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Effects of chronic exposure to toxic metals on haematological parameters in free-ranging small mammals

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Authors' Contributions

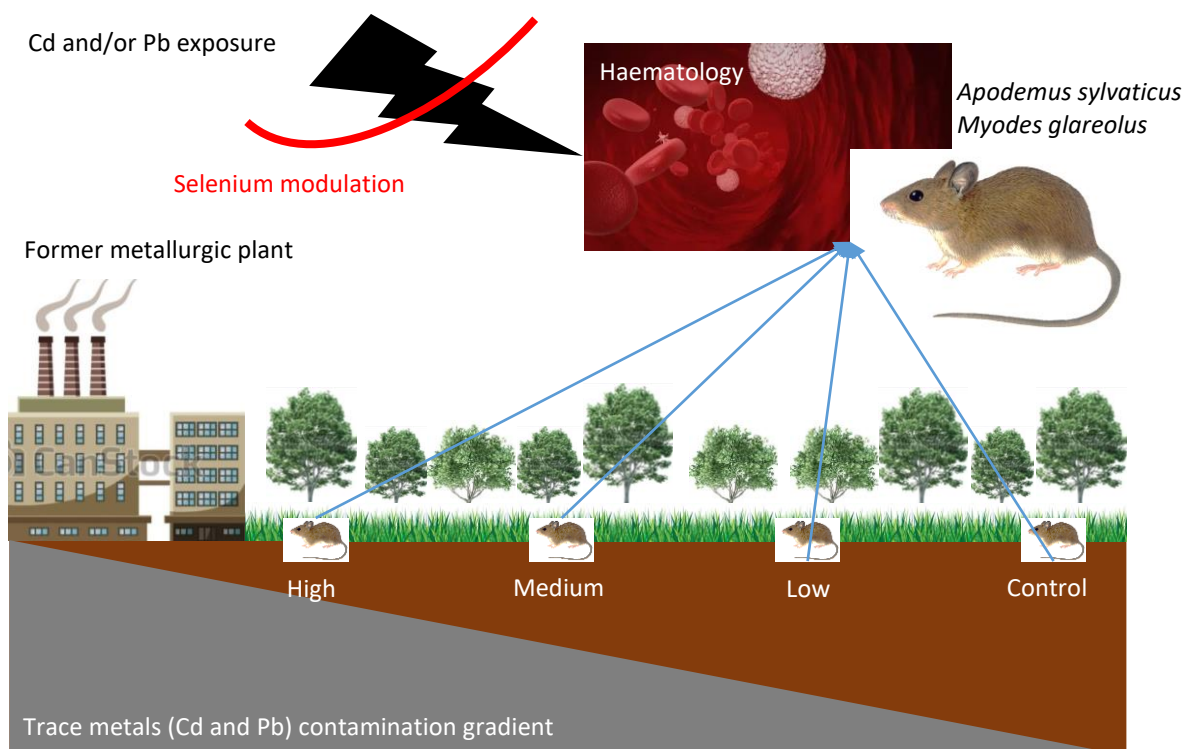
Clémentine Fritsch, Renaud Scheifler, Francis Raoul: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing. Thibaut Powolny: Data curation; Formal analysis; Validation; Visualization; Writing - original draft; Writing - review & editing. Michaël Coeurdassier: Conceptualization; Investigation; Methodology; Writing - review & editing.

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Highlights

- Haematological responses to trace metals chronic exposure were studied in rodents
- Non-essential metals were measured in tissues as a proxy of chronic exposure
- Some essential metals like iron and selenium were also measured
- Some erythrocyte indicators decreased with increasing Cd or Pb tissue concentration
- Selenium could modulate the effects of toxic metals on some erythrocyte indicators

Graphical abstract



Abstract

Blood circulates through the vascular system to carry oxygen, nutrients and metabolites to and away from tissues, and as such is a key-component of animal physiology. The impacts of metal pollution on blood, however, are poorly documented in free-ranging vertebrates. While the counteracting effect of selenium on mercury toxicity is well known in marine mammals, its potential role against the toxicity of other metals is less studied, especially on terrestrial wildlife. We explored the consequences of chronic exposure to two non-essential metals (cadmium and lead) along a pollution gradient in Northern France, on eleven haematological parameters in two free ranging small mammals, the wood mouse *Apodemus sylvaticus* and the bank vole *Myodes glareolus*. We hypothesized that haematology was related to metal concentrations in tissues, and that selenium might exert modulating effects. Concentrations of cadmium and lead in the tissues indicated an increased chronic exposure to and accumulation of metals along the gradient. Some haematological parameters were not explained by any measured variables while some others varied only with gender or age. Red blood cells, red blood cells distribution width, and blood iron concentration, however, decreased with increasing cadmium in the tissues in wood mice. Red blood cells and haemoglobin decreased with increasing renal lead and hepatic cadmium, respectively, in bank voles. Red blood cells distribution width in wood mice increased with cadmium concentrations in the liver but this was counteracted by high selenium levels in the same organ. An interaction of selenium and lead on red blood cells was also observed in bank voles. Further, selenium concentrations were associated with an increase of monocytes in wood mice. The present results show that toxic metals were related to haematology changes, particularly erythrocyte indicators, and that some essential elements like selenium should be measured as well since they may counteract toxic effects.

Keywords: haematology, biomonitoring, wildlife, inorganic pollutants, toxic elements

Introduction

Many ecosystems experience significant human-driven alterations (e.g. pollution, habitat changes, urbanization), especially since the beginning of the last century (Walther *et al.*, 2002; Halpern *et al.*, 2008). Limited knowledge exists about the effects of these anthropogenic changes on the physiological responses of wildlife, especially in mammals, and how individuals, populations, or species respond to these new environments remain unclear (Gottdenker *et al.*, 2014). While anthropogenic elements including metalloids and trace elements may impact many ecological parameters for a large diversity of taxa (van den Brink *et al.*, 2011; Eeva and Lehikoinen, 2000; Hamilton *et al.*, 2017), ecosystem and species conservation and management require the evaluation of the health risks of organisms by assessing the effects of contaminants on physiology (Herrera-Dueñas *et al.*, 2014; Kophamel *et al.*, 2022). Adoption of appropriate methods that enable a reliable estimation of health is therefore crucial to ecological, evolutionary and conservation research. Although there are various ways to investigate health, one of the most straightforward and less invasive assessments of the physiological and health status of a wild population of vertebrates is to analyse blood parameters (Ardia and Schat, 2008; Kophamel *et al.*, 2022; Maceda-Veiga *et al.*, 2015). Indeed, haematological parameters allow the detection of changes in physiological, pathological, ecological and environmental conditions in free-living populations (Beldomenico *et al.*, 2008; Sánchez-Guzmán *et al.*, 2004; Sarasola *et al.*, 2004). For instance, in wild bird populations, clinical haematology is one of the most informative procedures to assess health status (Totzke *et al.*, 1999; Quillfeldt *et al.*, 2004; Nadolski *et al.*, 2006), body condition (Alvarez *et al.*, 2006), and changes in nutritional state (Cherel *et al.*, 1988; Møriila and Svensson, 1995; Alonso-Alvarez and Ferrer, 2001) of individuals. Among the monitored parameters, haematocrit and leukocyte counts are the most frequently measured variables in wild animals because they reflect the efficiency of oxygen carrying capacity and the immune system status, respectively (Horak *et al.*, 1998; Ots *et al.*, 1998; Lobato *et al.*, 2005). However, the use of these two single parameters appears insufficient because of the large inter-individual or inter-species variability of the haematocrit (Fair *et al.*, 2007) or of the immunological variation following infection (Cutrera *et al.*, 2010). Among the various blood cell types, red blood cells (RBCs) and lymphocytes are relevant indicators of condition and fitness.

Low concentrations of RBCs, caused by blood loss, haemolysis or a decrease in erythrocyte production, result from deficient nourishment, infection or parasitism (Stockham and Scott, 2002) and other environmental stressors like pollutants. Small mammal RBCs have a short lifespan (20–30 days) (Feldman *et al.*, 2000), and anaemia may therefore appear rapidly. Lymphocytes, the effectors of acquired immunity, proliferate in response to antigenic stimuli and have a long-life span in blood, while their numbers decrease (lymphopenia) during immunosuppression by glucocorticoids or immunosuppressive infections (Feldman *et al.*, 2000; Stockham and Scott, 2002). Therefore, lymphocyte circulating levels may be useful indicators of immunological investment.

Blood parameters have been described from laboratory studies with significant effects of trace metals (Nikolić *et al.*, 2013; Hounkpatin *et al.*, 2013). In rabbit, exposure to lead (Pb) decreased RBC counts, haemoglobin concentration and haematocrit (Bersényi *et al.*, 2003). In laboratory mice, cadmium (Cd) was associated with a significant increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT), resulting in reduction of erythrocyte numbers, haemoglobin, haematocrit, and total leukocyte counts (Rehman *et al.*, 2017). Moreover, Pb influences haemoglobin production through the inhibition of the δ -aminolevulinic acid dehydratase enzyme (ALAD; Bergdahl *et al.*, 1998; Papanikolaou *et al.*, 2005), which catalyses the second step in the porphyrin and haem biosynthetic pathway. Surprisingly, few studies investigated the variability of haematological parameters in free living small mammals exposed to metal pollution (Nunes *et al.*, 2001; Rogival *et al.*, 2006; Tête *et al.*, 2015).

Recent studies have demonstrated the advantages of non-lethal methods for monitoring contaminants and health indicators in protected species (Keller *et al.*, 2004; Day *et al.*, 2007; van de Merwe *et al.*, 2010). By non-lethal methods, we here mean methods allowing assessing exposure to and effects of contaminants in wildlife, which do not require sacrificing studied individuals like e.g. the methods that require sampling tissues. In the present case, blood sampling only needs capturing, and anesthetizing individuals, which can then be released in their environment, with a minimal amount of stress and very low undesired mortality. Blood concentrations of some pollutants can be a proxy of recent exposure, while concentrations in keratinous tissues or organs reflect a longer-term signature because they incorporate elements over time (Powolny *et al.*, 2019). When paired with quantitative health assessments, these methods have the

potential to identify relationships between contaminants and physiological condition (Keller *et al.*, 2004; Day *et al.*, 2007), providing information on the conservation risks that a contaminated environment poses to natural populations.

Using a well-studied polluted site in Northern France where transfer in food webs and chronic exposure of wildlife to Cd and Pb have been shown (Fritsch *et al.*, 2010a; 2011a; 2011b; Fritsch *et al.*, 2012), we explored the relationships between these toxic non-essential metals and haematological parameters in two free ranging small mammal species inhabiting roughly the same habitat and sharing comparable diet but differing by their phylogeny and responses to metal pollution (Fritsch *et al.*, 2010a), the wood mouse *Apodemus sylvaticus* and the bank vole *Myodes glareolus*. The present study analysed the variations of 11 haematological parameters in natural populations with respect to intrinsic (sex, age) factors and exposure to toxic metals. Individual concentrations of Cd and Pb in some accumulation tissues, i.e. the liver and kidneys, and circulating concentrations of Cd and Pb in blood, were used as proxies of chronic exposure. Parameters associated with immunology (counts of total white blood cells, percentage of lymphocytes, monocytes, granulocytes), and markers of anaemia or oxygen transfer (total RBCs, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and RBC distribution width) were used as indicators of health status. The concentrations of iron (Fe) that play a crucial role in the haematological system and those of selenium (Se), an element known to counteract the impacts of some toxic elements like mercury (Scheuhammer *et al.*, 2008), have also been measured in tissues and blood of small mammals. We hypothesized differences in haematological parameters with higher signs of pathology, anaemia or other deficiencies for animals with higher internal concentrations of toxic metals, with possible counteracting effect of Se on variations of some haematological parameters. Variations related to age and gender are also expected, provided the changes in physiology during growth and due to hormonal balance. Differences between species are assumed, based on differences in their sensitivity to metal-induced stress (Fritsch *et al.*, 2010a).

Materials and Methods

Study site description

Fieldwork was conducted around the “Metaleurop Nord” former smelter (Hauts-de-France Region, northern France, 5025042N, 300055E). The smelter was established by the end of the 19th century and closed in 2003. The major emissions during this period were of Cd, Pb and zinc (Zn). Soil contamination levels in the woody habitats (the main habitats for the species studied in the present work) from this area are well documented with levels ranging from 0.1 to 2,402 µg/g dry mass for Cd, from 16 to 41,960 µg/g dry mass for Pb and from 44 to 38,760 µg/g dry mass for Zn (Fritsch *et al.*, 2010b). Cadmium, Pb and Zn concentrations in these soils are significantly correlated, with coefficients of determination ranging from 0.6 to 0.8. Concentrations decrease with the distance from the smelter and are related to the prevailing SW–NE winds (Fritsch *et al.*, 2010b), even if anthropogenic activities may locally disturb this global pattern (Douay *et al.*, 2009).

