Institute for Ecology of Industrial Areas in Katowice

Chosen physiological parameters analysis of *Miscanthus* x *giganteus* and *Spartina pectinata* cultivated on soil contaminated with heavy metals. Characterization of measuring equipments

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Presentation Plan

- Presentation of results form preliminary studies
  - Background
  - Aim of studies
  - Site description
  - Experiment design
  - Material & methods
  - Results
  - Conclusions

- Presentation of measuring equipment used in research by IETU Environmental Remediation Team:
  - Infra-Red Gas Analyser (IRGA) – „ Lc ProSD, ADC Bioscientific, UK”
  - Fluorimeter – „ Handy Plant Efficiency Analyser, Hansatech Instruments ltd., UK
  - Plant pigments content meter – „Dualex Scientific +, Force A, France”
  - Ceptometer (LAI-meter) – „LP-80, Decagon Device, USA”

Soil Contamination

- Soil can be contaminated with different compounds and elements which can indirectly influence human health
- Contaminated lands especially those arable must be excluded from food and fodder production
- Biomass production seems to be good alternative in contaminated arable land management
- Energy crop cultivation on contaminated arable land can be dually beneficial not only in lands management but also in phytoremediation

Energy Crops

- Despite the fact that energy crops are not hyperaccumulators, due to its huge biomass production ability they can extract totally a lot of heavy metals
- European Union Directive 2009/28/EC (RES) indirectly prescribe lands categories, where energy crops can be cultivated

Miscanthus x *giganteus*

Spartina pectinata

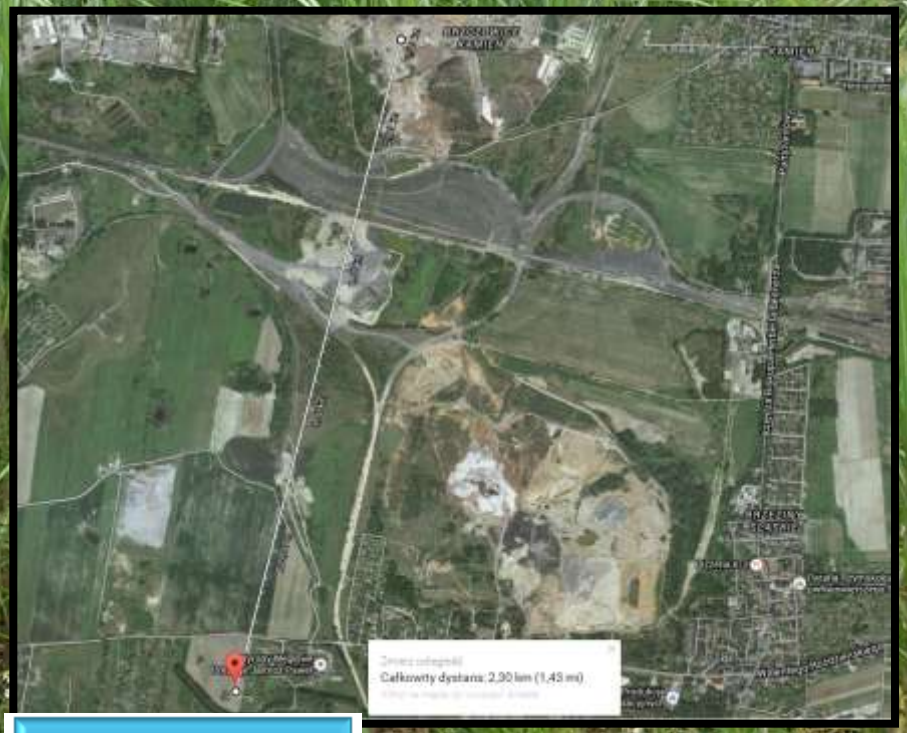
- ***Miscanthus* x *giganteus* and *Spartina pectinata* are second generation energy crops**

Aim of Study

The aim of preliminary studies was to analyze chosen physiological parameters, mostly associated with photosynthetic aparatus of *Spartina pectinata* and *Miscanthus* x *giganteus* cultivated on soil contaminated with Cd and Pb additionally treated with different fertilizers

Site description

Heavy metals contamination of arable land is present due to dust fall emitted by „Orzeł Biały” smelter in the last century

