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FACULTY OF SCIENCES

Department of Environmental Science and Management

Monitoring the influence of light intensity on the growth and mortality of duckweed (*Lemna minor*) through digital image processing

By

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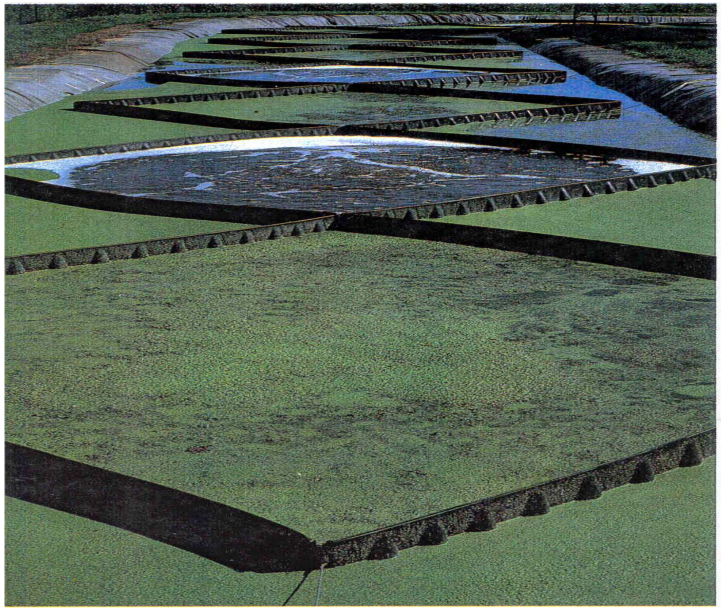
OUTLINE

1. INTRODUCTION
2. MATERIAL AND METHODS
3. RESULTS
4. CONCLUSION

INTRODUCTION

✓ Various techniques involved in the treatment of wastewater



✓ Floating macrophyte ponds (i.e. *Lemna minor*)

Excess or shortage may lead to dysfunctions

-> Optimal management

INTRODUCTION

General objective

To develop a mathematical model [Kinetic, Stoechiometry, Mass balances i.e. gas exchanges (O_2, CO_2)] of growth of floating macrophytes (*Lemna minor*) in the operation of floating macrophytes WSP (I, T, N, P, pH). A “Gujer-Petersen matrix" approach, should be obtained compatible with more general WSP models.

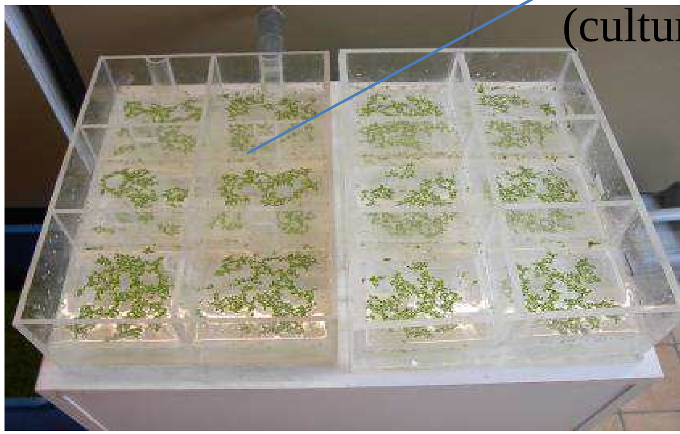
More specifically, in this study we will focus on the influence of light intensity on (i) the growth and (ii) the mortality of *Lemna minor* under a constant temperature.