Data collection

A 40 km² (8×5 km) study area around the former smelter was divided into 160 squares (500×500 m). Four sampling squares (named “sites” hereafter) across an environmental metal contamination gradient were studied (from control to highly contaminated sites) for the present study (Table S1, Fig. S1). In brief, the gradient represents heterogeneous but globally increasing concentrations of both Cd and Pb from control site (named hereafter “control”) to highly contaminated site (named “high”) with two intermediate (“low” and “medium”) sites (Fig. S1). Median soil Cd and Pb concentrations are of 1.6 and 110 µg/g dry mass, respectively, for “control” site, 4.0 and 261 for “low” site, 9.7 and 526 for “medium” site, and 59.3 and 1357 for “high” site (Table S1). From the 30th of September to the 10th of October 2014, 10 lines of 10 traps, spaced five meters apart, were placed in five to 10 (depending on availability/accessibility) woody habitats (woods, hedgerows) in each site for three consecutive nights. The sampling strategy thus represents a sampling effort of 300 trap/nights per 25 ha site, distributed in five to 10 habitats. Traps were non-lethal INRA traps with “dormitory” (i.e. small wooden boxes containing hay and food to ensure survival of the animals for at least 24 h). A total of 144 wood mice (78 ♂; 66 ♀) and 27 bank voles (15 ♂; 12 ♀) were trapped along the gradient of pollution (Table S2). In brief, 35, 38, 42, and 29 wood mice were captured

and processed in high, medium, low, and control sites, respectively, while 4, 4, 7 and 12 bank voles were captured along the gradient. For both species, individuals with a body mass inferior to 20g were considered as juveniles while individuals with a body mass superior to 20g as adults (adapted from Vandorpe and Verhagen (1980) and Tête *et al.*, (2014a)). Before animal sacrifice by cervical dislocation under anaesthesia following the American Veterinary Medical Association ethical guidelines (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists, 2016), blood was collected from the orbital sinus using a glass Pasteur pipette. To our knowledge, Pasteur pipettes are the only tools being sufficiently resistant and thin to be inserted into the orbital sinus without major risk of damaging the eye in so small mammals. Blood was immediately transferred into heparine-coated microtubes. Blood sampling were performed each morning between 9 and 12 am. Blood samples were stored at 4-8°C before assessment of blood cell counts and frozen at -20°C within the following 10 hours. Blood sample volume obtained (between 100 and 300 µL) did not allow both metal concentration and haematological analyses for all bank voles. Individuals were weighed, measured and sexed during dissection at the laboratory. Liver and kidneys were sampled, dried (65°C, drying oven) and stored at room temperature until analysis. Sampling authorisation was given by the Direction Régionale de l'Environnement, de l'Aménagement et du Logement (DREAL) of the Hauts-de-France Region. Experiments were performed under the authorization of the French National Ethical Committee (Project APAFIS N°5340) by skilled and experienced investigators from Chrono-environnement research department (EU0592), following directive 2010/63/EU on the protection of animals used for scientific purposes.

Haematological analyses

Haematological parameters were analysed within 8-10 hours following blood sampling. Counts of total white blood cells (WBC), percentage of lymphocytes (LYM), monocytes (MON), granulocytes (GRA), total red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cell distribution width (RDW) were determined using an automated Micros 60 Haematology Analyser (Horiba ABX Micros ES60, Montpellier, France; guaranteed CV of measurements 0.5-5%). Reagent kits were supplied by Horiba SAS. Repeatability (within-run precision) is measured automatically by the Micros device

(RSD). Reproducibility (run-to-run precision) of the measurements and quality control (accuracy) is certified by measuring 3 blood controls (provided by Horiba SAS; the 3 different controls referred to low, medium, high levels of each parameter) with known concentrations and blanks (saline solution) at the beginning of each session of analyses and every 30 runs in case of long-lasting measurement session. Each sample was analysed between two and four times and the average of replicates per individual was kept for statistical analyses.

Metal analysis

The concentrations of metals (Cd, Fe, Pb and Se) were determined in blood samples and organs for 151 individuals (144 wood mice and 7 bank voles) and only in the liver and in kidneys for 20 other bank voles (blood volume being too low for metal concentrations to be measured in these individuals). Blood samples were digested in 700 µl of nitric acid (HNO₃, 65%, analytical quality, Optima) for half a day in open tubes under fume hood, and then for 60 h in closed tubes at 60°C in an oven. After digestion, samples were diluted to 30 ml by the addition of ultra-pure water (Elga, 18.2 MV/cm²). Liver and kidneys dry samples were digested in nitric acid (HNO₃, 69%, analytical quality, Optima) and diluted by adding ultra-pure water (Elga, 18.2 MV/cm²) prior to analyses. Metals in blood and organs were analysed by Inductively Coupled Plasma—Mass Spectrometry (ICP-MS, ThermoFischer Scientific XSeries 2). For the values under quantification limits, values were replaced by the quantification limit divided by square root of two for statistical analysis (Helsel, 2010). Repeatability (within-run precision) and reproducibility of the measurements were surveyed (checking of RSD values and use of internal standards), and quality control (control solutions and blanks to check for the absence of drift) was conducted during measurements. Analysis accuracy was checked using certified reference materials (National Research Council Canada TORT-2 Lobster hepatopancreas, European Reference Material ERM[®]-CE196 Bovine blood). Blanks (acid + ultra-pure water) and certified reference materials were prepared and analysed using the same methods as the samples. Limits of quantification provided by the ICP-MS, certified values and average (upon 9 replicates) recoveries of the certified reference materials are given in Table S3. The concentrations of metals are expressed as µg/g dry mass in organs and as µg/L in blood.

Concentrations of metals in organs were compared to Lowest Observed Adverse Effect Levels (LOAELs) provided in the literature for small mammals (Shore and Douben, 1994a; 1994b). In absence of species-specific LOAELs in the literature, we used these that are supposed to apply to most of small mammal species. Individuals exhibiting levels that exceed these values either in the liver, in kidneys, or in both, may thus be subjected to noxious effects of Cd and/or Pb. The retained values are: 15 µg/g DW in the liver and 105 µg/g DW in kidneys for Cd, 10 µg/g DW in the liver and 25 µg/g DW in kidneys for Pb.

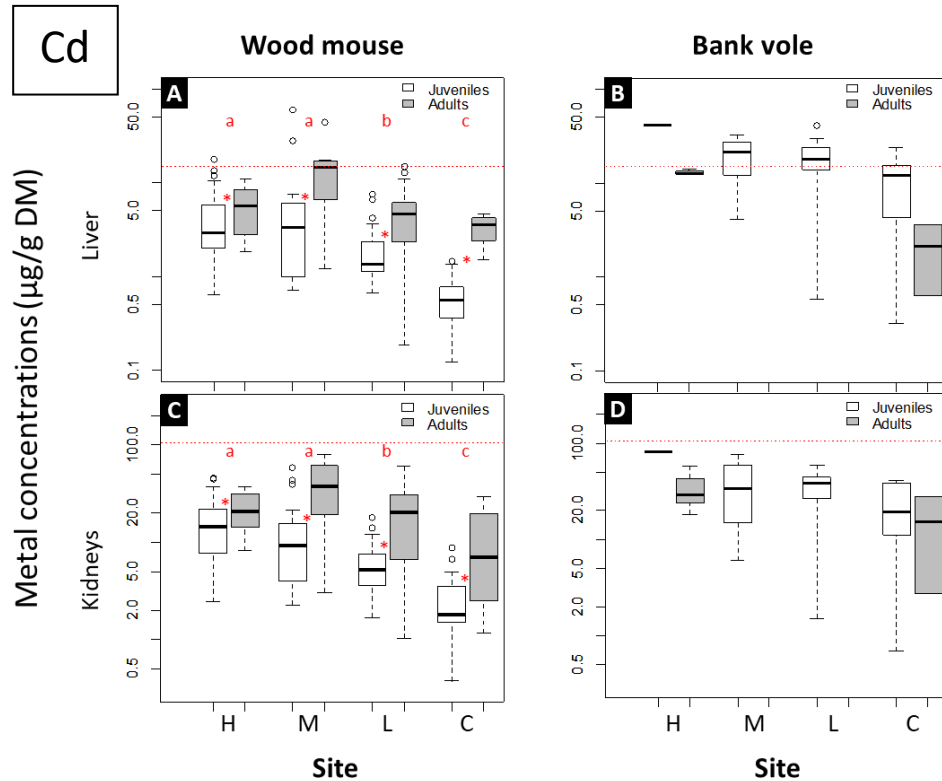
Statistical analyses

Exploratory analyses consisted in Spearman rank correlations between haematological parameters and metal concentrations in tissues. We then used general linear models (LMs) to investigate the effect of site of capture, species, individual parameters (sex and age) and their interactions on Cd and Pb concentrations in the liver and in kidneys. The full models tested were: Metal concentration (Cd or Pb) in tissues (liver or kidneys) ~ site + species + age + sex + site:species + site:age + site:sex + species:age + species:sex. Because metal concentrations were skewed, all tissue concentrations were log-transformed ($\log_{10}(x+1)$) to meet assumptions regarding normality of residuals and homoscedasticity of variance. Multiple comparisons among sites and between age classes or sex were investigated for each species separately using Tukey's Honest Significant Difference method. To investigate the potential effects of sex, age and Cd, Pb and Se concentrations in tissues on each of the haematological parameters measured (and Fe concentrations in blood to confirm possible anaemia) and for each of the species studied, we built a set of 41 LMs from model null to models integrating sex, age and the concentration of one metal in one tissue with their interactions (See Supplementary material for full list of models). Because data were skewed for some haematological parameters, corresponding data were log-transformed ($\log_{10}(x+1)$) to meet assumptions regarding normality of residuals and homoscedasticity of variance. Preliminary analyses showed that LMs were better or not different than general linear mixed models (LMMs) with site as random factor for most of the haematological parameters. LMs were therefore used for further analysis. Models were compared using Akaike information criterion (AIC) (Burnham and Anderson, 2002). The selection was based on small sample unbiased Akaike's Information Criterion (AICc) and Akaike weights (W_{ic}). The model with the smallest AICc has the most support given the data, and competing models were identified as those with

AICc values within two units of the best model (Burnham and Anderson, 2002). The most parsimonious model was retained among the competing models, if any. Models were checked graphically for homogeneity of variance, normality of error, and linearity/additivity. The leverage was checked plotting standardized residuals *versus* leverage. All analyses were performed with the software *R* v. 4.0.2 (R Development Core Team 2020).

Figure 1. Concentrations ($\mu\text{g/g}$ dry mass) of Cd in the liver and in kidneys of juvenile and adult wood mice and bank voles from Metaleurop Nord.

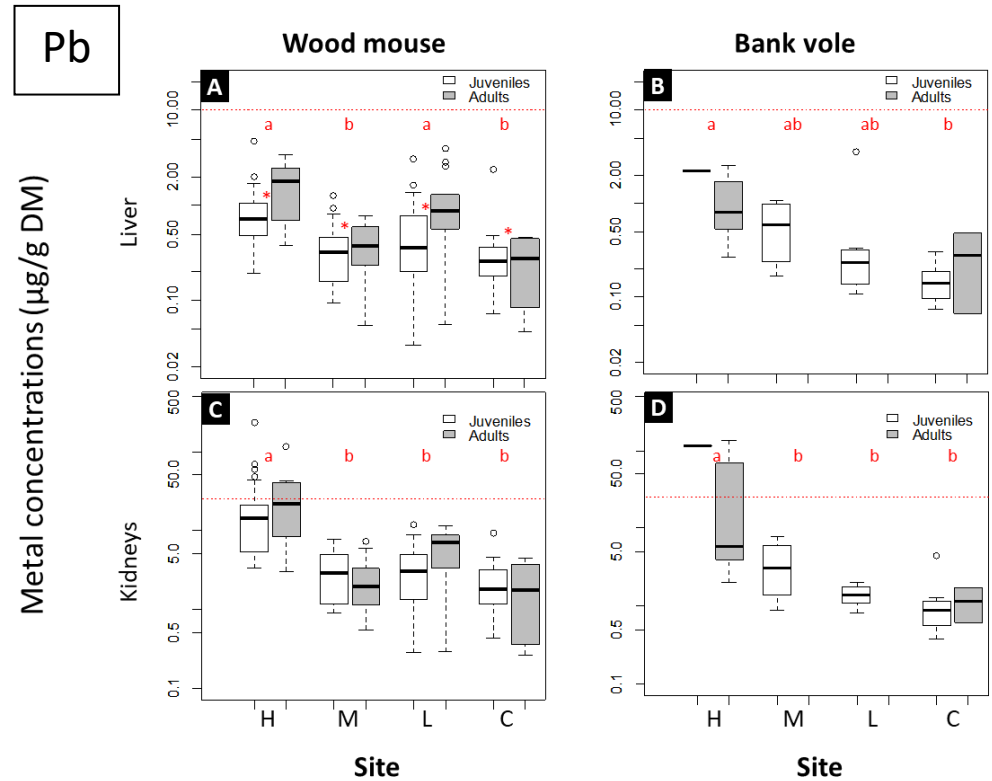
- (A) Cd in the liver of wood mice;
- (B) Cd in the liver of bank voles;
- (C) Cd in kidneys of wood mice;
- (D) Cd in kidneys of bank voles.



Abbreviations for the four sites are “H” for high, “M” for medium, “L” for low, and “C” for control. Dotted red lines represent the LOELs (Lowest Observed Adverse Effect Levels) for Cd (from laboratory derived thresholds for small mammals; Shore and Douben, 1994a; 1994b). Values are 15 $\mu\text{g/g}$ dry mass in the liver and 105 $\mu\text{g/g}$ dry mass in the kidneys. Asterisks present statistically significant differences between juveniles and adults. Statistically significant differences ($p < 0.05$) between sites, for each species, are indicated by red letters. Different letters indicate significant differences ($p < 0.05$).

Figure 2. Concentrations ($\mu\text{g/g}$ dry mass) of Pb in the liver and in kidneys of juvenile and adult wood mice and bank voles from Metaleurop Nord.

- (A) Pb in the liver of wood mice;
- (B) Pb in the liver of bank voles;
- (C) Pb in kidneys of wood mice;
- (D) Pb in kidneys of bank voles.



