TOOL WORKSHEET **NO.1**

Snails

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DESCRIPTION OF THE INDICATOR

Name of the indicator: Snails, bioindicators of bioavailability of contaminants on-site. SET index : Sum of Excess of Transfers

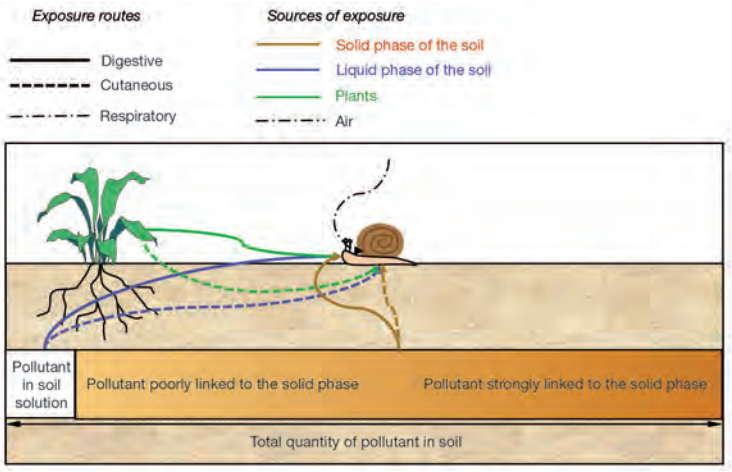


Figure 1 : Snails' exposure in the terrestrial ecosystem (from Scheifler, 2002).

Ecological role of the organism under test: Snails are macroinvertebrates living at the interface soil, plants and air. Those pulmonate gastropod molluscs are phytophagous and saprophagous (trophic level of primary consumers and detritivorous). They ingest vegetation and soil, crawl on the ground where they lay their eggs. Therefore, they integrate multiple sources and routes of contamination (see Fig. 1). Snails participate in exchanges with soil and are preyed upon by various consumers (invertebrates: glow-worms, ground beetle larvae, or vertebrates: birds, small mammals such as shrews, hedgehogs and humans).

The *Helix aspersa* or *Cantareus aspersus* species, nicknamed Petit Gris in French, or garden snail (photo here above), is widely distributed across the world[1]. Since farming is possible (see Annex in ISO 15952), snails with a known biological past can be used on the field to analyse the bioavailability of contaminants present in habitats (soil, plants, air) by measuring their accumulation in organisms caged for a determined period of time.

Type of indicator: Accumulation bioindicators: the analysis of internal concentrations is done in the viscera of snails exposed for 28 days on the site (= Static method[2]: 1 single exposure duration). Analyses are generally done in this part of their body which often concentrates contaminants (especially metals; see Gomot de Vaufleury & Pihan, ETC, 2002).

DESCRIPTION OF THE METHOD

Reference standards and/or protocols

In situ active biomonitoring: microcosms (25 cm in diameter x 25 cm in height stainless steel cylinders covered by a 0.5 or 1 cm mesh netting, maintained by 3 to 4 stainless steel pickets) are placed in the areas under study (Fig. 2).
Fifteen sub-adult (5-6 g) garden snails, farmed for the purpose of the project (see ISO 15952 Annex) or by local farmers, are placed in each microcosm. From the end of their breeding to their placement on the ground, they can be stored in dry wooden boxes (such as camembert boxes). They are humidified for revival a few hours before they are placed in microcosms where they will be exposed to soil as well as plants that have grown on-site and ambient air in order to be under natural exposure conditions (climate hazards).

1 For more information, ISO 15952 (2006) standard or website http://inpn.mnhn.fr/espece/cd_nom/199863/tab/taxo.

2 A kinetics study for contaminant assimilation modelling can also be undertaken: see http://www2.ademe.fr/servlet/KBaseShow?sort=-1&cid=96&m=3&catid=10143

Figure 2 : In situ exposure: Active biomonitoring using microcosms where snails are exposed (from left to right: sub-adult snail, total fresh mass 5-6 g, shell diameter: 25.6 mm (min/max 21.5-29.9 mm)); open microcosm; microcosms covered by a stainless steel netting (mesh size: 10 mm) securely fitted over the top of the microcosm by 4 pickets; microcosms on site).