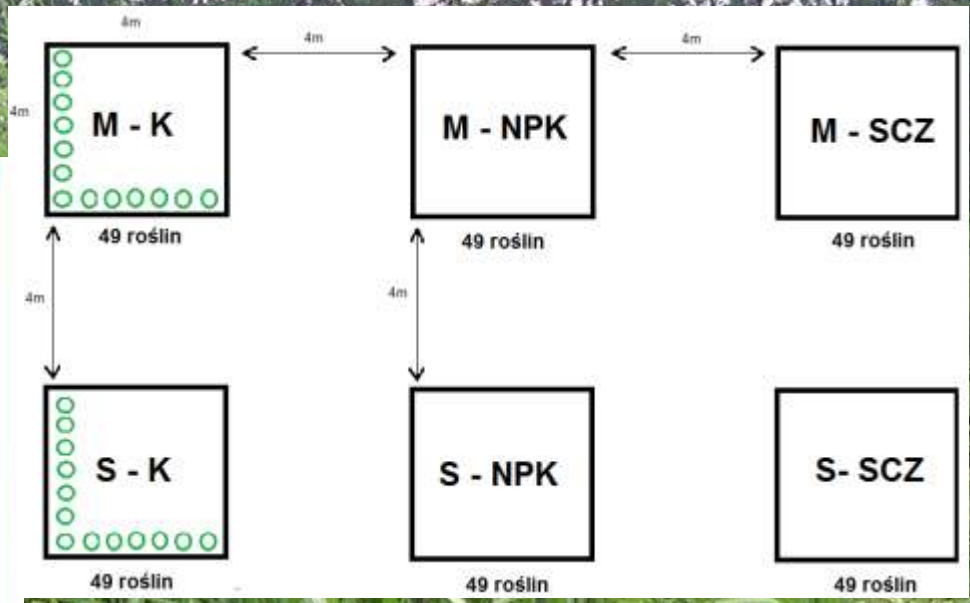


https://www.google.pl/maps

300 mm

17 °C

Experiment design



Two species of energy crop were tested:

- *Miscanthus* x *giganteus* (M)
- *Spartina pectinata* (S)

Three experimental options were set up:

- Control (K)
- Chemical fertilization NPK (NPK)
- Fertilization with commercialy alavailable microbial inoculum (SCZ)

Three randomly selected plants not exposed to edge effect from each plot were analyzed. (pseudo-replication)

Materials & Methods

- Soil characteristic (pH, EC, humidity)
- Soil Pb and Cd content(FAAS)
- Gas exchange measurments (LC Pro +, ADC Bioscientific)
- Chlorophyll content measurments (CL-01, Hansatech Instruments Ltd.
- Chlorophyll *a* fluorescence measurments (Pocket PEA, Hansatech Instruments Ltd.)
- Leaves Pb and Cd content (FAAS)

