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Optimization of the Sequential Sedimentation-Biofiltration System (SSBS) with application of sorptive materials

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LIST OF ABBREVIATIONS

CW: Constructed Wetland

DO: Dissolved Oxygen

ERCE: European Regional Centre for Ecohydrology

HF: Horizontal Flow System

HRT: Hydraulic Retention Time

IHP: International Hydrological Programme of UNESCO

LECA: Lightweight Expanded Clay Aggregates

RPM: Rotation Per Minute

SS: Suspended Solids

SSBS: Sequential Sedimentation-Biofiltration System

TN: Total Nitrogen

TP: Total Phosphorus

TSS: Total Suspended Solids

UNESCO: United Nations Educational, Scientific and Cultural Organization

VF: Vertical Flow System

VSS: Volatile Suspended Solids

WFD: Water Framework Directive

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1. Introduction

Over the past two centuries, the shift from a mechanistic to a more holistic view of nature has led man to abandon conventional technologies in favour of more integrated, environmentally beneficial and financially less costly methods.

This is how constructed wetlands (CWs) were created in the 1980s on the principles of the concept of Ecohydrology. Constructed wetlands are ecological systems that are artificially created, as opposed to natural wetlands whose purpose is largely to provide wildlife habitat. Constructed wetlands can be defined as engineering systems that use natural wetland ecology to remove impurities from wastewater or rivers.

The use of these true bioengineering tools over time has gained popularity due to its efficiency performance in depollution of suspended matter, organic matter, phosphorus, nitrogen and emerging contaminants. These contaminants, if exposed to receiving watercourses, generate health risks to the general public, aquatic organisms and the environment by creating severe eutrophication of inland waters.

Water treatment in artificial wetlands is therefore achieved by a combination of the following processes: sedimentation of suspended matter; physical trapping by plant roots and rocks, biological oxidation of organic matter and ion exchange/adsorption with organic litter and sediment.

Two main types of artificial wetlands are used for water treatment: the surface flow system and the subsurface flow system. Surface flow systems are shallow beds or channels filled with emerging macrophytes. The water surface is directly exposed to the atmosphere as the water flows between the plants.

Submerged flow systems are similar in construction except that they are filled with rock, gravel or sand. The porous medium supports the macrophytes root system and the water level is maintained below the top of the medium. Understanding and evaluating the mechanisms that lead to the removal of these contaminants for these two types of systems is therefore crucial.

For this reason, for the first part of the study, we carried out a complete diagnosis of the functioning of a biofiltration zone of a mature heterogeneous Sequential Sedimentation Biofiltration System (SSBS) having two subparts: a surface flow system and a groundwater flow system having a gravelly subsoil.

The objective of second part was to test different reactive materials in order to evaluate their phosphorus removal capacity. Indeed, one way to improve phosphorus reduction capacity in the wetland is to use more efficient filter materials than conventional materials generally used in the construction of CWs. After reviewing the literature, we therefore decided to test three reactive materials for phosphorus removal to assess their capacity: a modified material which is LECA® and two natural materials which are different types of dolomite. All this work therefore represents a major interest for research into improving the operating efficiency of constructed wetlands.

The presentation of the hosting institution is available in Appendice I. Moreover, this thesis will be structured in three parts: The first one will be devoted to the state of the art regarding the SBSS (definition, classification, interest in water treatment, classic operating mechanisms of an SSBS).

The second part will be devoted to carrying out the study on the mature SSBS (evaluation of the total efficiency performance of the biofiltration zone of the SSBS and its two sub-sections concerning different contaminants. Finally, the last one will be dedicated to testing substrates in batch experiment (quantity of materials tested, concentration of the phosphorus solution, contact time, pH).

2. Context of the study

2.1 The concept of Ecohydrology

In the 20th century, there is urgent need for a new approach for sustainability in the context of increasing human pressures, exacerbated by global climate and social change, with declining water quantity and quality, increasing pollution, and biodiversity (terrestrial and aquatic ecosystem).

Consequently, the major key challenges that Water Framework Directive (WFD) will face in terms of water management is to promote aquatic environment, improve the status of aquatic ecosystems, prevent and reduced water degradation and finally, mitigate the effects of floods and droughts which are likely to become more intense and more frequent in the future because of climate change.

Moreover, increasing progress in the environment sciences provided the background for the development of an integrative sustainability science which is ecohydrology.

Ecohydrology is a transdisciplinary science defined as a sub-discipline of hydrology focused on biological aspects of the hydrological cycle, therefore blending two often contradicting approaches to water resources management (hydrology vs ecology). Ecohydrology is a scientific concept applied to environmental problem-solving (Zalewski and al. 1997) which has been developed in the framework of the International Hydrological Programme of UNESCO (UNESCO-IHP), the mission statement of the ecohydrology program.

Its purpose is to quantify and explain the dual regulation between hydrological processes and biotic dynamics from molecular to a catchment scale (Maciej Zalewski 2011). In other terms, it means that hydrological processes are shaped by biological dynamics and that, ecological dynamics are also controlled by hydrological processes (Figure 1). Moreover, water quality is regulated by shaping biotic structures and communities in ecosystems and vice versa, the dynamics at the catchment scale is influenced by the regulation of hydrological mesocycle.

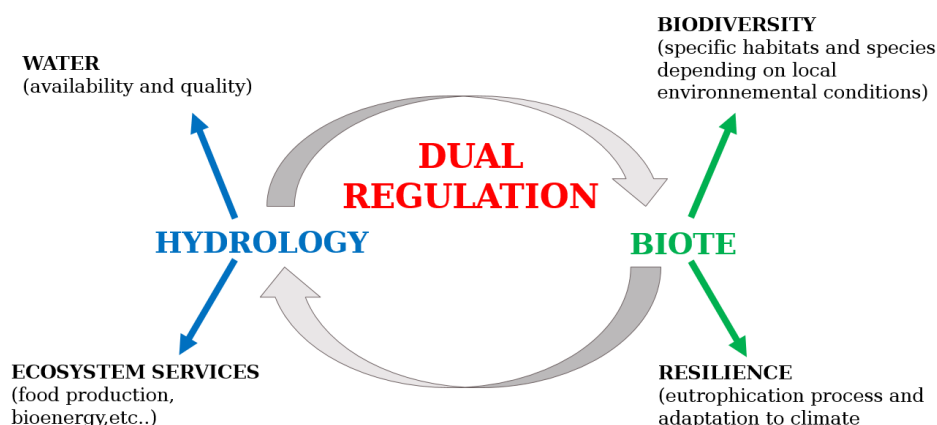


Figure 1: Diagram of the ecohydrology principle, a nature-based solution for the circular economy concept (Zalewski 2011)

As a result, ecohydrology provides scientific understanding of the hydrology/biota interplay, but also a systemic framework on how to use ecosystem processes as a new tool for management of water resources, complementary to already applied hydrotechnical solutions. The goal is to prevent and reduce ecological threats and therefore to enhance environmental sustainability.

To summarize, this concept is based upon the assumption that the sustainable water resources management can be achieved by:

- restoring and maintaining evolutionarily established processes of water and nutrient circulation and energy flows at a catchment scale;
- enhancing the carrying capacity of ecosystems against human impact based on evolutionarily established resistance and resilience to stress;
- using ecosystem properties as water management tools.

In addition, this approach aims at resolving technological issues faced by humanity but also societal and economical matters by taking into account the relationships between environment and cultural heritage.

This point of view has been highlighted by UNESCO IHP which now defines the ecohydrology programme as “a scientific programme to understand and elucidate the dynamic relationships between hydrological, ecological and social systems [...] and to seek new ways to balance human and environmental needs for water resources”(Unesco 2007) *in* (Zalewski 2011).

It is then obvious that scientists (hydrologists, ecologists and hydrological engineers, for example) cannot implement this approach by themselves and this is why the involvement of stakeholders is necessary and sharing the knowledge with the population too.

Many projects applying this concept have been implemented in Poland and on different continents within the framework of the UNESCO work (UNESCO demosites website, <http://ecohydrology-ihp.org/demosites/>). Each site project applies ecohydrology principles: (1) hydrological, by the quantification of the hydrological processes and threats at the basin scale, (2) ecological, by the enhancement of ecosystem resilience and (3) ecotechnological based on ecohydrological biotechnologies (Zalewski, 2010) in order to enhance its “carrying capacity” in the four dimensions: (1) water resources, (2) biodiversity, (3) ecosystem services and (4) resilience (Zalewski 2015)

2.2 Context

Rapid urbanization expansion, industrial and agricultural development in many developed and developing countries are often associated with large wastewater discharges, which require intensive treatment before being released to aquatic environments.

Although each technology has its own advantages and disadvantages, conventional technologies as oil and grit separators or settlement ponds (Haberl, Perfler, and Mayer 1995); (Saeed and Guangzhi 2012), also known as grey infrastructure (Jurczak et al. 2018), are not necessarily very attractive to implement because they require a significant supply of fossil fuels, whereas Nature-Based Solutions (European Commission and Directorate-General for Research and Innovation 2015) depend mainly on natural energies such as wind, solar radiation and biomass storage thereby reducing operation costs and can provide more complex and multifunctional benefits such as being able to fully integrate into Blue-Green Infrastructure (European Commission, 2013).

In urban watersheds, discharge of various industrial and domestic effluents (point source pollution), into surface waterways could, in the long term, seriously damage the environment. Although, in some cases, natural stream purification may be a viable means of maintaining the ecological health of watercourses (Shao et al. 2014); (Saeed et al. 2016), its efficiency could be extremely reduced when the quantity and concentration of the pollutant load of effluent discharges is too important.

Thus, the natural self-purification capacity of a waterway being limited, large inputs of pollutants, including nutrients (nitrogen and phosphorus from wastewater) linked to increasing urbanization and, as a result, a decrease in pervious surfaces, can lead to a severe and prolonged degradation of water

quality. Indeed, two major problems can occur: (a) the occurrence of toxic cyanobacterial blooms that may prevent city residents from using the reservoirs for recreational purposes (Jurczak et al. 2012) and (b) and often a decrease in dissolved oxygen (DO) concentration, resulting in the death of aquatic organisms.

Consequently, the natural wetland, a water-saturated area, either permanently or seasonally, is a low-cost green treatment that is a viable option for improving the pollutant removal capabilities of open water channels. Numerous studies (Haifeng 2014); (Shutes et al. 1997); (Hickey, Arnscheidt, and Eadaoin 2018) have reported the potential application of natural wetlands to improve water quality in polluted rivers.

However, the establishment of natural wetlands for the development of polluted surface waters in urban catchments is often difficult, due for example to the lack of land available on riverbanks, as they are often used in cities for infrastructure, such as roads or housing, or in more rural areas, for agricultural activities.

Thus, due to the limit of space within cities, it is not possible to implement solutions such as natural wetlands to purify water. We need solutions that require less space for at least same or better results: the constructed wetlands (CWs).

2.3 Constructed wetlands

2.3.1 Interest for water treatment

Under such circumstances and urged by the acceleration of climate change, constructed wetlands (CWs), a combination of engineering and biological measures, can provide a balance between the necessity of treating polluted river waters and shortage of land areas for industrial or agricultural activities in cities.

Indeed, the main interest of the establishment of artificial wetlands is based on the fact that they constitute a place where the same chemical and biological processes occur as in natural wetlands with the advantage of being dimensioned according to the precise purification needs.