Abbreviations for the four sites are “H” for high, “M” for medium, “L” for low, and “C” for control. Dotted red lines represent the LOELs (Lowest Observed Adverse Effect Levels) for Cd and Pb (from laboratory derived thresholds for small mammals; Shore and Douben, 1994a; 1994b). Values are 10 $\mu\text{g/g}$ dry mass in the liver and 25 $\mu\text{g/g}$ dry mass in the kidneys. Asterisks present statistically significant differences between juveniles and adults. Statistically significant differences ($p < 0.05$) between sites, for each species, are indicated by red letters. Different letters indicate significant differences ($p < 0.05$).

Results

Metal concentration in tissues

The concentrations of Cd and Pb in the liver and in kidneys (Table 1, Figs. 1 and 2) of wood mice and bank voles from Metaleurop Nord mostly varied according to sites, species, and age (See Supplementary material for model outputs). For Cd in both species, hepatic and renal concentrations were the highest in the sites closest to the smelter (H and M) and the lowest in the C site, though significantly in wood mice only (See Supplementary material for model outputs and post-hoc comparisons). Cadmium concentrations in both tissues were higher in bank voles than in wood mice. In both organs, Cd concentrations in wood mice were higher in adults than in juveniles, and also varied according with gender, with higher values in females than in males in wood mice only (Fig. S2). Lead concentrations in both species and for both organs were also the highest in the most polluted site (H) and generally, the lowest in the control site (Table 1, Figs. 1 and 2). Lead concentrations in the liver and in kidneys did not differ between the two species. Adults from both species exhibited higher concentrations of Pb than juveniles in the liver only. Concentrations of Pb in the liver of bank voles were higher in males than in females (Fig. S2). The same, but not significant, trend was observed in kidneys. There was no difference of Pb concentrations between genders in wood mice (Fig. S2). The values of LOAELs were exceeded for Cd in the liver for 12 out of 27 bank voles, and nine out of 144 wood mice. For Pb, LOAELs were exceeded in kidneys for 2 bank voles and 9 wood mice. The concentrations of Cd in kidneys and of Pb in the liver never exceeded the corresponding LOAELs. The individuals exhibiting concentrations above the toxic thresholds came mostly from the two polluted sites “H” and “M” (24 individuals, representing 83%), and a few from the “L” site (5 bank voles), while no individual from the control site were above LOAELs.

Table 1. Mean \pm standard deviation (SD) of concentrations of Cd and Pb in the liver and in kidneys of wood mice ($n = 144$) and bank voles ($n = 27$) captured along a gradient of contamination (high, medium, low contamination and control site) near the former Metaleurop Nord smelter.

	Wood mouse (<i>Apodemus sylvaticus</i>)				Bank voles (<i>Myodes glareolus</i>)			
	High	Medium	Low	Control	High	Medium	Low	Control
Cd _{liver}	5.15 \pm 4.10a	8.93 \pm 12.23a	3.14 \pm 3.30b	1.02 \pm 1.09c	20.22 \pm 14.34a	19.86 \pm 11.90a	19.56 \pm 13.05a	9.42 \pm 8.09a
Cd _{kidneys}	18.42 \pm 12.21a	22.19 \pm 22.04a	11.44 \pm 13.23b	3.90 \pm 5.40c	46.95 \pm 28.69a	37.66 \pm 30.49a	34.55 \pm 18.58a	19.98 \pm 15.11a
Pb _{liver}	1.10 \pm 0.97a	0.39 \pm 0.27b	0.82 \pm 0.87a	0.34 \pm 0.41b	1.47 \pm 1.11a	0.60 \pm 0.44ab	0.68 \pm 1.26ab	0.17 \pm 0.12b
Pb _{kidneys}	27.26 \pm 42.28a	3.01 \pm 2.04b	4.33 \pm 3.18b	2.41 \pm 1.82b	63.98 \pm 69.69a	3.75 \pm 3.12b	1.43 \pm 0.45b	1.16 \pm 1.11b

Statistically significant differences ($p < 0.05$) between sites, for each species, are indicated by letters. Different letters indicate significant differences ($p < 0.05$).

Table 2. Haematological parameters (minimum, median and maximum, mean \pm standard deviation, SD) for wood mice ($n = 144$) and bank voles ($n = 27$) captured along a gradient of contamination (high, medium, low contamination and control site) near the former Metaleurop Nord smelter.

Parameter (abbreviation, unit)	Wood mouse (<i>Apodemus sylvaticus</i>)				Bank vole (<i>Myodes glareolus</i>)			
	Min	Median	Max	Mean \pm SD	Min	Median	Max	Mean \pm SD
White blood cells number (WBC, $10^3 / \text{mm}^3$)	0.35	3.20	25.50	4.36 \pm 4.12	1.10	2.70	14.25	3.56 \pm 2.72
Lymphocytes (LYM, %)	52.20	80.15	100.00	79.67 \pm 9.34	51.70	89.05	98.25	85.20 \pm 10.72
Monocytes (MON, %)	0.00	13.25	32.00	13.37 \pm 5.86	1.50	8.55	30.50	9.72 \pm 6.23
Granulocytes (GRA, %)	0.00	5.87	30.10	6.95 \pm 5.04	0.25	2.67	18.40	5.08 \pm 5.36
Red blood cells number (RBC, $10^6 / \text{mm}^3$)	2.88	8.73	11.97	8.69 \pm 1.20	6.68	9.43	11.65	9.54 \pm 1.04
Haemoglobin (HGB, g / dL)	1.13	13.03	16.17	12.92 \pm 1.62	10.07	13.33	16.35	13.27 \pm 1.42
Haematocrit (HCT, %)	14.47	41.84	54.70	41.59 \pm 5.08	30.53	42.85	53.95	42.94 \pm 4.70
Mean corpuscular volume (MCV, μm^3)	40.00	48.00	57.50	48.11 \pm 2.65	41.00	45.00	51.00	45.09 \pm 2.33
Mean corpuscular haemoglobin (MCH, pg)	3.93	14.85	18.35	14.91 \pm 1.45	12.55	13.90	16.50	13.93 \pm 0.90
Mean corpuscular haemoglobin concentration (MCHC, g / dL)	7.83	31.00	38.40	31.00 \pm 2.37	29.30	30.73	32.93	30.89 \pm 0.78
Red blood cell distribution width (RDW, %)	13.00	15.65	21.40	15.72 \pm 1.20	13.25	14.60	16.97	14.81 \pm 1.05

Haematological parameters

The values measured for the different haematological parameters are summarized in Table 2. Haematological parameters were related either to biological individual parameters such as sex and age or to metal concentrations in the liver or in kidneys (Table 3). For readability purposes, Table 3 gives the main findings of this study, while Tables S4 and S5 provide full statistical results.

*Table 3 Summary of the effect of explanatory variables on haematological parameters in the two species studied, the wood mouse *Apodemus sylvaticus*, and the bank vole *Myodes glareolus*.*

Haematological parameter	Variables influencing haematological parameters	
	<i>Apodemus sylvaticus</i>	<i>Myodes glareolus</i>
WBC	Sex	No variable
LYM	Sex	No variable
MON	↗ with ↗ [Se_{liver}]	No variable
GRA	Sex	No variable
RBC	↘ with ↗ [Cd _{kidneys}]	↘ with ↗ [Pb_{kidneys}], modulated by ↗ [Se_{kidneys}]
HGB	No variable	↘ with ↗ [Cd _{liver}]
HCT	No variable	No variable
MCV	Age	Age
MCH	Age	Age
MCHC	No variable	No variable
RDW	↗ with ↗ [Cd_{liver}], modulated by ↗ [Se_{liver}]	No variable
[Fe _{blood}]	↘ with ↗ [Cd _{kidneys}]	No variable

↗ stands for an increase, ↘ for a decrease. [Metal_{organ}] stands for the concentrations of a given metal in a given organ. Variables are in bold when Se concentrations influence a haematological parameter. Only variables included in a model different from the null model are said influential in this Table (see Table S4 for details).

In the wood mouse, HGB, HCT and MCHC were not correlated to any of the variables tested. WBC, LYM and GRA, and MCV and MCH were only and weakly related to sex and age, respectively (Fig. S3). MON were best explained by a model including the interaction between Pb and Se concentrations in the liver but only the factor Se was significant, with MON increasing with increasing Se concentrations (Fig. 3A). RDW was related to the interaction between Cd and Se concentrations in the liver: RDW increased with Cd concentrations but this was counteracted by high Se levels in the same organ (Fig. 3B). Both RBC and Fe

concentrations in blood decreased with increasing Cd concentrations in kidneys (Fig. 3C and D). In bank voles, 8 out of 12 parameters were not related to any of the tested variables (potentially due to low sample size). As in wood mice, MCV and MCH varied according to age (Fig. S4). In both species, adults showed higher levels than juveniles.

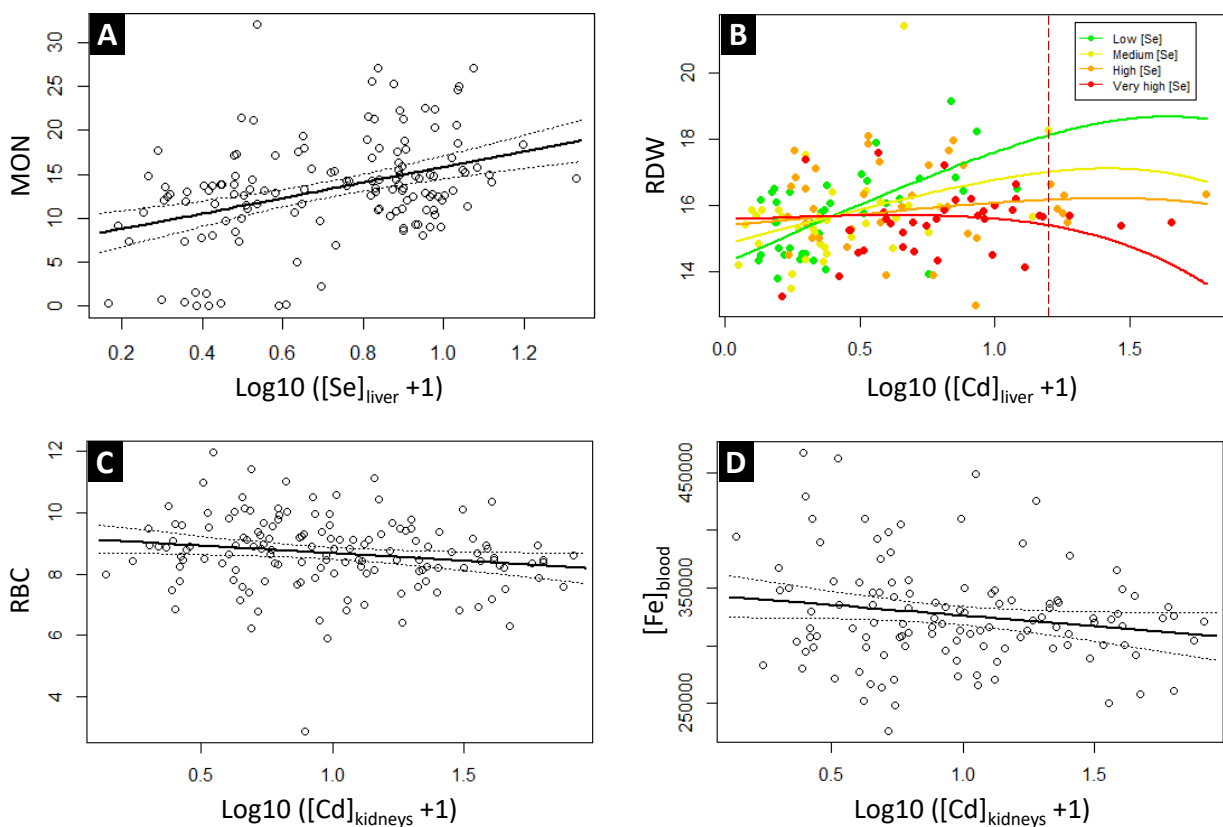
Figure 3 Relationships between haematological parameters and metal concentrations ($\mu\text{g/g}$ dry mass) in organs of wood mice from Metaleurop Nord.

(A) Percentage of monocytes in relation with Se concentrations in the liver (solid line represents the model, dotted lines 95%-confidence intervals);

(B) RDW in relation with Cd concentrations in the liver according to levels of Se in the same organ (low: $[\text{Se}] \leq 2.04$ ([Min - 1st Qu.], medium: $2.04 < [\text{Se}] \leq 5.45$ (1st Qu. - Median), high: $5.45 < [\text{Se}] \leq 7.41$ (Median - 3rd Qu.), very high: $[\text{Se}] > 7.41$ (3rd Qu. - Max]). Red dashed vertical bar represents the LOAEL value ($15 \mu\text{g/g}$ dry mass) for Cd in the liver in small mammals (from laboratory derived thresholds for small mammals; Shore and Douben, 1994a; 1994b);

(C) RBC in relation with Cd concentrations in kidneys (solid line represents the model, dotted lines 95%-confidence intervals);

(D) Blood Fe concentrations in relation with Cd concentrations in kidneys (solid line represents the model, dotted lines 95%-confidence intervals).

