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M. Le Guédard, J.J. Bessoule, Catherine Boutin, H. Budzinski, K. Le Ménach, et al.. The Omega-3 Index of macrophytes to improve the assessment of the treatment performance of constructed wetlands receiving treated wastewater. SETAC Europe 29th annual meeting, May 2019, Helsinki, Finland. pp.1, 2019. hal-02609358

HAL Id: hal-02609358

<https://hal.inrae.fr/hal-02609358>

Submitted on 26 May 2021

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The Omega-3 Index of macrophytes to improve the assessment of the treatment performance of constructed wetlands receiving treated wastewater.

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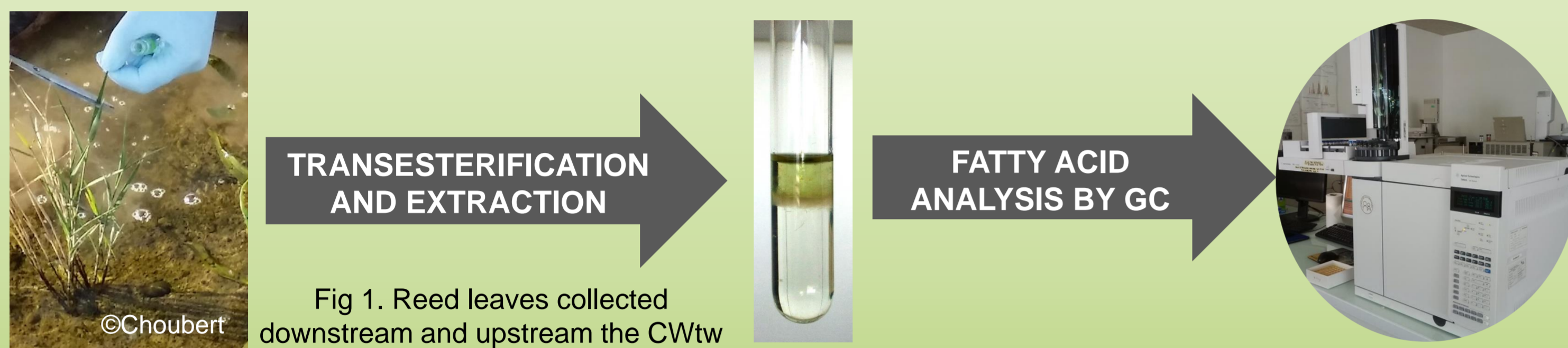
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Introduction

Effluents from wastewater treatment plants (WWTP) contain chemicals that are not removed (metals, pharmaceuticals, hormones...) and are therefore considered as a source of pollution for the aquatic environment. Constructed wetlands receiving treated wastewater (CWtw), built between the WWTP and the receiving environment, can help to limit the impact of WWTP discharge on the natural environment. The commonly methods used to evaluate the treatment performance of these processes are mainly based on physicochemical parameters. However, the wide variety of contaminants that can be encountered and their variable concentrations over time (sometimes below the limit of detection of equipments) constitute a limit of the use of such tools. Moreover, these methods do not provide information on the toxic impact of the treated water on the recipient ecosystem that is one expectation of the WFD. Plants respond to environmental changes and exhibit signs of stress in polluted conditions. They reflect the impact of the overall toxic contaminants on the ecosystem and by this way can fill the gap left by physicochemical analyses concerning the toxic impact of environmental realistic contaminant mixture on ecosystem. The Omega-3 Index, a standardized biological tool based on plant stress response, has been proven it worth to assess soil quality (ISO/FDIS 21479). In this study, the Omega-3 Index of reed leaves was tested as a biological tool to assess the treatment performance of CWtw and, as a consequence, the improvement of water quality. This biological tool was implemented on the experimental sites of the MARGUERITTES and BIOTRYTIS projects located in the suburb of Nimes and Bordeaux metropolises. These projects aimed at studying the operations and the true effectiveness for the removal of conventional pollutants and micropollutants of CWtw with different configurations (ponds, meadows, ditches built with soil and adsorbing materials).