MATERIAL AND METHODS

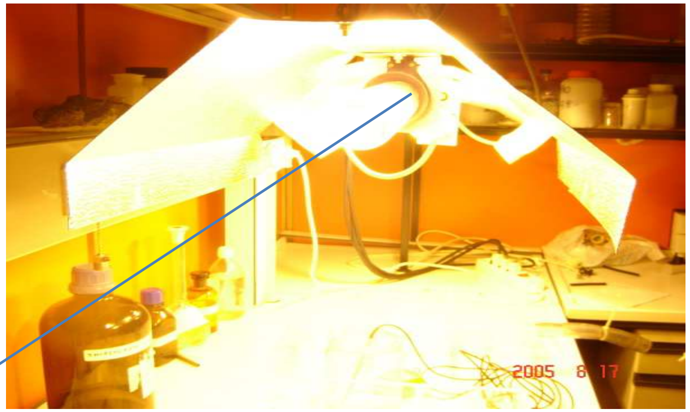
- Bertrix WSP ⟹ Phytotron

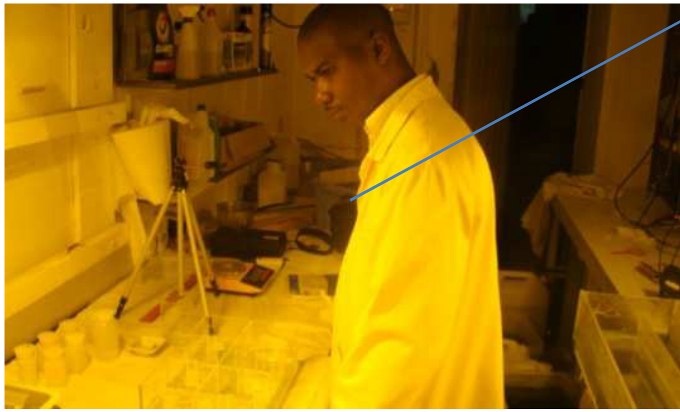
Storage of Lemna

Lab scale Pilot plant
(cultures media)



Phytotron





Sodium lamps
(400 watts)

L. minor growth monitoring in laboratory

MATERIAL AND METHODS

Experimental set up of duckweed growth monitoring (t = 4 days)

Test (Midst)	*$[N\text{-}NH_4^+]_i$ (mg.L^{-1})*	*$[P\text{-}PO_4^{3-}]_i$ (mg.L^{-1})*	*Light intensity (µmole.m^{-2}.s^{-1})*	*Temperature (°C)*	*Photoperiod (h/h)*
1	*5 - 15*	*1*	***200***	***20.6***	*12/12*
2	*5 - 15*	*1*	***250***	***20.9***	*12/12*
3	*5 - 15*	*1*	***300***	***21.2***	*12/12*
4	*5 - 15*	*1*	***350***	***21.5***	*12/12*
5	*5 - 15*	*1*	***400***	***21.7***	*12/12*
6	*5 - 15*	*1*	***450***	***21.9***	*12/12*

Tank 1 (5 mg.$N\text{-}NH_4^+$.L^{-1}); Tank 2 (7 mg.$N\text{-}NH_4^+$.L^{-1}); Tank 3 (9 mg.$N\text{-}NH_4^+$.L^{-1}); Tank 4 (11 mg.$N\text{-}NH_4^+$.L^{-1}); Tank 5 (13 mg.$N\text{-}NH_4^+$.L^{-1}); Tank 6 (15 mg.$N\text{-}NH_4^+$.L^{-1})

MATERIAL AND METHODS

BIOMASS MONITORING

- ✓ **Fresh weight (FW) :** less accurate and difficult to duplicate
- ✓ **Dry weight (DW):** more reliable but destructive method
- ✓ **Images processing (Area, A):** continuous monitoring and non-destructive (Camerawork and Processing)
- ✓ **Relationship Biomass (DW/FW)-Covered area (A %):**

$$- FW\ (g.m^{-2}) = f\ (A)$$

$$- DW\ (g.m^{-2}) = f\ (A)$$

MATERIAL AND METHODS

- **Steps of *L. minor* camerawork**

- Nikon® digital camera COOLPIX L120

[14.1 MP: 4320 x 3240 pixels; Focal length (integrated objective): 25-525 mm]

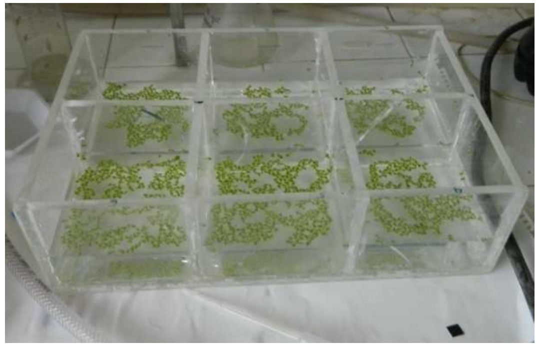
- Around 1g of fresh biomass in each tank

