TOOL WORKSHEET NO.17

Functioning of photosynthetic systems

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DESCRIPTION OF THE INDICATOR

Name of the indicator: Functioning of the photosynthetic apparatus of superior plants, bioindicator of soil contamination.

Principles of the bioindicator:

Photosynthesis is a central route of cellular metabolism. It is the biological process that allows superior plants, in the presence of light, to fix atmospheric CO_2 to produce organic matter (Fig. 1). It takes place in 2 steps:

- the first one consists in capturing light energy and converting it into chemical energy in order to synthesise NADPH and ATP, essential elements for cellular metabolism,
- the second one consists in using those energy molecules in order to reduce carbon, fixed as CO_2, and to transform it into organic compounds.

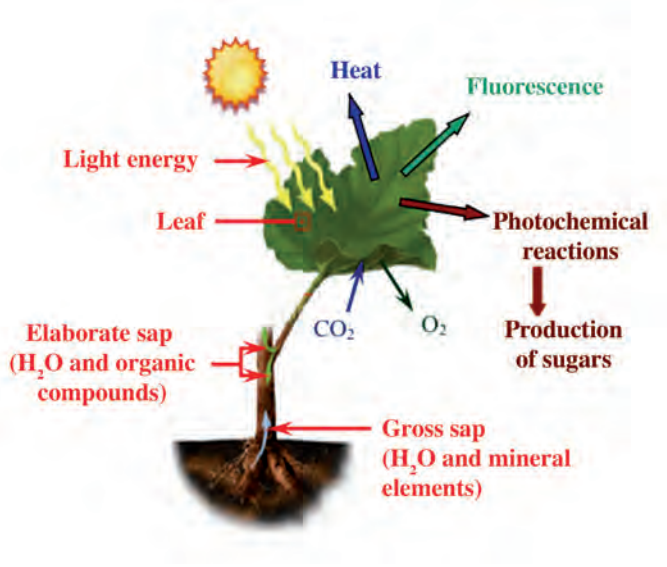

Figure 1: Simplified mechanism of the photosynthesis process

Light energy absorbed by the photosynthetic apparatus is not totally transformed into chemical energy. Therefore, if photosynthetic activity is disrupted by an adverse environment, energy dissipated as heat and chlorophyll fluorescence will have to compensate this decrease. Fluorescence of chlorophyll a is considered as a precise intrinsic indicator of the first stages of photosynthesis, and its intensity is inversely linked to photosynthetic yield and thus vitality of plants. The characterisation parameters of the functioning of the photosynthetic apparatus, and in particular photosystems II (PSII), are sensitive to plants' environmental conditions (Buonasera et al., 2011) and have been proposed as a "biomonitoring" method in strategies of improvement of agricultural productions (Baker and Rosenqvist, 2004; Bourrié, 2007) and in ecotoxicology (Bi Fai et al., 2007). Recently, the technique of measurement of chlorophyll fluorescence in modulated light has gained recognition to characterise the performance of photosynthetic apparatuses due to its rapidity, its simplicity, its reliability and above all its non-invasive character and the possibility to conduct in situ measurements routinely.

DESCRIPTION OF THE METHOD

Analyses are conducted in situ by professionals specialised in plant systematics and ecophysiology. They are systematically conducted during the vegetation climax between May and June. The following steps should be followed:

Step 1: For each site, modalities differing in the level of soil pollution or in the nature of vegetation occupation have been characterised. Then, test modalities with relatively low to no pollution have been identified. A selection of 5 dominant plant species, preferably common in the modalities of each site, has been collected.

Step 2: Study of the functioning of the photosynthetic system (Fig. 2).

- Measurements of Net photosynthesis (Pn): measurements have been conducted using a portable Li-Cor device 6400 model (Lincoln, NE, USA) (Fig. 2). The device includes a console containing electronics and flow rate adjustment machinery. The console is linked to pliers that hold the leaf on a 2x3 cm^2 watertight chamber. Air hygrometry is regulated at 30% of relative humidity by passing through Drierite. Content in CO_2 is adjusted at the value of 360 µmol $CO_2.l^{-1}$ using a CO_2 injector (LI-Cor 6400-01, Lincoln, NE, USA) which uses cylinders of high pressure liquefied CO_2. Concentrations in H_2O and CO_2 at the entrance and at the exit of the chamber are determined by two infrared spectrophotometers directly placed in the pliers. The temperature of the chamber is fixed at 23°C; it is controlled by two thermoelectric cooling systems (Peltier effect) and measured by a thermocouple placed under the leaf. The leaf is lit by a light source made of the LI-Cor 6400-02 LED (Lincoln, NE, USA) module. It receives 1500 µmol.$m^{-2}.s^{-1}$. Light intensity is measured by a silicon photodiode sensor directly placed in the source. During a measurement, the analyser regularly records the pump flow and variations of CO_2 and H_2O rates in the circuit. A measurement on the leaves of each plant has been realized. 6 measurements by plant species and by modality have been conducted.