Materials and methods



Leaf fatty acid composition analysis: fresh foliar tissues were placed in 1 ml of methanol containing 2.5% sulfuric acid. After 1 hour of heating at 80 °C, 0.75 ml of hexane and 1.5 ml of water were added. After vigorous shaking and centrifugation, methyl esters extracted in hexane phase (upper phase) were analysed by gas chromatography (Agilent 7890A). The percentage of each fatty acid was determined and the Omega-3 index was then calculated. **The Omega-3 index decreases when the soil is polluted and gives information on the health status of plants.**

First test with the Omega-3 index on a CW using reed bed filters receiving sewage from a village

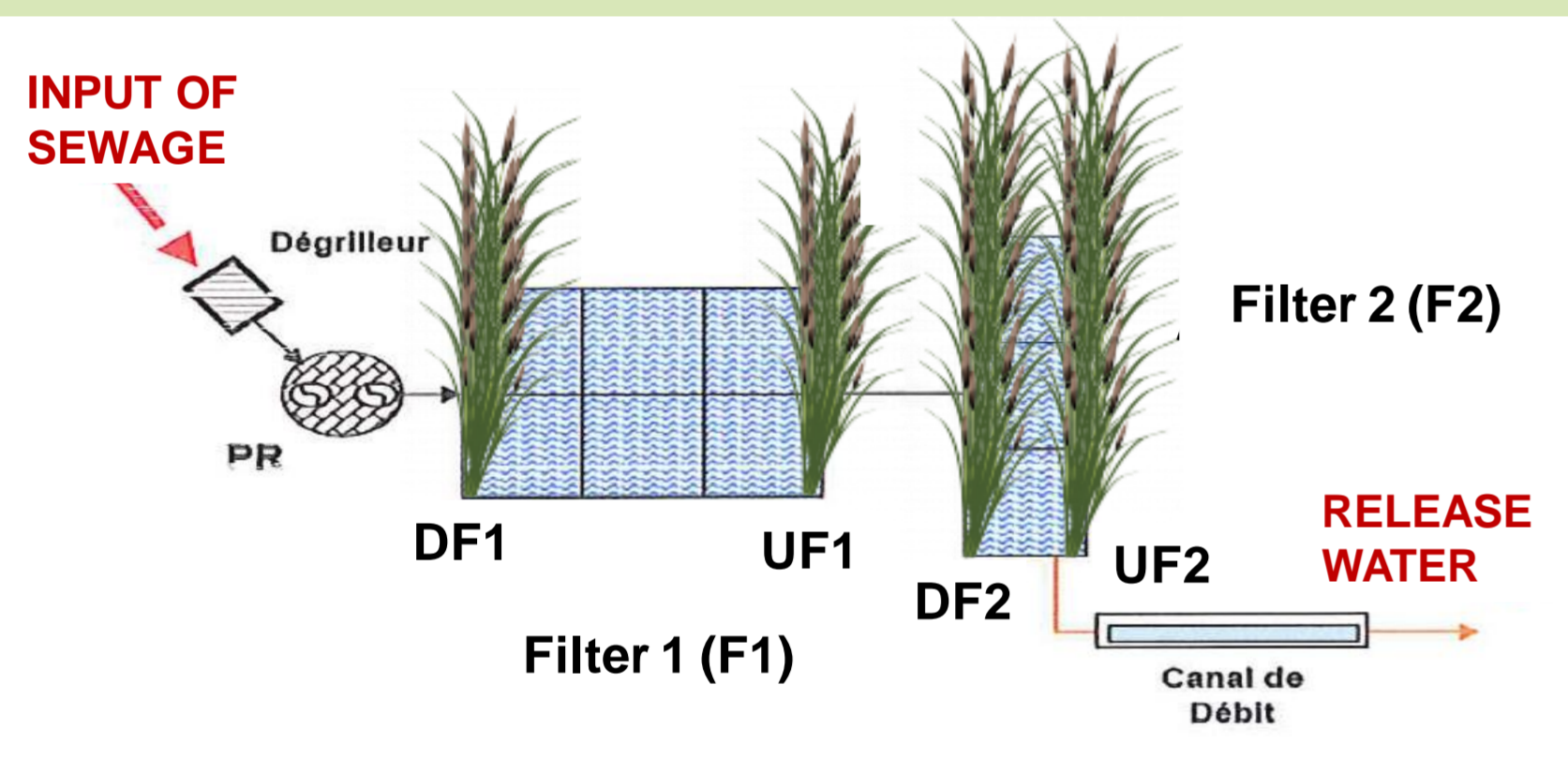


Fig. 2. Diagram of the reed bed filters with two stages planted with *Phragmites australis*. Reed leaves were harvested from upstream and downstream of the first stage (UF1, DF1) and of the second stage (UF2, DF2)