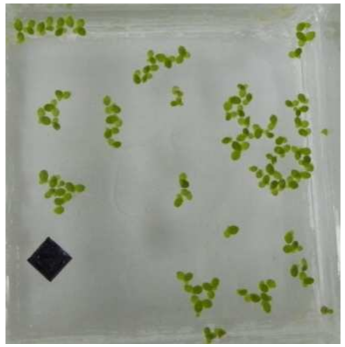
- A floating colored control (1cm x 1cm)

- Natural light in the lab (no flash, neon tubes turned off)

-Automatic option (default): good resolution compensating ‘no zoom’ effect

- Tripod (45 cm above the water)

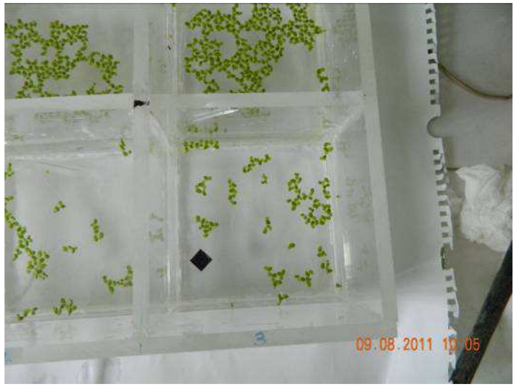


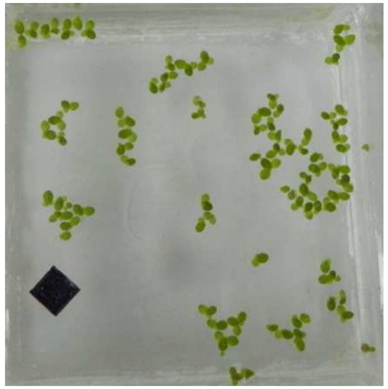


MATERIAL AND METHODS

- **Images processing**

-ACD-see® : Pre-processing → Area covered by plants





- Image Pro-Plus® : Processing → Counting and determining the geometrical characterics of *L. minor* (area, major axis, minor axis, perimeter color)

MATERIAL AND METHODS

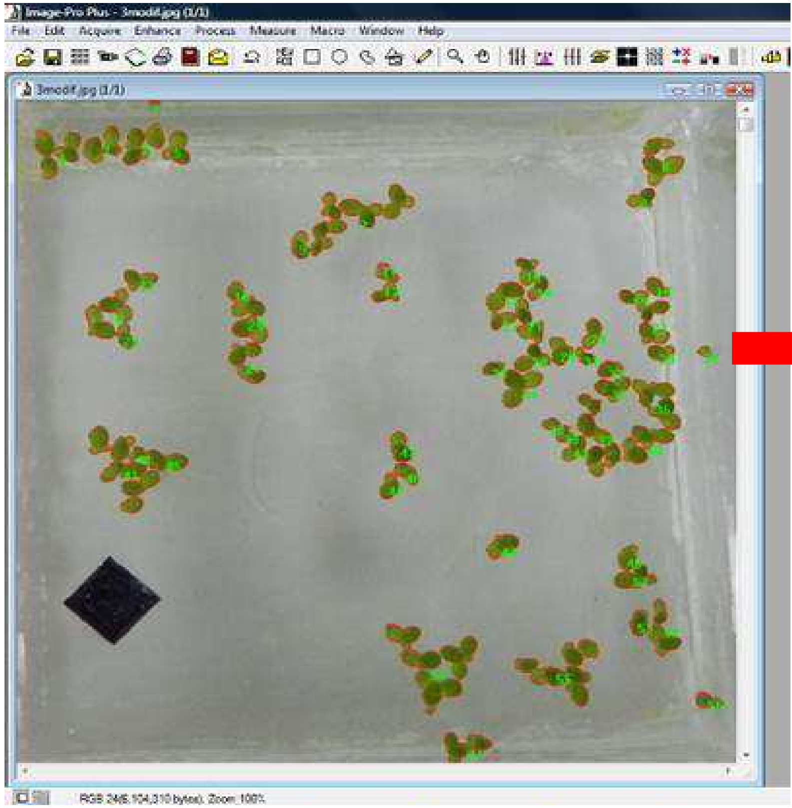
- ✓ **Automatic count based on color:**

  Green color (*RGB between 100-255*) -> living duckweed
  White color (*RBG between 200-255*) -> dead duckweed

