TOOL WORKSHEET NO.2

Omega-3 fatty acid

M. Le Guédard, J.-J. Bessoule / LEB Aquitaine Tranfert / ADERA ;
UMR CNRS 5200, Villenave d'Ornon
Contact : **marina.leguedard@u-bordeaux2.fr**

DESCRIPTION OF THE INDICATOR

Name of the indicator: Omega-3 fatty acid: plant fatty acids, diagnostic and surveillance tool for soil contamination.

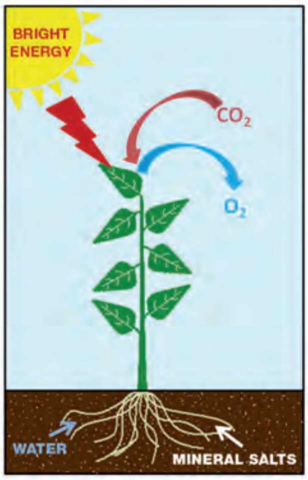

Figure 1

Ecological role of the organism under test: In the terrestrial ecosystem, plants are primary producers because they are completely independent from the rest of biosphere for their carbon and energy supplies. They create their own organic matter through photosynthesis only by using light and mineral matter (water, CO_2 and mineral salts, Fig 1). Photosynthesis reaction enables plant growth and results in the release of oxygen, essential to our lives.

In addition to their role as oxygen producers, plants also bring organic matter, used as a necessary source of energy and carbon for other trophic levels, thus forming the first link in the food chain. They are also used as shelters and food by fauna. Moreover, they bring elements required by soil bacteria that participate in the improvement of soil fertility. Therefore, they ensure the sustainability of terrestrial ecosystems. Furthermore, through their root systems, plants aerate, build and protect the soil by limiting erosion and crusting.

Type of indicator: The Omega-3 fatty acid indicator is a biochemical biomarker that highlights the exposure of plants to various soil contaminants (metals and organic). More precisely, this indicator shows the state of degradation of chloroplastic lipids exposed to soil contaminants. In fact, chloroplasts (Fig 2) contained in higher plants' leaf cells ensure chloroplastic membrane integrity and play a very important role in photosynthetic activity. Linolenic acid (C18:3) is a fatty acid mostly associated with chloroplastic lipids. Chloroplasts contain up to 90% of this Omega-3 fatty acid. Lipid degradation driven by the presence of contaminant(s) is evaluated by measuring the fatty acid content of plants' leaves and by calculating the Omega-3 fatty acid indicator, reported as the ratio between C18:3 content and other 18 carbon atoms-fatty acids. This indicator decreases in the presence of contaminants (Fig 3).

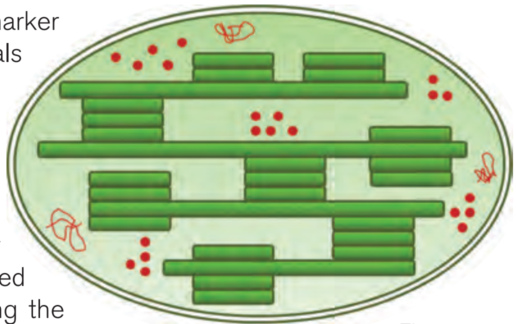

Figure 2

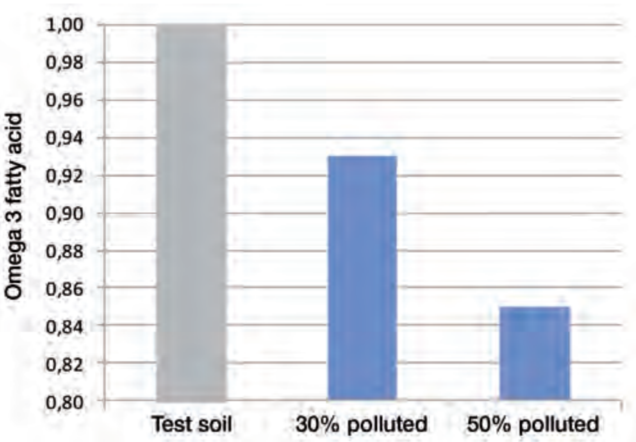

Figure 3

The Omega-3 fatty acid indicator can be used at the laboratory (AFNOR XP-X31 233 standard) or on the field. It allows for a global appreciation of the state of health of the ecosystem, an evaluation integrated over time and space of phytoavailable pollutants as well as an early detection of contaminants' effects. Measured effects on plants are even observed with contaminants present at "sub-acute" doses, which alter neither plant germination nor plant growth, and which even do not lead to any particular "visual" phenotype (chlorosis, yellowing of the leaves…).