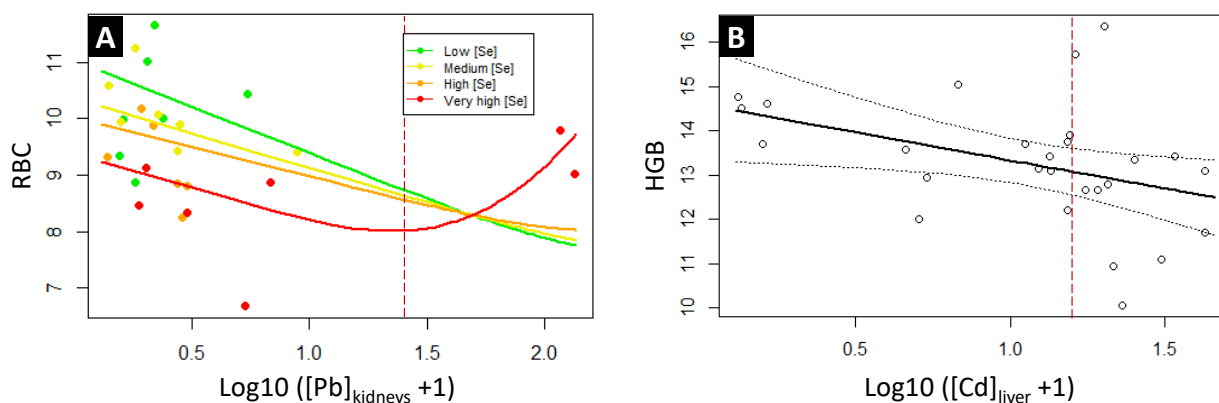


RBC decreased with increasing Pb concentrations in kidneys but, at very high concentrations of Pb, high Se levels allowed maintaining RBC levels (Fig. 4A). This result, however, is linked to only 2 individuals exhibiting very high Pb concentrations in kidneys and should be interpreted cautiously. Haemoglobin decreased with increasing Cd concentrations in the liver (Fig. 4B). In the wood mouse, concentrations of Se in the liver were positively correlated with Cd concentrations in both kidneys and the liver and with Pb concentrations in kidneys, while Se concentrations in kidneys were only correlated with Pb in the same organ (Fig. S5). In the bank vole, Se concentrations in the liver correlated with Pb only, both in kidneys and in the liver, while Se concentrations in kidneys correlated with both Cd and Pb concentrations in the two organs (Fig. S5). Correlations between metal concentrations in the organs, between haematological parameters and between metal concentrations and haematological parameters are shown in Fig. S5.

Figure 4 Relationships between haematological parameters and metal concentrations ($\mu\text{g/g}$ dry mass) in organs of bank voles from Metaleurop Nord.

(A) RBC in relation with Pb concentrations in kidneys according to levels of Se in the same organ (low: $[\text{Se}] \leq 4.23$ ([Min - 1st Qu.], medium: $4.23 < [\text{Se}] \leq 4.85$ (1st Qu. - Median), high: $4.85 < [\text{Se}] \leq 6.41$ (Median - 3rd Qu.), very high: $[\text{Se}] > 6.41$ (3rd Qu. - Max)). Red dashed vertical bar represents the LOAEL value ($25 \mu\text{g/g}$ dry mass) for Pb in kidneys in small mammals (from laboratory derived thresholds for small mammals; Shore and Douben, 1994a; 1994b);

(B) HGB in relation with Cd concentrations in the liver (solid line represents the model, dotted lines 95%-confidence intervals). Red dashed vertical bar represents the LOAEL value ($15 \mu\text{g/g}$ dry mass) for Cd in the liver in small mammals.



Discussion

In this study, we investigated the impacts of chronic exposure to toxic non-essential metals (Cd and Pb) on haematological parameters, an important component of health, in two common species of free-ranging small mammals along a pollution gradient. Chronic exposure was assessed individually using Cd and Pb concentrations in the liver and in kidneys and potential antagonistic effects of Se concentrations in those organs were also studied. Concentrations of Cd and Pb in tissues of wood mice and bank voles showed that chronic exposure and thus accumulation increased relatively linearly along the gradient, in agreement with our previous findings on the same species and the same sites (Fritsch *et al.*, 2010a; Tête *et al.*, 2015). The comparison of these data with toxicological thresholds (i.e. LOAELs for Cd and Pb from laboratory derived thresholds for small mammals; Shore and Douben, 1994a; 1994b) suggested that 6 and 44% of wood mice and bank voles, respectively, were above toxic levels and might therefore exhibit deleterious health effects possibly including variation of haematological parameters. Seven out of the 11 haematological parameters (plus Fe blood concentrations) were not explained by any of the measured variables in the bank vole, probably because of a low sampling size (27 individuals). This was true for only three parameters in the wood mouse for which sample size was much higher (144 individuals).

White blood cells counts and composition

Leukocytes counts did not correlate with any of the measured variables in the bank vole and WBC, LYM and GRA were only related to sex in the wood mouse. Overall, WBC count is traditionally subject to controversial interpretation. A high value has been considered as a signal of good health status (Gustafsson *et al.*, 1994). For instance, Snoeijs *et al.* (2004) found a higher immunological response including WBC for birds (great tits) living in lowly polluted sites along a metal contamination gradient and Tête *et al.* (2015) found a decreased of leukocytes with increasing Cd concentrations in organs in wood mice. Conversely, a high level of WBC may also be due to an increase in circulating granulocytes under stressing situation in both birds and mammals (Apanius, 1998; Beldomenico *et al.*, 2008). In the present study, both total WBC and granulocytes were only related to sex in the wood mouse and a relation between granulocytes and Pb in kidneys was suspected in the bank vole. Low WBC counts represent not only the level of circulating

granulocytes, but also low lymphocyte numbers, which could indicate low infection levels (Norris and Evans, 2000). In our study, monocytes were the only leukocyte type that was related to any of the studied metals. Indeed, in the wood mouse, monocytes were related to Pb in the liver, although not significantly, and to Se in the same organ, showing a clear and strong increase with Se. It is well known that Se plays a key role in redox regulation through its incorporation into selenoproteins as the 21st amino acid selenocysteine (Liao *et al.* 2018). Selenium deficiency is also known as being a cofactor in the anaemia associated with chronic inflammatory diseases. In such anaemia, a so-called stress erythropoiesis predominates “normal” steady-state erythropoiesis and Se has recently been shown (i) to promote stress erythroid cell expansion and differentiation and (ii) to act on monocytes by inducing their differentiation to erythroblastic island macrophages (Liao *et al.* 2018). The increase of monocytes with increasing Se concentrations in the liver observed in the present study is different from the role of Se in differentiating monocytes into macrophages (that were not measured here). While Se has been shown to play a role in differentiation and proliferation of T cells (Gombart *et al.* 2020), we failed to find in the literature any article reporting a direct link between Se and an increase of monocytes. Moreover, an increase of monocytes is also diagnostic of tissue damage (which have been observed in the same species from the same study sites in our previous research, see Tête *et al.* 2014b), chronic inflammation, and infection (Maceda-Veiga *et al.* 2015). As pathogens were not monitored in our study, it is challenging to disentangle responses due to potential individual differences of infection status from those due to stress induced by metals. Nevertheless, based on the article from Liao *et al.* (2018) and on the relationship between monocytes and Se observed in the present work, it could be hypothesized that Se might have a functional role in mitigating the impacts of toxic metals such as Cd or Pb through e.g. stress erythropoiesis, to compensate for the anaemia observed (see below).

Erythrocyte indicators

Our results showed that RBCs were negatively related with Cd in kidneys of wood mice and with Pb in the same organ of bank voles. In wood mice only, Fe concentrations in blood also dropped with increasing concentrations of Cd in kidneys while, in bank voles only, an interaction between Pb and Se suggested a counteracting role of this essential metal on the decrease of RBCs. In bank voles, haemoglobin was also

negatively related to Cd in the liver. In wood mice, the results suggest that the increase of RDW with Cd in the liver is modulated by Se concentrations in the same organ. Besides, both haematocrit and haemoglobin levels showed low values compared to other studies on the same species (Tables S6 and S7). Taken together, these results strongly suggest that (i) there are some signs of anaemia (but the combination of parameters does not allow specifying the type of anaemia that the two species may suffer from) and that (ii) Se plays a role in modulating the impacts of chronic exposure to toxic metals on some haematological parameters. These results are in agreement with other studies showing decreases in RBCs, haematocrit, haemoglobin, MCV and/or MCH with increasing metal concentrations in the environment, in tissues and/or in blood in birds (Nyholm, 1998; Geens *et al.* 2010) and small mammals (Rogival *et al.*, 2006; Tête *et al.* 2015). Under experimental exposure to Pb through drinking water, Marques *et al.* (2006) also described a reduction of RBCs, haematocrit and MCH in the Algerian mouse *Mus spretus*. Discrepancies between studies in the type and/or number of parameters significantly related to pollution may be partly explained by differences in puncture sites, which may impact blood parameter estimations (Bonnet *et al.* 2016), and the season of sampling. Seasonal variations in haematology have indeed been reported for fish species (de Pedro *et al.*, 2005), birds (Ots *et al.*, 1998) or small mammals (Perez-Suarez *et al.*, 1990; Beldomenico *et al.*, 2008), and may be attributed to changes in diet (Thompson *et al.*, 1997), physiological needs for preparing (or during) breeding season (Rai and Haldar, 2003), or higher metabolic rate in response to their acclimatization to low temperature during winter (Pérez-Suarez *et al.*, 1990). Further, reproductive status (Ferrer, 1990; Nunes *et al.*, 2001), diurnal time (Ots *et al.*, 1998), periods of population growth or decline (Tarakhtii *et al.*, 2007a; 2007b), or infection status (Maceda-Veiga *et al.*, 2015) modify haematological parameters as well. For instance, during pregnancy of wood mice, Perez-Suarez *et al.* (1990) found sexual differences in haematocrit, haemoglobin or total RBC number. In the present study, differences between males and females as well as between juveniles and adults were detected in both species (Fig. S2 and S3). We did not detect variations of MCV, MCH or MCHC with metal concentrations in the tissues for both species. Variations of one or the other of these parameters have however been reported previously on the Algerian mouse exposed to metals (Nunes *et al.*, 2001; Marques *et al.*, 2006).

The antagonistic effects of Se against Pb have been observed in rats experimentally exposed to Se through drinking water, Pb (by cutaneous application) or both metals (Rastogi *et al.*, 1976). In the latest study, Se has been shown to maintain at a normal level growth rate and food consumption as well as ALAD activity in whole blood, liver and kidney and liver P-450 enzyme activity. In a more recent but relatively similar experimental study, the effect of Se in counteracting Cd effects on cytokines, antioxidant enzymes such as reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid peroxidation malondialdehyde (MDA), as well as biochemical markers of liver and kidney functions, has been found (El-Boshy *et al.*, 2015). A recent review found that Se may exert a protection against Cd and Pb toxicity by several mechanisms such as boosting up antioxidant defence, lowering lipid peroxidation, limiting mitochondrial damage, or modulating signal transduction pathways of apoptosis (Rahman *et al.*, 2019). Most of the studies cited above are cell or *in vivo* (lab) studies and very few, if any, field works have studied the possible modulatory effect of Se on metal toxicity, an area that probably deserves more attention for conservation purposes in polluted environments. Apart from a possible compensatory effect of Se on some haematological parameters, the relatively modest impact of metals on haematological parameters in the present study can also be linked to the fact that only few animals in our sample presented values higher than LOAELs for Pb and/or Cd, suggesting a relatively low risk of toxicity due to these two metals. According to our sample size, only 6% of wood mice presented Cd concentrations above LOAELs in the liver whereas this percentage was higher (14%) in 2011, with, besides, higher concentrations above the LOAEL values than in the present work (Tête *et al.*, 2015). This result suggests changes in terms of metals bioavailability and transfer over time on the site of Metaleurop Nord whose industrial activity and most emission ceased in 2003, an issue that is undergoing investigations in our Department. This also suggests that the use of LOAELs (threshold initially mostly established from laboratory species under experimental conditions) may be useful for the study of wildlife in (metal) polluted environments in the purpose of conservation.

Conclusion

This study highlights variations of haematological parameters in relation to chronic exposure to environmental metal pollution, and thus advocates for the use of lowly invasive methods adapted from veterinary sciences to survey the impacts on human-induced disturbances on wildlife health. We acknowledge that haematology does not provide a full assessment of health in wildlife but provides valuable information about one important component of animal health, which is, contrarily to more invasive techniques such as histopathology, lowly invasive. Showing that the responses of animals to toxicant-induced stress could be mediated by Se, our results argue for the need to involve the measurement of essential elements when addressing the effects of toxic metals on organisms and consider the ecological and biological determinants of levels of essentials. More broadly, these results show that biomarkers should be interpreted within a multi-criteria approach, and that frameworks inspired from epidemiological sciences such as weight of evidence approaches may be valuable to further study and manage the effects of chemical pollution in ecosystems.

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Supplementary material

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Supplementary material – Tables S1 to S6

Table S1

Characteristics of the study squares (distance from the former smelter, median, minimum and maximum soil concentrations of Cd and Pb) along a gradient of contamination (high, medium, low contamination and control site) near the former Metaleurop Nord smelter.

		High	Medium	Low	Control
Distance from Metaleurop Nord (median, km)		0.6	1.4	3.0	11.2
Soil metal concentrations (µg/g dry mass)	Cd	59.3 (15.3 – 236.5)	9.7 (3.6 – 19.5)	4.0 (1.5 – 6.0.)	1.6 (1.3 – 2.0)
	Pb	1357 (755 – 6809)	526 (245 – 1260)	261 (149 – 333)	110 (73 – 182)

Table S2

Summary of small mammal sample size in the study sites (squares) in the vicinity of the former Metaleurop Nord smelter used in this work.