Colors are identified by the operator

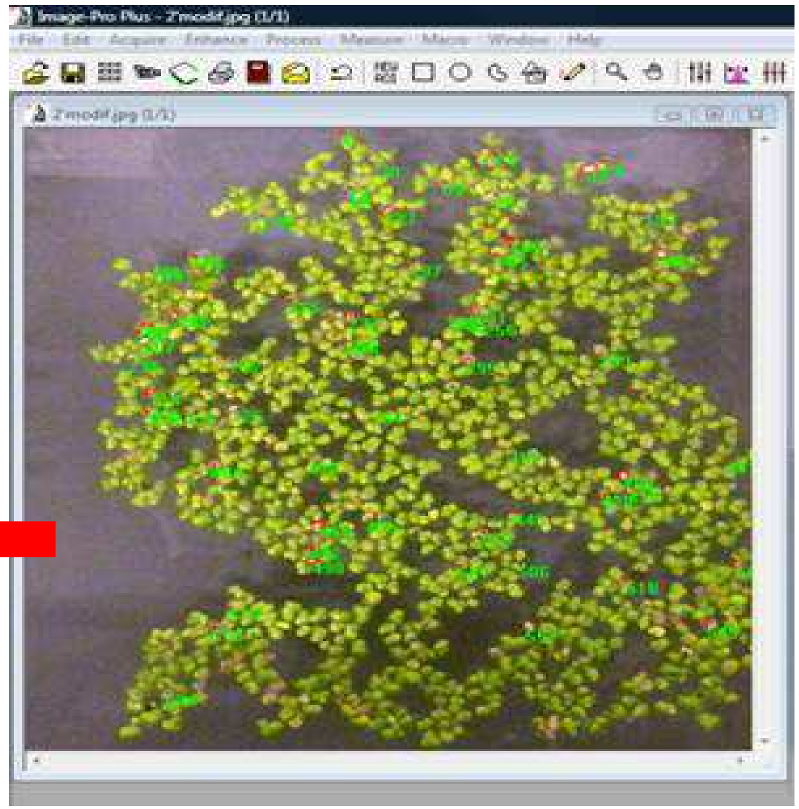
- ✓ Table of statistical characteristics automatically generated by the software.

- ✓ Expecting future automatic recognition method (i.e. neural network) for the determination of lemna objects and their color.



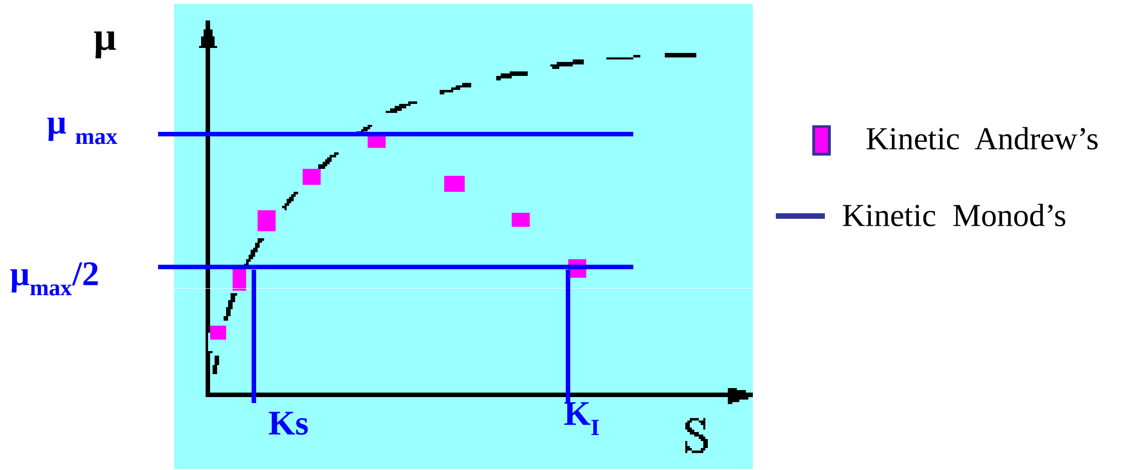
Statistics Living	Area (cm²)	Major axis (cm)	Minor axis (cm)	Perimeter (cm)
Min	*0.0083*	*0.151*	*0.071*	*0.373*
(Object #)	*1*	*11*	*1*	*1*
Max	*0.674*	*1.462*	*0.886*	*5.560*
(Object #)	*54*	*7*	*54*	*7*
Range	*0.666*	*1.312*	*0.815*	*5.187*
Mean	*0.133*	*0.539*	*0.305*	*1.767*
Std Deviation	*0.123*	*0.271*	*0.152*	*1.224*
Sum	***7.449***	***30.184***	***17.087***	***98.952***
Samples	*56*	*56*	*56*	*56*

