

		Control	Low	Medium	High
F=3.97 p=0.01 Df=3	Mo				
	Control				
	Low	0.20			
	Medium	0.57	0.005		
	High	0.96	0.50	0.29	

		Control	Low	Medium	High
F=123.9 p<0.001 Df=3	Se				
	Control				
	Low	0.28			
	Medium	p<0.001	p<0.001		
	High	p<0.001	p<0.001	0.05	

		Control	Low	Medium	High
F=1.6 p=0.19 Df=3	Ti				
	Control				
	Low	0.19			
	Medium	0.90	0.5		
	High	0.46	0.97	0.82	

		Control	Low	Medium	High
F=4.49 p=0.01 Df=3	Zn				
	Control				
	Low	0.03			
	Medium	0.96	0.004		
	High	0.99	0.08	0.85	

		Control	Low	Medium	High
F=14.62 p<0.001 Df=3	Cd				
	Control				
	Low	0.04			
	Medium	p<0.001	0.006		
	High	p<0.001	0.033	0.98	

		Control	Low	Medium	High
F=8.48 p<0.001 Df=3	Mn				
	Control				
	Low	p<0.001			
	Medium	0.18	0.11		
	High	p<0.001	0.97	0.06	

		Control	Low	Medium	High
F=12 p<0.001 Df=3	Pb				
	Control				
	Low	0.004			
	Medium	0.87	0.03		
	High	p<0.001	0.20	p<0.001	

		Control	Low	Medium	High
F=1.01 p=0.39 Df=3	Sn				
	Control				
	Low	0.79			
	Medium	0.41	0.92		
	High	0.46	0.93	0.99	

		Control	Low	Medium	High
F=10.28 p<0.001 Df=3	Tl				
	Control				
	Low	0.99			
	Medium	0.99	0.99		
	High	p<0.001	p<0.001	p<0.001	

Fig. S2. Relationship between blood (in  $\mu\text{g/L}$ ) and kidneys (in  $\mu\text{g/g}$ ) concentrations for 9 trace metals (Se, Cd, Fe, Tl, Mo, Pb, Zn, Ti and Cu).