http://ppsystems.com

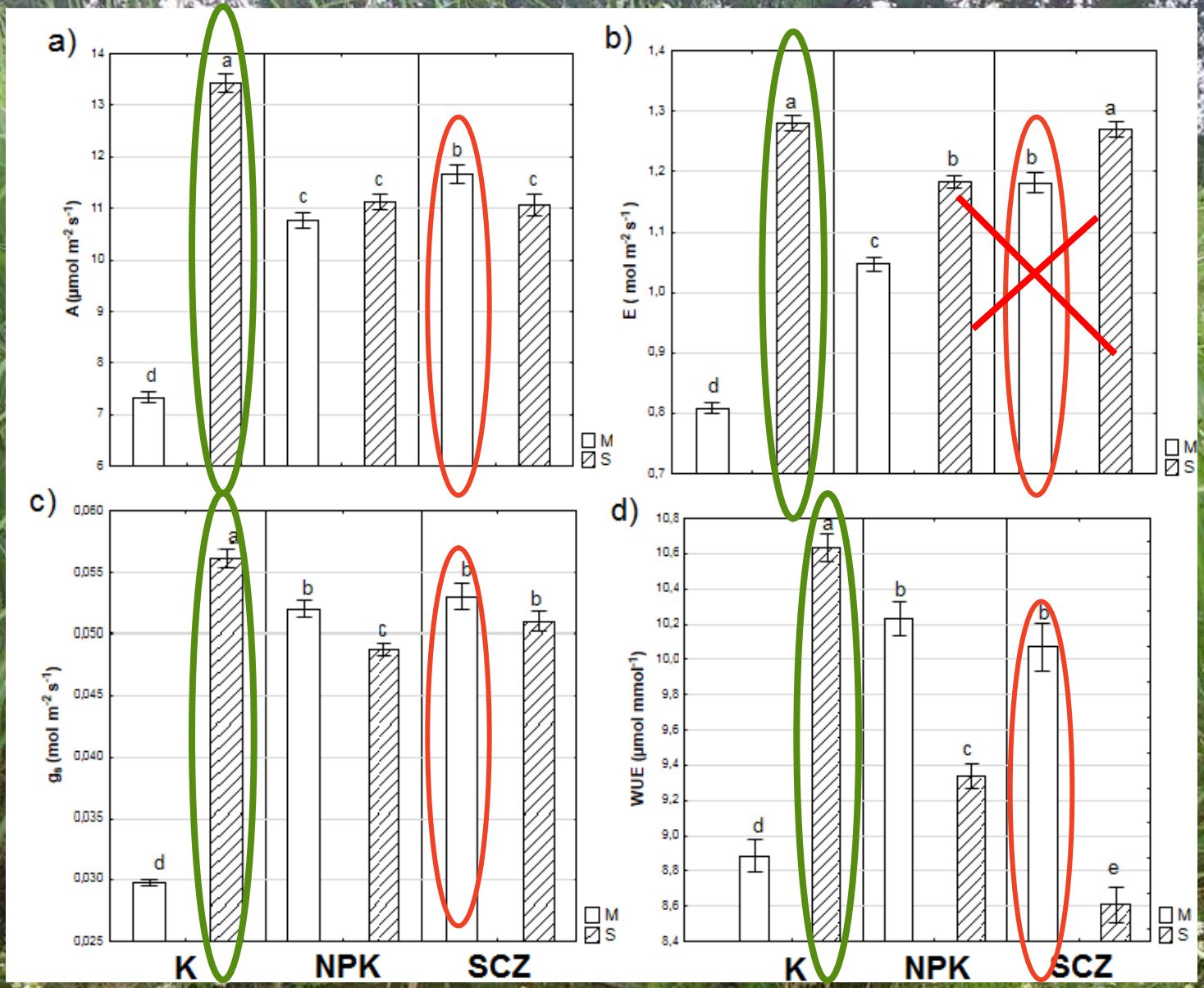
Soil characteristic

Parameters	EXPERIMENT VARIANTS					
	M-K	M-NPK	M-SCZ	S-K	S-NPK	S-SCZ
Total Cd and Pb content (mg kg^{-1})						
Cd	18,32 ± 0,99a	19,49 ± 0,65a	19,37 ± 0,59a	12,64 ± 0,53b	13,04 ± 0,42b	12,96 ± 0,53b
Pb	429,89 ± 4,27a	466,29 ± 12,35a	441,51 ± 19,41a	282,13 ± 14,69b	287,49 ± 8,57b	278,45 ± 10,11b
Bioavailable Cd and Pb content (mg kg^{-1})						
Cd	0,72 ± 0,06b	1,03 ± 0,03a	0,76 ± 0,07b	1,00 ± 0,09a	1,00 ± 0,07a	1,02 ± 0,02a
Pb	1,17 ± 0,04b	1,38 ± 0,05a	1,36 ± 0,07a	1,38 ± 0,02a	1,45 ± 0,05a	1,49 ± 0,04a
Soil physico-chemical parameters						
Humidity(%)	4,54 ± 0,56b	6,06 ± 1,05ab	6,20 ± 1,32ab	8,21 ± 0,61a	6,86 ± 0,63ab	7,11 ± 0,60a
pH	6,97 ± 0,06a	6,72 ± 0,06b	7,07 ± 0,10a	6,66 ± 0,08b	6,45 ± 0,03c	6,40 ± 0,02c
EC (µS)	79,88 ± 7,12a	79,30 ± 5,24a	90,20 ± 8,95a	75,39 ± 1,34a	83,83 ± 2,05a	79,48 ± 0,41a