Indeed, these technologies can be optimized to reach a given treatment level for a specific parameter (carbon pollution, nitrogen, phosphorus...). Thus, with a good system maintenance, constructed wetlands can therefore achieve higher performance than natural wetlands for water purification.

In addition, for a wetland to be as efficient as possible, the wastewater flow must be evenly distributed over the entire surface. Of course, this condition can be easily met in artificial wetlands by controlling the water level and using appropriate devices for water supply and drainage, whereas this is more difficult to achieve in natural wetlands.

Indeed, natural wetlands are conditioned by the initial terrain configuration and topography that create preferential flow paths. Hydrodynamic studies using tracers have shown that the effective surface area for water purification does not exceed 10% of the surface area in natural wetlands (White and al. 2011).

These technologies have many advantages that make them an interesting alternative to conventional treatment methods. In particular, these biological treatment systems are economical and easy to operate and maintain. Thus, artificial wetlands can operate without external energy input and can be operated by personnel with no special skills. Moreover, they show a high level of reliability and efficiency in the literature.

2.3.2 Definition and classification

CWs are saturated or unsaturated artificial wetlands composed of one or more types of emergent, floating or submerged macrophytes planted in the substrate and one or more media, both selected for optimal pollution control effectiveness.

In addition, the colonization of microorganisms that develops around suspended roots, forming a complex network of roots and biofilms creates a "mesh" to increase the net pollutant removal efficiency as water passes through the network. It was created for the purpose of treating anthropogenic discharge such as municipal or industrial wastewater or stormwater runoff.

Then, it is important to notice that it exists two basic types of constructed wetlands: surface flow systems and subsurface/groundwater flow systems (Shaeed 2012); (Hainming and al. 2014).

Firstly, surface-flow wetlands shown on Figure 2 are similar to natural wetlands, with shallow systems where wastewater flows over the top of the system and infiltrates down through the media.

Secondly, subsurface flow wetlands (Figure 2) allows water to come into contact with microorganisms that live on the surface of plant roots and substrate.

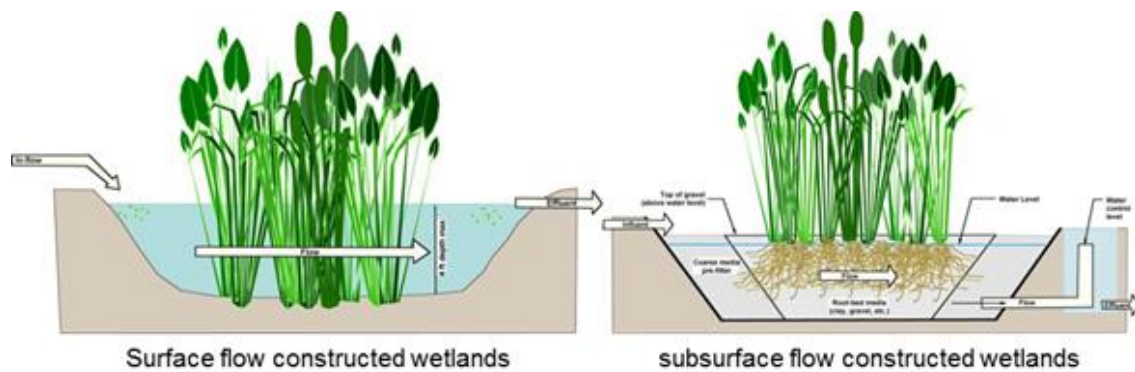


Figure 2: Scheme emphasises the differences between surface-flow and subsurface wetlands (theadvocateproject.eu)

In addition, wetlands constructed by groundwater flow are divided into two main groups (1) vertical flow (VF) systems and (2) horizontal flow (HF) systems. Both are generally more effective than surface flow wetlands in terms of massive removal of pollutants per m² of system surface area because the active part of the wetland is below ground so consequently, they are more effective at filtration and microbial processes and they can stay above freezing temperatures.

Each system has its own specificities, but only the analysis of the advantages and disadvantages of the HF system is presented in the following Table 1 since the VF system will not be the subject of our study.

Table 1: Advantages and disadvantages of HF wetlands. (Shaeed 2012)

Advantages	Disadvantages
<ul style="list-style-type: none">• Long flowing distance• Nutrients gradients can be established• Efficient on the removal of SS and organics• Denitrification possible• Formation of humic acids for N, P removal	<ul style="list-style-type: none">• High area demand• Clogging problem• Sulphur transformation can effect nitrification• Loss of P removal performance

2.4 Review of the Sequential Sedimentation-Biofiltration Systems

2.4.1 The different SSBS zones and their functions

The shift from a "mechanistic" to a more holistic view of nature has moved us from conventional technologies to more integrated, environmentally beneficial and financially less costly methods, based on the principles underlying the concept of ecohydrology (Zalewski et al. 1997, 2011).

One of the examples of ecohydrological solutions is the Sequential Sedimentation-Biofiltration System (SSBS), a bioengineering tool resulting from the optimization of biotic and abiotic parameters of conventional wetlands (optimization of the system's feeding mode, hydrological, ecohydrological and microbiological parameters).

SSBS can be considered as three-phase reactors (solid/liquid/gas) in which physical, chemical and biological phenomena occur. Thus, raw urban river water undergoes different purification processes through these reactors:

- physical filtration of suspended solids (SS),
- biological degradation by bacteria developed on rhizomes;
- physicochemical transformations (sorption, precipitation, mineralization)

One SSBS was therefore built on the Sokolowka River (during a SWITCH project) and another in the Arturówek Park (during Eh-Rek, Life + project) in the city of Lodz (Poland). The first system aims at purifying a small urban river whose hydrological regime is dominated by stormwater and meltwater while the second system aims at retaining and purifying stormwater runoff from a street that run directly to a cascade of recreational reservoirs. These are surface flow constructed wetlands during the normal and flow periods, but during winter the systems functions rather like a wetland constructed in the HF subsurface (Szklairek and al. 2018).

Each SSBS is composed of 3 zones, each of which has a specific function: a sedimentation zone with added structures to improve sedimentation, a geochemical barrier consisting of a dolomite/limestone deposit and a biofiltration zone (Case of the SSBS of the Sokolowka River, Figure 3):

- The sedimentation zone is an area with concrete structures and lamellar structures that reduce the energy of the inflow and improve the percentage of sedimentation. The barrier is fixed to the ground and covered with biodegradable geofibre material (400 g/m²) to increase efficiency of the sedimentation process.
- An area of intensive biogeochemical processes (with groundwater flow) in the limestone gabion (Sokolowka river)/ dolomite (Bzura river): an area that increases the intensity of biological processes, has an additional filtration function and adsorbs PO₄³⁻.
- A biofiltration zone (with surface runoff):
In the case of the Sokolowka River, a wetland with *Phragmites australis*, *Typha angustifolia*, *Acorus calamus* planted side by side (in strips 6 m, 10 m and 8 m long respectively) in a sandy-gravelly subsoil (conventional environments) 0.35 m deep. It is also interesting to note that 8 m long PVC sheet piles were installed perpendicular to the direction of flow, which increased the length of the water flow path by about 25-30%.

In the case of the Bzura River, a small floating island (6 m²) located in the outflow part of the SSBS planted with the following aquatic vegetation: *Typha angustifolia*, *Carex riparia*, *Glyceria maxima*, *Iris*, and *Ceratophyllum demersum*.

In the case of the Sokolowka River, the main channel of the river functions as a "diversion channel" to prevent problems that can arise when hydraulic conductivity is too high: erosion and discharge of pollutants from the wetland into the watercourse.

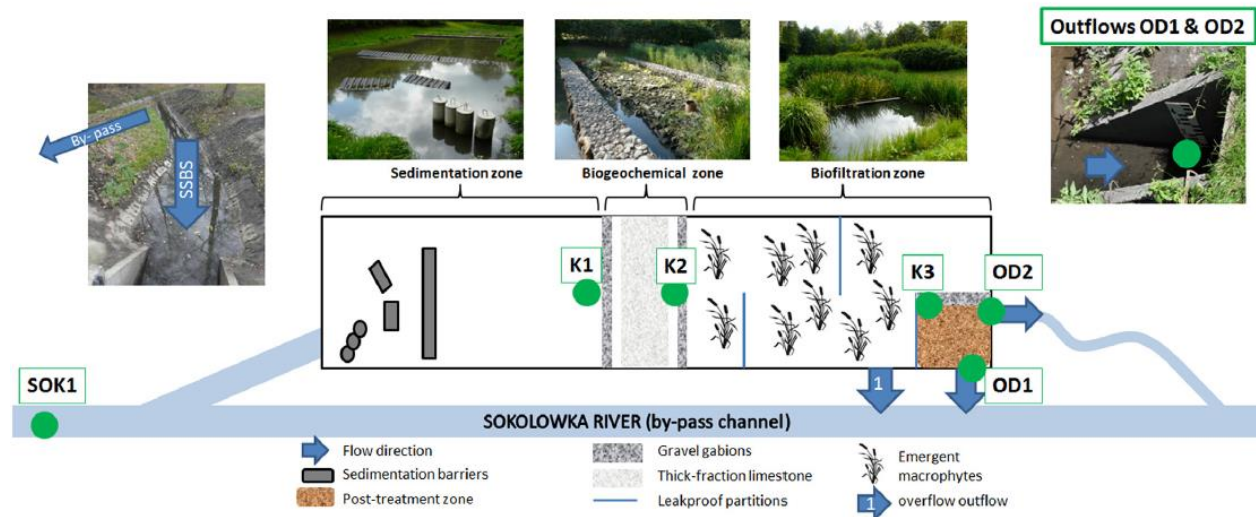


Figure 3: Scheme of the different parts of the SSBB (Szklaek and al., 2018)

Finally, it should be noted that the specificity of SSBSs lies in the fact that it has, in addition to the traditional biofiltration zone, different separate beds with their own function (sedimentation zone and geochemical barrier), which aim to increase the pollution elimination rate.

2.4.2 Abiotic and biotic processes in the biofiltration zone

Efficiency analyses of biofiltration zones published in 2018 by Szklaek et al (Sokolowka river) and Jurczak et al, 2018 (Bzura river), show that the efficiency share of the biofiltration zone to remove pollutants in general (except NH_4^+ and NO_2^- for SSBS in the Bzura river) is relatively low compared to that of the sedimentation zone in particular (Figure 4).

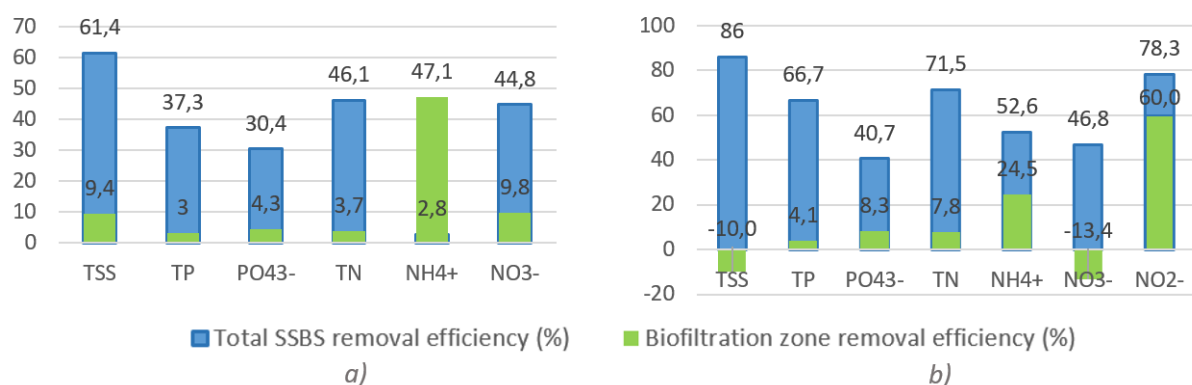


Figure 4: Overall removal rate and biofiltration zone removal rate: a) Sokolowka river and b) Bzura river (Szklaek and al. 2018); (Jurczak and al. 2018).