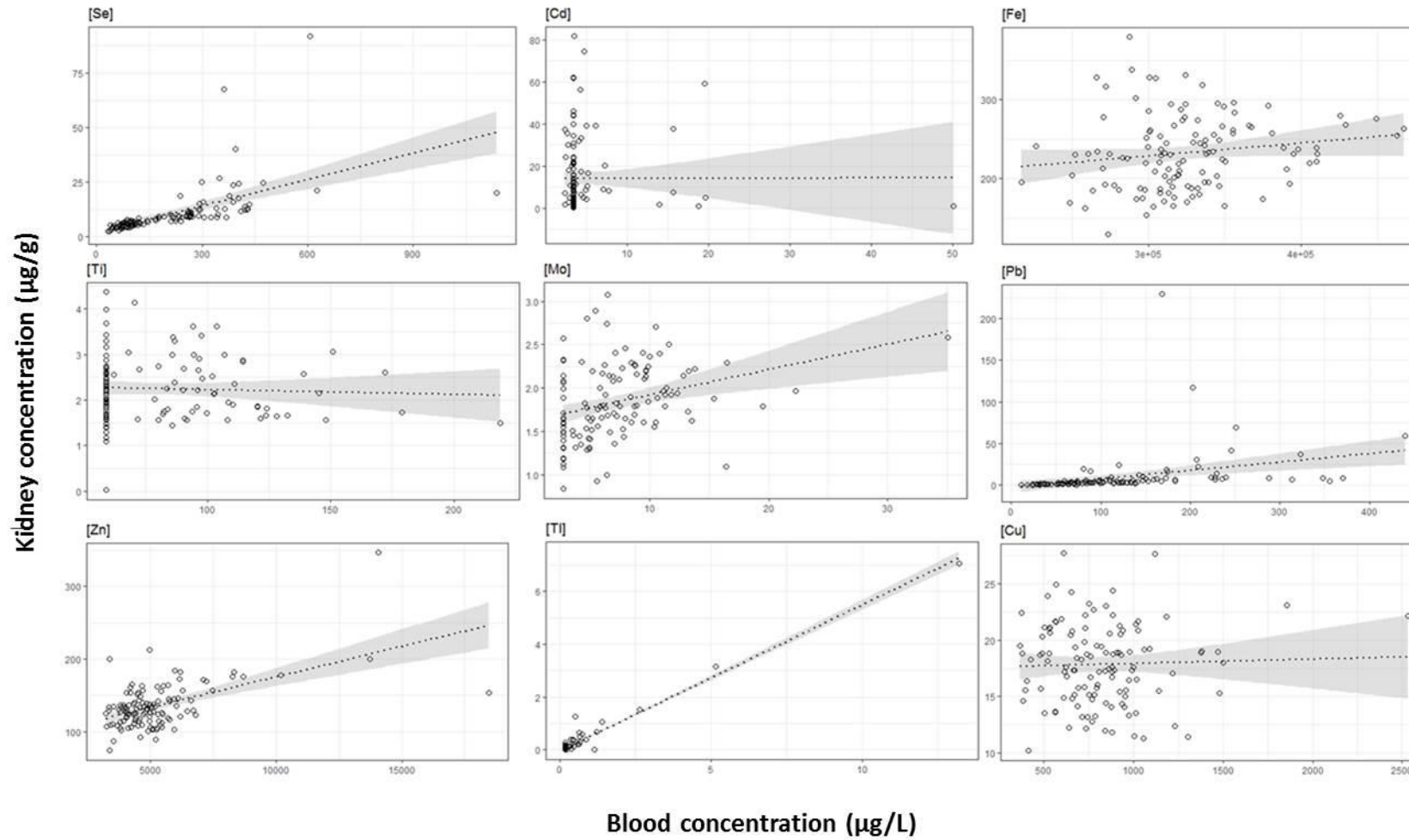