	Study squares				
	High	Medium	Low	Control	Total
<i>Apodemus sylvaticus</i>	35	38	42	29	144
Adult	8	12	13	4	37
<i>Female</i>	1	4	9	2	16
<i>Male</i>	7	8	4	2	21
Juvenile	27	26	29	25	107
<i>Female</i>	15	8	12	15	50
<i>Male</i>	12	18	17	10	57
<i>Myodes glareolus</i>	4	4	7	12	27
Adult	3			2	5
<i>Female</i>	2			1	3
<i>Male</i>	1			1	2
Juvenile	1	4	7	10	22
<i>Female</i>		3	4	2	9
<i>Male</i>	1	1	3	8	13
Total	39	42	49	41	171

Table S3

Limits of quantification of ICP-MS analysis of metals, certified values and average recoveries of the certified reference materials used in this study (the number of reference samples analyzed for each certified reference material is 13).

Parameter	Cd	Pb	Se	Fe
Limit of quantification in µg/L	0.005	0.011	0.089	0.729
TORT-2 certified values (and uncertainty: 95 confidence limits) in µg/g	26.7(0.6)	0.35(0.13)	5.63(0.67)	105(13)
Recovery (%)	98	120	109	75
ERM-CE196 certified values (and uncertainty: 95 confidence limits) in µg/L	12.33(0.20)	772(11)	NS ¹	NS
Recovery (%)	77	79	ND ²	ND

¹NS: not specified; ²ND: not determined

Table S4

Models retained and statistical outputs from selected models to analyse haematological parameters in wood mice and bank voles captured along a gradient of contamination (high, medium, low contamination and control site) near the former Metaleurop Nord smelter in function of age, sex, and concentrations of Cd, Pb or Se in the liver or in kidneys (list of models available in Supplementary material).

	<i>Apodemus sylvaticus</i>		<i>Myodes glareolus</i>	
	Model retained	Statistical parameters	Model retained	Statistical parameters
Log ₁₀ (WBC+1)	Sex	F _{1,137} =8.58, AdjR ² =0.05, p=0.004	Null model	
LYM	Sex	F _{1,137} =5.50, AdjR ² =0.03, p=0.020	Log ₁₀ (Pb _{kidneys} +1), not ≠ from null	
MON	Log ₁₀ (Pb _{liver} +1) x log ₁₀ (Se _{liver} +1)	F _{3,135} =8.72, AdjR ² =0.14, p<0.001	Log ₁₀ (Pb _{liver} +1), not ≠ from null	
Log ₁₀ (GRA+1)	Sex	F _{1,137} =4.37, AdjR ² =0.02, p=0.038	Log ₁₀ (Pb _{kidneys} +1), not ≠ from null	
RBC	Log ₁₀ (Cd _{kidneys} +1)	F _{1,142} =4.67, AdjR ² =0.03, p=0.032	Log ₁₀ (Pb _{kidneys} +1) x log ₁₀ (Se _{kidneys} +1)	F _{3,23} =5.22, AdjR ² =0.33, p=0.007
HGB	Null model		Log ₁₀ (Cd _{liver} +1)	F _{1,25} =4.84, AdjR ² =0.13, p=0.037
HCT	Null model		Log ₁₀ (Cd _{liver} +1), not ≠ from null	
MCV	Age	F _{1,142} =7.72, AdjR ² =0.05, p=0.006	Age	F _{1,25} =13.64, AdjR ² =0.33, p = 0.001
MCH	Age	F _{1,142} =5.38, AdjR ² =0.03, p=0.022	Age	F _{1,25} =13.5, AdjR ² =0.32, p=0.001
MCHC	Null model		Null model	
RDW	Log ₁₀ (Cd _{liver} +1) x log ₁₀ (Se _{liver} +1)	F _{3,140} =6.93, AdjR ² =0.11, p<0.001	Age, not ≠ from null	
Fe _{blood}	Log ₁₀ (Cd _{kidneys} +1)	F _{1,115} =3.83, AdjR ² =0.02, p=0.053	Null model (but only 7 data available)	

Table S5

Coefficients and associated standard errors of explanatory variables from selected models to analyse haematological parameters in wood mice and bank voles captured along a gradient of contamination (high, medium, low contamination and control site) near the former Metaleurop Nord smelter in function of age, sex, and concentrations of Cd, Pb or Se in the liver or in kidneys (list of models available in Supplementary material).

		<i>Apodemus sylvaticus</i>		<i>Myodes glareolus</i>	
	Variables	Coefficient, standard error		Variables	Coefficient, standard error
Log ₁₀ (WBC+1)	Sex (male)	0.12, 0.04		None	
LYM	Sex (male)	-3.67, 1.56		None	
MON	Log ₁₀ (Pb _{liver} +1)	-11.28, 9.18		None	
	Log ₁₀ (Se _{liver} +1)	5.13, 3.14			
	Interaction	17.40, 12.44			
Log ₁₀ (GRA+1)	Sex (male)	0.11, 0.05		None	
RBC	Log ₁₀ (Cd _{kidneys} +1)	-0.51, 0.24		Log ₁₀ (Pb _{kidneys} +1)	-4.15, 1.60
				Log ₁₀ (Se _{kidneys} +1)	-6.88, 1.89
				Interaction	4.07, 1.19
HGB	None			Log ₁₀ (Cd _{liver} +1)	-1.27, 0.58
HCT	None			None	
MCV	Age (juvenile)	-1.38, 0.49		Age (juvenile)	-3.49, 0.95
MCH	Age (juvenile)	-0.63, 0.27		Age (juvenile)	-1.34, 0.36
MCHC	None			None	
RDW	Log ₁₀ (Cd _{liver} +1)	4.11, 1.02		None	
	Log ₁₀ (Se _{liver} +1)	1.67, 0.82			
	Interaction	-4.17, 1.27			
Fe _{blood}	Log ₁₀ (Cd _{kidneys} +1)	-18,841, 9,623		None	

Table S6

Haematological parameters (mean ± standard deviation) for wood mice (n = 144) captured along a gradient of contamination (high, medium, low contamination and control site) near the former Metaleurop Nord smelter (present study) and mean (for all sites studied, when parameters varied with pollution, this is discussed in the Discussion section of the present article) or range values found in the literature.

	Present study	Gorritz et al., 1996	Tersago et al., 2004	Pérez-Suárez et al., 1990	Rogival et al., 2006
	France, polluted and control areas, autumn	Spain, polluted and control areas, 1 year long study	Belgium, polluted and control areas, spring	Spain, non-polluted areas, 1 year long study	Belgium, polluted and control areas, spring
Parameter (abbreviation, unit)	Mean±SD			Mean	
White blood cells number (WBC, 10 ³ / mm ³)	4.36 ± 4.12	1.37	1.66		6.06
Lymphocytes (LYM, %)	79.67 ± 9.34				
Monocytes (MON, %)	13.37 ± 5.86				
Granulocytes (GRA, %)	6.95 ± 5.04				
Red blood cells number (RBC, 10 ⁶ / mm ³)	8.69 ± 1.20	8.51	8.45	9.46	9.88
Haemoglobin (HGB, g / dL)	12.92 ± 1.62	16.44		14.59	17.27
Haematocrit (HCT, %)	41.59 ± 5.08	46.71		46.59	46.81
Mean corpuscular volume (MCV, µm ³)	48.11 ± 2.65	57.33		48.96	47.89
Mean corpuscular haemoglobin (MCH, pg)	14.91 ± 1.45	20.16		15.39	17.96
Mean corpuscular haemoglobin concentration (MCHC, g / dL)	31.00 ± 2.37	35.83		31.88	37.17
Red blood cell distribution width (RDW, %)	15.72 ± 1.20				

References

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Table S7

Haematological parameters (mean ± standard deviation) for bank voles (n = 27) captured along a gradient of contamination (high, medium, low contamination and control site) near the former Metaleurop Nord smelter and mean (for all sites studied, when parameters varied with pollution, this is discussed in the Discussion section of the present article) or range values found in the literature.

	Present study	Nieminen et al. 2015	Lipowska et al. 2019	Miska-Schramm et al. 2017	Tarakhtii & Davidova 2007	Tarakhtii et al. 2007
	France, polluted and control areas, autumn	Finland, non-polluted areas, spring	Lab experiment	Lab experiment	Central Ural, Russia, non-polluted areas, all seasons	Central Ural, Russia, non-polluted areas, all seasons
Parameter (abbreviation, unit)	Mean±SD	Mean				
White blood cells number (WBC, 10 ³ / mm ³)	3.56 ± 2.72	3.24			3.00	2.53
Lymphocytes (LYM, %)	85.20 ± 10.72	80.33				
Monocytes (MON, %)	9.72 ± 6.23	6.91				
Granulocytes (GRA, %)	5.08 ± 5.36	12.76				
Red blood cells number (RBC, 10 ⁶ / mm ³)	9.54 ± 1.04	10.19	12.05		9.13	6.90
Haemoglobin (HGB, g / dL)	13.27 ± 1.42	14.35	14.97	12.33	16.28	15.83
Haematocrit (HCT, %)	42.94 ± 4.70	47.03	52.72	39.17	45.94	46.56
Mean corpuscular volume (MCV, μm ³)	45.09 ± 2.33	46.64	44.24		63.53	81.21
Mean corpuscular haemoglobin (MCH, pg)	13.93 ± 0.90	14.21	12.59		21.32	26.21
Mean corpuscular haemoglobin concentration (MCHC, g / dL)	30.89 ± 0.78	30.51	28.41		35.30	33.88
Red blood cell distribution width (RDW, %)	14.81 ± 1.05	15.56				

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Supplementary material – Figures S1 to S4

Figure S1

Sampling design of the present study. The map top left is the Hauts-de-France region, located in Northern France. The rectangle on the map shows where the study site is. The zoom on the rectangle allows seeing distance of high (H), medium (M), low (L), and control (C) sites from the former smelter of Metaleurop Nord (in grey). These four squares of 500m over 500m (i.e. 25 ha) constitute the pollution gradient. Levels of Cd and Pb concentrations along the gradient are presented in Table S1. The zoom of one of these square allows seeing where the 10 traps lines of ten traps each (each line is here represented by three white dots) were set in the various (here five) woody habitats available in the study square.

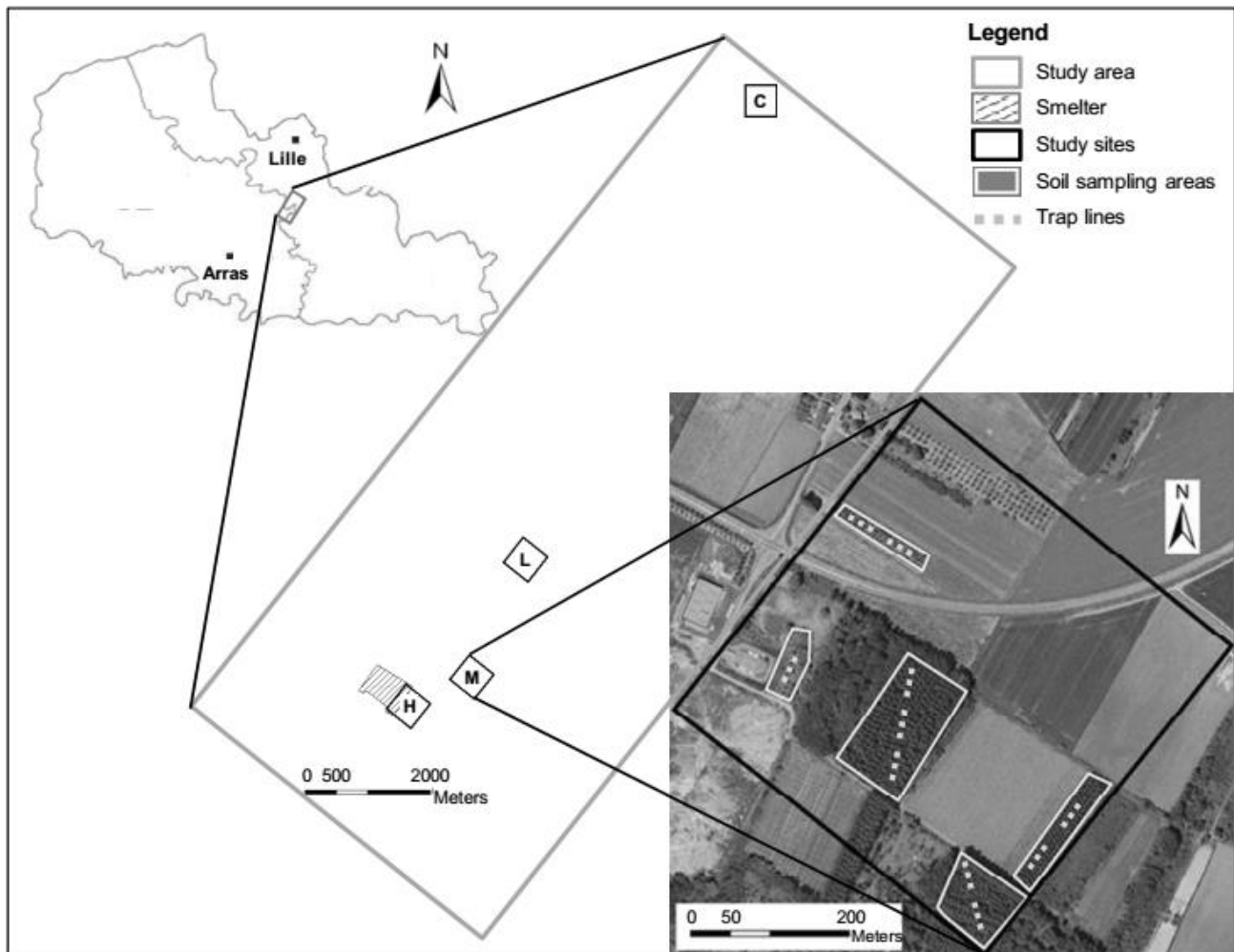
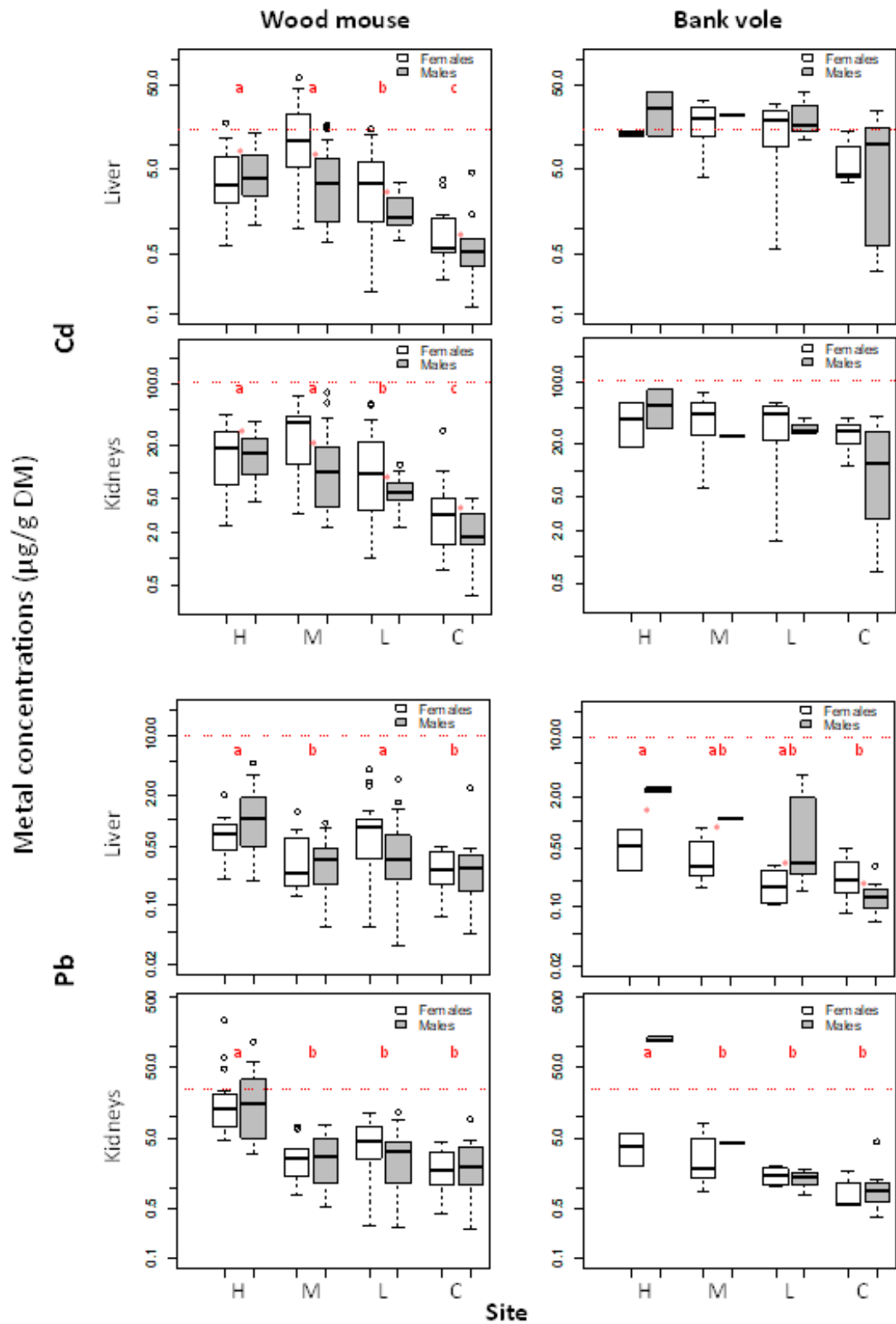