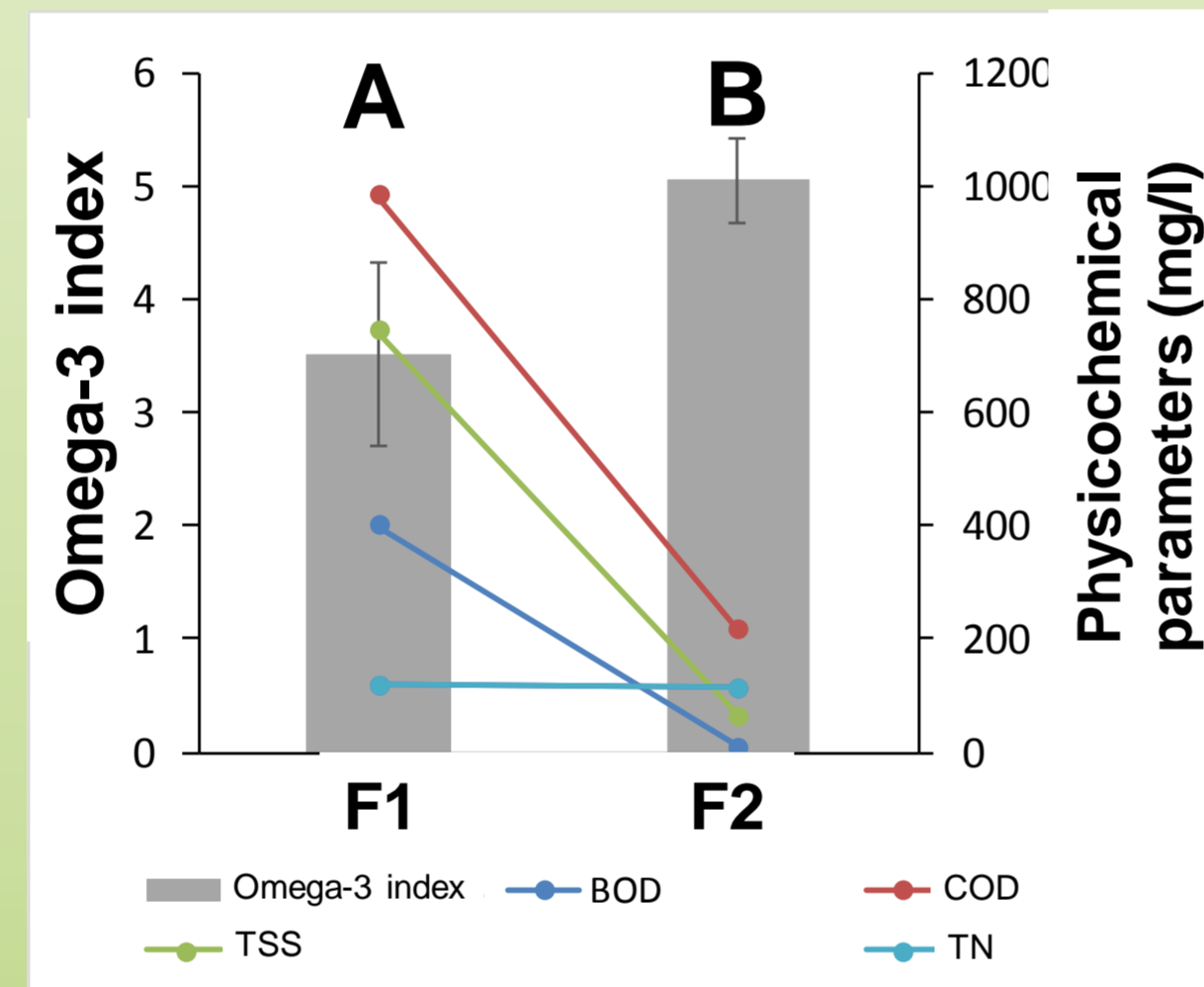


Fig. 3. Omega-3 Index measured on reeds harvested in the first stage and in the second stage of the reed bed filters and water quality parameters of the two stages: biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total nitrogen (TN)

Omega-3 Index negatively correlated to physicochemical parameters
⇒ Retention of pollutants in the 1st stage of reed-bed filters

⇒ **Omega-3 index can be used to assess water quality in CW**

What about CWtw?