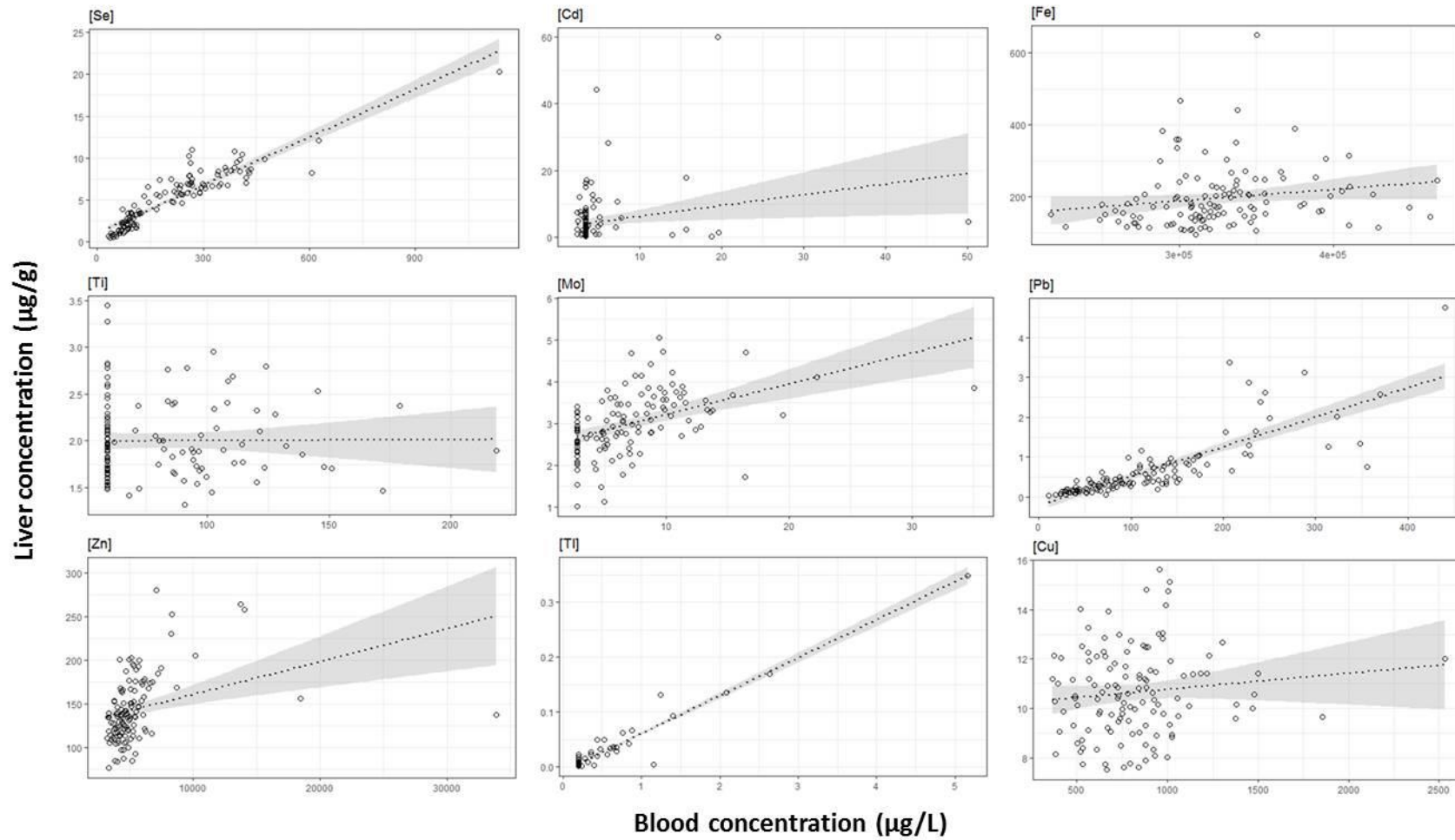