Figure S2

Concentrations ($\mu\text{g/g}$ dry mass) of Cd and Pb in the liver and in kidneys of female and male wood mice and bank voles from Metaleurop Nord.



Abbreviations for the four study squares are "H" for high, "M" for medium, "L" for low, and "C" for control. Dotted red lines represented the LOAELs (Lowest Observed Adverse Effect Levels) for Cd and Pb (from laboratory derived thresholds for small mammals; Shore and Douben, 1994a; 1994b). Values are: 15 $\mu\text{g/g}$ DM in the liver and 105 $\mu\text{g/g}$ DM in kidneys for Cd, 10 $\mu\text{g/g}$ DM in the liver and 25 $\mu\text{g/g}$ DM in kidneys for Pb. Asterisks present statistically significant differences between females and males. Statistically significant differences between sites, for each species, are indicated by red lower-case letters. Different letters indicate significant differences ($p < 0.05$).

Figure S3

Variation of some haematological parameters in wood mice from Metaleurop Nord according to sex (F: females, M: males) or age (J: juveniles, A: adults). Asterisks present statistically significant differences between gender or age class.

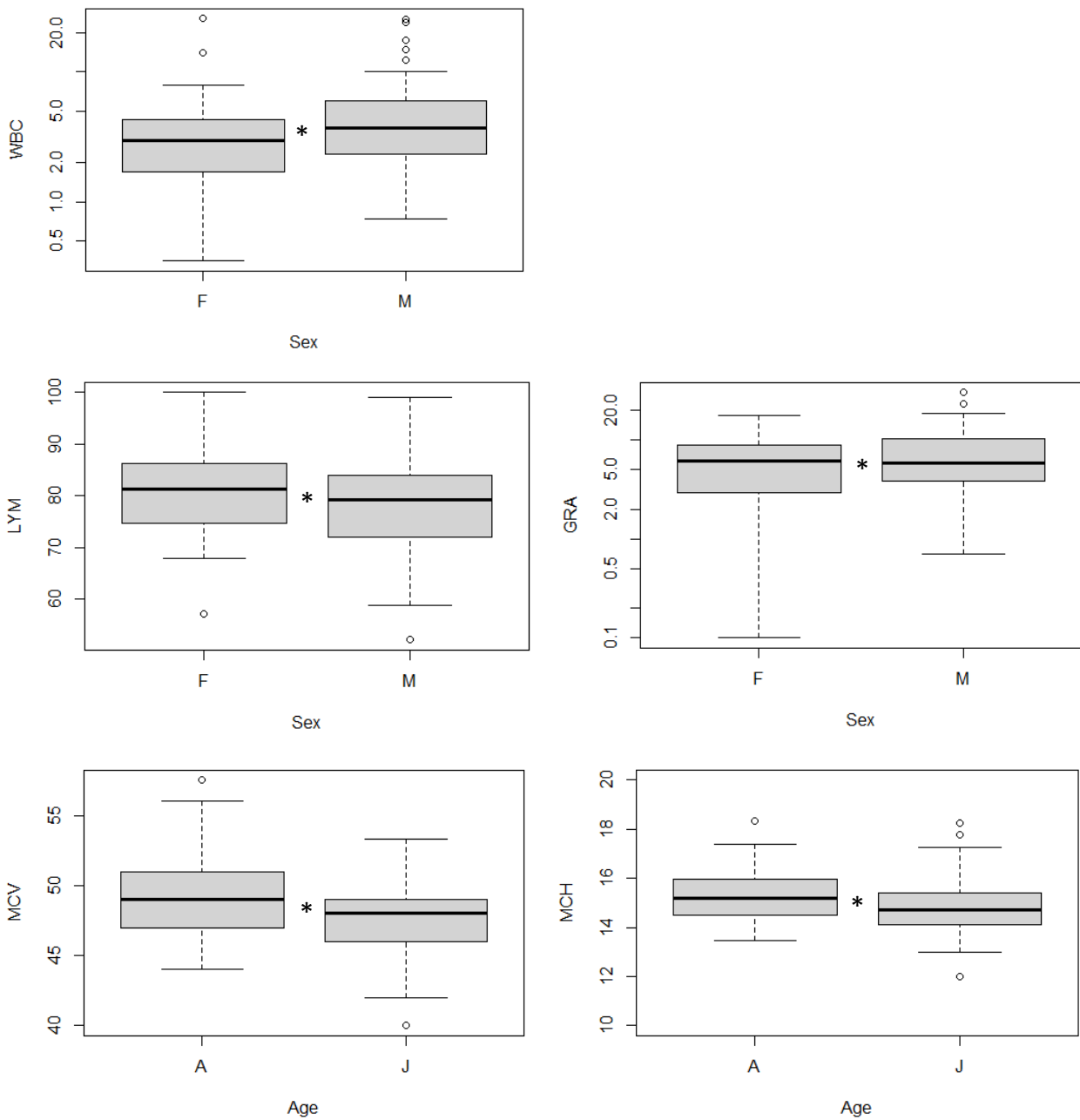


Figure S4

Variation of some haematological parameters in bank voles from Metaleurop Nord according to age (J: juveniles, A: adults). Asterisks present statistically significant differences between age class.

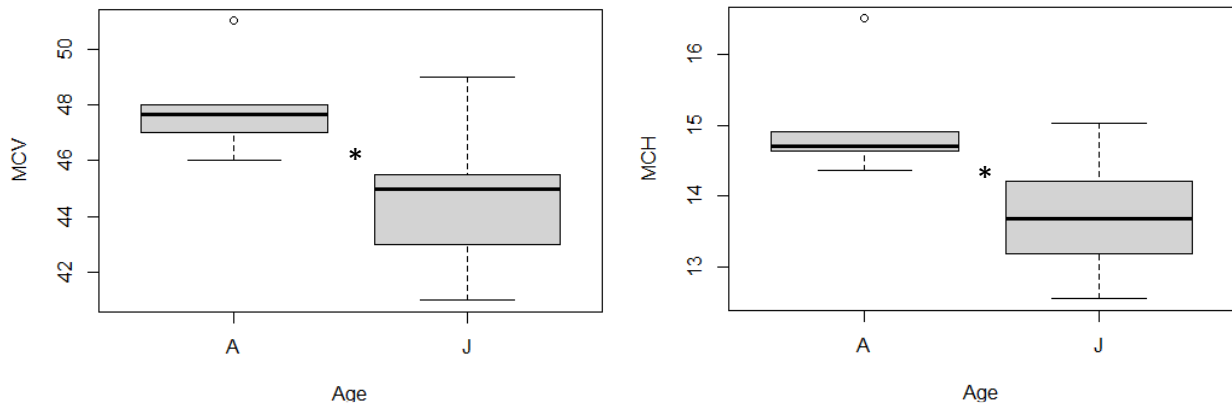
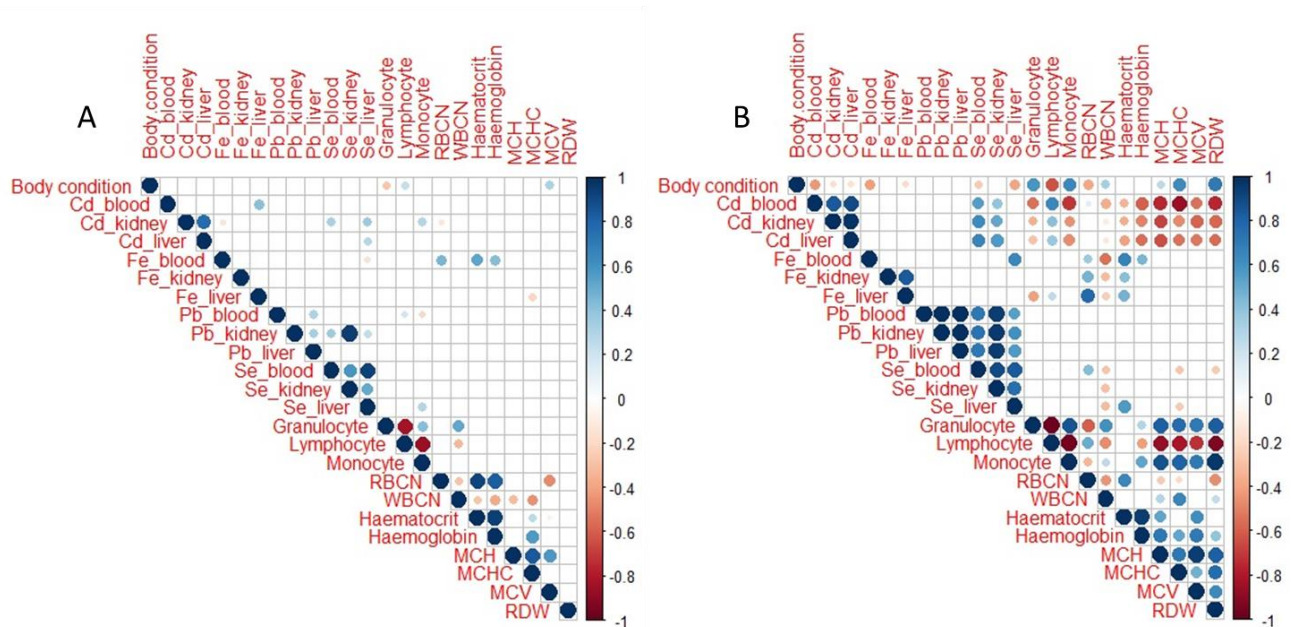


Figure S5

Spearman rank correlation plot showing correlations between haematological parameters and metal concentrations in tissues (liver, kidneys, in $\mu\text{g/g}$ dry mass) and blood (in $\mu\text{g/L}$), and body condition (as calculated as the residuals of an ordinary least square regression of mass upon size) for wood mice (A, $n = 144$) and bank voles (B, $n = 27$).



RBCN: Red blood cells number; WBCN: White blood cells number; RDW: Red blood cell distribution width; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration. Lymphocyte, monocyte and granulocyte are expressed in percentage of the total white blood cells. Only significant correlations ($P < 0.05$) are dotted. Color (blue positive and red negative) and plot size (proportional to correlation coefficient values) indicate the correlation coefficient. The graphical visualization of correlation matrices was done with the 'corrplot' package in R.