Evaluation of the treatment efficiency of a pond CWtw: MARGUERITTES SITE

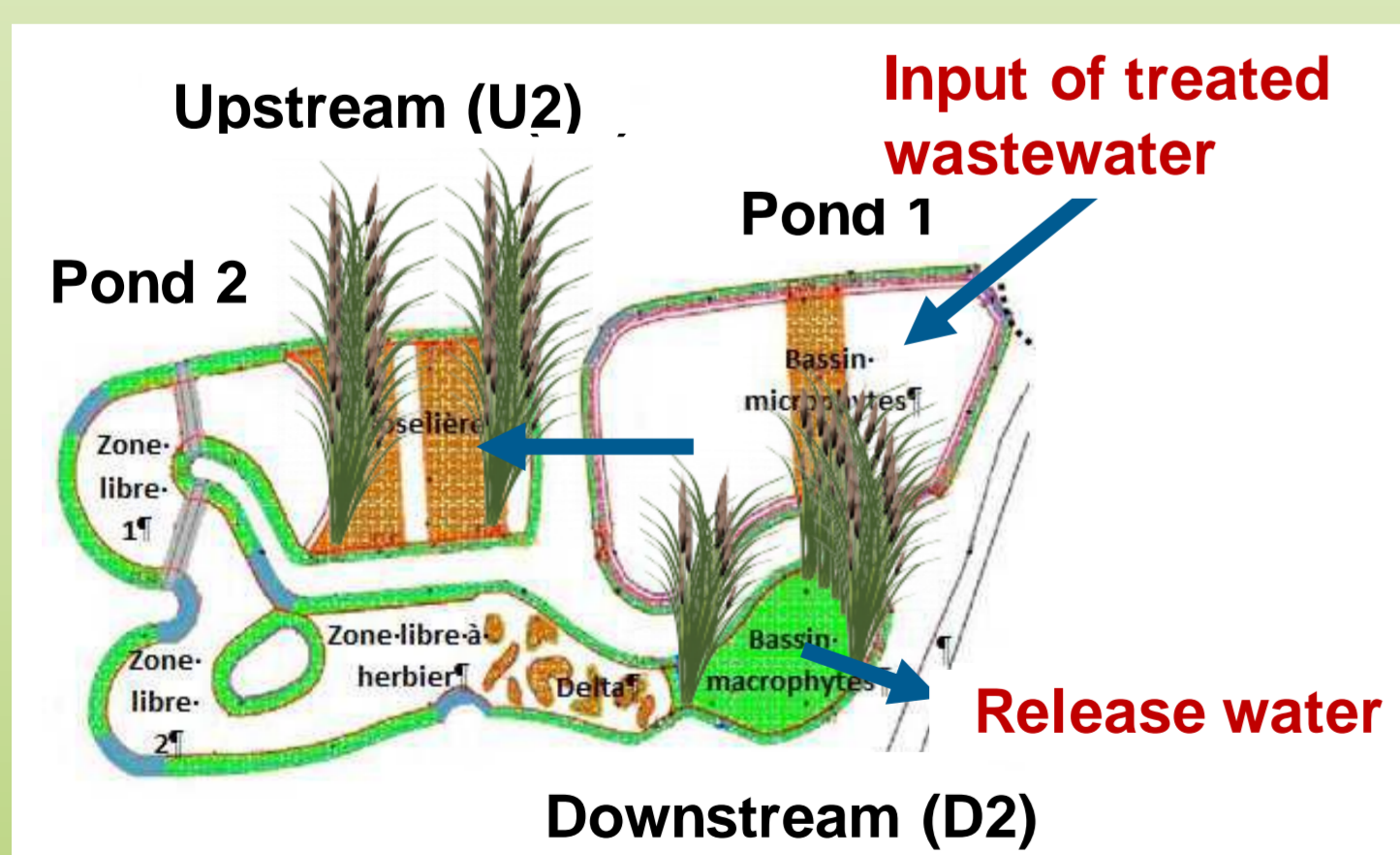


Fig. 4. Diagram of the CWtw of MARGUERITTES site with two ponds. Reed leaves were harvested from upstream (U2) and downstream (D2) of the second pond