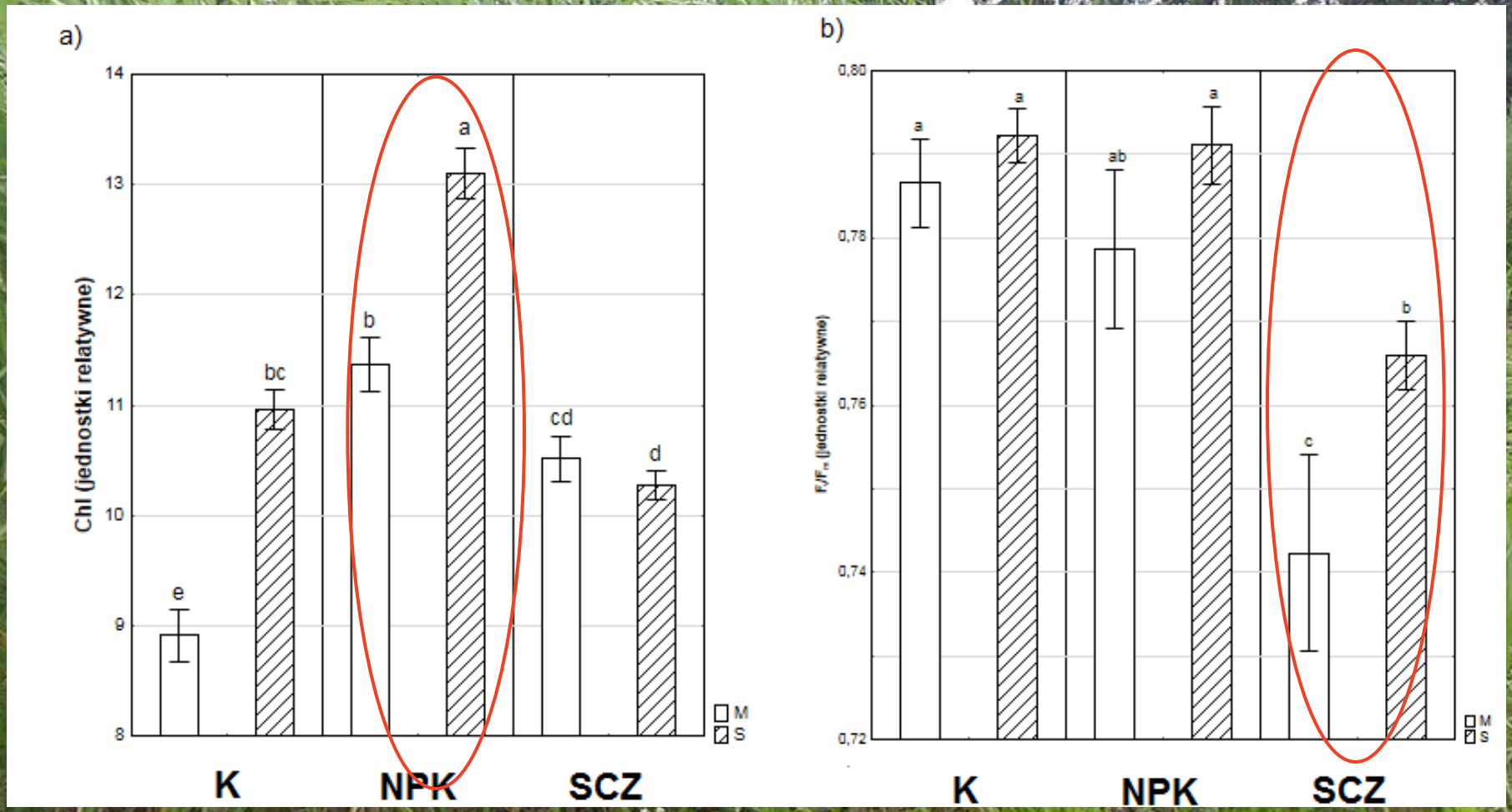
Leaves Pb and Cd accumulation

	EXPERIMENT VARIANTS					
	M-K	M-NPK	M-SCZ	S-K	S-NPK	S-SCZ
Cd (mg kg^{-1})	5,09 ± 0,14b	5,10 ± 0,14b	5,84 ± 0,30a	4,44 ± 0,21c	4,97 ± 0,35b	4,90 ± 0,25b
Pb (mg kg^{-1})	75,39 ± 5,96a	75,55 ± 2,12a	75,32 ± 6,07a	41,21 ± 3,84b	50,20 ± 4,78b	65,97 ±2,76a

Gas exchange parameters



Leaves chlorophyll content and general plant vitality (Fv/Fm)