DESCRIPTION OF THE METHOD

Figure 4

Reference standards and/or protocols: Analysis method standard: AFNOR XP-X31 233 - Soil quality - Effects of contaminated soil on foliar fatty acid content of Lactuca sativa.

Sampling plan and method: Sampling is easy to undertake by non-specialised staff and can take from ½ day up to one day depending on the number of modalities (i.e. lots) to be studied. The various modalities under study are prospected and 4 to 6 species, to the extent possible, common to all modalities are identified. It is important when sampling to avoid collecting plants under hydric (drought) or biotic (pathogens) stress.

A piece of leaf (25mm²) from each identified plant species is cut with scissors (Fig 4) and collected foliar tissues are placed in glass tubes containing 1 ml of methanol and 2.5% of sulphuric acid (Fig 5). If the leaf is wet because of dew or rain, it needs to be dried before placing in the tube.

Figure 5

Sampling storage and pre-treatment: The samples can be stored for several days in methanol / sulfuric acid (2.5%) at room temperature prior analysis.

Simplified description of the measurement method: Providing that gas chromatography is available (approximately 30.000 €), the method is cheap, quick (2 days) and easy to implement by non-specialised staff.

Samples are heated 1 hour at 80°C (Fig 6). Then, tubes are quickly chilled in ice and, after adding 0.75 ml of hexane and 1.5 ml of water, they are stirred and centrifuged. The hexane phase (green superior phase, Fig 7) is subsequently collected, and methyl esters are analysed using gas chromatography (Fig 8).

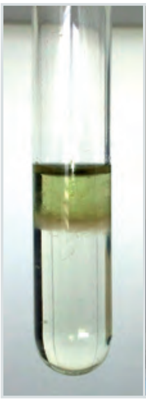

Figure 7

Figure 8

Figure 6

Measured parameters:

The percentages of each fatty acid are then determined for each plant and the ratio "(C18:3)/(C18:0 +C18:1 + C18:2)" is calculated. This ratio is normalised to 1 for all species. To this end, the ratio values of individuals from a specific species are divided by the highest individual value achieved by this same species on the whole site (all modalities taken together). To determine if the observed differences between modalities are significant, statistical tests are undertaken. If distribution follows a normal rule then a t-test (2 modalities) or an ANOVA (k modalities) are run. If distribution does not follow a normal rule, non-parametric tests are applied (Mann-Whitney (2 modalities) or Kruskal-Wallis (k modalities) followed, if necessary, by Tukey's 2 by 2 comparison test).

INTERPRETATION OF RESULTS

Need for a local reference system:

The Omega-3 fatty acid indicator varies between 0 and 1, 1 showing the modality with the fewest effects. Data received are interpreted in relation to a reference situation chosen for the studied site. If, in the best-case scenario, a test modality has been identified, then data is compared to the test modality with a relative value of 1. This indicator allows us to rank modalities.

Based on our experience, the relative values determined for a modality are interpreted as follows: In relation to the modality with a relative value of 1:

- 1 > Relative value > 0.93: No effect of modality
- 0.93 ≥ Relative value ≥ 0.85: Average effect of modality
- Relative value > 0.85: Strong effect of modality

Based on data collected in the BIO2 program (Fig 9), the Omega 3-fatty acid indicator has a variation range of 0.89 to 1 on agricultural sites and of 0.74 to 1 on contaminated sites. Such results show that the Omega 3-fatty acid indicator is sensitive to specific cultivation practices (ex: tillage system) and pollution (metal and organic).

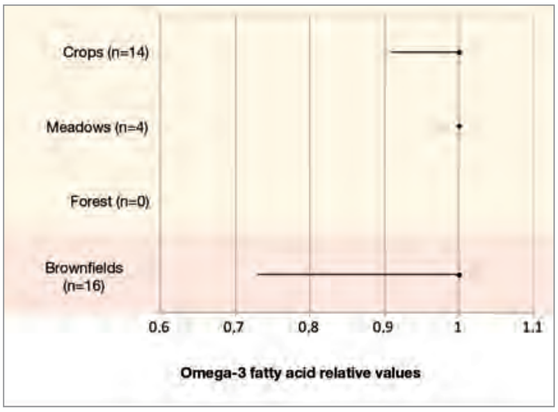

Figure 9

EXEMPLE OF APPLICATION

Auzon site: 7 modalities under study on this former phytosanitary manufacturing plant

Location	Modalities	Distribution Parameters	As	Pb	Sb
Outside the site	CtW	Median MAD	123^{a} 3	60^{a} 1	12^{a} 1
	CtG	Median MAD	115^{a} 8	52^{a,b} 2	7^{a} 1
Within the Site	CoW	Median MAD	339^{b} 23	104^{a} 4	38^{b} 7
	CoWW	Median MAD	3285^{a} 1490	4575^{a} 2800	3930^{d,e} 2827
	CoWa	Median MAD	1087^{d,e} 530	1834^{a} 1448	2222^{d} 1864
	CoWH	Median MAD	661^{c} 216	282^{c} 145	176^{c} 100

Table 1 : Total contents (mg/kg) in As, Pb and Sb in soils of different modalities. On the same column, the letters indicate significant differences between modalities (p<0.05 ,Tukey's test).