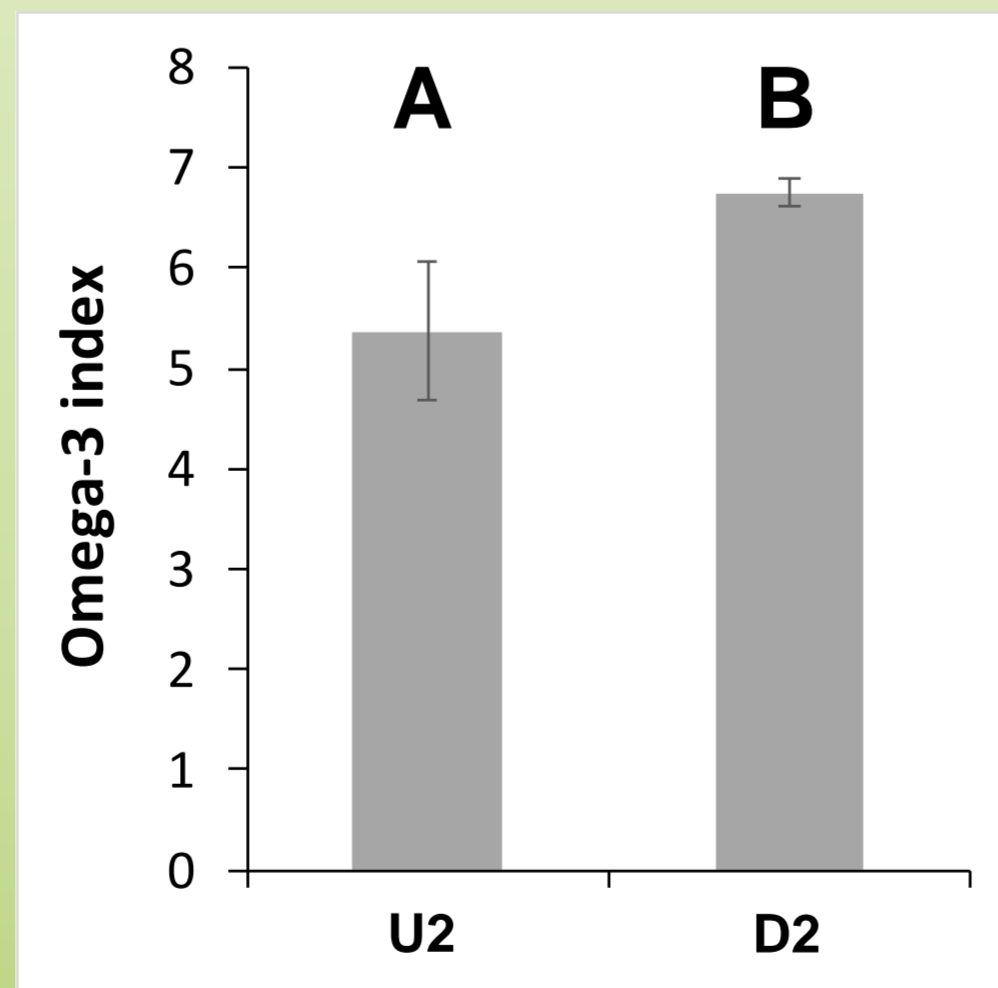


Fig. 5. Omega-3 Index measured on reeds harvested from upstream and downstream of the second pond

⇒ Retention of pollutants upstream of the pond 2
⇒ **Omega-3 index ⇒ bioindicator of CWtw performance**

Comparison of the treatment efficiency of several CWtw : BIOTRYTIS project



Fig. 6. CWtw with different configurations (meadows, ditches built with soil and adsorbing materials) studied during the BIOTRYTIS project