These results could be partially explained by the spatial location of the biofiltration zone in space. In fact, it is located after the two previous barriers, especially behind the sedimentation zone which has higher yields in terms of suspended solids and nutrient removal because it first captures much of the

pollution that is easier to remove. These results raise questions about the role of substrate and vegetation in nutrient removal in the SSBS zone.

2.4.2.1 Phosphorus removal:

In SSBS, particulate and inorganic phosphorus removal occurs by physical retention by filtration through the porous medium while others parts of phosphorus can be assimilated by plants or microorganisms forming a biofilm on the root surface (Figure 5).

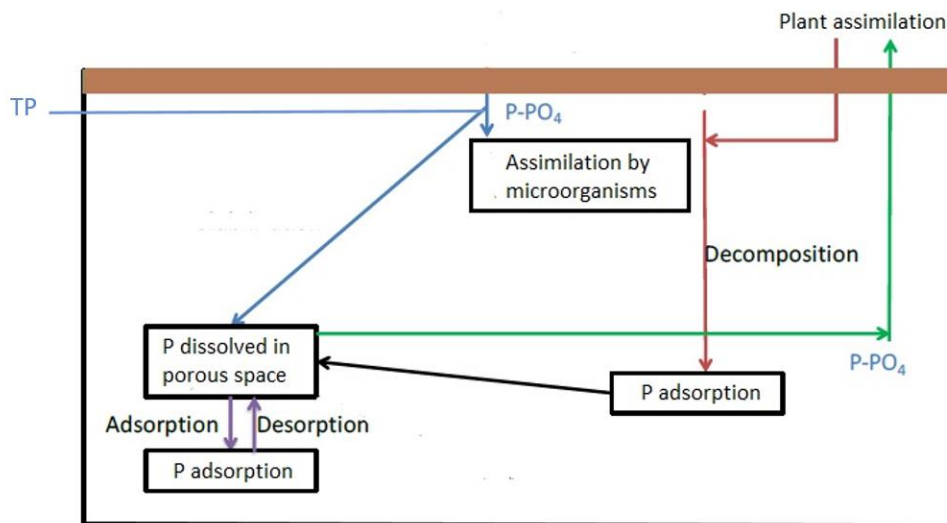


Figure 5: Simplified diagram of the phosphorus cycle taking place in the SSBS biofiltration zone; adapted from Prigent 2012

Two main mechanisms allow phosphorus removal in SSBS:

➤ Physico-chemical mechanisms:

Sorption is a phenomenon that occurs when inorganic soluble phosphorus $P-PO_4^{3-}$ attaches to a porous inorganic (SSBS substrate) and/or organic (biofilm, roots) support (Prigent 2012). Phosphorus sorption depends mainly on the concentration of $P-PO_4^{3-}$ in the environment and the substrate ability to accumulate $P-PO_4^{3-}$ to saturation.

In addition, the sorption of $P-PO_4^{3-}$ on the substrate may be reversible when environmental conditions change, this is what is called a desorption phenomenon.

➤ Biological mechanisms:

- Plant assimilation: All plants assimilate phosphorus for their growth through the root system and accumulate it in their different parts. One way to remove phosphorus is therefore to mow the aerial part of these plants and extract cut plants. For emerging macrophytes, export of P by plant mowing is estimated to be low, between 30 and 150 kg P.ha⁻¹.yr⁻¹ (IWA, 2000). Thus, since it would take a huge surface area to achieve an interesting phosphorus removal rate, phosphorus removal by mowing plants does not seem to be a good pathway to be developed.

- Microbial assimilation: Dephosphatation by microorganisms is based on an overaccumulation of phosphorus by biomass. This biological phosphorus removal pathway requires demanding operating conditions such as alternating aerobic/anaerobic conditions and adapted hydraulic residence times (Prigent 2012). Under optimal conditions, bacteria can absorb about 40% of the total phosphorus present in raw wastewater (Strickler and Hédut 2010) but the smooth running of this type of process is difficult to control and to implement sustainably in SSBSs.

2.4.2.2 Nitrogen removal

While planted wetlands are not extremely effective in removing phosphorus (Abdelhakeem et al. 2016), they are effective in removing unwanted nutrients such as nitrogen and organic matter (Huett 2005); (Dan et al. 2011). Eight nitrogen transformation mechanisms (biological or chemical) have been identified in constructed wetlands (Figure 6). It should be mentioned that the three main purification mechanism in SSBSs is biological and refers to nitrification, denitrification and plant assimilation (range of 0.5 to 40.0% total nitrogen removal) (Shamir et al. 2001); (Drizo et al. 1997); (Meers et al. 2008).

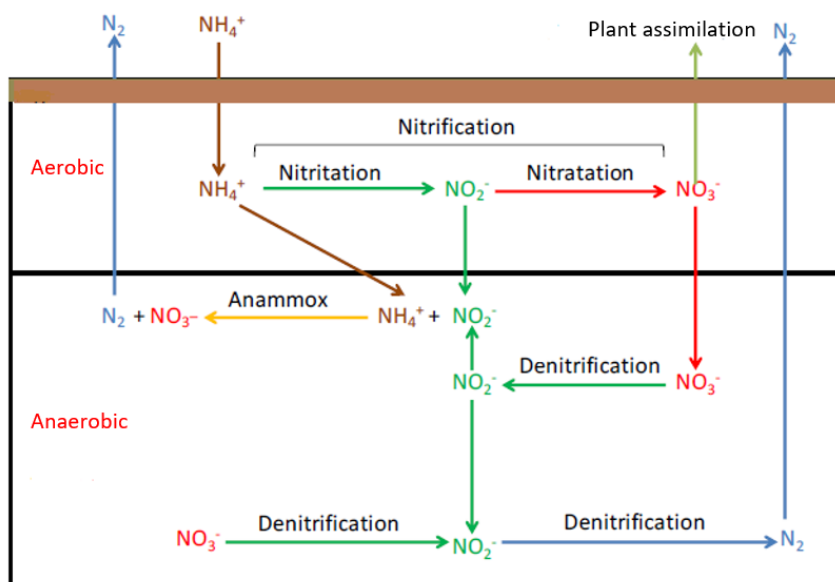


Figure 6: Simplified diagram of the nitrogen cycle taking place in the SSBS biofiltration zone; adapted from Prigent 2012

Indeed, more specifically with regard to elimination performance, planted wetlands: (a) provide surfaces and oxygen for the growth of micro-organisms in the rhizosphere that perform nitrification and denitrification but also degrade pesticides or other substances partially attached to the environment (documentation.pole-zhi.org); and (b) provide carbon from root exudates (due to fixed carbon photosynthesis, in a range of 5 to 25% C), and optimize the removal of organic matter by overhead biomass that acts as a filter, excretes oxygen and reduces water velocity, thereby promoting sedimentation.

Obviously, nitrogen assimilation by plants differs according to different parameters: hydraulic parameters such as retention time (HRT), hydraulic load, system configurations, incoming pollutant load and environmental conditions (season, temperature, oxygen, mineralizable carbon) (zh.assoc.pagespro-orange.fr). However, it should be noted that decomposition of plant matter may also increase the concentration of nutrients in the effluent through discharge or leaching (Brodrick, Cullen, and Maher 1988).

2.5 Objectives of the study

The objective of the study is to better understand, evaluate and if possible optimize the complex biotic and abiotic processes that occur in constructed wetlands (HF system) and in particular those that occur in the third SSBS zone: the biofiltration zone. The study will be divided into two different parts.

The first part will aim at carrying out a complete inventory of processes in the biofiltration zone of a prototype SSBS at the Tresta field station, the ERCE experimental station. The measurement of the organic matter (SOM) and carbon content of the soil will be conducted. In addition, the total removal capacity of the biofiltration zone according to various parameters (Total suspended solids (TSS), Volatile Suspended Solids (VSS), Total Nitrogen (TN) and Total Phosphorus (TP)) will be carried out.

The biofiltration zone is heterogeneous because its first part presents a surface flow while the second part presents a subsurface/groundwater flow. The difference in performance between these two different parts will be studied for TN and TP in order to determine the most effective part concerning the elimination of these parameters.

In parallel, a verification of a difference or absence of difference in elimination performance (TSS, VMS, TN and TP) over time (day against night) will be performed for the whole SSBS biofiltration zone and its two sub-divided parts. Indeed, during the night, the water temperature decreasing and the available DO concentration increasing, the elimination performance (TSS, VMS, TN and TP) can be dependent on the variations of these parameters.

Finally, the last investigation aims to highlight a correlation or an absence of correlation between the TP/TN concentration and the DO concentration, in the whole of the SBSS and in these two sub-divided parts (the surface flow system and the subsurface flow system). Specifically, since aerobic microorganisms consume O₂ to oxidize TN/TP, we assume that the higher is the TN/TP concentration, the lower is the DO concentration and vice versa.

Summary of the objectives of the first part:

- **Objective 1:** Characterization of sediments type in the SSBS biofiltration zone through sediments organic matter (SOM) and sediments carbon assessment
- **Objective 2:** Evaluation of the total removal capacity of the biofiltration zone according to various parameters (Total suspended solids (TSS), Volatile Suspended Solids (VMS), Total Nitrogen (TN) and Total Phosphorus (TP))
- **Objective 3:** Evaluation of the elimination capacity of each subpart (surface flow system and subsurface flow system) of the SSBS biofiltration zone for TN and TP.
- **Objective 4:** Identification of a difference or absence of difference in removal performance (TSS, VMS, TN and TP) over time (day versus night) of the SSBS biofiltration zone and its two sub-sections.
- **Objective 5:** Identification of a correlation or absence of correlation between TP/TN concentration and DO concentration, in the whole SBSS biofiltration zone and in these two sub-divided parts.

The second part deals only with the abiotic process of retention of soluble inorganic phosphorus on an inorganic substrate by physical and/or chemical adsorption which is the main mechanism of phosphorus removal in HF systems. Indeed, since the SSBS dates from 2013-2014, it is hypothesized that the adsorption sites of the gravelly substrate in the second part presenting a groundwater flow saturated after so many years of operation (Prigent 2012) and therefore that the abiotic process of phosphorus retention no longer takes place.

Consequently, the objective of my second part is to test the capacity of modified reactive materials (LECA) or natural materials (two types of dolomite: dolomite A and dolomite B) in order to evaluate their phosphorus removal capacity. To test these materials in the best possible way, experiments should normally be conducted at different scales, from the smallest to the largest and therefore

generally from the simplest to the most complicated. Indeed, each time the scale is increased, new parameters are integrated to better model the functioning of the substrate and its interactions with biotics in a real constructed wetland.

The three envisaged stages are the following:

- The first step, very frequent in this field of research, is to carry out a batch experiment in which only the intrinsic properties of the material with respect to phosphorus adsorption are studied as a function of the input phosphorus load, the contact time of the temperature and the pH.
- The second step consists in testing these materials in a hydroponic system (pilot unit) not planted or planted (*Spinacia Olereacea*) by a model organism modelling the macrophytes of a large-scale conventional system in order to evaluate whether the phosphorus elimination rate is increased by biological mechanisms (plant and microbial assimilation) that can take place as a function of the polluting phosphorus load (HRT), the hydraulic load and the configuration of the system.
- The final step would be to inject these materials into a real constructed wetland and monitor their performance over time, but also according to environmental conditions.