Statistics Dead	Area (cm²)	Major axis (cm)	Minor axis (cm)	Perimeter (cm)
Min	*0.001*	*0.036*	*0.019*	*0.066*
(Object #)	*30*	*198*	*446*	*198*
Max	*0.021*	*0.194*	*0.137*	*0.647*
(Object #)	*39*	*39*	*39*	*237*
Range	*0.019*	*0.158*	*0.117*	*0.581*
Mean	*0.004*	*0.086*	*0.053*	*0.217*
Std Deviation	*0.004*	*0.037*	*0.031*	*0.130*
Sum	***0.266***	***5.664***	***3.486***	***14.292***
Samples	*66*	*66*	*66*	*66*



MATERIAL AND METHODS

Kinetic parameters: *μ, μ*



Monod kinetic equation

$$\mu = \mu_{\max} \times \left(\frac{S}{S + K_S} \right)$$

Andrews kinetic equation

$$\mu = \mu_{\max} \times \left(\frac{1}{1 + \frac{K_S}{S} + \frac{S}{K_I}} \right)$$

MATERIAL AND METHODS

- Growth rate (μ)

$$X = X_0 . e^{\mu t} \longrightarrow \ln X = \mu . t + c$$

- Mortality rate (b)

$$\frac{dXm}{dt} = b.Xv \longrightarrow b = \frac{1}{Xv} . \frac{\Delta Xm}{\Delta t}$$