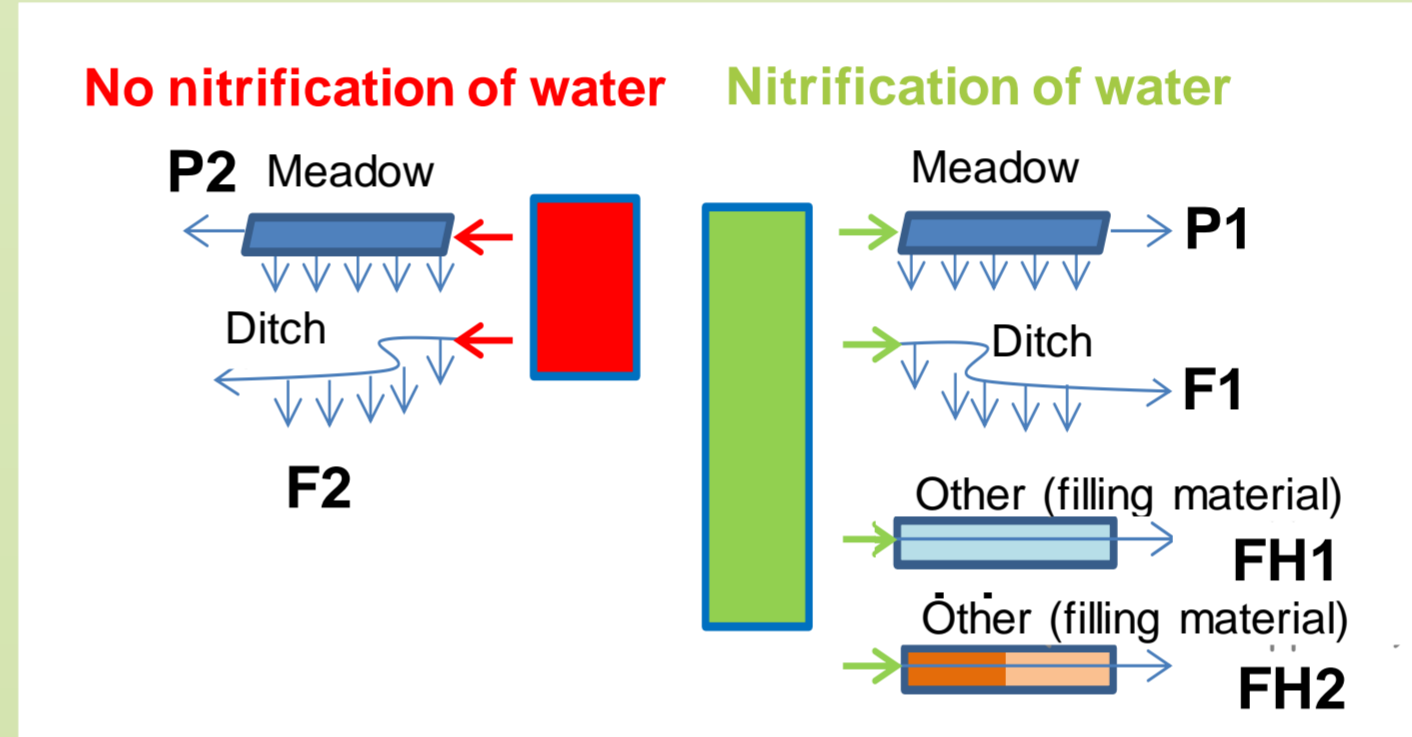


Fig. 7. Diagram of the CWtw built for the BIOTRYTIS project with different configurations (meadows, ditches built with soil and adsorbing materials). Reed leaves were harvested from upstream to downstream of each CWtw

2 years after putting the CWtw into service

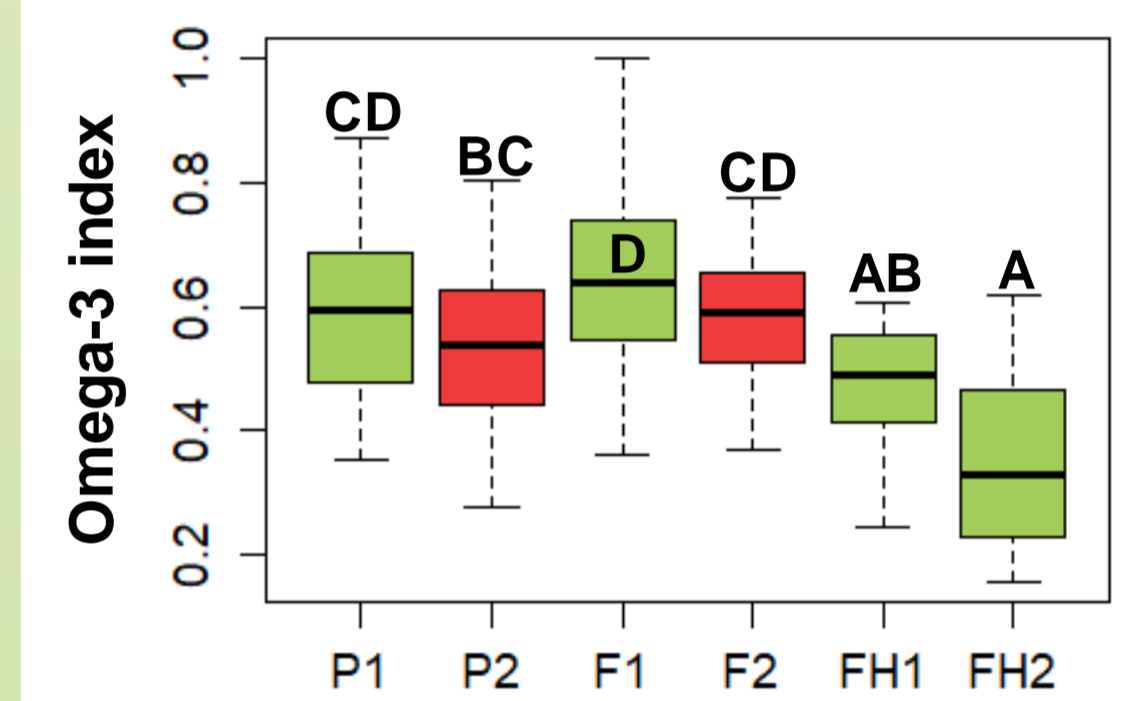


Fig. 8. Omega-3 index measured on reeds harvested from the different CWtw

Tab. 1. Removal efficiency (%) of some micropollutants analysed in water (Red <30%; 30%<Orange<70%; 70%<Green)

