TOOL WORKSHEET NO.16

Small mammals

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DESCRIPTION OF THE INDICATOR

Name of the indicator: Bioaccumulation of metallic elements by small mammals.

Ecological role of the organism under test: small mammals form a group of morphologically-similar small mammals but taxonomically and ecologically different. In France, we distinguish between 2 main sub-families within the Order of rodents (Rodentia): Arvicolinae, which include voles, and Murinae, which include rats, mice and field mice. Shrews also are small mammals from the Soricomorpha order (formerly Insectivora) and their diet is more carnivorous than rodents. Small mammals are globally found in all terrestrial ecosystems and some species are preys to a number of predators, especially birds of prey, all protected in France.

Type of indicator: Accumulation bioindicators: the analysis of internal concentrations in metallic trace elements (ETMs) is conducted in small mammals' target (kidneys) or accumulation (liver) organs.

DESCRIPTION OF THE METHOD

Reference standards and/or protocols

Sampling protocol and sample processing are published in Environmental Pollution (Fritsch et al. 2010) and in C. Fritsch's thesis:
http://tel.archives-ouvertes.fr/docs/00/52/45/16/PDF/ThA_se_C_Fritsch.pdf

Sampling plan and method: Sampling is conducted from standard methods also allowing for the measurement of the structure of ommunity and populations. Lines of 10 or 34 traps (mouse traps), 3 m from each other and baited, are displayed for 3 nights in a row in the habitats to be sampled and daily checked. Sampling can be done at the beginning of spring or at the end of autumn, the latter being generally the season with the highest trapping rate.

Sampling storage and pre-treatment: Trapped small mammals can immediately be dissected after catch or be frozen at -20°C for a few weeks or months. Species are for the most part easy to identify with the naked eye thanks to visible morphological criteria or with a manual magnifying glass for dental criteria. During dissection, the individual's genus is written down, the liver and kidneys are removed and dried (page 2) in a 60°C oven to a constant weight. After dissection, dosages can be conducted externally as a service provided by certified laboratories (~ 50 € without tax / sample for 6 metals).

In order to evaluate the relative age of rodents, the eyes are removed and placed for at least 2 weeks in a 2% formaldehyde solution. Lenses are subsequently extracted with caution using narrow pliers and then dried in a 45°C oven to a constant weight. Their dry weight rounded down to 1/10th of mg gives an estimate of the individuals' age.

Simplified description of the measurement method: Standard equipment: mousetraps, bait (mix of peanut butter and flour), small resealable plastic bags, ranging poles, scales (precision: 0.1 mg), narrow pliers and dissection scissors, oven for drying and digestion of samples, 50 ml tubes, acid of analytical grade for digestion of tissues. Specific equipment: for analyses of ETMs after acid digestion; SAA, ICP (MS or OES).

Estimated time: Trapping: 4 days; preparation and analyses about 6 days for 50 individuals = dissection 15 mn per individual; dry-freezing or oven-drying 24 to 48h; mineralisation 24h; dosage ½ day with calibration of the machine (for metals).

Measured parameters

Concentrations of ETMs (mg/kg dry mass) in liver and/or kidneys.

INTERPRETATION OF RESULTS

Need for a global reference system using a database

The publication of a number of studies conducted on various species provides ETM concentrations measured on small mammals from non contaminated sites (table 1). These values can serve as a reference to use internal concentrations measured on a given site as a transfer indicator that integrates exposure and bioavailability.

Species	ETMs	Concentrations (mg/kg MS)	References
Wood mouse	As	0.03 – 1.75	1,2,3,4,5,6,7,
(sub-family Murinae)	Cd	0.26 – 3.87	1,3,4,8,9,10,11
	Pb	0.07 – 9	1,3,4,8,9,10,11
Field vole	As	0.03 – 1.8	1,2
(sub-family Arvicolinae)	Cd	0.2 – 7.8	1,11,12,13
	Pb	0.54 – 6.1	1,11,12,13

Table1: Bibliographic summary of hepatic concentrations (minimum and maximum values) in ETMs measured on the wood mouse and the field vole in non-contaminated sites.