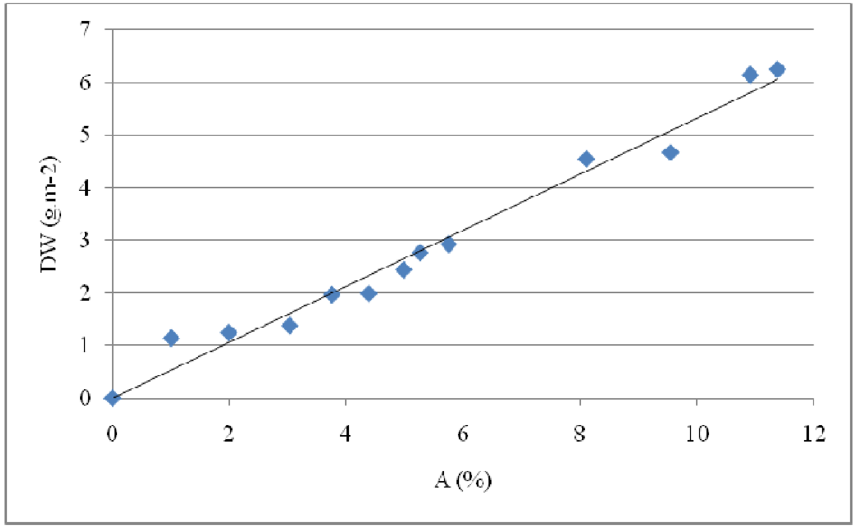
With Xm: Dead biomass

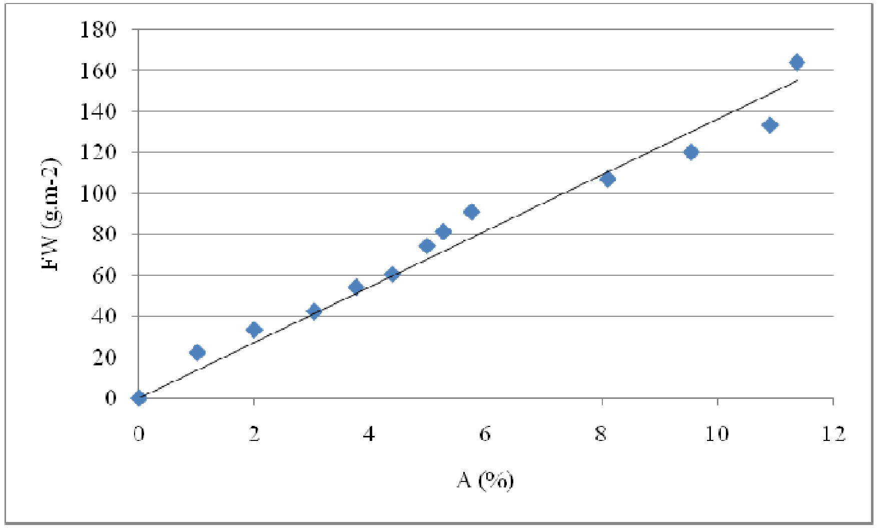
Xv: Living biomass

- $\mu_{tot.} = \mu + b$

RESULTS

RELATIONSHIPS BIOMASS Vs % COVERED AREA





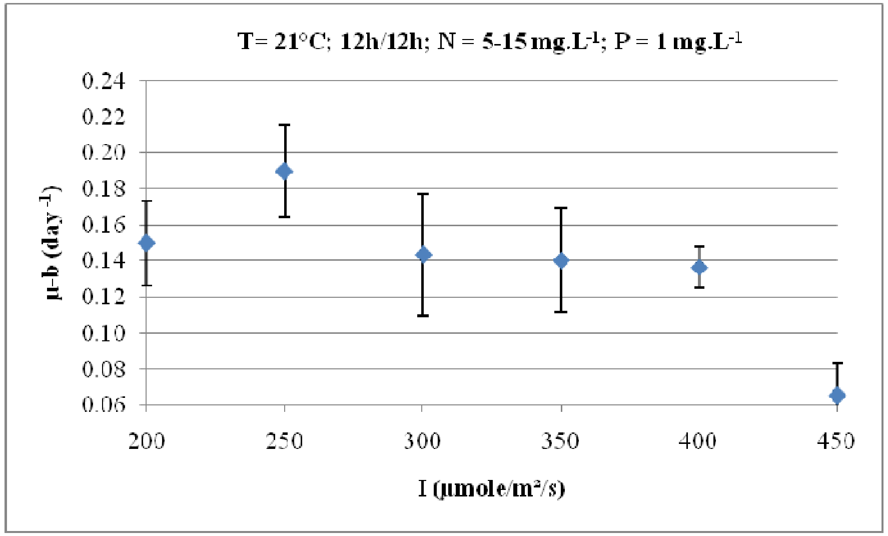
DW (g.m^{-2}) = 0.5325*A (%), R^2 = 0.98

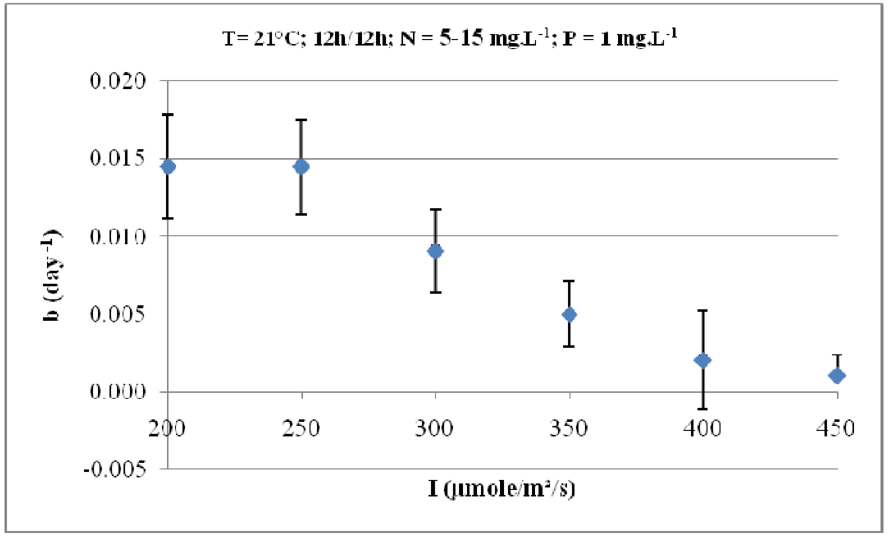
FW (g.m^{-2}) = 13.629*A (%), R^2 = 0.97