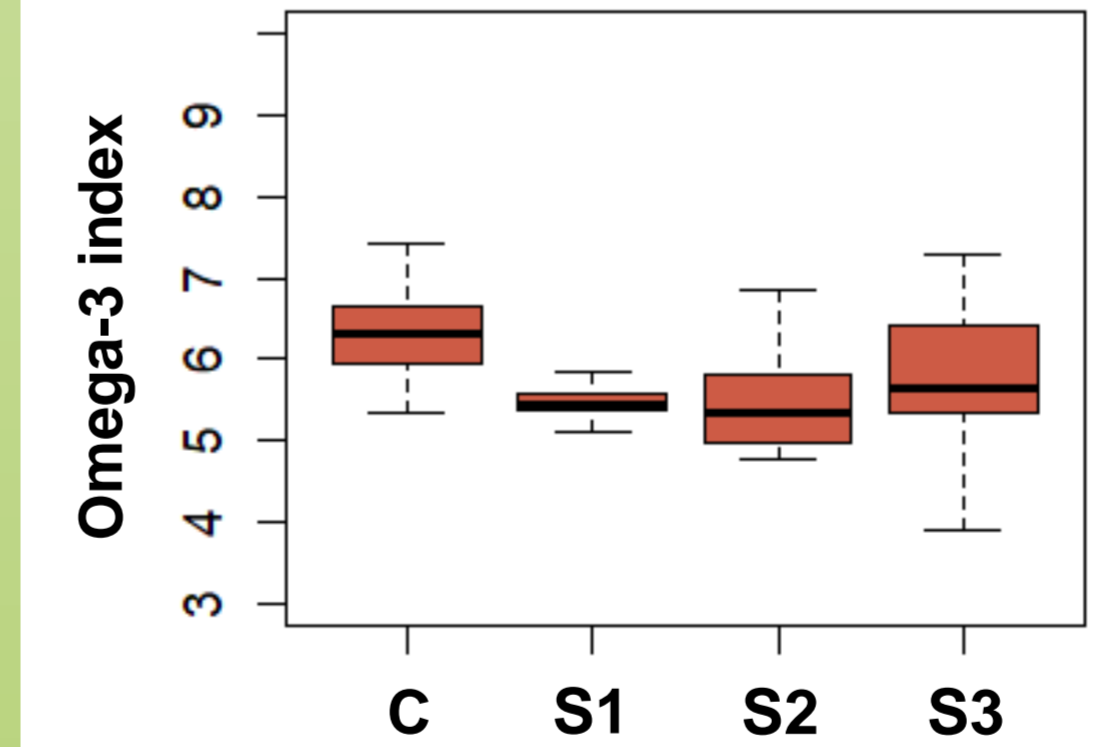
	P1	P2	F1	F2	FH1	FH2
Atrazine	Red	Red	Red	Red	Red	Red
Atrazine 2 hydroxy	Red	Red	Red	Red	Red	Red
Diuron	Red	Red	Red	Red	Red	Red
AMPA	Red	Red	Red	Red	Red	Red
Glyphosate	Red	Red	Red	Red	Red	Red
Hexazinone	Red	Red	Red	Red	Red	Red
Amisulpiride	Red	Red	Red	Red	Red	Red
Carbamazépine	Red	Red	Red	Red	Red	Red
Propranolol	Red	Red	Red	Red	Red	Red
Venlafaxine	Red	Red	Red	Red	Red	Red
Cr	Red	Red	Red	Red	Red	Red
As	Red	Red	Red	Red	Red	Red
Cd	Red	Red	Red	Red	Red	Red