The purpose of this second part was to carry out the first and second stages.

The purpose of the first stage will be more precisely:

- **Objective 1:** To examine the ability of the three selected materials to remove phosphorus in % based on the initial concentration.
- **Objective 2:** To examine the ability of the three selected materials to remove phosphorus by calculating the maximum sorption of experimental phosphorus in $\text{mg P-PO}_4^{3-} \cdot \text{g}^{-1}$ of materials.

Unfortunately, the second stage could not be completed. Although germination of spinach started relatively early and went smoothly, hydroponic culture with the nutrient solution (Appendice II: Conditions for germination and culture of spinach) was not as effective as expected with growth difficulties, drying and yellowing of leaves.

Thus, among the three materials tested only in the batch experiment, the one with the best phosphorus removal capacity will be selected.

Having assumed from the literature that this material has a higher phosphorus sorption rate than the material used in the second part with groundwater flow (gravel), it can not only (1) be injected into the system instead of gravel during a subsequent SSBS restoration project but also (2) generally replace conventional materials (sand, gravel) used in the construction in Europe of subsurface flow wetlands HF or even VF.

3. Materials and methods

3.1 Study of an SSBS in Tresta station

3.1.1 Description of the SSBS and sampling sites

The SSBS constructed wetland studied is located on the experimental station belonging to the ERCE (Tresta station) located 80 km from Lodz and 5 km from the Sulejow reservoir. The entire constructed wetland area (SSBS) is 30 m long and 1.10 wide, the flow rate from the SSBS was measured at 1.75 L/s and the estimated water depth at $h=30$ cm. Our study covers only part of the biofiltration zone which is an HF system (Figure 7). The entire biofiltration zone measures a total surface of 15.9 m² and is composed of two subparts:

- The first part has a surface flow and is composed exclusively of the macrophyte *Acorus calamus* L. belonging to the family Acoraceae. The surface area is 8.5 m² (7.70m × 1.10 m).
- The second part has a subsurface flow and is composed exclusively of the macrophyte *Glyceria maxima* belonging to the Poaceae family. The surface area is 7.4 m² (6.70 m × 1.10m).

The determination of the two dominant species was carried out on the basis of my botanical knowledge and specified by the use of macrophyte and helophyte recognition guide (Deat 2014).

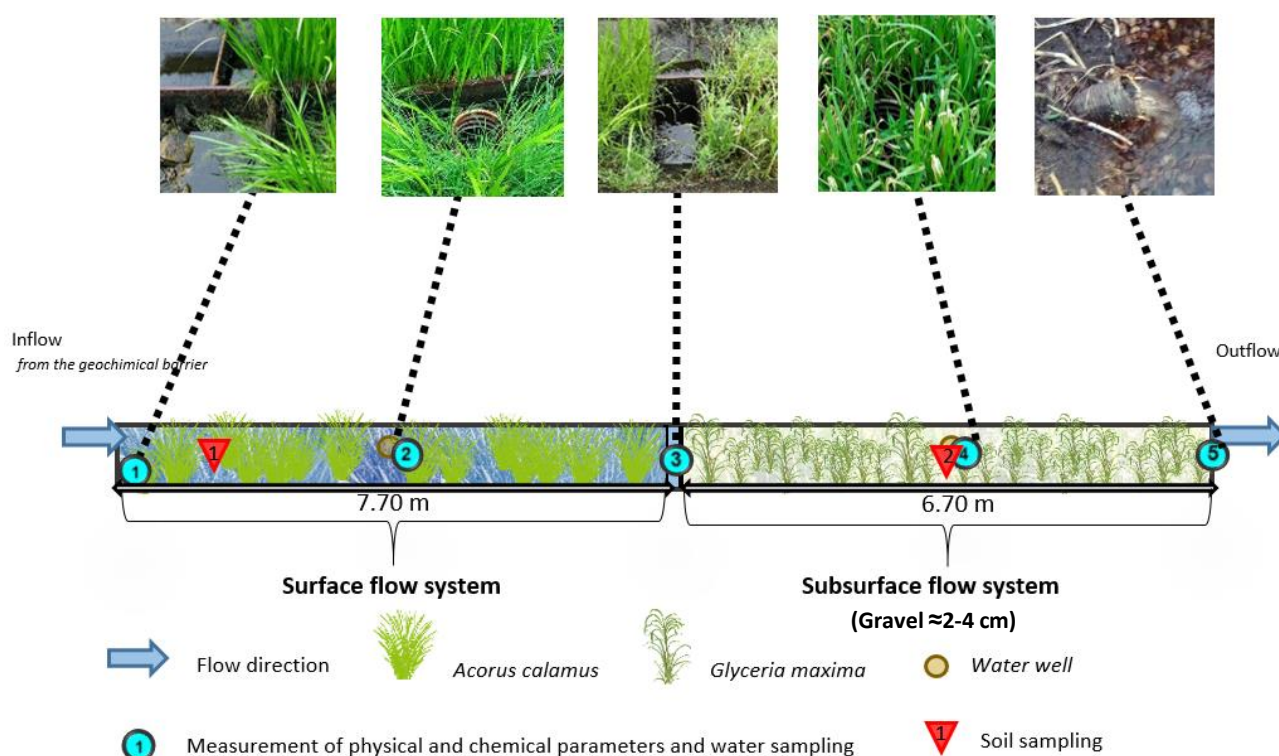


Figure 7: Presentation of the SSBS biofiltration zone studied and different sampling points

Every 4 hours during 24 hours, water samples were taken from five different locations on the SSBS (figure 7). Each water sample was taken with the measurement of classical physico-chemical parameters. Furthermore, to determine the sediments organic matter concentration, two sediments samples were also taken (beginning of the 1st bed - middle of the 2nd bed) in the first soil horizons (0-

20 cm) in order to obtain a result representative of the whole SSBS, in the case where the soil content was very heterogeneous from one bed of macrophytes to the other.

3.1.2 Determination of Total Suspended Solids and Volatile Suspended Solids in water

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) are commonly water quality parameters used to assess the quality of a sample of any type of water, in the same way as TN or TP, for example. Both were determined only for station 1 (inflow) and station 5 (outflow) every 4 hours for 24 hours in order to be able to evaluate the removal rate of TSS (%) by the biofiltration zone over time.

➤ Total Suspended Solids (TSS)

Total suspended solids (TSS) is the dry weight of suspended particles in a water sample that can be trapped by a filter using a filtration device (Figure 8).

Thus, the TSS were determined by pouring a carefully measured volume of well mixed water (usually half a litre, as the particle density was high) through a pre-weighed Whatman filter (glass fibres) with a pore size of 0.7 µm. Following this, the filter was placed in a 105°C oven during 5-6 hours to remove all the water on the filter. The TSS in g.L-1 were calculated according to the following formula:

$$TSS = \frac{\text{dry weight of residue and filter (after 105°C)} - \text{dry weight of filter only, in g}}{\text{sample volume (mL)}}$$

➤ Volatile Suspended Solids (VSS) or Volatile organic compounds (VOC)

After the Total Suspended Solids value was determined, the determination of Volatile Suspended Solids (VSS) was performed (Figure 8). The filter used for the determination of total suspended solids (TSS) is placed in a muffle furnace at 550° C for 6-5 hours. Most of the organic matter was oxidized, while the inorganic matter remained in the form of ash. The VSS in g.L-1 (organic matter content), was calculated using the following formula:

$$VSS = \frac{\text{dry weight of ash and filter (after 500°C)} - \text{dry weight of filter and residue(after 105°C), in g}}{\text{sample volume (mL)}}$$

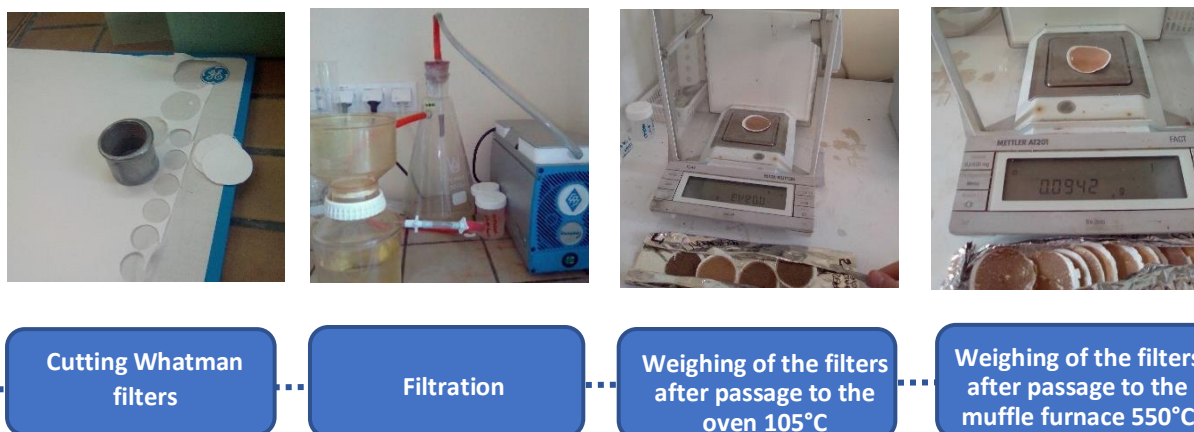


Figure 8: Different steps needed to determine TSS and VSS

3.1.3 Determination of soil organic matter (SOM)

In order to evaluate the percentage of organic matter present in the SSBS biofiltration zone, two soil samples taken (beginning of the 1st bed - middle of the 2nd bed) were taken. About 3g of well mixed sediments were deposited in a container, the whole was weighed precisely then passed in the oven at 105°C during 24 hours. Once out of the oven, the whole was weighed, then passed to the muffle oven at 500°C for 24 hours and finally weighed one last time (Figure 9). The analyses were performed in triplicate and the standard deviation calculated. Organic matter content (%) in soil was calculated using the following formula:

$$\text{Organic matter content (\%)} = \frac{[(\text{container} + \text{soil}(\text{after } 500^{\circ}\text{C}) - \text{container} + \text{soil}(\text{ after } 105^{\circ}\text{C})) \times 100]}{\text{dry soil mass (g)}}$$

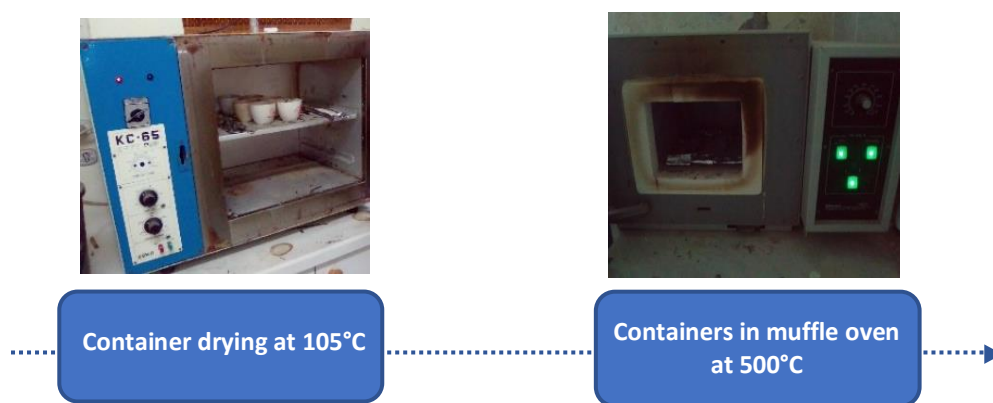


Figure 9: Determination of organic matter in soil

3.1.4 Nutrient Analysis

TP (inorganic and organic phosphorus) analyses were performed using the ascorbic acid method (with mineralisation) and the absorbance of the blue- colored antimony-phospho-mobylate complex is measured at $\lambda = 690$ nm using a spectrophotometer (Figure 10). The results were obtained directly in absorbance and the results in mg.L-1 were then determined using the calibration curve previously performed (Appendice III).