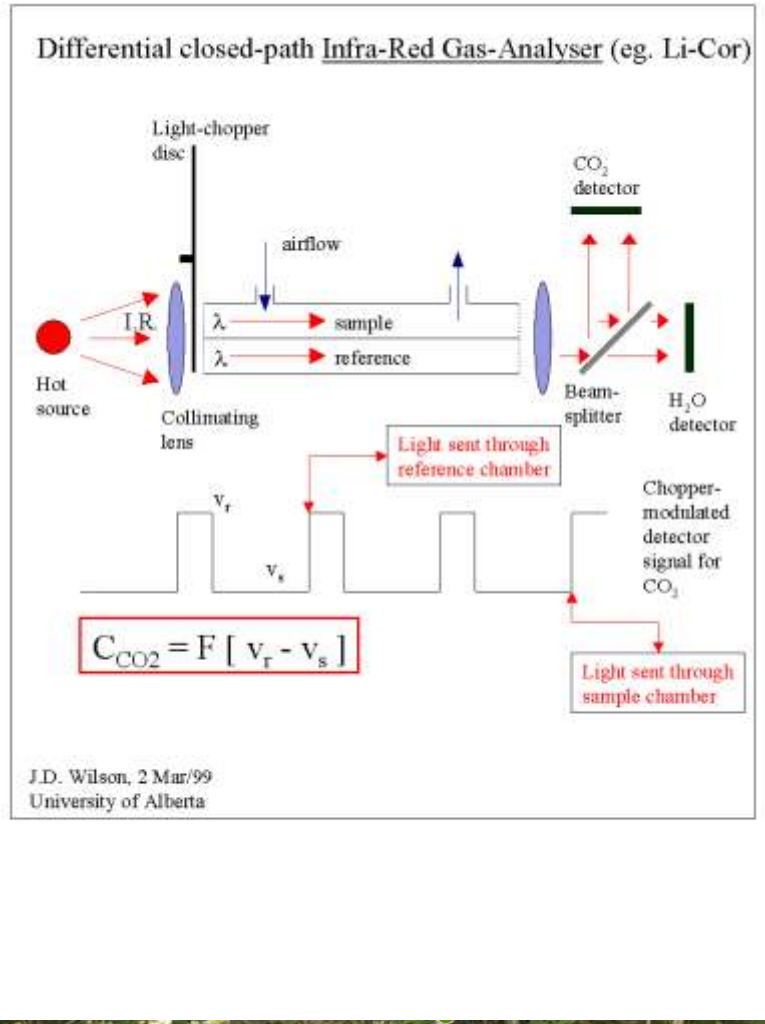


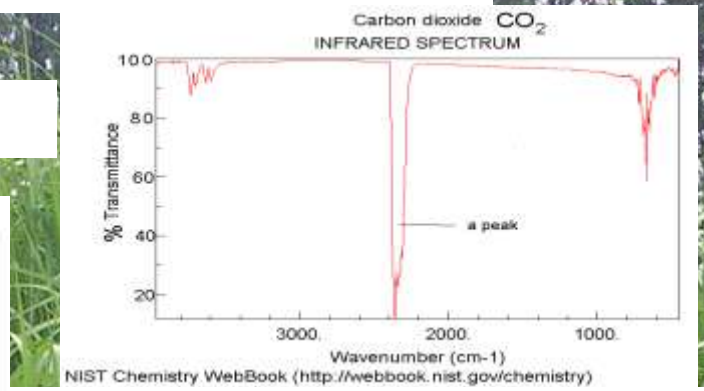
Conclusion

* Influence of fertilizers on plants is species specific, photosynthesis process is more intensive in fertilized *Miscanthus* x *giganteus* when compare to control, however for *Spartina pectinata* opposite phenomenon was observed
* Microbial inoculum caused higher Cd leaves accumulation in both plant species
* Chemical fertilization significantly increase chlorophyll content in both species
* *Miscanthus* x *giganteus* accumulates more Cd and Pb in leaves than *Spartina pectinata*
* Despite the Miscanthus accumulate more Pb and Cd in leaves there were no visible effects of those elements toxicity on photosynthetic apparatus

Infra-Red Gas Analyzer

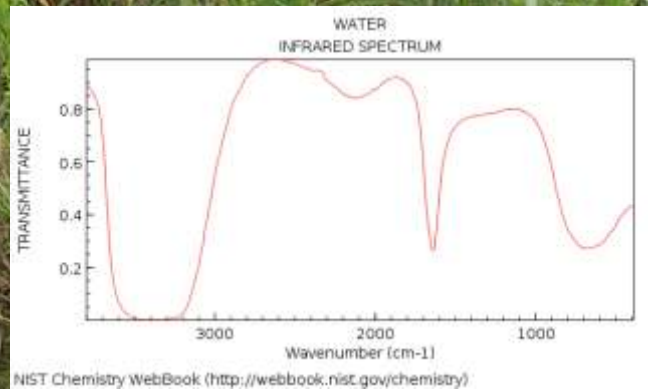
What informations are obtained ?