As expected, total content in As, Pb and SB is significantly higher on soils of modalities located within the former manufacturing plant's walls than on soils of modalities located outside the site. Based on these analyses, within the site, it seems that modalities CoWW and CoWa are highly contaminated in As, Pb and Sb, while modalities CoW and CoWH are moderately contaminated.

As for the Omega-3 fatty acid indicator, relative values are significantly lower on modalities located within (in red, Fig 10) the former manufacturing plant's walls than on those located outside (in green). This indicator confirms the results of physico-chemical analyses as it distinguishes between contaminated and non-contaminated modalities. However, the ranking of different modalities in relation to the measured level of contamination with total content in As, Pb and Sb or the Omega 3-fatty acid indicator is not exactly the same.

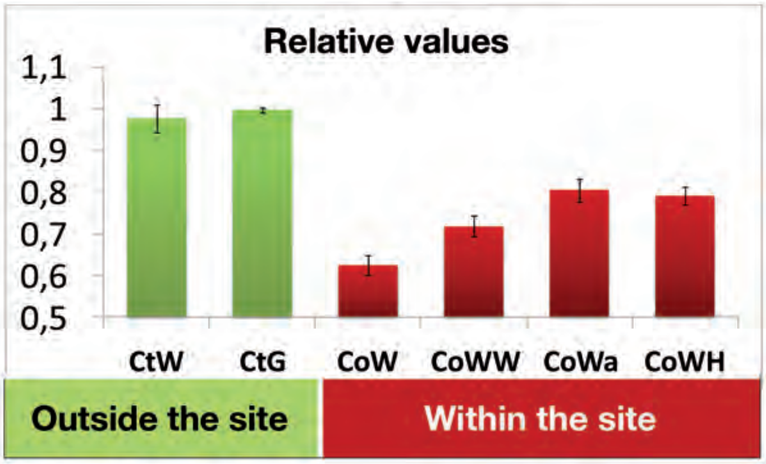

Figure 10

In fact, among the contaminated modalities, the highest effect on the Omega 3 indicator is found on CoW, even though it shows low contamination in As, Pb and Sb, according to total content in soil. Results derived from the Omega 3 indicator can thus be explained by the presence in CoW modality soils of higher herbicide content (especially di-ATR, fenuron and isoproturon) than in other modalities. Such results can also be explained by the fact that the Omega-3 fatty acid indicator evaluates the global stress level of the environment by integrating in particular the possible combined effects of synergy or antagonism between contaminants, which are not taken into consideration by physico-chemical analyses. Results therefore highlight the interest of the Omega-3 fatty acid indicator as a complement of soil physico-chemical analyses.

INTERESTS AND LIMITS OF THE INDICATOR

Interest of the Omega-3 fatty acid indicator:

- Evaluating the "quality of agricultural and contaminated soils";
- Ranking modalities against one another;
- Indicator complementary to physico-chemical analyses;
- Sensitive test: fatty acid content is altered for contaminant doses (organic and metal) that do not lead to any visual phenotype;
- Robust, replicable and reproducible method of analysis;
- Cheap, quick and easy to implement by non-specialised staff.

Limits of the Omega-3 fatty acid indicator:

- The Omega-3 fatty acid indicator is a comparison tool. It cannot be used on a site with only one modality;
- Only for agricultural sites: need to grow the same plant on all tested modalities;
- Sampling dates depend on the presence of vegetation on modalities, and plants with leaves that are not altered by hydric and biotic stresses.

LEB Aquitaine Transfert is a technology transfer centre managed by the ADERA (Association for the Development of Research and Education in Aquitaine) and backed by the Laboratory of Membrane Biogenesis (UMR 5200 CNRS-University of Bordeaux). LEB Aquitaine Transfert provides industrials, public actors and collectivities technological services adapted to their needs and innovative in the field of lipid-induced environmental impacts evaluation. In particular, LEB Aquitaine Transfert provides its monitoring, diagnosis and decision-support tools (AFNOR XP X31 233 Standard) to all actors aiming at improving the environmental quality of their territories and developing an ecological procedure of control and follow-up. To accompany the development of R&D projects, LEB Aquitaine Transfert, always in search of innovation, relies on the effective skills of its research teams and the scientific equipment of its partner laboratory.

CONTACT

marina.leguedard@u-bordeaux2.fr

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