TN (inorganic and organic nitrogen) analyses were performed using the persulfate oxidation technique (Figure 11). Indeed, alkaline digestion of persulfate converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after digestion to remove interference from halogen oxides. Then the nitrate reacts with chromotropic acid under strongly acidic conditions to form a yellow complex. Absorbance is measured at $\lambda = 410$ nm using a spectrophotometer. The results were obtained directly in mg.L-1. The protocol of the method is available in Appendice IV.



Figure 10: Absorbance measurement with spectrophotometer



Figure 11: Kit 17 and 21 needed to determine TN

3.1.5 Physical and chemical water measurement

Every 4 hours during 24 hours, for each water sample taken, six classical physical and chemical parameters (Table 2) were measured using a pH meter.

Table 2: Physico-chemical parameters measured during each sampling in the SSBS biofiltration zone

Station n	Physical and chemical parameters					
	Water T° (Celsius °C)	SPC (mg.L-1)	TDS (mg.L-1)	O2		pH
				%	mg.L-1	

3.1.6 Statistical analysis

In order to meet objective n°5 formulated in the first part of the 2.5 Objectives of the study, statistical tests were carried out. Indeed, the objective was to show a correlation or absence of correlation between the TP and TN concentration and the DO concentration in the whole SBSS and in these two subdivisions (the surface flow system and the groundwater flow system). To do this, the hypothesis of **H0** and **H1** was formulated:

H0: There is no correlation between the TN/TP concentration and the DO concentration in the whole SSBS, and therefore in the two different subsections (the surface flow system and the groundwater flow system).

H1: There is a correlation between the TN/TP concentration and the DO concentration in the whole SSBS, and therefore in the two different subsections (the surface flow system and the groundwater flow system).

To measure the dependence or absence of nonparametric statistical dependence between two quantitative variables on a sample $n < 50$, Spearman (r_s) tests were performed using PAST PAleontological STatistics Version 3.20 software.

The results were expressed using the critical values table of the classified Spearman correlation coefficient (Appendix V) with a 95% confidence interval of $\alpha = 0.05$.

3.2 Batch sorption experiment (P)

3.2.1 Choice of tested material

In order to determine which materials will be interesting to test in our study, an overall analysis of the literature was performed.

The objective was not only to select materials with good phosphorus retention potential but also to select also materials that were inexpensive, reusable, available in the country of study and that did not present an environmental toxicity risk (unlike slag, waste products for example).

Following this first work, a collection of data from the literature concerning mainly the batch tests performed with LECA, dolomite and their phosphorus reduction capacity was carried out (Table 3).

Table 3 : Collection of data from the literature: LECA, and dolomite phosphorus-binding capacity

Material	Use	Experiment type	Material amount (g)	C in (mg L ⁻¹)	P reduction (%)	P sorption [mg.g ⁻¹]	Author
LECA	Filter	Column	n.d	200	n.d	0.29–0.55 (kg m ⁻³)	(Johansson 1997)
		Batch experiment	5	1-50	–	–	(Karczmarczyk and Bus 2014)
			n.d	0–320	n.d	0,04 -0,6	(Zhu and al. 1997)
Dolomite	Soil amendment and filter	Batch experiment	1	1,4 5,6	14,6 7,6	0,02 0,04	(Karczmarczyk and Bus 2014)
			1	0.01	n.d	0,007	(Al-Anbari and al. 2008)

–: result not significant; n.d: data not available

3.2.2 Characteristics of tested materials

An overview of the appearance of the three materials (two different types of dolomite) is visible Figure 12.



Figure 12: Overview of the appearance of the three materials

3.2.2.1 LECA

Lightweight Expanded Clay Aggregates (LECA®) are modified clay product formed by expanding special clay minerals at high temperature (> 1100°C) to produce lightweight pebbles. The LECA® was specially developed in Scandinavia for P-PO₄³⁻ sorption and now available on the market. Its surface is high and the main mechanism of removal of P is precipitation of oxides of aluminium, calcium and iron. This material is commonly used in civil engineering, geo-engineering, agriculture, horticulture and environmental protection. The one used for experiments was obtained from Leca Polska Sp. Z oo (<https://leca.pl/produkty/izolacje>).

3.2.2.2 Dolomite

Dolomite is a sedimentary rock composed mainly of skeletal fragments of marine organisms such as coral and molluscs for example. Its main materials are magnesium and minerals calcite and aragonite, which are different crystalline forms of calcium carbonate (CaCO₃). Dolomite has many uses: as a building material, an essential component of concrete, as an aggregate for the base of roads, as a white pigment or filler in products such as toothpaste or paints, as a chemical raw material for the production of lime, as a soil conditioner or as a popular decorative addition to rock garden. Crushed or powdered

dolomite is widely used as a calcium-rich soil amendment to reduce soil acidity and enhance P retention. It may also be a suitable material for sorbing P in a farm drainage wetland—either as a soil amendment (crushed) or a filter material—because of its high Ca content and associated ability to facilitate P precipitation. The two different types of dolomite powder used for the experiments were obtained respectively from <http://www.jksm.pl/> and <http://www.wapno-magnezowe.pl/>.

Table 4: Characteristics of tested materials

Material	LECA®	Dolomite	
		(A)	(B)
Grain size [mm]	10-20	0.1-1	0.1-1
Chemical composition	n.d	CaO: 30%	MgO: 55%
pH	9,5 ± 0,5	9 - 9.6	
Free bulk density	246-333 kg. m ⁻³	1600 kg. m ⁻³	
Porosity	External (open pores): 42% Internal (closed pores): 84%		
Humidity	<4%		
Price	400 zł-m ³	60 zł-m ³	

3.2.3 Experimental conditions

There are three standard assessment methods for assessing material retention capacity: batch, column and pilot/full scale. In order to carry out a first test of the retention capacity of these three substrates, a batch experiment was carried out in this study because this method has the advantage of being easy to use (bottles, small quantity of material, stirring table) and is inexpensive.

3.2.3.1 Concentration of tested artificial P solutions

Initially, as reported quite frequently in the literature, various high phosphorus concentrations (5 mg.L⁻¹, 15 mg.L⁻¹ and 25 mg.L⁻¹) were tested in a pre-experiment. This pre-work allowed us to determine that the evaluation of such concentrations by the ascorbic acid method (TP assay) was impossible. Indeed, although in the literature, it is reported that this method is usable for concentrations < 25 mg.L⁻¹ under our experimental conditions, the method could only accurately estimate concentrations < 5 mg.L⁻¹.

As a result, much lower values (0.25 mg P-PO₄³⁻ L⁻¹, 0.5 mg P-PO₄³⁻ L⁻¹ and 1 mg P-PO₄³⁻ L⁻¹), much more similar to the natural phosphorus concentrations that can be found in urban watercourses, were tested. In order to do this, 4.389 mg KH₂PO₄³⁻ were diluted in 1 L deionized water to obtain a 1000 mg stock solution P-PO₄³⁻ L⁻¹. From this solution, the 0.25 mg P-PO₄³⁻ L⁻¹, 0.5 mg P-PO₄³⁻ L⁻¹ and 1 mg P-PO₄³⁻ L⁻¹ (Figure 8) were made per dilution.

3.2.3.2 Substrates quantity, contact time and table shaker parameters

200 ml of the different solutions prepared were mixed in Erlenmeyer glass bottles, each containing 5 g LECA®. Indeed, although generally the quantity of substrate tested reported in scientific articles is 1g, Nair et al (1984) in (Ádám et al. 2007), it is recommended, in the case of the LECA® to carry out the experiment with 5g (Karczmarczyk and Bus 2014). Indeed, clay balls being large and having a size varying between 10 and 20 mm, it could be that the sample is not representative.

In the case of dolomite, although the 1g test of each substrate was reported in the vast majority of scientific papers that conducted batch experiments (Al-Anbari and al. 2008); (Karczmarczyk and Bus

2014) (Johansson 1999), the experiment was performed with 3g (substrate/solution ratio: 1/66) with the objective of not having a substrate/solution ratio too low.

Phosphorus concentration decrease was measured at different times (1h, 2h, 3h, 5h, 8h, 24h, 48h) during a total contact time of 48h and a constant shaker table agitation speed (120 rpm and amplitude 9) (Figure 11).

3.2.3.3 Controls and monitoring of physical parameters

The analyses were conducted in duplicate (for LECA®) and three controls were used. First of all, the microbiological activity was verified with a control using sterilized substrates and a concentration of 0.5 mg P-PO₄³⁻ L⁻¹ (for LECA), because in batch assays, only the physico-chemical effect wants to be evaluated (C1 control).

To achieve this, the substrates were sterilized in autoclave Sano (20 min at 105 °C) (Domínguez et al., 2004). The second, desorption P-PO₄³⁻ was analyzed with a concentration of 0 mg P-PO₄³⁻ L⁻¹ (C2 control). Third one, in order to verify that the P-PO₄³⁻ concentration remains the same over time in the absence of substrate a control was performed with only 0.25 mg P-PO₄³⁻ L⁻¹ concentration (C3 control) Room temperature and pH were measured at the beginning and end of the 48-hour experiment (Figure 13).

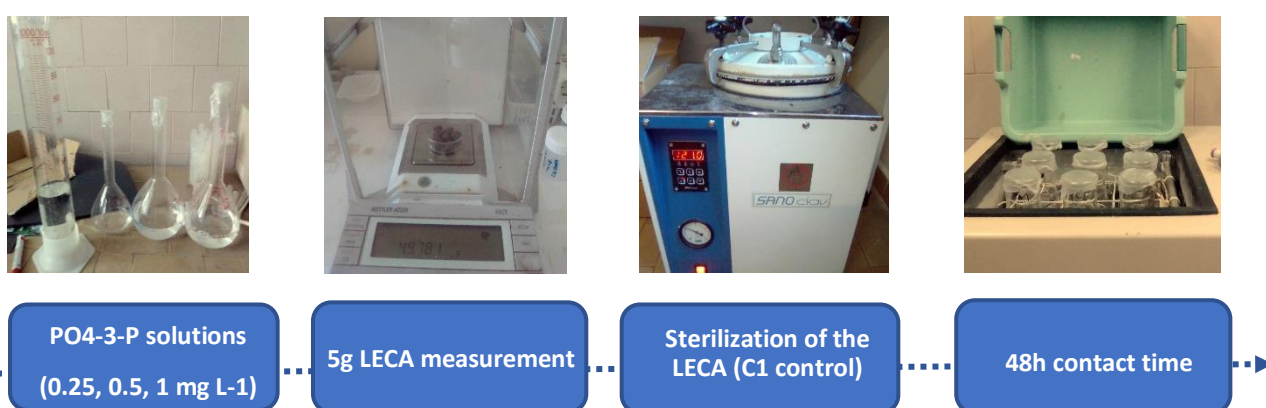


Figure 13 : Different stages in the realization of the experiment (in this case, LECA test)

The phosphorus analyses were performed in the same manner as in 3.1.4.2 Analysis of Total Phosphorus (TP) without the mineralization step since the phosphorus source added was inorganic.

