TOOL WORKSHEET NO.3

Plant communities

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DESCRIPTION OF THE INDICAT0R

Name of the indicator: Foliar content in trace elements (TEs) in plant communities, contaminant phytoavailability indicator, Phytomet index.

Ecological role of the organism under test: Herbaceous and woody plants form communities of sessile organisms whose mineral nutrition, and hence content in TEs, strongly depends on the properties of soils on which they grow. As primary producers, plants are also an essential link in trophic chains, and thus represent a potential transfer route of contaminants towards primary and secondary consumers.

On non-contaminated soils, metal (Cd, Cr, Cu, Ni, Pb, Zn) and metalloid (As) concentrations measured on plants' leaves vary depending on species and elements under examination, but they always remain relatively low, within a well-defined range. On contaminated sites on the other hand (Fig. 1), measured foliar concentrations can reach "unusually" high values, if contaminants are easily available for plants. Foliar metal element content of a site's vegetation therefore tells us not only about the presence of contaminants, but above all about their phytoavailability, i.e. their ability to move within the soil's matrix to reach a biological target (vegetation "compartment").

Figure 1: Plant communities growing on contaminated sites show foliar metal concentrations that shed light on the phytoavailability of trace elements.

Type of indicator : Accumulation bioindicators. Foliar metal concentrations on a range of species from the same plant community are considered to be representative of the phytoavailability of metals on the site. The measured values enable us, for the elements under consideration, to calculate the excess of metal charge of a plant community under exposure, and thus to evaluate the phytoavailability of each contaminant. A single indicator, the PhytoMet index, which integrates the phytoavailability and potential toxicity of main ETMs, can also be calculated and makes it possible to clearly discriminate between multicontaminated sites in relation to risks of transfers towards primary consumers.

DESCRIPTION OF THE METHOD

Reference standards and/or protocols

Reference protocol was defined in the Bioindicateurs II program. Composite samples, representative of the plant community in place, are prepared by collecting leaves on the most abundant species observed on the site under study (Fig. 2). Each composite sample is then mineralised and analysed by atomic emission spectrometry, in order to determine their content in TEs (As, Cd, Cr, Cu, Ni, Pb and Zn). The comparison between obtained values and reference values, determined on non-contaminated soils, allows us to calculate the PhytoMet index.

Sampling design and method: Sampling is undertaken at vegetation optimum, i.e. in June-July. In order to build composite samples ("pools"), 10 to 50 g of fresh matter (leaves for woody plants, leaves and possibly chlorophyllian stems for herbaceous plants) are collected on three to five different species grown within the same area (approximately 10 to 100 m^2 depending on specific richness and vegetation cover). In general, five composite samples are collected on total surfaces from 100 to 1000 m^2 (in each corner and in the middle, if possible by using mixes of different species between each replicate), but the number of samples can be raised depending on the surface and on observed heterogeneity of the area under study. Site prospection and sample collection takes two to three hours (at the research engineer level) and requires no specific knowledge in botany (taxonomic recognition of species is not necessary).

Figure 2 : Collection, mineralisation and analysis of plant samples

Sampling storage and pre-treatment: After being collected, samples of each pool-constitutive species are thoroughly rinsed in running water, dried until complete dehydration (in the open air or a 40°C oven), before they are individually grinded (<2 mm). Composite sample ("pool") is then prepared by mixing 100 mg (DW) of each pool-constitutive species. Each composite sample is then mineralised by acid attack. For the analysis of one site, total preparation time for five composite samples amount to approximately two working days (at the technician level).

Simplified description of the measurement method: The analyses of elemental composition of mineralised samples are undertaken by atomic emission spectrometry (ICP-AES). About a hundred samples can be analysed in a day of work (at the technician level).

Based on analytical data, determination of the PhytoMet index is based on a comparison between distributions of contents in metal elements on samples from the studied site with those measured in control samples, collected on non-contaminated sites (data available in the Bioindicateurs II program database; see below).