Measured parameters:

- **Photosynthesis intensity (A)**
- **Substomatal CO_2 (C_i)**
- **Transipration rate (E)**
- **Stomatal conductance (g_s)**
- **Photosynthetic active radiation (PAR)**



Infra-Red Gas Analyzer

How we measure ?

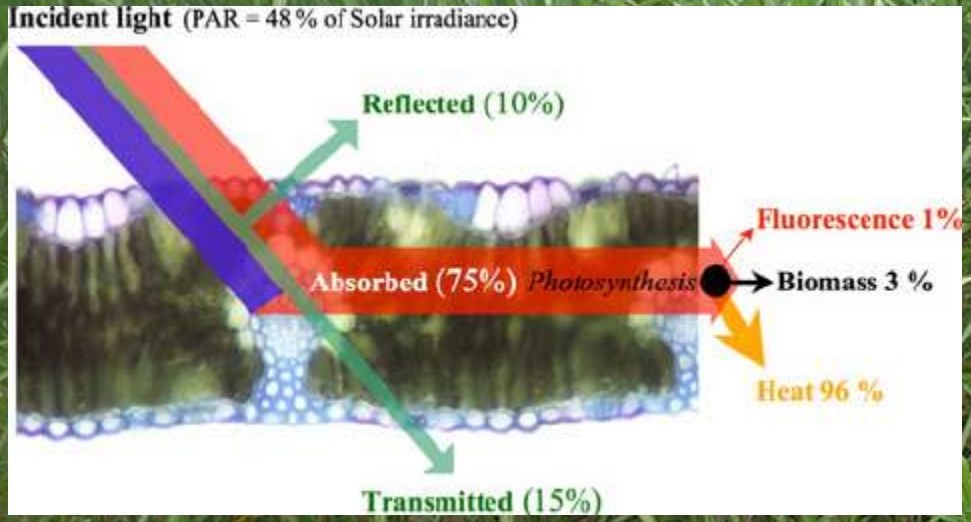


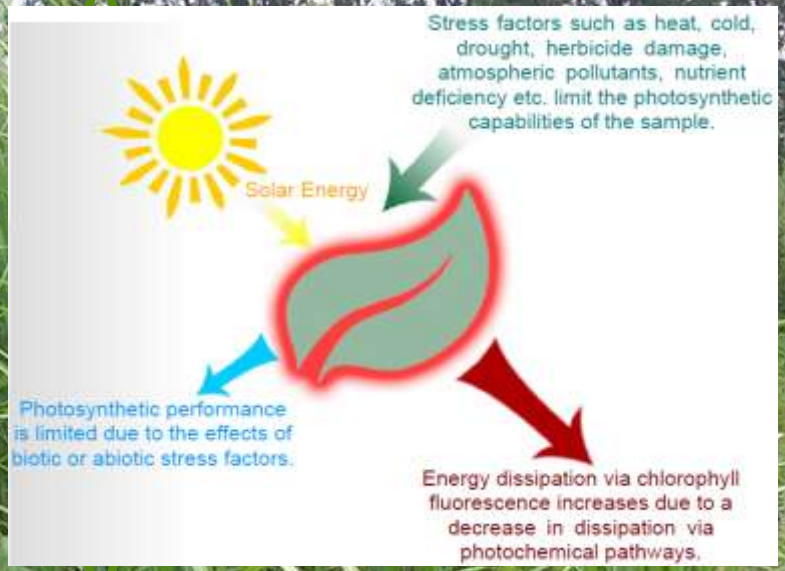


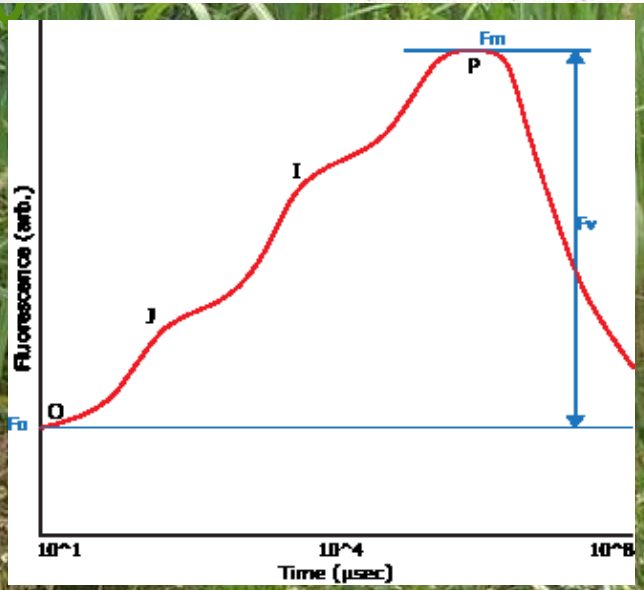

- Possibility to control : Light intensity, CO_2 concentration, temperature in leaf chamber
- Possibility to measure all parameters on leaves with different size and morphological structure
- Measurment is performed on leaf sample *in situ*

Fluorimeter (Chlorophyll *a* fluorescence)

What informations are obtained ?