[1] Sheffield *et al.* 2001 In Shore & Rattner ; [2] Erry *et al.* 2005 ; [3] Tersago *et al.* 2004 ; [4] Rogival *et al.* 2007 ; [5] Ismail & Roberts 1992 ; [6] Erry *et al.* 1999; [7] Erry *et al.* 2000 ; [8] Talmage et Walton 1991 ; [9] Fritsch *et al.* 2010 ; [10] Beernaert *et al.* 2007 ; [11] Sanchez-Chardi *et al.* 2007 ; [12] Ainsworth et Cooke 1991 ; [13] Beardsley *et al.* 1978

Furthermore, the existence of critical internal concentrations for specific elements (Cd, Pb…) in target tissues enables a toxicological interpretation of measured concentrations and a risk assessment for small mammals species under study (table 2).

Element	Organ	Critical concentrations listed in literature	
		Lowest value	Median value
Cd	Liver	0.9	15
	Kidney	3.5	112
Pb	Kidney	25	51

Table 2: Example of critical internal concentrations in Cd and Pb (mg/kg MS) in micromammals' livers and kidneys (based on Wijjnhoven et al. 2008).

Database availability/access

The benchmarks reported in tables 1 and 2 come from scientific articles published in international journals (see references cited)

Necessary supplementary information (ex: climate, use, type of soil…)

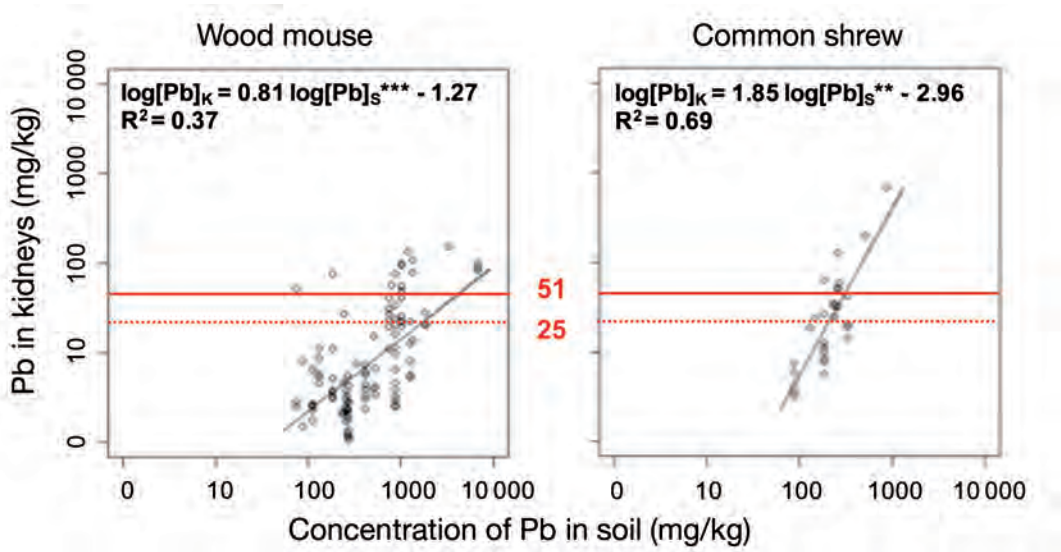
The age of individuals is important to consider as it influences internal concentrations measured in small mammals' tissues. Besides, some features of sampled habitats can help interpret transfer data (Fritsch 2010, Fritsch et al. 2011a).

EXAMPLE OF APPLICATION

Case of the former Metaleurop smelter (STARTT - ADEME/ANR program)

Liver and kidney concentrations in Cd and Pb have been measured on wood mouse and common shrew populations along a gradient of Cd and Pb pollution close to the former foundry of Metaleurop (Nord-Pas de Calais,, Northern France). In all cases, internal concentrations increase with the level of contamination of soils. Figure 1 shows results for Pb dosed in the kidneys of 2 small mammals species. The age of individuals and landscape composition near catching sites are complementary factors explaining variations of internal concentrations (Fritsch et al. 2011a).