Internal contaminant concentrations are measured:

- At t0: 6 snails are used for the analysis of contaminant concentration before exposure (the median values of As, Cd, Cr, Cu, Mo, Ni, Pb, Sb, Zn concentrations in snails used for the Bioindicateurs 2 program were respectively 0.32-0.90-2.61-105-2.9-3.15-0.72-0.027-872 mg.kg-1 DW). For AQ calculations, if the CIRef is not available, it is possible to use concentrations of farmed snails at t0 instead.
- After 28 days, for a static study of transfers: 6 snails are collected after their exposure on site in order to analyse contaminants (metals for ex.). The remaining 9 snails (if no mortality occurred) can be used to analyse other contaminants.

Sampling plan and method: Exposures must occur to avoid extrems climatics conditions (frost, drought…). Once on the field, about 20 minutes are needed to set up a microcosm, place the snails, the pieces of tiles used as shelters (Fig. 2) and finally the netting and pickets.

Sampling storage and pre-treatment: After exposure, the collected snails are weighed before they are brought back to the laboratory for a fasting period of 48 hours. During the fast, feces are removed every 24 hours. Snails are then freezed at -80°C. After defrosting, the soft body is removed from the shell; the viscera and foot (Fig. 2) are separated and then dried in the oven at 60°C to a constant weight. For the analyses of organic contaminants, tissues are freeze-dried.

Simplified description of the measurement method: General equipment: snails; wooden storage boxes; microcosms (with netting and pickets); scales, plastic boxes for fasting; elastic strips; oven for drying and then mineralisation of samples, 50 ml tubes; acid for tissue mineralisation or solvents for the extraction of organic pollutants. Specific equipment: for analyses of metal elements after acid mineralisation; SAA or multi-elements, ICP (MS or AES). For organic analyses: CG-ECD, HPLCMS/MS.

Estimated time: exposure 28 days; fasting 2 d; preparation and analyses approximately 3 d (dissection 10 min for 5 snails; freeze-drying or oven-drying 24 to 48 h; digestion 24 d; dosage ½ day with calibration of the apparatus for the analysis of metals).

Measured parameters:

- Metal element concentrations (mg.kg^{-1} in dry mass) in viscera.
- Calculation of Accumulation Quotient per metal (AQ) = ratio of measured concentrations and internal concentrations of reference (CIRef see here below); if CIRefs are not available for the contaminant under study, you can refer to the concentration of the contaminant in snails before exposure on the ground. A AQ higher than 1 characterises an excessive transfer of the contaminant under observation.

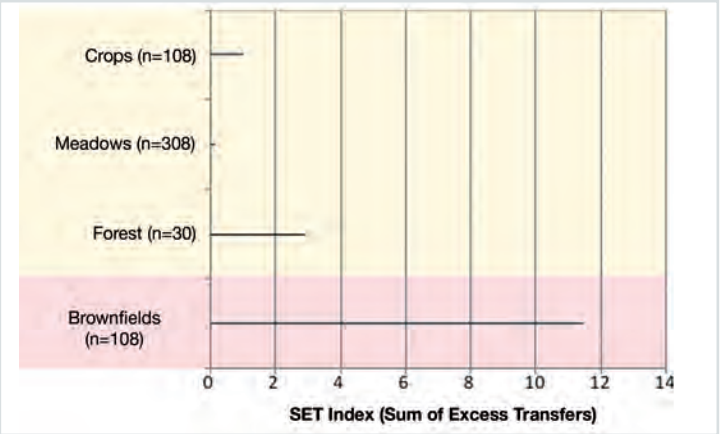


Figure 3 : Range of variation of the SET Index modality on the sites of Bioindicateurs 2 program.