For each analysed element, the parameters to take into considering are (figure 3):

- Distribution median on control sites, MedT.
- Distribution upper whisker on control sites (3rd quartile + 1.5 * interquartile distance), V.
- Distribution median observed on control site, MedObs.
- Frequency of outliers (values > whisker) in the observed distribution, in relation to control distribution, FreqOut (for ex. in the case study presented on Fig. 2, four out of five measurements are higher than the whisker, FreqOut = 4/5).

Based on this data, the calculation of PhytoMet index takes four steps:

- **Step 1 :** calculate for each metal(loid) relative deviation (DRMet), in absolute value, from the median observed on site (MedObs) to the median on control site (MedT).

DRMet = ABS(MedObs – MedT) / MedT

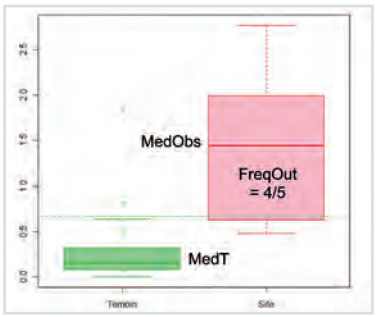

Figure 3: Example of foliar content in Cd distribution within plant communities grown on non-contaminated site (Test) and studied site (Site). The dotted line depicts the distribution upper whisker on test site.

- **Step 2:** correct calculated relative deviations by taking into consideration the frequency of outliers (FreqOut) on the observed distributions (enables to account for the number of abnormal values and to keep the index at 0 when observed deviations in relation to the control median are within the range of values observed on non-contaminated sites).

  DRMet/Cor = DRMet X FreqOut

  (Note: DRMet/Cors represent, for each metal, the excessive metal charge within the studied plant community. They provide information on individual phytoavailability of metals in comparison with test situations, on non-contaminated sites.)

- **Step 3:** calculate the risk indicator (IRMet) associated with each element, by multiplying each DRMet/Cor by a constant characteristic of potential toxicity for each metal (kMet).

  IRMet = DRMet/Cor X kMet

  The selected kMets are those used for calculation of the Metox index used by water agencies to evaluate aquatic contaminations (but other constants can be chosen depending on suspected targets):

  kAs = 10; kCd = 50; kCr = 1; kCu = 5; kNi = 5; kPb = 10; kZn = 1

- **Step 4 :** calculate the site vegetation PhytoMet index.

PhytoMet = Σ IRMet

INTERPRETATION OF RESULTS

Need for a global reference system using a database

The calculation of "excessive metal charge" and PhytoMet index for contaminated sites requires knowledge of "usual" distribution parameters of TEs foliar content, for control situations, on non-contaminated sites. It is therefore necessary to have a database as accurate as possible, which brings these values together for analysed metals.

Database availability/access

A database was started during the Bioindicateurs II program. For instance, figure 4 shows the PhytoMet index variation range for various soil use types studied in the Bioindicateurs II program.

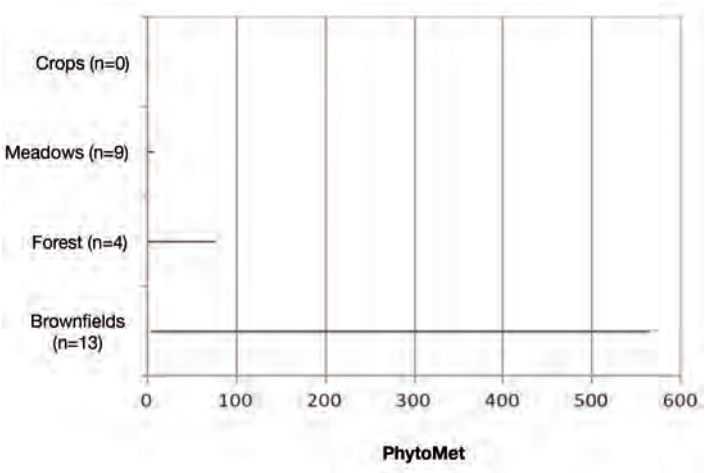

Figure 4: PhytoMet index variation range on Bioindicateurs II program sites

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

No additional information is necessary.