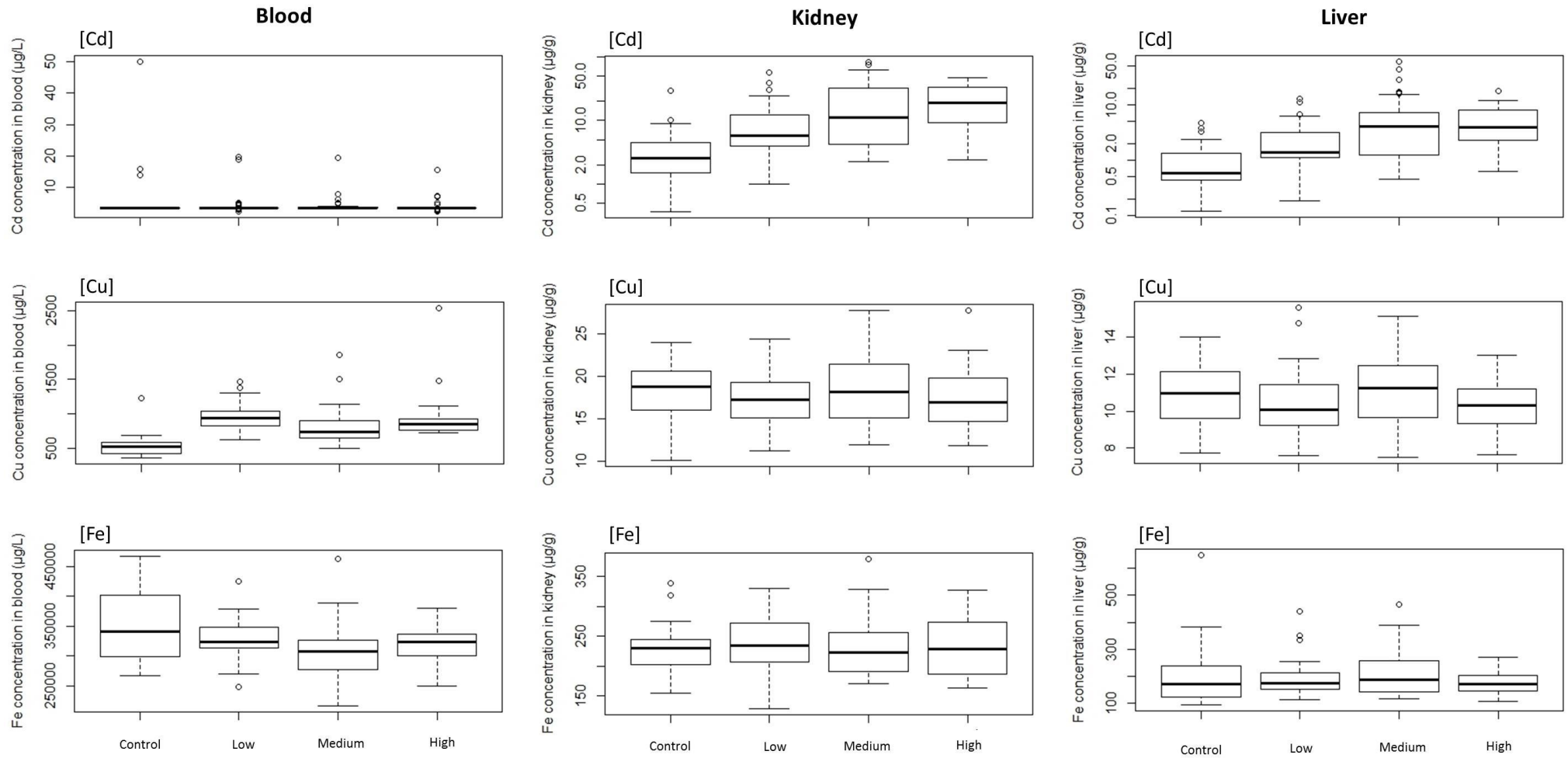