- Calculation of the Sums of Excess Transfers[1] of metals per modality ($SET_{modality}$) = sum of AQs - 1 (= AQ metal1 - 1 + AQ metal2 -1 +…). See Fig. 3. When SET > 0 it characterises an excessive transfer of at least one of the metals.
- Calculation of the Index SET_{site} = (∑ SET modality)/ number of modalities on-site.

INTERPRETATION OF RESULTS

Need for a global reference system using a database

Internal concentrations of reference, determined in the viscera of snails exposed for 28 days on sites not contaminated by metal elements, are available[1] (CIRef, Table 1).

[mg.kg^{-1} DW]	As	Cd	Co	Cr	Cu	Hg	Mo	Ni	Pb	Tl	Zn
CIRef snail	0.307	2.27	4.68	2.01	184.7	0.19	4.40	5.17	12.9	0.25	1490
"Soil threshold"*	25	0.45	23	90	20	0.10	1.59	60	62	1.7	100

Table 1 : Values of internal concentrations of reference established on the basis of measurements made in snails exposed to non-contaminated soils of the Bioindicateurs 2 program. The "soil threshold" concentrations refer to "normal" values on soils from RMQS or ASPTITET data (Baize et al, 2007, Villaneau et al., 2008).

Database availability/access

CIRef values are available for the metal elements of table 1.

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

None. However, the following informations can help data interpretation: soil contamination and parameters influencing transfers (ex: pH; CEC; texture etc (Pauget et al. 2012 and thesis https://chrono-environnement.univ-fcomte.fr/IMG/pdf/These_B_PAUGET.pdf)), extreme climatic conditions that can influence snails' activities under exposure; presence of shadow; mortality; possibly the abundance and nature of plants in microcosms.

EXAMPLE OF APPLICATION

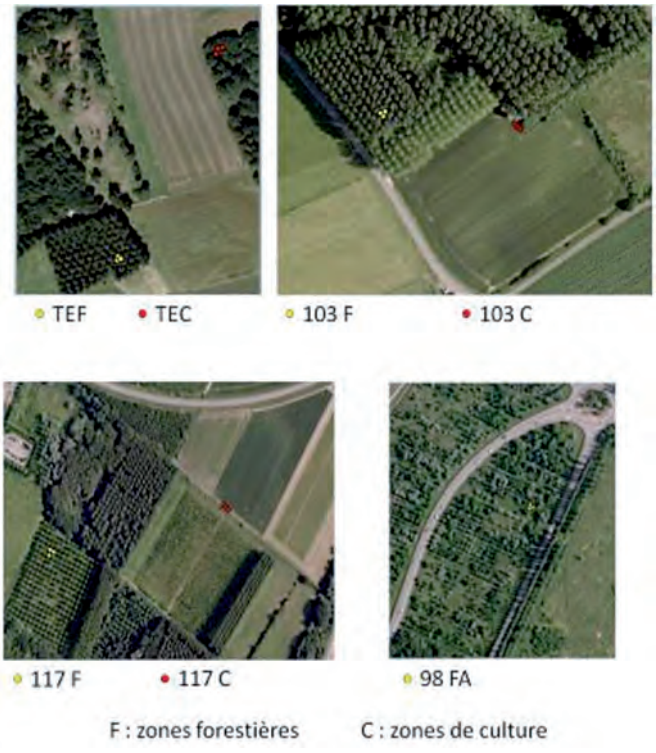
Metaleurop site: 7 modalities under study on this former metallurgical site.

3 microcosms 6 analysed snails (2/microcosm) per modality (Table 2).

Results :

The most contaminated site (98FA with a pH_{water} of 8 and approximately 8% organic matter) with a forest use (wood planted about 30 years ago) shows high bioavailability of contaminants with a SET Index value of 11.47 (Table 3).

However, the ranking of sites according to the SET Indexes does not always follow the soil metal concentration gradient.