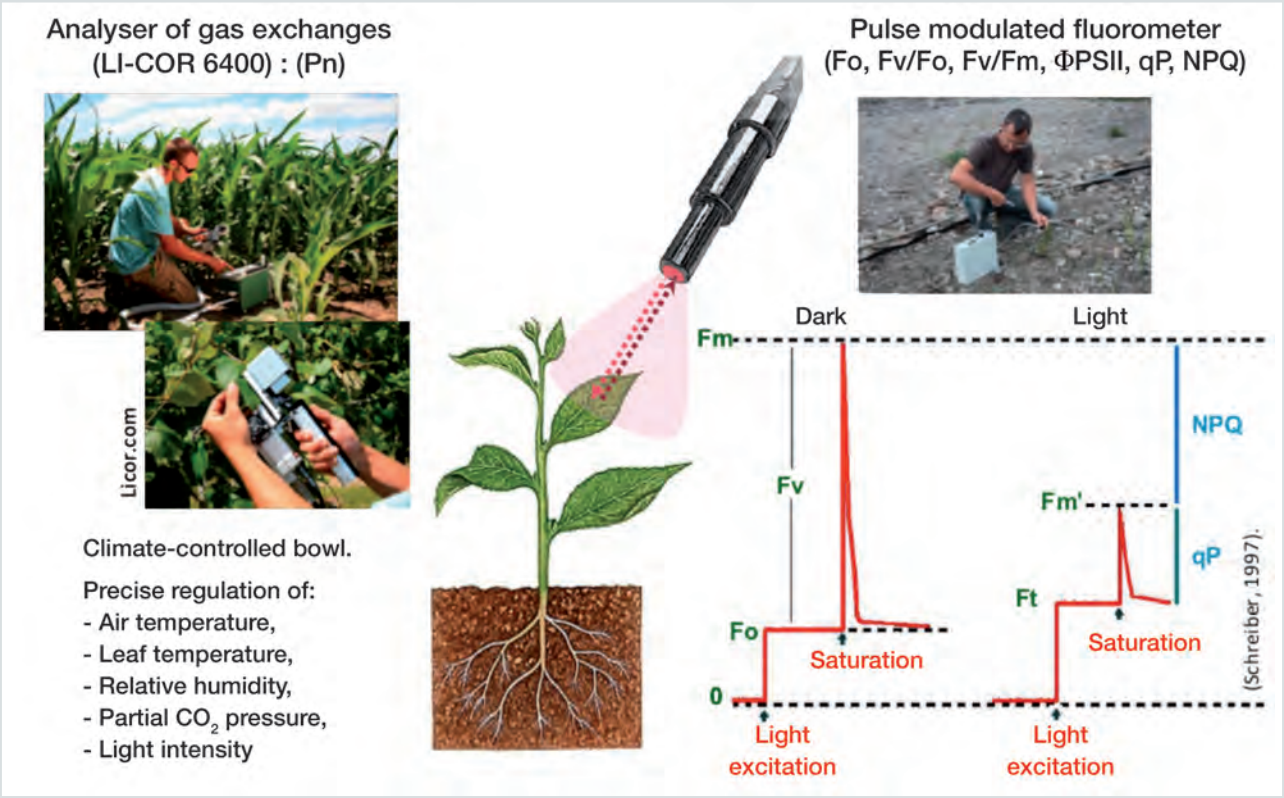

Figure 2: Measurement tools of parameters characterising the functioning of plant photosynthetic systems.

- Measurement of fluorescence of chlorophyll a: measured parameters allow determining the functioning of PSII, especially the collecting antenna and the transmission of light energy in potential electrochemical energy. Measured parameters are achieved on intact leaves using a PAM FMS 1 "Pulse Amplitude Modulation Fluorescence Monitoring System 1" (Hansatech Instruments, Norfolk, UK) system. This measurement system relies on the use of continuous and very low intensity light, actinic light, modulated light, and a saturating flash of light. Different parameters are monitored. We will present the parameters we propose as potential bioindicators of soil quality. They are:

- **Fo: initial-stage fluorescence.** This parameter evaluates the intensity of fluorescence when all reaction centres of PSII are open (oxidised quinines). It is the minimum fluorescence achieved when plants are adapted to darkness..
- **Fv/Fo: maximum primary production of PSII.** It characterises the potential quantum yield of PSII, which enables the estimation of photosynthetic capacity of leaves.
- **Fv/Fm: maximum photochemical efficiency of PSII.** This parameter shows the efficiency of PSII in using light for photochemical conversion. Its reference value is close to 0.8 for a healthy plant.