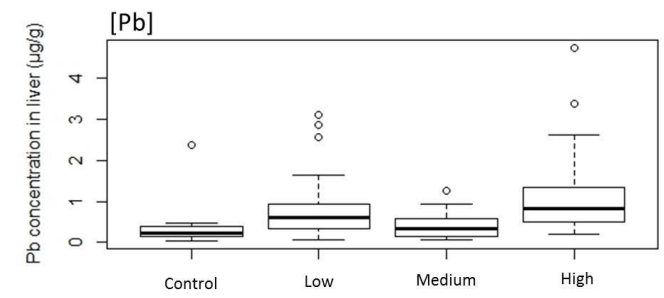
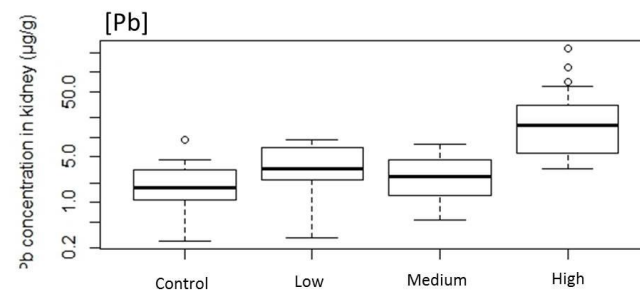
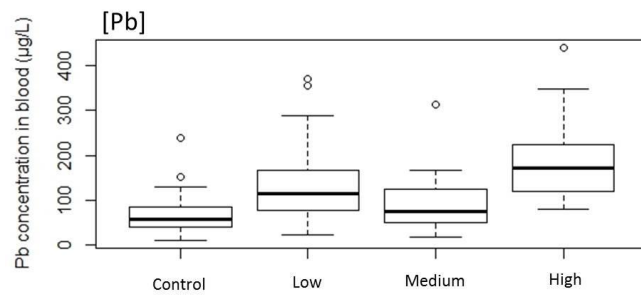
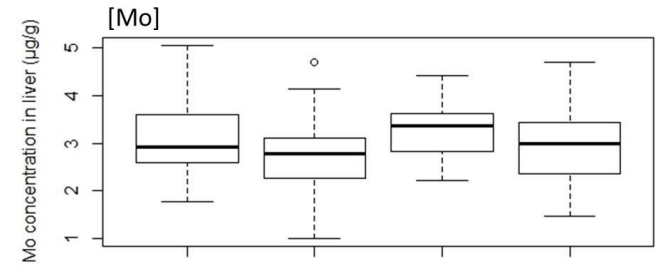
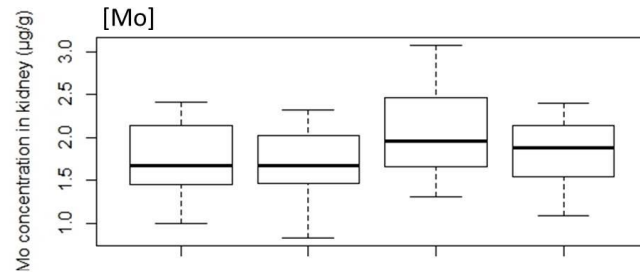
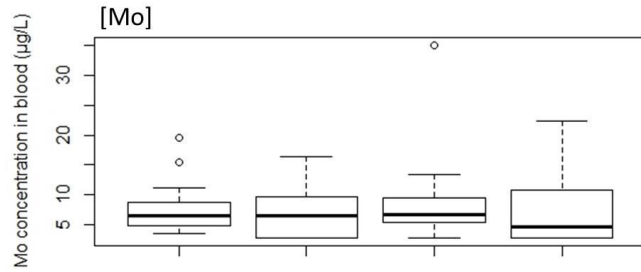
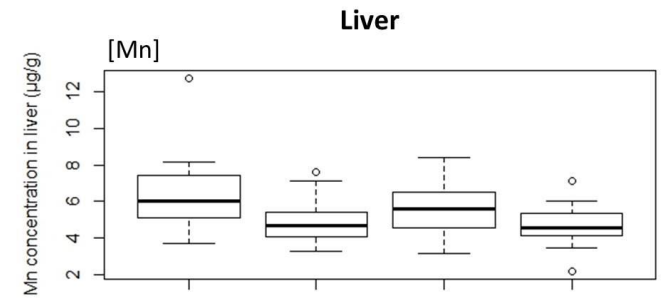
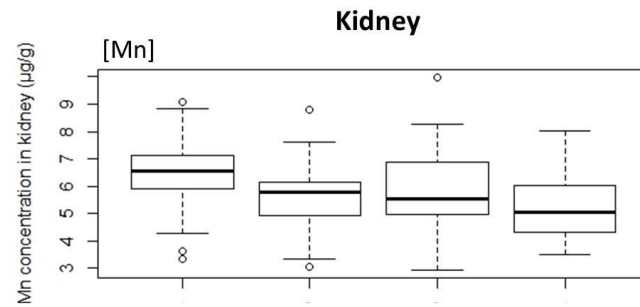
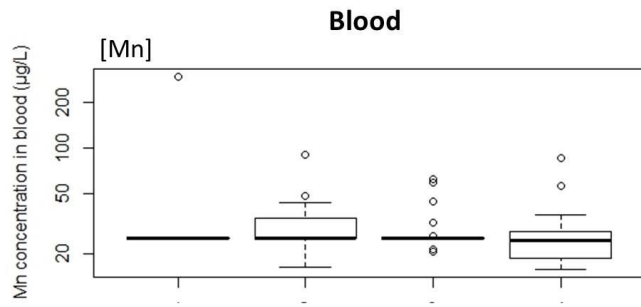
Fig. S3. Relationship between blood ( $\mu\text{g/L}$ ) and liver ( $\mu\text{g/g}$ ) concentrations for 9 trace metals (Se, Cd, Fe, Tl, Mo, Pb, Zn, Ti and Cu).

515 Fig. S4. Concentrations in blood, kidneys and liver along the pollution gradient based on soil concentrations for Cd, Pb and Zn, for 11 trace metals (Cd, Cu, Fe, Mn, Mo, Pb,  
 516 Se, Sn, Ti, Tl and Zn).



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