In order to obtain the retention rate (%) and the maximum phosphorus sorption rate (mg g⁻¹) for each material tested, the following formulas were applied:

$$\text{Removal efficiency (\%)} = \frac{C_n - C_{n+1}}{C_n} \times 100$$

with C_n = initial P-PO₄³⁻ L⁻¹ concentration (mg L⁻¹)

C_{n+1} = P-PO₄³⁻ L⁻¹ concentration after 48h hours - when an equilibrium (asymptotic value) is reached

$$\text{P sorption (mg. g}^{-1}\text{)} = \frac{(C_n - C_{n+1}) \times \text{Volume of P - PO}_4\text{ - solution in each vial}}{M_{\text{substrat}}}$$

with Volume of P-PO₄³⁻ L⁻¹ solution in each vial (200ml)

with M_{substrat} (g)

4. Results and discussion

4.1 Assessment of biotic and abiotic processes: Study of a mature SSBS

4.1.1 Quantification of sediments organic matter and carbon

Soil organic matter (SOM) and carbon content are key indicators of soil quality and are correlated to a number of important soil processes that occur in constructed wetlands such as respiration, denitrification, and phosphorus sorption. Knowledge and understanding of the functioning of SSBS biofiltration zone soil, and in particular its organic matter and carbon content, is therefore a major challenge.

Thus in the SSBS biofiltration zone studied, the percentages of organic matter in sediments for the first macrophyte bed (*Acorus calamus* L.) and the second macrophyte bed (*Glyceria maxima*) are $49.33 \pm 0.53\%$ and $56.62 \pm 1.01\%$ respectively (Figure 14). This slight difference can possibly be explained by the fact that the main organic compounds of *Acorus calamus* L. are perhaps less easily biodegradable than those of *Glyceria maxima*.

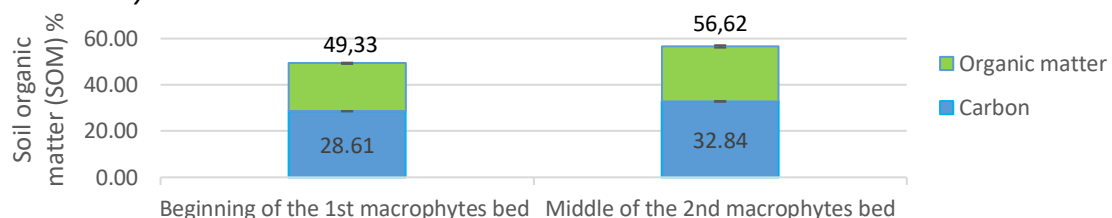


Figure 14: Percentage of organic matter and carbon in biofiltration zone soil

However, the average sediments organic matter content of the SSBS biofiltration zone is $52.98 \pm 4.06\%$ while its average carbon content, defined as $OM/C = 1.724$ (Baize, 2016), is $30.73 \pm 0.20\%$. These extremely high levels of organic matter and carbon found in the first soil horizon can easily be explained.

In permanently submerged soils, as is the case in SSBS biofiltration zone, organic matter no longer or only slightly decomposes due to very low biological activity due to oxygen deficiency. Indeed, these asphyxiating conditions (anaerobiosis), due to permanent submersion, cause a slowdown in biological activity which results in a very slow and very partial mineralization of the litter. (Pollet 2009).

Thus, we obtain on the surface of the in SSBS biofiltration zone a soil characterized by an accumulation of undecomposed organic matter (organic matter rate $>40\%$ and carbon rate $>20\%$) very swampy (Legros 2007): peat. Peat is a particular and fragile substrate whose characteristics make it a real carbon well, because there is more synthesis of organic matter than degradation.

More precisely, when peat is formed by accumulation of organic matter composed almost exclusively of plant debris (the other tiny part being animal debris (soil microfauna, amphibians, etc..), it is called black peat. Black peat (Figure 15) is a compact, humified material resulting from the relatively advanced decomposition of biofiltration zone macrophytes containing fewer fibres and whose structure is described as sapric structure. (<http://www.pole-tourbieres.org/>)



Figure 15: Black peat with sapric structure found in the 2nd bed of macrophytes

4.1.2 Overall pollutant removal performance

4.1.2.1 Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

The removal efficiency of SSBS biofiltration zone for TSS and VSS is presented Figure 16 while the raw data table is available in Appendice VI.

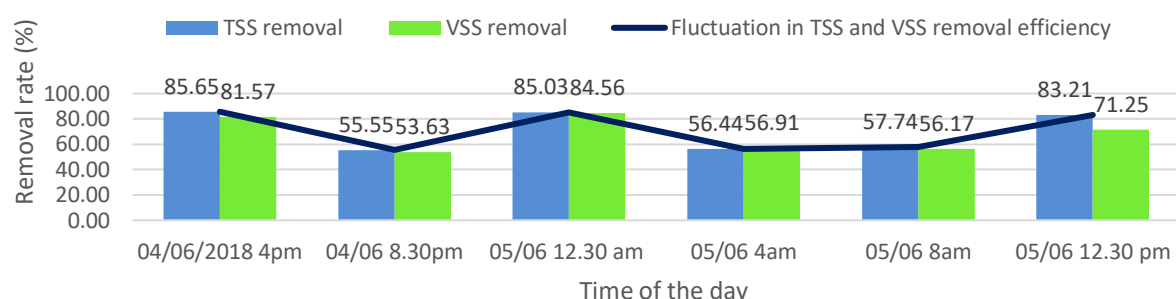


Figure 16: TSS and VSS removal rate (%) in the SSBS biofiltration zone

➤ Total Suspended Solids (TSS)

Based on these results, the SSBS biofiltration zone removes between 55.55% (minimum) and 85.64% (maximum) TSS for an average highly concentrated incoming hydraulic load of 94.43 mg L⁻¹.

This range of efficacy values corresponds to different results from scientific articles. Indeed, according to the results presented in section 2.4.2 Abiotic and biotic processes in the biofiltration zone of the different SSBS on the Sokolowka River (Szklairek et al. 2018) and on the Bzura River (Jurczak et al. 2018), the elimination rates of all SSBS (and not those of the biofiltration zone only) are respectively 61.4 and 81 %, which is of the same order of magnitude as our results.

In addition, Manios et al. 2003 conducted a two-year study on various underground (HF) systems and obtained an average cleaning efficiency of 82% TSS for an outgoing load of 5 mg/L. These values indicate the achievement of effective biotic and abiotic processes in the SSBS biofiltration zone concerning to TSS removal.

Surprisingly, the SSBS biofiltration zone allows TSS removal which can vary with sampling time. Indeed, on 04/06 at 4 pm, 12h30 am and 05/06 at 12h30 pm, the elimination rate is on average $84.62 \pm 1.27\%$ while on 04/06 at 8.30 pm and 05/06 at 4 pm and 8 am, the elimination rate is on average $56.6 \pm 1.10\%$.

This difference in results obtained depending on the time of sampling is difficult to explain because it does not seem to have any particular logic. There is no link to establish with these variations and particular times of the day (day/night) and therefore no correlation to establish with the parameters themselves according to the time of day (temperature, dissolved oxygen). Finally, these results are also not explained by a change in the hydraulic conditions, the retention time (HRT), the hydraulic load and the configuration of the system having remained constant throughout the experiment.

However, the two different parts of the SSBS biofiltration zone (surface flow system and groundwater flow system) have different approaches to suspended solids removal, although based on the same principles, so it is interesting to study in more detail the suppression mechanisms for both systems.

The SSBS surface drainage system normally removes suspended solids primarily by the following means: (1) sedimentation /flocculation and (2) aerobic biological interception and treatment.

Sedimentation (1) can be divided into two categories: the decantation of discrete particles and the decantation of flocculating particles. Discrete particle decantation is the removal of particles of the same size and origin without contact with other types of particles. (Ouriemi n.d). On the other hand, flocculent decantation involves the interaction of particles between them. The sedimentation rate of the particles therefore increases during decantation due to agglomeration of the particles. It can therefore be assumed that coarse particles would be removed rapidly in the first few metres of the surface flow system, while smaller particles would be removed more slowly in the last few metres of the surface flow system.

In general, the interception and adsorption (2) of TSS on surfaces of aerial plant systems plays another important role. Wetland plant surfaces are covered with an active biofilm layer called periphyton that can absorb colloidal and soluble materials. These solids can then be metabolized and converted to gas or biomass (Norton n.d).

The SSBS groundwater flow system generally removes suspended solids primarily by filtration. Indeed, TSS in this zone is adsorbed mainly on gravels (2-4 cm) and macrophyte rhizomes. It has been found that, through these mechanisms, 60 to 75% of TSS removal in wetland flow (HF) occurs in the first third of the wetland (EPA 1999). However, one of the main problems that can occur in 4-5 years old SSBS is the clogging of the filter media. When TSS passes through gravel, it can clog pores over time and reduce the hydraulic conductivity of the medium.

This last element may allow us to make an assumption regarding the identified variation in TSS removal efficiency over time (dark blue curve figure 16). Indeed, we can assume that when the substrate is clogged (formation of a TSS pool), the percentage of removal efficiency increases. This mechanism occurs until a certain threshold value is reached beyond which the mechanism reverses. The TSS pool is detached and the TSS are released into the environment, thereby lowering the percentage of system elimination efficiency. This assumption, as well as the different approaches to the removal of TSS from these two subsystems, could not be verified. Further experiments would be necessary to confirm or refute our proposals

➤ Volatile Suspended Solids (VSS)

For VSS, also called VOCs, the SSBS biofiltration zone eliminates between 53.63% (minimum) and 81.55% (maximum) for an average incoming pollutant load of 52.34 mg L⁻¹ highly concentrated, more than half the average load of 94.43 mg L⁻¹. These values show, as for TSS, a good elimination of VOC and thus, the realization of effective biotic and abiotic processes in the SSBS biofiltration zone. VSS results, follow the same trend as described above for TSS, which seems logical since VOCs are contained in TSS with a classic TSS/VOC ratio in the order of 1.80.

The SSBS biofiltration zone therefore allows VOC removal that varies with sampling time. Indeed, on 04/06 at 4 pm, 12.30 am and 05/06 at 12.30 pm, the elimination rate is on average $79.13 \pm 6.98\%$ while on 04/06 at 8.30 pm and 05/06 at 4 am and 8 am, the elimination rate is on average $55.57 \pm 1.71\%$. This difference in the results obtained as a function of the sampling time can be explained by the same hypothesis concerning filtration in the second part of the surface flow system previously developed for VSS.

The physical VOC removal mechanisms are normally similar to those used to remove TSS in the surface flow portion (sedimentation/flocculation) and in the subsurface flow portion (filtration).

With regard to the biological means of VOC removal in the surface flux part and in the groundwater flux part of the SSBS biofiltration zone, the mechanisms are similar.

Thus, in both parts, decomposition plays a major role in the elimination of organic matter (Norton n.d). Heterotrophic microorganisms forming biofilms on the surface of macrophytes break down organic matter (sorption, volatilization) to produce new biomass, reproduce and remain alive. The degradation of organic matter can be done by aerobic reactions (O₂ as oxidant - more efficient for energy production), anoxic and anaerobic (CH₄ as oxidant - less efficient for energy production).