⇒ Reeds health impacted in pilots FH1 and FH2

⇒ Higher retention of micropollutants in pilots FH1 and FH2

Pilot P2

Sampling campaign for 2015



Sampling campaign for 2017

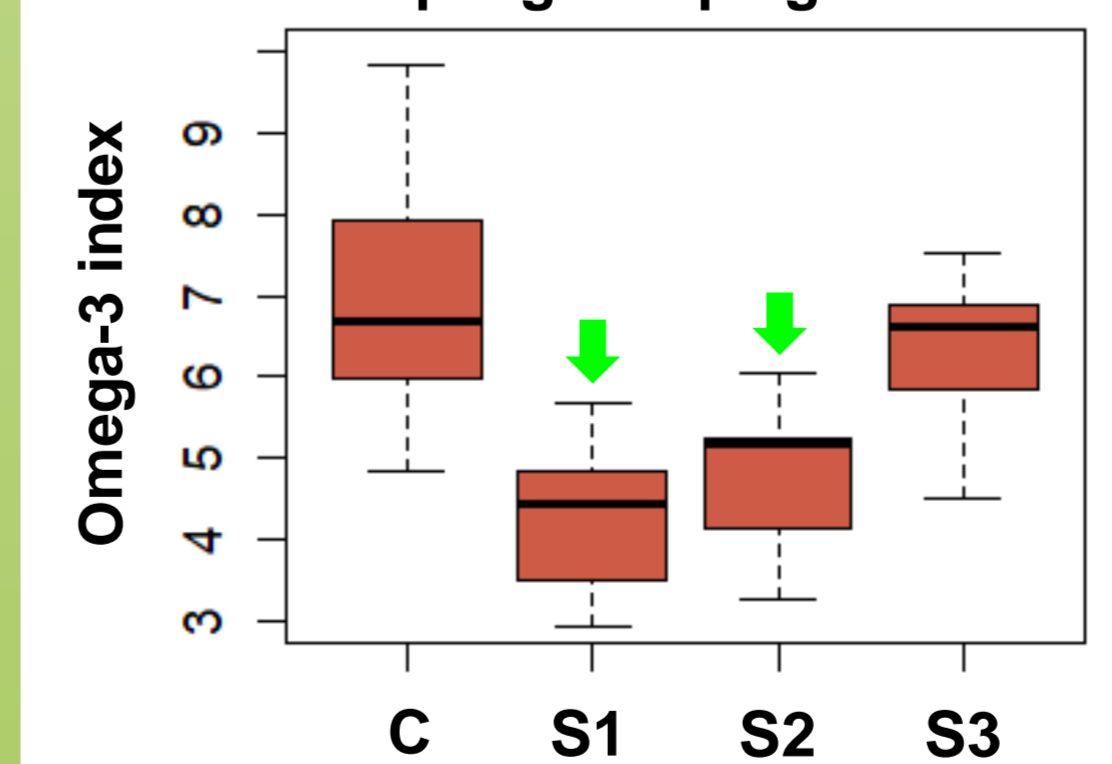


Fig. 9. Omega-3 index measured on reeds harvested in the pilot P2 before the release of the treated wastewater (C) and in 3 spots located from upstream to downstream (S1, S2, S3) after the release of treated wastewater

⇒ Reeds health is improved from upstream to downstream

⇒ Impact on reeds health is evident 2 years after putting the CWtw into service

Tab. 2. Concentration of some micropollutants in reeds harvested upstream and downstream the pilot P2 (ng.g⁻¹)

		Upstream	Downstream
Diuron (ng.g ⁻¹)	Roots	1,50	0,90
	Leaves	3,61	4,08
Carbamazépine (ng.g ⁻¹)	Roots	17,56	15,93
	Leaves	47,59	24,11
Propranolol (ng.g ⁻¹)	Roots	89,10	17,33
	Leaves	71,18	76,10
Venlafaxine (ng.g ⁻¹)	Roots	35,86	<lq
	Leaves	14,92	32,32
Amisulpiride (ng.g ⁻¹)	Roots	154,81	30,54
	Leaves	1,69	<lq
Cr (µg.g ⁻¹)	Roots	0,58	0,58
	Leaves	7,31	4,60
Cu (µg.g ⁻¹)	Roots	10,8	8,30
	Leaves	47,7	17,17
Zn (µg.g ⁻¹)	Roots	37,2	42,9
	Leaves	91,6	35,10

Omega-3 index ⇒ could be used as complementary tool for CWtw water quality management decisions

Conclusions

All our results obtained show that plant health is positively correlated with the improvement of water quality from downstream and therefore the Omega-3 index seems to be a relevant biological tool to assess CWtw performance. **From a regulatory standpoint, this bioindicator could help to better evaluate the treatment performance of CWtw and to assess the complex effects of pollutants present in this type of process in mixture and at very low concentrations. The ISO standard of this tool used in soil quality will be published very soon (ISO/FDIS 21479) and could be extended to water quality assessment.**