EXAMPLE OF APPLICATION

The comparison of four standard situations, studied during the Bioindicateurs II program, highlights the interest of the PhytoMet index. Presented examples refer to plots contaminated in various ways by TEs, located on the workshop sites of Metaleurop (plot MeTeF), GISFI (plot GHM), St Etienne (StEtA) and Auzon (AuzFCo). Table 1 shows total soil concentrations in TEs from the various plots and compares them with concentration limits observed on non-contaminated sites (RMQS whiskers).

Metal	RMQS Whisker	Plot under study			
		MeTeF	GHM	AuzFco	StEtA
As	60	7,1	58,5	1087	73,3
Cd	0,67	1,1	1,2	6,7	21,0
Cr	116	41,5	171,5	52,3	982
Cu	42,7	12,3	45,3	139,8	1555
Ni	61,5	13,7	26,9	25,7	685
Pb	62,3	48,8	309,0	1834	2525
Zn	161	101,7	323,0	173,2	2830

Table 1: Soil total content (mg/kg-1) in TEs for four contaminated plots in relation to RMQS whiskers. Values in green (<RMQS whisker) are considered non-abnormal, values in red (>RMQS whisker) are considered abnormal.

The study of table I shows that the MeTeF plot is the least contaminated and only shows a slight Cd anomaly. However, the GHM and AuzFCo plots are moderately to highly contaminated, with strong anomalies for five of the seven analysed metals. The most contaminated plot is StEtA, for which all analysed metals show highly anomalous concentrations. Therefore, soil analyses tend to show that the MeTeF plot presents a lesser potential hazard than the StEtA plot, regarding biological receivers likely to be exposed; the other two plots (GHM and AuzFCo) present intermediary potential hazards.

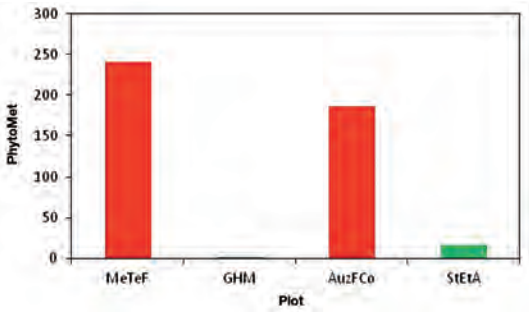

Figure 5 : PhytoMet indexes of four contaminated plots of the Bioindicateurs II program

However, the question remains whether metals identified as anomalous are indeed bioavailable and therefore likely to interact with living organisms. The calculation of the PhytoMet index (Figure 5) brings some answers, at least regarding vegetation compartment. In fact, quite unexpectedly, final results show that the MeTeF plot, even though it is the least contaminated, presents the highest index. Conversely, the GHM and StEtA plots, which are very contaminated, present very low or nil indexes. As for the AuzFCo plot, it shows a high index, but still lower than in MeTeF.

These results suggest that, despite very low contamination, the MeTeF plot presents very important Cd phytoavailability. Given the high toxicity of this metal, the risk associated with its transfer to plants is high, as shown by the high value of the PhytoMet index. Conversely, despite very important multimetal contaminations, metals present on the GHM and StEtA plots show very little phytoavailability, and risks associated with their transfer to plants and primary consumers are very small.

INTERESTS AND LIMITS OF THE INDICATOR

+ Brings information on contaminant phytoavailability (very hard to predict on chemical extractions).
+ Brings information on the exposure of higher consumers and associated risks.
– The database of reference values only provides information on a limited number of contaminants (As, Cd, Cr, Cu, Ni, Pb, Zn).
– Does not provide information on the bioavailability of organic contaminants.

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