Thus, we can assume that slight differences between TSS and VOC removal efficiency may be due to decomposition of organic matter by heterotrophic microorganisms within the SSBS. As this phenomenon has not been observed, further experiments would be necessary to prove or disprove this hypothesis.

4.1.2.2 Total Nitrogen Removal (TN) and Total Phosphorus Removal (TP)

The removal efficiency of the entire SSBS for TN and TP is presented Figure 17:

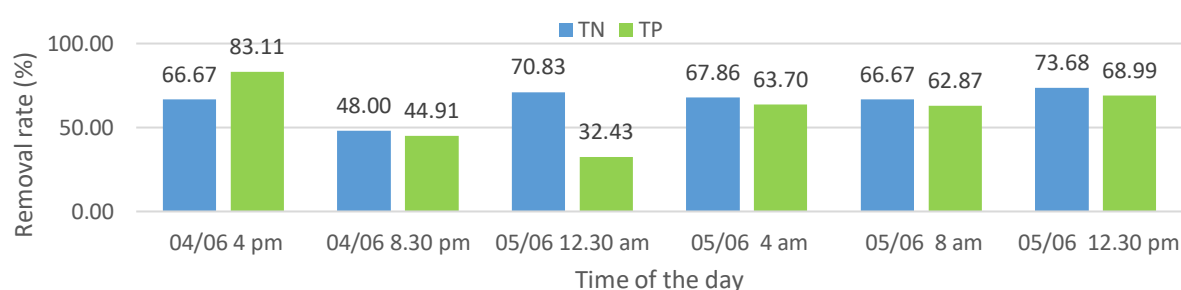


Figure 17: TN and TP removal rate (%) in the entire SSBS biofiltration zone

The whole biofiltration zone of the SSBS (surface flow and subsurface flow) seems to allow an almost constant elimination of TN as a function of time (except for the result on 04/06 at 8.30 pm where the elimination percentage is only 40%) with an average of $65.62 \pm 9.05\%$. These results are similar to those obtained for whole SSBS (not biofiltration zone) on the Sokolowka river (Szklairek and al. 2018) and the Bzura river (Jurczak and al. 2018) with a TN removal percentage of 46.1 and 71.5% respectively.

Concerning the elimination of TP, the whole SSBS seems to allow a time-dependent elimination. More precisely, the elimination is variable during the afternoon and evening of 04/06 but stable during part of the morning of 05/06 until noon. Indeed, on 04/06 at 4 pm then at 8.30 pm then on 05/06 at 12.30 am, the elimination rate decreases with the respective values of 83.11%, 44.91% and 32.43%. However, from 4 am until 12.30 pm the next day, the elimination rate remains constant with an average of $65.18 \pm 3.32\%$.

This difference in performance results obtained according to the time of sampling does not seem to be related to particular times of the day (day/night). There is therefore no correlation to be established with the parameters themselves depending on the time of day (temperature, dissolved oxygen).

Finally, nitrification, denitrification, plant assimilation, ammonia adsorption and volatilisation, organic nitrogen burial, and ANAMMOX are all processes that reduce the amount of nitrogen in planted constructed wetlands (see 2.4.2.2 Nitrogen removal). However, in order to have a better knowledge of these mechanisms normally occurring within the SSBS biofiltration zone, the analysis of dissolved chromatographic forms could have been performed.

4.2 Surface and subsurface flow systems removals comparison

4.2.1 Total Nitrogen Removal (TN) and Total Phosphorus Removal (TP)

However, as for TSS, the two different parts of the SSBS biofiltration zone (surface flow system and groundwater flow system) have different processes for the removal of TN and TP. It is therefore interesting to study the removal rates of TN (Figure 18) and TP (Figure 19) separately for the two subsections of the biofiltration zone.

➤ Total Nitrogen Removal (TN):

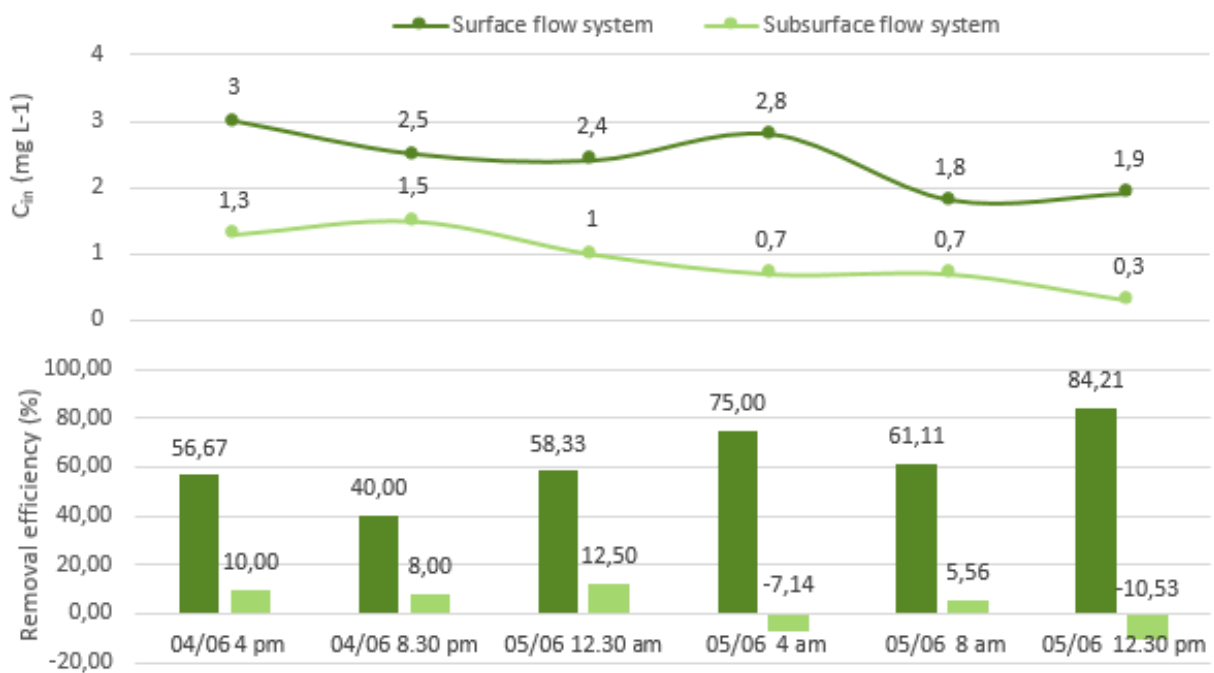


Figure 18: Comparison of TN efficiency for surface and subsurface flow systems (with incoming concentrations)

The results show that for the surface flow system, an average TN removal of $62.55 \pm 15.41\%$ is achieved with an average input concentration of 2.40 ± 0.48 mg L⁻¹. The subsurface flow system, on the other hand, allows an average TN elimination (without negative release values) of $9.01 \pm 2.95\%$ for an average incoming concentration of 0.92 ± 0.44 mg L⁻¹. The surface flow system therefore seems to be on average more than 7 times more efficient than subsurface flow for higher incoming concentrations. Moreover, we can note that in addition to being much less efficient, we can see that per time period, the subsurface flow releases TN into the environment, which the surface flow system does not do. Indeed, during two sampling operations (05/06 4 am and 05/06 12.30 pm), the subsurface flow rejects $8.83 \pm 2.39\%$ of the TN entering the receiving medium.

Thus, it can be said that the surface flow is largely responsible for the good elimination results of the SSBS elimination zone (detailed in 4.1.2.2 Total Nitrogen Removal (TN) and Total Phosphorus Removal (TP)). Indeed, without the surface flow system and taking into account the release episodes of the subsurface flow system, the percentage efficiency of the SSBS biofiltration zone would be almost zero.

Regarding to variables that may vary the TN removal rate, neither the initial incoming concentration nor the time of day appear to be these variables.

Indeed, for the elimination of the surface flow system according to the time of day, the elimination remains relatively constant, whereas for the groundwater flow system, the results vary. However, this difference in performance results obtained as a function of the time of sampling does not appear to be related to particular times of the day (day/night). There would therefore be no correlation to establish with the parameters themselves according to the time of day (temperature, dissolved oxygen). However, in order to fully assert this absence of correlation, it would be more prudent to repeat the experiment with triplicates.

Finally, as mentioned above, it would have been interesting, in order to carry out a more in-depth study of the TN elimination mechanism within the two sub-parts of the SSBS biofiltration zone, to carry out analyses of the dissolved forms by ion chromatography (nitrates, nitrites and ammonium).

➤ Total Phosphorus Removal (TP) :

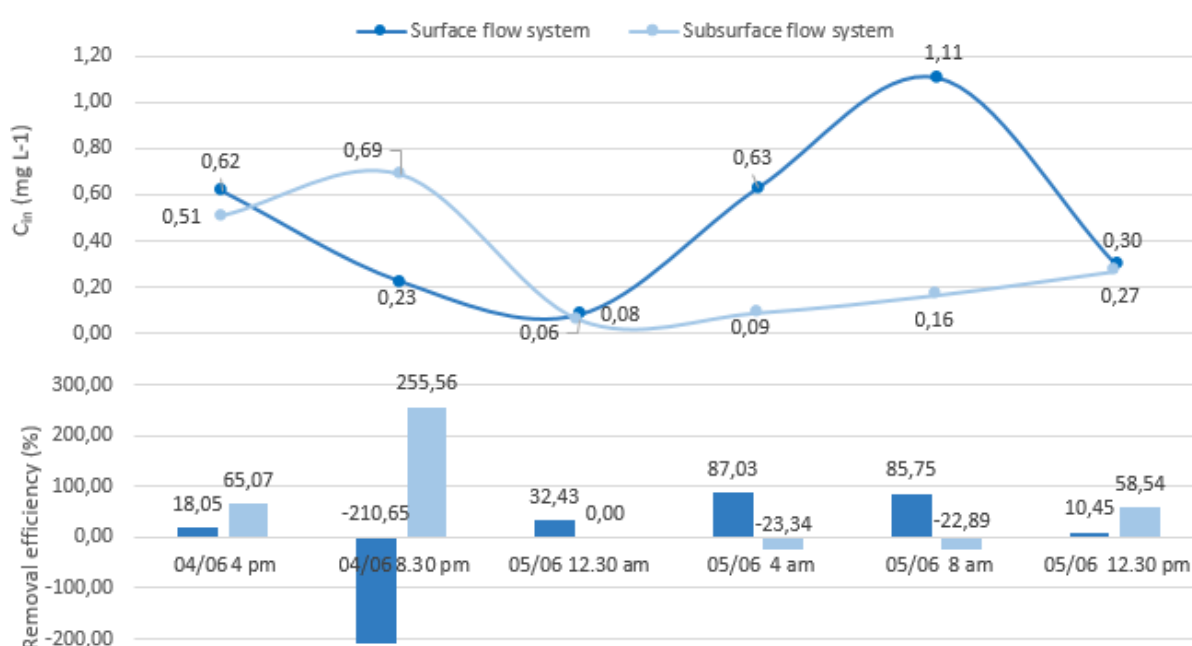


Figure 19: Comparison of TP efficiency for surface and subsurface flow systems (with incoming concentrations)

For the surface flow system, without the 04/06 8h30 pm value, the removal of TP is between 10.45 (minimum) and 87.03% (maximum) for incoming concentrations of 0.30 and 0.63 mg L⁻¹ respectively. The average is $46,74 \pm 33,14$ %. The subsurface flow system allows an elimination of $61,8 \pm 4,61$ % outside its discharge periods. During these two release periods (05/06 4 am and 05/06 8 am), it releases approximately $23.11 \pm 0.32\%$ of the incoming TP to the external environment.