Fluorescence : Heat = const. ratio

Fluorimeter

How we measure ?

- **Samples required dark-adaptation (25 – 40 min)**
- **108 measurments in 1 sec**
- **Derived informations about light-depending photosynthesis phase**

Chlorophyllmeter

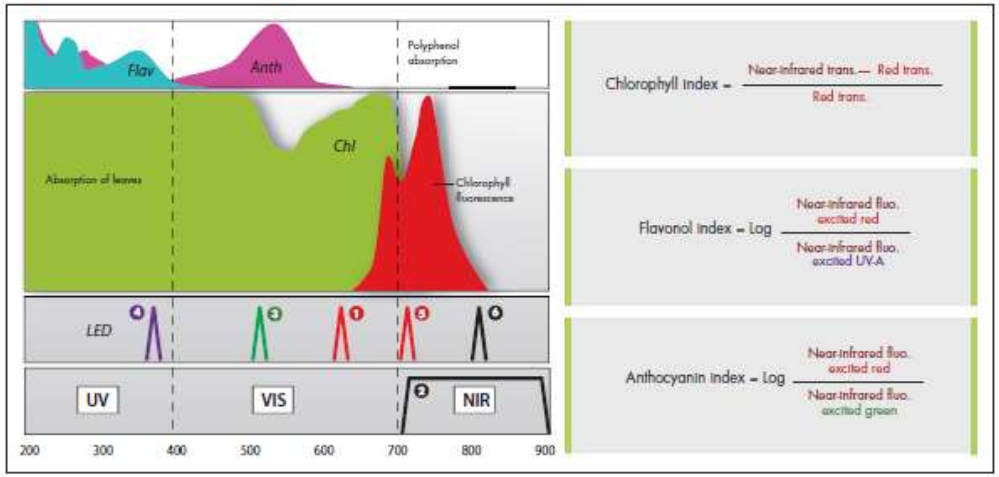
What informations are obtained ?

POLYPHENOLS measurement

Near-infrared chlorophyll fluorescence ❷ is measured under a first reference excitation light ❶ not absorbed by polyphenols. It is compared to a second sampling light specific to a particular type of polyphenols (e.g. green ❸ for anthocyanins or UV-A ❹ for flavonols). Only a fraction of this light reaches the chlorophyll in the mesophyll and can generate near-infrared fluorescence.