- **ϕPSII: effective photochemical efficiency of PSII under a given lighting**. This parameter depends on the fraction of PSII open and shows the efficiency of PSII to use luminous energy.
- **NPQ: non photochemical quenching.** It evaluates the implementation of photoprotection mechanisms which leads to excess energy dissipation as heat.
- **qP:** photochemical quenching. This parameters make it possible to have an estimate of relative concentration of open centres and thus to determine their capacity to initiate the photochemical process.

Except for Fv/Fm, none of the characterisation parameters of the functioning of photosynthetic systems shows values of reference, hence the necessity of a "reference" modality in each site. 6 measurements by plant species have been conducted.

$$IPSP = \frac{Pn}{Fv/Fm + (NPQ/qP)}$$

Step 3 : Normalisation of data: Measured parameters have allowed developing an index of performance of photosynthetic systems (IPSP):

In order to get past the issue of a different flora in the modalities of a single site, the protocol of normalisation of data developed by Le Guédard and Bessoule (2011) has been applied on IPSP data and enabled to give a relative note to each modality of each workshop site. IPSP varies between 0 and 1; the closer the value is to 1, the more the physic-chemical characteristics of soil have a moderate effect on the activity of photosynthetic systems.

INTERPRETATION OF RESULTS

To demonstrate the relevance of monitoring the functioning of plants photosynthetic apparatuses for the evaluation of soil pollution associated risks, measurements have been conducted in May 2009 on the experimental site of Homécourt (54), in May 2010 in the "Vieille Usine" brownfield in Auzon (43) and on an industrial tip near Saint-Etienne (42). These sites are characterised by polymetallic pollutions (Pb, Sb, Zn, As...). Different modalities have been defined for each site depending on the level of pollution and/or the nature of vegetation.

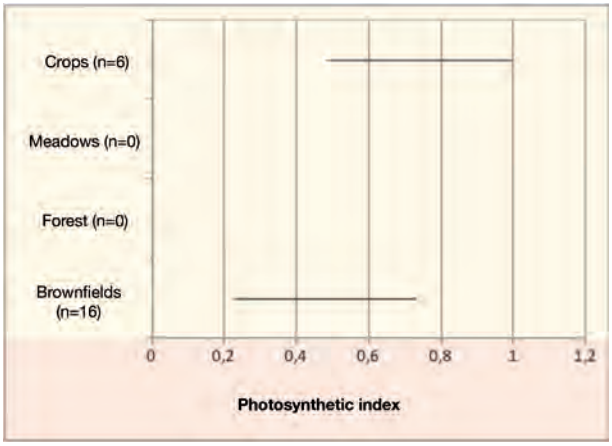

Figure 3: Variation range of the index of performance of photosynthetic systems of dominant plant species in workshop sites of the "Bioindicateurs 2" program.

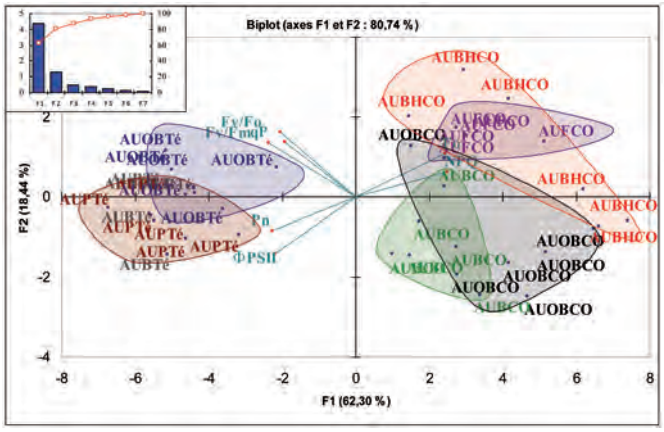

Figure 4: Correlation circles showing the distribution of parameters of photosynthetic activity on Rubus sp. plants from the Auzon site. Red, black, green and purple: contaminated modalities. Blue, brown and grey: test modalities.
BH: Hydromorphic wood, **B:** Wood, **OB:** Wooded border,
F: Fallow land, **P:** Meadow

Results collected on the 7 identified modalities on the Auzon site are represented in figure 4. Correlations between net photosynthesis (Pn), parameters of fluorescence of chlorophyll a and levels of pollution and nature of vegetation show that the functioning of photosynthetic systems for developed plants differs on test and polluted modalities. This experiment shows that the efficiency of photosynthetic systems for brambles is insensitive to soil occupation. Studying the performance of photosynthetic systems for evening primrose patches spontaneously developed in the modalities of the Saint-Etienne and Homécourt sites allows us to identify the different modalities of each of the studied workshop sites (Fig. 5).