Supplementary material – Details about statistics

List of linear models used to analyse each of the haematological parameters (HP) in wood mice and bank voles captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter. Amongst HP, only WBC and GRA have been log10 transformed for analysis.

```

LM1<-lm(HP~1)
LM2<-lm(HP~sex)
LM3<-lm(HP~age)
LM4<-lm(HP~sex + age)
LM5<-lm(HP~sex * age)
LM6<-lm(HP~sex + age + log10(L_Cd+1))
LM7<-lm(HP~sex + age + log10(K_Cd+1))
LM8<-lm(HP~sex + age + log10(L_Pb+1))
LM9<-lm(HP~sex + age + log10(K_Pb+1))
LM10<-lm(HP~sex + log10(L_Cd+1))
LM11<-lm(HP~sex + log10(K_Cd+1))
LM12<-lm(HP~sex + log10(L_Pb+1))
LM13<-lm(HP~sex + log10(K_Pb+1))
LM14<-lm(HP~ age + log10(L_Cd+1))
LM15<-lm(HP~ age + log10(K_Cd+1))
LM16<-lm(HP~ age + log10(L_Pb+1))
LM17<-lm(HP~ age + log10(K_Pb+1))
LM18<-lm(HP~sex + log10(L_Cd+1) + sex:log10(L_Cd+1))
LM19<-lm(HP~sex + log10(K_Cd+1) + sex:log10(K_Cd+1))
LM20<-lm(HP~sex + log10(L_Pb+1) + sex:log10(L_Pb+1))
LM21<-lm(HP~sex + log10(K_Pb+1) + sex:log10(K_Pb+1))
LM22<-lm(HP~ age + log10(L_Cd+1) + age:log10(L_Cd+1))
LM23<-lm(HP~ age + log10(K_Cd+1) + age:log10(K_Cd+1))
LM24<-lm(HP~ age + log10(L_Pb+1) + age:log10(L_Pb+1))
LM25<-lm(HP~ age + log10(K_Pb+1) + age:log10(K_Pb+1))
LM26<-lm(HP~sex + age + log10(L_Cd+1) + sex:log10(L_Cd+1) + age:log10(L_Cd+1))
LM27<-lm(HP~sex + age + log10(K_Cd+1) + sex:log10(K_Cd+1) + age:log10(K_Cd+1))
LM28<-lm(HP~sex + age + log10(L_Pb+1) + sex:log10(L_Pb+1) + age:log10(L_Pb+1))
LM29<-lm(HP~sex + age + log10(K_Pb+1) + sex:log10(K_Pb+1) + age:log10(K_Pb+1))
LM30<-lm(HP~log10(L_Cd+1))
LM31<-lm(HP~log10(K_Cd+1))
LM32<-lm(HP~log10(L_Pb+1))
LM33<-lm(HP~log10(K_Pb+1))
LM34<-lm(HP~log10(L_Cd+1) + log10(L_Pb+1))
LM35<-lm(HP~log10(L_Cd+1) * log10(L_Pb+1))
LM36<-lm(HP~log10(L_Cd+1) * log10(L_Se+1))
LM37<-lm(HP~log10(L_Pb+1) * log10(L_Se+1))
LM38<-lm(HP~log10(K_Cd+1) + log10(K_Pb+1))
LM39<-lm(HP~log10(K_Cd+1) * log10(K_Pb+1))
LM40<-lm(HP~log10(K_Cd+1) * log10(K_Se+1))
LM41<-lm(HP~log10(K_Pb+1) * log10(K_Se+1))

```

Statistical outputs of models performed to analyse Cd concentrations ($\mu\text{g/g}$ dry mass) in the liver of wood mice and bank voles captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter

```
>LM_L_Cd1<-
lm(log10(L_Cd+1)~site+species+age+sex+site:species+site:age+site:sex+species:age+species:
sex,data=tab)
> summary(LM_L_Cd1)
Call:
lm(formula = log10(L_Cd + 1) ~ site + species + age + sex +
    site:species + site:age + site:sex + species:age +
    species:sex, data = tab)
Residuals:
    Min       1Q   Median       3Q      Max
-0.95313 -0.14135 -0.00215  0.13619  0.89075
Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      0.83239    0.12573   6.620 5.71e-10 ***
siteM             0.48542    0.16209   2.995 0.003205 **
siteL            -0.07879    0.14991  -0.526 0.599924
siteC            -0.19617    0.17706  -1.108 0.269621
speciesMYGL      0.21585    0.18540   1.164 0.246147
ageJ             -0.14162    0.11541  -1.227 0.221675
sexM             -0.03476    0.09673  -0.359 0.719850
siteM:speciesMYGL -0.57395    0.27674  -2.074 0.039760 *
siteL:speciesMYGL -0.31128    0.25221  -1.234 0.219018
siteC:speciesMYGL -0.36375    0.21925  -1.659 0.099147 .
siteM:ageJ       -0.28197    0.15186  -1.857 0.065270 .
siteL:ageJ       -0.10635    0.15130  -0.703 0.483190
siteC:ageJ       -0.23344    0.17282  -1.351 0.178751
siteM:sexM       -0.32067    0.13454  -2.383 0.018378 *
siteL:sexM       -0.08164    0.12709  -0.642 0.521606
siteC:sexM       -0.08214    0.13342  -0.616 0.539058
speciesMYGL:ageJ  0.74147    0.22086   3.357 0.000993 ***
speciesMYGL:sexM  0.17439    0.13433   1.298 0.196168
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.2827 on 153 degrees of freedom
Multiple R-squared:  0.5627,    Adjusted R-squared:  0.5141
F-statistic: 11.58 on 17 and 153 DF,  p-value: < 2.2e-16
> anova(LM_L_Cd1)
Analysis of Variance Table
Response: log10(L_Cd + 1)
      Df Sum Sq Mean Sq F value    Pr(>F)
site     3  3.8028  1.2676 15.8650 4.945e-09 ***
species  1  7.1036  7.1036 88.9072 < 2.2e-16 ***
age      1  1.5408  1.5408 19.2843 2.092e-05 ***
sex      1  0.5014  0.5014  6.2759 0.0132864 *
site:species  3  0.2270  0.0757  0.9471 0.4194788
site:age    3  0.5699  0.1900  2.3774 0.0720982 .
site:sex    3  0.7517  0.2506  3.1362 0.0272335 *
species:age  1  1.0967  1.0967 13.7263 0.0002949 ***
species:sex  1  0.1347  0.1347  1.6854 0.1961684
Residuals 153 12.2245  0.0799
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Statistical outputs of models performed to analyse Cd concentrations ($\mu\text{g/g}$ dry mass) in kidneys of wood mice and bank voles captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter

```
>LM_K_Cd1<-
lm(log10(K_Cd+1)~site+species+age+sex+site:species+site:age+site:sex+species:age+species:
sex,data=tab)
> summary(LM_K_Cd1)
Call:
lm(formula = log10(K_Cd + 1) ~ site + species + age + sex +
    site:species + site:age + site:sex + species:age +
    species:sex, data = tab)

Residuals:
    Min       1Q   Median       3Q      Max
-1.04791 -0.21306  0.03012  0.20928  0.55405

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    1.34893    0.14475   9.319  <2e-16 ***
siteM           0.34705    0.18661   1.860  0.0648 .
siteL          -0.10827    0.17259  -0.627  0.5314
siteC          -0.34990    0.20383  -1.717  0.0881 .
speciesMYGL     0.16056    0.21344   0.752  0.4531
ageJ           -0.17915    0.13287  -1.348  0.1795
sexM           -0.02663    0.11136  -0.239  0.8113
siteM:speciesMYGL -0.46107    0.31860  -1.447  0.1499
siteL:speciesMYGL -0.16805    0.29035  -0.579  0.5636
siteC:speciesMYGL -0.04250    0.25241  -0.168  0.8665
siteM:ageJ      -0.29175    0.17483  -1.669  0.0972 .
siteL:ageJ      -0.19438    0.17418  -1.116  0.2662
siteC:ageJ      -0.20506    0.19895  -1.031  0.3043
siteM:sexM      -0.26804    0.15489  -1.731  0.0856 .
siteL:sexM      -0.05657    0.14631  -0.387  0.6995
siteC:sexM      -0.19993    0.15360  -1.302  0.1950
speciesMYGL:ageJ  0.58621    0.25426   2.306  0.0225 *
speciesMYGL:sexM  0.03434    0.15465   0.222  0.8246
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3254 on 153 degrees of freedom
Multiple R-squared:  0.5079,    Adjusted R-squared:  0.4532
F-statistic: 9.288 on 17 and 153 DF,  p-value: 2.722e-16

> anova(LM_K_Cd1)
Analysis of Variance Table

Response: log10(K_Cd + 1)
      Df Sum Sq Mean Sq F value    Pr(>F)
site    3  6.3284   2.1095  19.9205 5.926e-11 ***
species 1  4.8048   4.8048  45.3736 3.102e-10 ***
age     1  2.8097   2.8097  26.5331 7.871e-07 ***
sex     1  0.6819   0.6819   6.4398  0.01216 *
site:species 3  0.4633   0.1544   1.4583  0.22819
site:age  3  0.4982   0.1661   1.5683  0.19938
site:sex  3  0.5167   0.1722   1.6264  0.18557
species:age 1  0.6115   0.6115   5.7748  0.01745 *
species:sex 1  0.0052   0.0052   0.0493  0.82457
Residuals 153 16.2018  0.1059
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Statistical outputs of models performed to analyse Pb concentrations ($\mu\text{g/g}$ dry mass) in the liver of wood mice and bank voles captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter

```
>LM_L_Pb1<-
lm(log10(L_Pb+1)~site+species+age+sex+site:species+site:age+site:sex+species:age+species:
sex,
+ data=tab)
> summary(LM_L_Pb1)
```

```
Call:
lm(formula = log10(L_Pb + 1) ~ site + species + age + sex +
site:species + site:age + site:sex + species:age +
species:sex, data = tab)
```

Residuals:

```
Min      1Q   Median      3Q      Max
-0.30740 -0.07065 -0.01810  0.05042  0.44864
```

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	0.300447	0.056921	5.278	4.4e-07	***
siteM	-0.159729	0.073383	-2.177	0.0310	*
siteL	0.030373	0.067868	0.448	0.6551	
siteC	-0.165888	0.080157	-2.070	0.0402	*
speciesMYGL	-0.044135	0.083935	-0.526	0.5998	
ageJ	-0.085077	0.052250	-1.628	0.1055	
sexM	0.100556	0.043791	2.296	0.0230	*
siteM:speciesMYGL	0.069230	0.125286	0.553	0.5814	
siteL:speciesMYGL	-0.036258	0.114179	-0.318	0.7513	
siteC:speciesMYGL	-0.098702	0.099258	-0.994	0.3216	
siteM:ageJ	0.081886	0.068752	1.191	0.2355	
siteL:ageJ	-0.042643	0.068495	-0.623	0.5345	
siteC:ageJ	0.074337	0.078237	0.950	0.3435	
siteM:sexM	-0.103996	0.060909	-1.707	0.0898	.
siteL:sexM	-0.138293	0.057538	-2.404	0.0174	*
siteC:sexM	-0.126510	0.060403	-2.094	0.0379	*
speciesMYGL:ageJ	-0.004798	0.099988	-0.048	0.9618	
speciesMYGL:sexM	0.143902	0.060814	2.366	0.0192	*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.128 on 153 degrees of freedom
Multiple R-squared: 0.3651, Adjusted R-squared: 0.2946
F-statistic: 5.176 on 17 and 153 DF, p-value: 7.207e-09

```
> anova(LM_L_Pb1)
Analysis of Variance Table
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
site	3	0.89591	0.298638	18.2367	3.612e-10	***
species	1	0.00469	0.004693	0.2866	0.593207	
age	1	0.14827	0.148274	9.0545	0.003065	**
sex	1	0.01260	0.012598	0.7693	0.381796	
site:species	3	0.06322	0.021073	1.2868	0.280991	
site:age	3	0.11761	0.039202	2.3939	0.070600	.
site:sex	3	0.10348	0.034495	2.1065	0.101716	
species:age	1	0.00332	0.003317	0.2026	0.653280	
species:sex	1	0.09169	0.091692	5.5993	0.019220	*
Residuals	153	2.50548	0.016376			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Statistical outputs of models performed to analyse Pb concentrations ($\mu\text{g/g}$ dry mass) in kidneys of wood mice and bank voles captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter

```
>LM_K_Pb1<-
lm(log10(K_Pb+1)~site+species+age+sex+site:species+site:age+site:sex+species:age+species:
sex,
+           data=tab)
> summary(LM_K_Pb1)
```

```
Call:
lm(formula = log10(K_Pb + 1) ~ site + species + age + sex +
    site:species + site:age + site:sex + species:age +
    species:sex, data = tab)
```