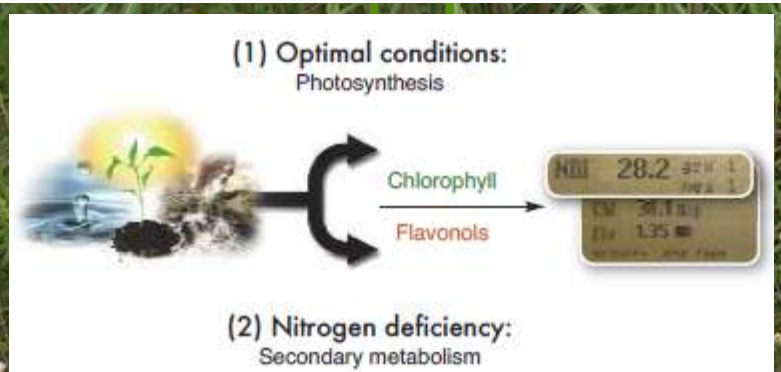
CHLOROPHYLL measurement

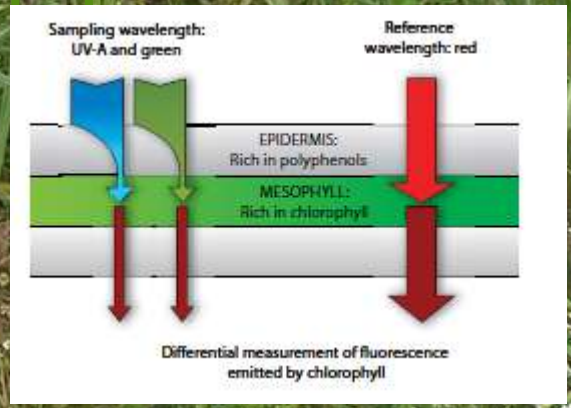
The leaf chlorophyll content can rapidly and accurately be assessed from light transmission. A first wavelength very close to the red ❸ quantifies the chlorophyll and a second in the near-infrared ❺ can take into account the effects of leaf structure.



Measured parameters:

- Chlorophyll content (Chl)
- Flavonoids content (Flav)
- Anthocyanins content (Anth)
- Nitrogen Balance Index (NBI)





Chlorophyllmeter

How we measure ?

- 1 measurement = 1 sec
- 4 parameters = 1 measurement
- Included GPS (< 2,5 m resolution)
- 10 000 measurements memory

3 main rules to respect to compare treatments :

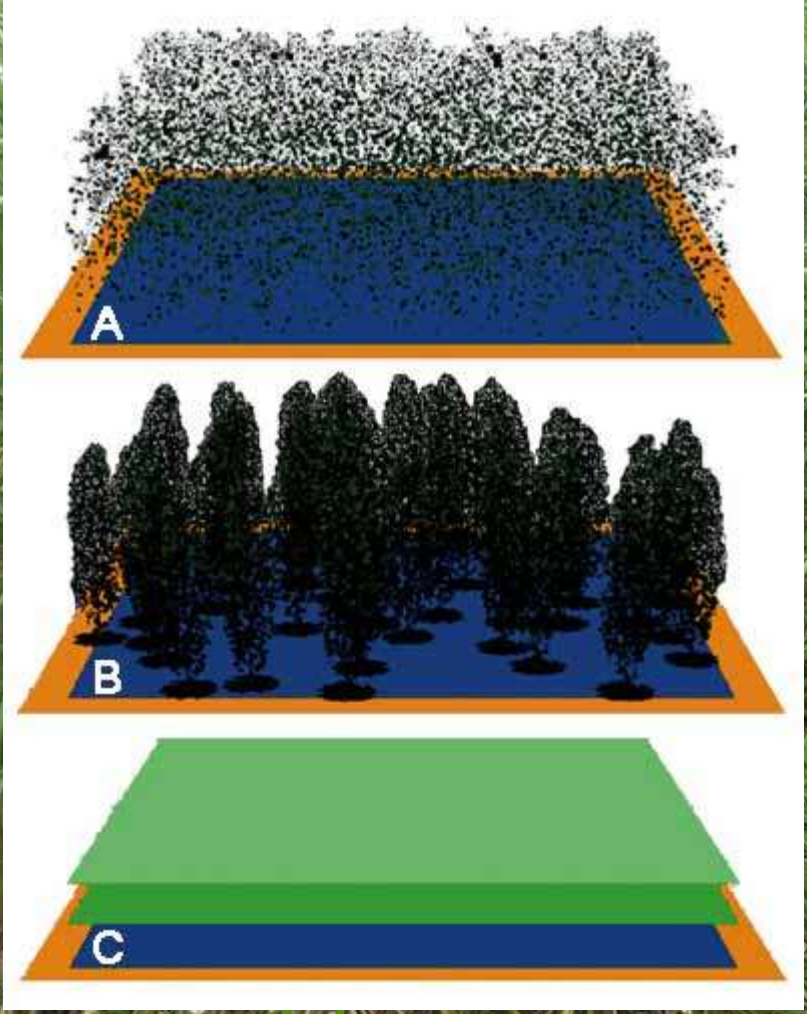
① - Make readings on leaves with the same physiological age. Prefer well developped young leaves.

② - Make readings on the same position of the leaves, for all leaves

③ - The entire Dx window must be covered by the leaf.

Ceptometer (LAI-meter)

What informations are obtained ?



- **LAI is defined as: total leaf area per unit ground area**
- **Indication of Canopy density, biomass**

Ceptometer (LAI-meter)

How we measure ?

- **80 photodiod PAR sensors included**
- **Possibility to measure with or without external sensor**
- **Possibility to control how many sensors are used (min 10)**

Thank you for your attention

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