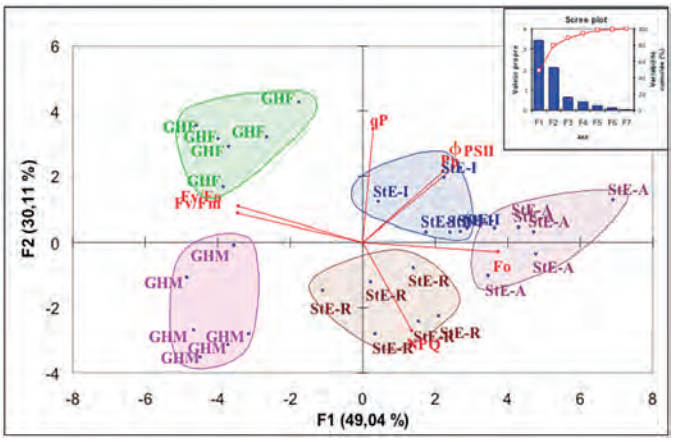

Figure 5: Correlation circles showing the distribution of parameters of photosynthetic activity on Oenothera biennis L. plants native to modalities of the Saint-Etienne (StE-A, Ste-R and Ste I) and Homécourt (GHM and GHF) sites.

Modalities	IPSP index	(ETM)soil (mg/kg)		Soil classification (RMQS whisker)
		As	Pb	
AUPTE	1	115[a]	52[a]	C2
AUOBTE	0,81	63[a]	30[a]	C2
AUBTE	0,80	123[a]	60[a]	C2
AUOBCO	0,39	891[b]	581[c]	C3
AUFCO	0,37	1149[c]	2033[d]	C3
AUBCO	0,30	341[a]	105[b]	C3
AUBHCO	0,26	3643[c]	4422[e]	C3

Table 1: Relation between the IPSP relative notes achieved on the different modalities of the Auzon site, the total contents in As and Pb in the soils of modalities and their classification in accordance with the level of contamination. Letters indicate for a parameter significant differences ($p<0.05$, Test of Mann and Whitney).

Level of pollution:
- **C2 : moderately contaminated site**
- **C3 : highly contaminated site**
- **TE : test modality**
- **CO : contaminated modality**

Vegetation occupation:
- **P : Meadow**
- **B : Wood**
- **BH : Hydromorphic wood**
- **OB : Wooded border**
- **F : Fallow land**

Total contents in As and Pb in soils of the 7 Auzon modalities show very high levels of pollution in contaminated areas. However, contents in test modalities are higher than the impact statement values for As (37 mg/Kg). Relative notes of the IPSP indexes are lower in contaminated modalities than in test modalities. The contaminated wood modality (AUBCO) shows a low relative note of the IPSP index (0.3), reflecting a significant inhibition of the functioning of photosynthetic apparatuses of the modality's indigenous plants. Nevertheless, the soil physic-chemical characterisation shows that this modality is the least contaminated within the site. In order to explain this result, it would be relevant to study the impact of specific organic pollutants on the functioning of photosynthetic systems. Higher concentrations in di ATR, fenuron and isoproturon have been determined in the soil of this modality. The IPSP index seems to describe the global state of plants environment and its action on their development - and not only metallic trace elements (ETM) pollution of soils. Achieved results confirm the relevance to use this index to define soil quality additionally to traditional physic-chemical analyses of soil.

INTERESTS AND LIMITS OF THE INDICATOR

Interest of the IPSP index:

- Measurements of parameters conducted in situ using portable device.
- Quick measurements, non-destructive of vegetation material.
- Index enabling classification of modalities depending on the presence or absence of soil contamination.
- Index complementary to physic-chemical analyses.
- Sensitive test since it allows characterising soil quality before the appearance of stress symptoms at the level of plants.

Limits of the IPSP index:

- Compulsory reference modality with optimal conditions for plant development.
- Field topography and climatic conditions must be suited for transportation and use of measurement materials.
- Specialised user in the field of plant ecophysiology and systematics (Bac+2; Bachelor's degree) required.

Références Bibliographiques

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