```
Residuals:
    Min       1Q   Median       3Q      Max
-0.70262 -0.19842 -0.01748  0.17478  1.19805
```

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.204211   0.135578   8.882 1.67e-15 ***
siteM        -0.695187   0.174788  -3.977 0.000107 ***
siteL        -0.392419   0.161652  -2.428 0.016362 *
siteC        -0.672890   0.190921  -3.524 0.000560 ***
speciesMYGL  -0.042478   0.199920  -0.212 0.832018
ageJ         -0.040216   0.124453  -0.323 0.747026
sexM         0.057639   0.104304   0.553 0.581338
siteM:speciesMYGL -0.185182   0.298413  -0.621 0.535815
siteL:speciesMYGL -0.490208   0.271958  -1.803 0.073433 .
siteC:speciesMYGL -0.494368   0.236417  -2.091 0.038173 *
siteM:ageJ    0.114569   0.163757   0.700 0.485224
siteL:ageJ   -0.112250   0.163145  -0.688 0.492473
siteC:ageJ    0.009359   0.186350   0.050 0.960012
siteM:sexM   -0.073518   0.145077  -0.507 0.613057
siteL:sexM   -0.181438   0.137046  -1.324 0.187506
siteC:sexM   -0.114015   0.143872  -0.792 0.429310
speciesMYGL:ageJ  0.174739   0.238158   0.734 0.464247
speciesMYGL:sexM  0.304475   0.144849   2.102 0.037190 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.3048 on 153 degrees of freedom
Multiple R-squared:  0.543,    Adjusted R-squared:  0.4922
F-statistic: 10.69 on 17 and 153 DF,  p-value: < 2.2e-16
```

```
> anova(LM_K_Pb1)
Analysis of Variance Table
```

```
Response: log10(K_Pb + 1)
      Df Sum Sq Mean Sq F value Pr(>F)
site    3 14.9631  4.9877  53.6874 < 2e-16 ***
species 1  0.2480  0.2480   2.6692 0.10437
age     1  0.0752  0.0752   0.8090 0.36982
sex     1  0.0043  0.0043   0.0464 0.82981
site:species 3  0.5195  0.1732   1.8638 0.13807
site:age  3  0.3516  0.1172   1.2614 0.28971
site:sex  3  0.1841  0.0614   0.6607 0.57746
species:age 1  0.1323  0.1323   1.4238 0.23462
species:sex 1  0.4105  0.4105   4.4185 0.03719 *
Residuals 153 14.2141  0.0929
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Statistical outputs of models performed to analyse Cd concentrations ($\mu\text{g/g}$ dry mass) in the liver of wood mice captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter in function of sites, age and sex, and post-hoc comparisons

```
> APSY_L_Cd<-lm(log10(L_Cd+1)~site + age + sex, data=tabAPSY, na.action=na.omit)
> anova(APSY_L_Cd)
Analysis of Variance Table

Response: log10(L_Cd + 1)
      Df Sum Sq Mean Sq F value    Pr(>F)
site    3  5.1486  1.71619   25.398 3.814e-13 ***
age     1  2.4501  2.45013   36.260 1.479e-08 ***
sex     1  0.8967  0.89669   13.270 0.0003807 ***
Residuals 138  9.3250  0.06757
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(aov(APSY_L_Cd))
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = APSY_L_Cd)

$site
      diff          lwr          upr      p adj
M-H  0.08052051 -0.0778577  0.23889872 0.5504479
L-H -0.18008897 -0.3348090 -0.02536895 0.0154387
C-H -0.43975144 -0.6095042 -0.26999870 0.0000000
L-M -0.26060948 -0.4119616 -0.10925740 0.0000916
C-M -0.52027195 -0.6869608 -0.35358315 0.0000000
C-L -0.25966248 -0.4228795 -0.09644549 0.0003510

$age
      diff          lwr          upr      p adj
J-A -0.2947755 -0.3928026 -0.1967484      0

$sex
      diff          lwr          upr      p adj
M-F -0.155417 -0.2413816 -0.06945239 0.0004836
```

Statistical outputs of models performed to analyse Cd concentrations ($\mu\text{g/g}$ dry mass) in kidneys of wood mice captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter in function of sites, age and sex, and post-hoc comparisons

```
> APSY_K_Cd<-lm(log10(K_Cd+1)~site + age + sex, data=tabAPSY, na.action=na.omit)
> anova(APSY_K_Cd)
Analysis of Variance Table

Response: log10(K_Cd + 1)
      Df Sum Sq Mean Sq F value    Pr(>F)
site    3  7.8675   2.6225  29.449 8.847e-15 ***
age     1  3.5615   3.5615  39.994 3.304e-09 ***
sex     1  0.9268   0.9268  10.407 0.001568 **
Residuals 138 12.2890   0.0891
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(aov(APSY_K_Cd))
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = APSY_K_Cd)

$site
      diff          lwr          upr      p adj
M-H -0.02410663 -0.2059220  0.15770874 0.9858512
L-H -0.25512484 -0.4327407 -0.07750901 0.0015427
C-H -0.62220718 -0.8170803 -0.42733406 0.0000000
L-M -0.23101822 -0.4047677 -0.05726873 0.0039964
C-M -0.59810056 -0.7894563 -0.40674478 0.0000000
C-L -0.36708234 -0.5544525 -0.17971214 0.0000067

$age
      diff          lwr          upr p adj
J-A -0.3553964 -0.4679297 -0.242863    0

$sex
      diff          lwr          upr      p adj
M-F -0.1580017 -0.2566876 -0.05931591 0.0019037
```


Statistical outputs of models performed to analyse Pb concentrations ($\mu\text{g/g}$ dry mass) in the liver of wood mice captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter in function of sites, age and sex, and post-hoc comparisons

```
> APSY_L_Pb<-lm(log10(L_Pb+1)~site + age + sex, data=tabAPSY, na.action=na.omit)
> anova(APSY_L_Pb)
Analysis of Variance Table

Response: log10(L_Pb + 1)
      Df Sum Sq Mean Sq F value    Pr(>F)
site    3  0.66576  0.221921  13.1419 1.342e-07 ***
age     1  0.16264  0.162643   9.6315 0.002321 **
sex     1  0.00068  0.000684   0.0405 0.840802
Residuals 138  2.33035  0.016887
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> APSY_L_Pb<-lm(log10(L_Pb+1)~site + age, data=tabAPSY, na.action=na.omit)
> anova(APSY_L_Pb)
Analysis of Variance Table

Response: log10(L_Pb + 1)
      Df Sum Sq Mean Sq F value    Pr(>F)
site    3  0.66576  0.22192  13.2332 1.192e-07 ***
age     1  0.16264  0.16264   9.6984 0.00224 **
Residuals 139  2.33104  0.01677
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(aov(APSY_L_Pb))
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = APSY_L_Pb)

$site
      diff          lwr          upr      p adj
M-H -0.15322308 -0.23211634 -0.07432982 0.0000081
L-H -0.06564012 -0.14271112  0.01143088 0.1243084
C-H -0.17484346 -0.25940274 -0.09028419 0.0000019
L-M  0.08758296  0.01218965  0.16297628 0.0157072
C-M -0.02162038 -0.10465341  0.06141265 0.9056251
C-L -0.10920334 -0.19050695 -0.02789973 0.0035506

$sage
      diff          lwr          upr      p adj
J-A -0.07594767 -0.1247793 -0.02711604 0.0025341
```

Statistical outputs of models performed to analyse Pb concentrations ($\mu\text{g/g}$ dry mass) in kidneys of wood mice captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter in function of sites, age and sex, and post-hoc comparisons

```
> APSY_K_Pb<-lm(log10(K_Pb+1)~site + age + sex, data=tabAPSY, na.action=na.omit)
> anova(APSY_K_Pb)
Analysis of Variance Table

Response: log10(K_Pb + 1)
      Df Sum Sq Mean Sq F value Pr(>F)
site    3 11.2146   3.7382  41.5288 <2e-16 ***
age     1  0.0907   0.0907   1.0076  0.3172
sex     1  0.0735   0.0735   0.8170  0.3676
Residuals 138 12.4221   0.0900
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> APSY_K_Pb<-lm(log10(K_Pb+1)~site, data=tabAPSY, na.action=na.omit)
> anova(APSY_K_Pb)
Analysis of Variance Table

Response: log10(K_Pb + 1)
      Df Sum Sq Mean Sq F value Pr(>F)
site    3 11.215   3.7382  41.581 < 2.2e-16 ***
Residuals 140 12.586   0.0899
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(aov(APSY_K_Pb))
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = APSY_K_Pb)

$site
      diff      lwr      upr      p adj
M-H -0.65544486 -0.83809497 -0.47279475 0.0000000
L-H -0.55985858 -0.73828988 -0.38142728 0.0000000
C-H -0.72308554 -0.91885336 -0.52731772 0.0000000
L-M  0.09558628 -0.07896092  0.27013348 0.4866242
C-M -0.06764068 -0.25987501  0.12459364 0.7969343
C-L -0.16322696 -0.35145741  0.02500348 0.1138580
```

Statistical outputs of models performed to analyse Cd concentrations ($\mu\text{g/g}$ dry mass) in the liver of bank voles captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter in function of sites, age and sex

```
> MYGL_L_Cd<-lm(log10(L_Cd+1)~site + age + sex, data=tabMYGL, na.action=na.omit)
> anova(MYGL_L_Cd)
Analysis of Variance Table

Response: log10(L_Cd + 1)
      Df Sum Sq Mean Sq F value Pr(>F)
Site    3  0.9805  0.32684   1.8484 0.16937
age     1  0.5560  0.55597   3.1441 0.09071 .
sex     1  0.0105  0.01052   0.0595 0.80968
Residuals 21  3.7134  0.17683
---
Signif. codes:  0  '***'  0.001  '**'  0.01  '*'  0.05  '.'  0.1  ' '  1
```

Statistical outputs of models performed to analyse Cd concentrations ($\mu\text{g/g}$ dry mass) in kidneys of bank voles captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter in function of sites, age and sex

```
> MYGL_K_Cd<-lm(log10(K_Cd+1)~site + age + sex, data=tabMYGL, na.action=na.omit)
> anova(MYGL_K_Cd)
```

Analysis of Variance Table

Response: log10(K_Cd + 1)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	3	0.8692	0.289743	1.3281	0.2919
age	1	0.1250	0.125034	0.5731	0.4574
sex	1	0.0741	0.074098	0.3396	0.5662
Residuals	21	4.5815	0.218165		

Statistical outputs of models performed to analyse Pb concentrations ($\mu\text{g/g}$ dry mass) in the liver of bank voles captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter in function of sites, age and sex, and post-hoc comparisons

```
> MYGL_L_Pb<-lm(log10(L_Pb+1)~site + age + sex, data=tabMYGL, na.action=na.omit)
> anova(MYGL_L_Pb)
Analysis of Variance Table
```

```
Response: log10(L_Pb + 1)
      Df Sum Sq Mean Sq F value Pr(>F)
site   3 0.26035 0.086784  4.6682 0.01189 *
age    1 0.00320 0.003197  0.1720 0.68257
sex    1 0.09460 0.094598  5.0885 0.03488 *
Residuals 21 0.39040 0.018591
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> MYGL_L_Pb<-lm(log10(L_Pb+1)~site + sex, data=tabMYGL, na.action=na.omit)
> anova(MYGL_L_Pb)
Analysis of Variance Table
```

```
Response: log10(L_Pb + 1)
      Df Sum Sq Mean Sq F value Pr(>F)
site   3 0.26035 0.086784  4.8804 0.009471 **
sex    1 0.09699 0.096994  5.4546 0.029037 *
Residuals 22 0.39120 0.017782
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> TukeyHSD(aov(MYGL_L_Pb))
  Tukey multiple comparisons of means
    95% family-wise confidence level
```

```
Fit: aov(formula = MYGL_L_Pb)
```

```
$site
      diff          lwr          upr      p adj
M-H -0.16313302 -0.4249667  0.09870062 0.3327199
L-H -0.19266470 -0.4247554  0.03942602 0.1274111
C-H -0.28883803 -0.5026243 -0.07505176 0.0056374
L-M -0.02953168 -0.2616224  0.20255904 0.9844652
C-M -0.12570501 -0.3394913  0.08808126 0.3817658
C-L -0.09617333 -0.2722807  0.07993407 0.4450824
```

```
$sex
      diff          lwr          upr      p adj
M-F 0.1116722 0.004565155 0.2187792 0.0417405
```

Statistical outputs of models performed to analyse Pb concentrations ($\mu\text{g/g}$ dry mass) in kidneys of bank voles captured along a gradient of contamination (high, medium, low contamination and control site) from the former Metaleurop Nord smelter in function of sites, age and sex, and post-hoc comparisons

```
> MYGL_K_Pb<-lm(log10(K_Pb+1)~parcelle + age + sex, data=tabMYGL, na.action=na.omit)
> anova(MYGL_K_Pb)
Analysis of Variance Table

Response: log10(K_Pb + 1)
      Df Sum Sq Mean Sq F value    Pr(>F)
parcelle  3  3.6784  1.22614  11.5242 0.0001118 ***
age       1  0.1726  0.17258   1.6220 0.2167300
sex       1  0.3280  0.32797   3.0825 0.0937141 .
Residuals 21  2.2343  0.10640
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> MYGL_K_Pb<-lm(log10(K_Pb+1)~parcelle, data=tabMYGL, na.action=na.omit)
> anova(MYGL_K_Pb)
Analysis of Variance Table

Response: log10(K_Pb + 1)
      Df Sum Sq Mean Sq F value    Pr(>F)
parcelle  3  3.6784  1.22614  10.312 0.0001705 ***
Residuals 23  2.7349  0.11891
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(aov(MYGL_K_Pb))
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = MYGL_K_Pb)

$parcelle
      diff          lwr          upr       p adj
M-H -0.77381663 -1.4485750 -0.09905825 0.0205393
L-H -0.99760890 -1.5957183 -0.39949952 0.0006561
C-H -1.07597072 -1.6269086 -0.52503280 0.0000955
L-M -0.22379227 -0.8219017  0.37431711 0.7307654
C-M -0.30215408 -0.8530920  0.24878383 0.4436587
C-L -0.07836181 -0.5321994  0.37547576 0.9632346
```