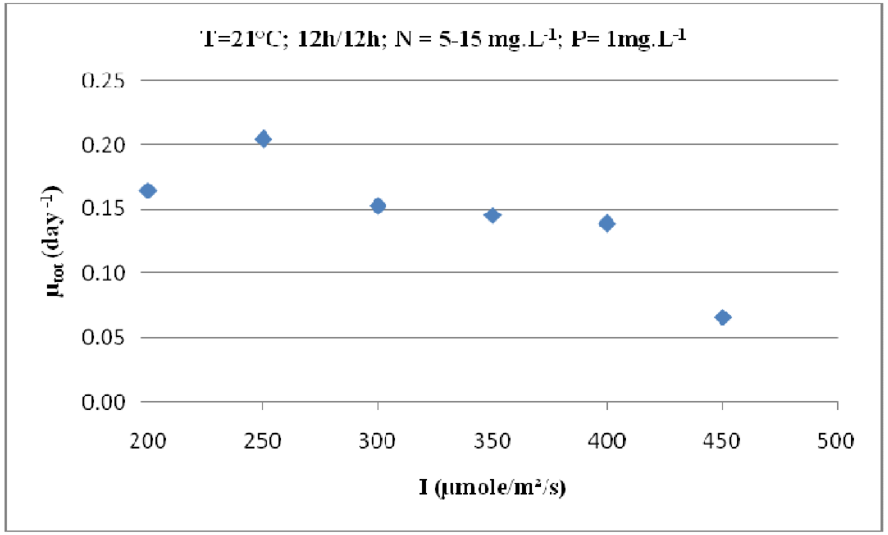
- ✓ Good relationships between the dry or fresh weight and the area covered by duckweeds in the pilot
- ✓ In natural conditions, weights depending on available nutrients

RESULTS

μ = f(I) and b = f(I)







RESULTS

Kinetic model of *Lemna minor* growth

$$\mu = \mu_{\max} \times f(I) \times f(T) \times \left(\frac{1}{1 + \frac{K_{S,N}}{[N-NH_4^+]} + \frac{[N-NH_4^+]}{K_{I,N}}} \right) \times \left(\frac{1}{1 + \frac{K_{S,P}}{[P-PO_4^{3-}]} + \frac{[P-PO_4^{3-}]}{K_{I,P}}} \right)$$

with $f(I) = A_I \frac{I}{I_M} \exp\left(1 - \frac{I}{I_M}\right)$ (Vatta *et al.*, 1995)

By Iteration: $\boldsymbol{I_M = 286.55\ \mu mol.m^{-2}.s^{-1}}$

$A_I = 1$; $T = 21°C$; $\mu_{max} = 0.19\ d^{-1}$ ($\boldsymbol{I_{exp.} = 250\ \mu mol.m^{-2}.s^{-1}}$)

$[N\text{-}NH_4^+] = 10$ mg/l; $K_{S,N} = 3.83$ mg.L^{-1}; $K_{I,N} = 204.27$ mg.L^{-1}

$[P\text{-}PO_4]^{3-} = 1$ mg.L^{-1}; $K_{S,P} = 1.26$ mg.L^{-1};*et* $K_{I,P} = 13.33$ mg.L^{-1} (Tangou *et al.*, 2013)

Optimum intensity for the growth of L. minor ranged between 250 and 300 µmol.m-^{2}.s^{-1}
Saturation observed at 400 µmol.m^{-2}.s^{-1}.

CONCLUSIONS

- **Image processing method**

- Interesting for the biomass determination : continuous and non destructive method

-Empirical-based relationships -> valid in the range of our experimental conditions

- **Modeling**

The model takes into account the influence of light at constant temperature. It calculates a production of living and mortality of *Lemna*. It could be combined with a system of natural WSP.

Thank you