Figure 1 : Internal concentrations of Pb in 2 small mammal species caught along a gradient of soil pollution close to the former Metaleurop smelter (based on Fritsch et al. 2010). Red lines represent critical internal concentrations for Pb in small mammals' kidneys (see table 2: lowest value (25 mg/kg) and median (51 mg/kg)).



Case of the Auzon brownfield (Bioindicateurs II - ADEME program)

The Auzon brownfield shows specifically-localised arsenic contamination of soils. Standardised rodent sampling, especially field mice (*Apodemus* sp.), has been conducted on 3 sites along a gradient of As pollution, a highly contaminated site (site F), a moderately contaminated site (site M) and a control site (site C) without proven contamination. Relative densities of field mice are statistically higher in site F than in site C, which suggests that this endpoint is not a relevant indicator in biomonitoring, as it is probably more strongly influenced by both structure and quality of habitat than by soil contamination.

Here too, the measurement of hepatic concentrations is relevant to discriminate between sites depending on their level of contamination (figure 2). In sector C, no individual exceeds the maximum concentration listed for the soil of a non contaminated site, i.e. 1.75 mg/kg (table 1). In sector M, a single individual exceeds this value whereas close to 25% of field mice caught in sector F exceed this reference.

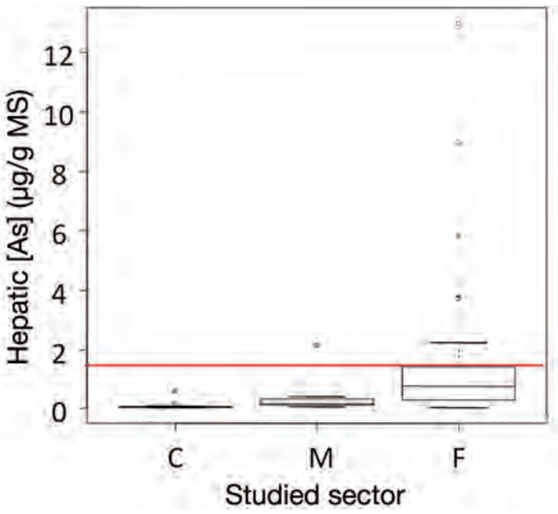


Figure 2: Hepatic As concentrations in caught field mice in studied sectors of the Auzon site. The red line indicates a concentration of 1.75 mg/kg which is the highest value listed in literature for a non contaminated site.

INTERESTS AND LIMITS OF THE INDICATOR

The use of small mammals has recently prompted a surge of interest within the field of biomonitoring. Some of the traits that make small mammals potential bioindicators include:

- the abundance of some ubiquitous species (wood mice…) or more locally of some shrews.
- their large home range (from tens to hundreds of m^2 depending on species) compared to invertebrates and their low migration. Therefore, mobile species such as wood mice integrate environmental contamination at a large yet local scale.
- Murinae have an omnivorous diet, they are good integrators of contamination from different compartments of their environment. As predators, shrews are relevant bioindicators for high biomagnification potential substances.
- the use of some species (rat and mouse) as models in toxicology, pharmacology and medicine implies that (i) their farming and experiments under controlled conditions are conductible routinely; (ii) their physiology is globally well known.
- the possibility to use ETM concentrations in small mammals' whole bodies to evaluate risks for their predators.

Currently, the proposed methods require the sacrifice of animals. Non-lethal and little-invasive investigation methods (concentrations of pollutants in hair or blood, blood biochemistry, body condition index…) are subject to current developments which are not yet fully operational. Other individual responses (biomarkers…) and/or ecological (structure of populations and communities) must be tested and/or validated in order for their routinely use in biomonitoring to be considered (Sheffield et al. 2001, Fritsch 2010, Fritsch et al. 2011b). Furthermore, interpretation frames of reference for internal concentrations need to be more precisely redefined.

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