1 Pauget, B., Gimbert, F., Coeurdassier, M., Crini, N., Pérès, G., Faure, O., Douay, F., Hitmi, A., Beguiristain, T., Alaphilippe, A., Guernion, M., Houot, S., Legras, M., Vian, J.-F., Hedde, M., Bispo, A., Grand, C., de Vaufleury, A., 2013. Ranking field site management priorities according to their metal transfer to snails. Ecol. Indic. 29, 445–454.

Modality	As	Cd	Cr	Pb	Cu	Zn
	(Soil concentrations in mg.kg^{-1})					
98F	39	34.4	48.6	2485	68	1885
117F	30	13.2	50.3	730	27	745
117C	18	8.5	61.5	481	28	508
103F	9	5.4	51.9	319	19	331
103C	8	3.1	49.4	142	15	226
TEF	7	1.1	41.5	48	12	101
TEC	8	0.9	45.1	41	14	91

Tableau 2 : Arsenic, cadmium, chrome, lead, copper and zinc concentrations of Metaleurop soils.

The control forest site (TEF) shows a high transfer of Cd (which could not have been drawn from its total content in soils), while highly-contaminated modalities (117C and F with 8.2 pHs, among the highest of the site) show limited transfers with a SET value even inferior to that of in-site control areas (TEF and TEC).

Modality	Concentration in snail (mg.kg^{-1} DW)						CIRef (mg.kg^{-1} DW)						Accumulation Quotient (AQ)						SET modality
	As	Cd	Cr	Pb	Cu	Zn	As	Cd	Cr	Pb	Cu	Zn	As	Cd	Cr	Pb	Cu	Zn	
98FA	0.241	10.9	0.03	112	168	1181							1.00	**4.80**	1.00	**8.67**	1.00	1.00	**11.47**
117F	0.196	6.03	0.213	34.8	136	993							1.00	**2.66**	1.00	**2.69**	1.00	1.00	**3.35**
117C	0.237	1.76	0.581	14.3	141	916							1.00	1.00	1.00	**1.11**	1.00	1.00	**0.11**
103F	0.25	9.94	0.588	48.7	153	1304	0.31	2.27	2.01	12.9	185	1490	1.00	**4.38**	1.00	**3.78**	1.00	1.00	**6.15**
103C	0.301	4.73	0.313	61.4	161	1599							1.00	**2.53**	1.00	**4.75**	1.00	**1.07**	**5.36**
TEF	0.369	8.24	0.03	13.8	136	1651							**1.20**	**3.63**	1.00	**1.07**	1.00	**1.11**	**3.01**
TEC	0.383	2.3	0.03	9.95	106	887							**1.25**	**1.15**	1.00	1.00	1.00	1.00	**0.40**

Table 3: Calculation of indexes Sum of Excess of Transfer ($SET_{modality}$) on the basis of accumulation quotients (AQ) established by comparing reference internal concentrations (CIRef) with internal concentrations of snails exposed for 28 days on the 7 modalities.

The analysis of bioaccumulation in snails therefore reveals variable bioavailability of metal elements in the plots under study, which is not always possible to do on the sole basis of soil total concentrations and physico-chemical characteristics such as pH, organic matter content for example.

INTERESTS AND LIMITS OF THE INDICATOR

+ Integrates all factors modulating bioavailability of soil metal contaminants for snails.
+ Brings information on the exposure of consumers of higher level (direct exposure and exposure through food).
+ Snail farms are active in France and Europe, which can help getting sub-adult snails.
– CIRefs are currently only available for 14 metal contaminants.
– Allows for the measurement of non-degraded contaminants accumulation only.
– The analysis after only one exposure period can give a partial vision of accumulation because we do not know whether internal concentrations have reached their point of equilibrium or not (a kinetics study is more precise but also more costly).

UMR Chrono-Environnement in Besançon; other laboratories such as UMR BIOGECO (M Mench) have already used microcosms.

PUBLICATIONS : ***Scheifler et al., Env Poll 2003 ; Gimbert et al., Env Poll 2008 ; Fritsch et al, Ecotoxicol. 2011 ; de Vaufleury et al. 2011 ; Pauget et al, 2013a and b, Ecol.Indic.***

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