Thus, since the surface flux does not seem to release a lot of TP (only for 04/06 8.3 pm value), which the subsurface flux system seems to do more frequently, we can think that it is the surface flow system that allows a better overall elimination of TP. Consequently, the surface flow system is thus mainly responsible for the good removal results of TP from the SSBS removal zone (see 4.1.2.2 Total Nitrogen Removal (TN) and Total Phosphorus Removal (TP)). Indeed, without the surface flow system and taking into account the discharge episodes of the groundwater flow system, the percentage efficiency of the SSBS biofiltration zone would be about 15% (without 04/06 8h30 pm) and extremely variable.

The fact that the subsurface flow system presents a variable elimination of pollutants with an alternation of elimination/relargage periods (probably linked to a clogging and unclogging mechanism as for TSS and VSS) tends to validate the hypothesis previously made in section 2.5

Objectives of the study. Indeed, the SSBS being several years old, we had previously formulated the hypothesis that the adsorption sites of the gravelly substrate were saturated and therefore inactive for the fixation of TP in order to look for a material allowing an effective sorption of TP. This hypothesis therefore seems to be validated.

Regarding to variables that may vary the rate of removal of TP, neither the initial incoming concentration nor the time of day appear to be these variables for the same reasons as those explained above for the removal of TN. However, in order to be able to assert this last absence of correlation, it would be more prudent to repeat the experiment with triplicates.

4.3 Correlation between nutrients and dissolved oxygen

In order to show a correlation or absence of correlation between the TN/TP concentration and the DO concentration, in the whole SBSS and in these two subdivided parts (the surface flow system and the groundwater flow system), non-parametric Spearman tests were carried out (95% confidence interval). The values of the r_s coefficients obtained were then compared with the critical values table (Appendice V) in order to validate or refute H_0 .

- If $r_{s_{obs}} < r_{s_{table}}$ -> Hypothesis H_0 is accepted: there is no correlation between TN/TP and DO
- If $r_{s_{obs}} > r_{s_{table}}$ -> Hypothesis H_1 is accepted: there is a correlation between TN/TP and DO

4.3.1 Total Nitrogen Removal (TN) in SSBS biofiltration zone, surface and subsurface flow systems

The table of Spearman's test results for the SSBS biofiltration zone and its two sub-parts is visible Table 5.

Table 5: Table of Spearman's test results for the three zone tested for TN and DO correlation investigation

	Sample size(n)	Observed r_s value (absolute value)	Value of r_s in table	Comparison	Conclusion
Biofiltration zone	29	0.75	0.312	$0.75 > 0.312$	Correlation
Surface flow system	18	0.84	0.401	$0.84 > 0.401$	Correlation
Subsurface flow system	12	0.42	0.503	$0.51 < 0.503$	No correlation

➤ SSBS biofiltration zone

According to the Spearman correlation coefficient $r_s = -0.75$, there is a negative linear correlation between the two variables tested: TN and DO. Thus, this means that overall, in the SSBS biofiltration zone, the higher the TN concentration, the lower the DO concentration.

In order to learn more about the role played by each subpart of the SSBS, the same was done for the surface flow system and the subsurface flow system.

➤ Surface flow and subsurface system

For the surface flow system (figure 20 a)) with $r_s = -0.84$, there is a correlation between TN and DO while for the subsurface flow system (figure 20 b)) with $r_s = -0.51$, there is no correlation (Figure 20).

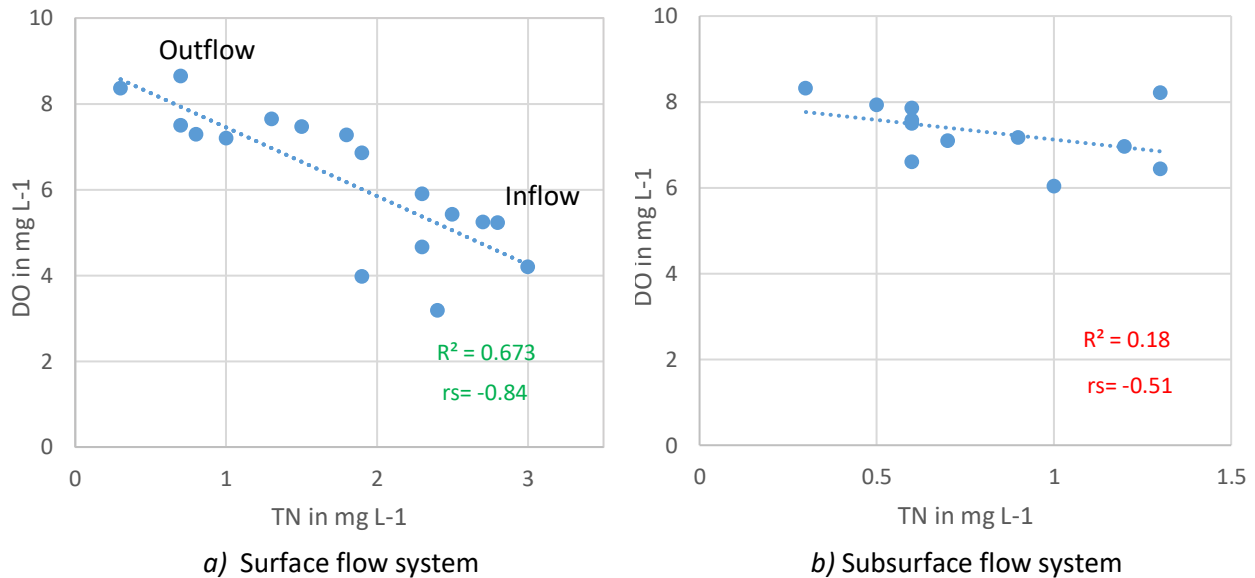


Figure 20: Linear regression between TN and DO for the two-subparts of the SSBS biofiltration zone

Thus, the dependence between these two variables for the surface flow system lies in the fact that the lower is the DO concentration, the higher is the TN concentration.

In addition to this information, the concentration of TN is gradually decreasing within the surface flow system (concentration gradient: inflow: high - outflow: lower).

Thus, we can assume that the DO is low and the TN high because at the entrance of the surface flow system the higher is the TN concentration and the more organisms consume O₂ to try to decompose it.

Progressively, the TN being decomposed, its concentration will decrease throughout the subsurface flow system with a decrease in the decomposition activity of these heterotrophic organisms; this results in a concentration of DO which rises progressively.

It would thus be processes of nitrification (transformation of ammonium into nitrates) and denitrification (transformation of nitrates into volatile nitrogen), both very expensive in oxygen consumption which would take place within the surface flow system. Indeed, nitrification consumes 4.3 mg of oxygen per g of oxidized ammonia (Vymazal 2006) and denitrification consumes 2.86 g of oxygen per gram of oxidized nitrate (Norton n.d).

Following this, we can therefore assume that the different forms of TN present in the water column in the presence of a sufficient concentration of oxygen are mainly nitrites and nitrates. It would therefore be interesting, in order to validate these assumptions and carry out an even more complete study, to evaluate the quantity of these two forms dissolved by ion chromatography (nitrates, nitrites and ammonium).

4.3.2 Phosphorus Removal (TP) in SSBS biofiltration zone, surface and subsurface flow systems

The table of Spearman's test results for the SSBS biofiltration zone and its two sub-parts is visible Table 6.

Table 6: Table of Spearman's test results for the three zone tested for TP and DO correlation investigation

	Sample size(n)	Observed rs value	Value of rs in table	Comparison	Conclusion
Biofiltration zone	30	0.13	0.306	$0.13 < 0.306$	No correlation
Surface flow system	18	0.11	0.401	$0.11 < 0.401$	No Correlation
Subsurface flow system	12	0.31	0.503	$0.31 < 0.503$	No correlation

There is no correlation (positive or negative) with $r_s = 0.13$ at the global scale of the SSBS biofiltration zone. This means that there is no link between the two TP and DO variables at the global scale of the SSBS biofiltration zone. Logically, there is also no correlation for the two sub-parties.

This means that the TP removal process throughout the SBSS biofiltration zone is not governed by oxygen consuming processes. For the surface flow (high TP elimination) and the subsurface flow (low TP elimination) presenting, this elimination is mainly achieved by biological mechanisms. Indeed, for the subsurface system, we have previously ruled the absence of proper functioning of the physical filtration mechanisms on the gravelly substrate for the removal of TP.

Thus, biological mechanisms possibly occurring within the SSBS would be assimilation by macrophytes and anaerobic microbial activity. These hypotheses would deserve other types of analyses to be verified or disproved.

4.4 Assessment of an abiotic process: Phosphorus removal capacity

4.4.1 LECA®

Figure 21 shows the maximum percentage P removal and P kinetic adsorption achieved by LECA.

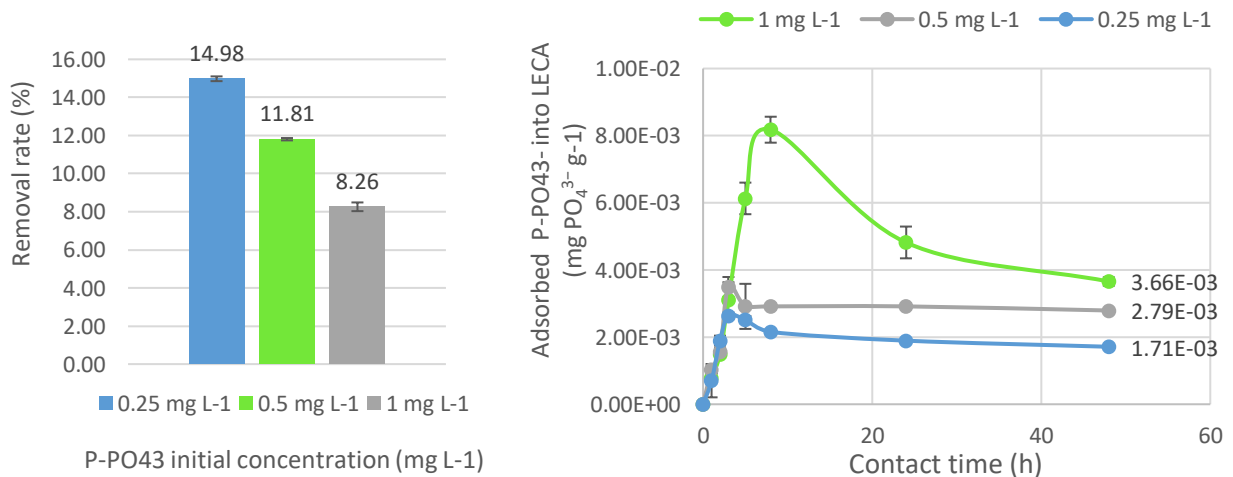


Figure 21: Phosphorus removal rate (%) and kinetic adsorption achieved by LECA ($\text{mg PO}_4^{3-} \text{g}^{-1}$)

The experiment was performed at a temperature between 22.2 (start)-22.9° (end) and pH between 6.57 (start) - 7.1 (end).

The elimination percentage (on the left) for a low concentration of 0.25 mg L⁻¹, medium 0.5 mg L⁻¹ and 1 mg L⁻¹ are respectively $14.98 \pm 0.25 \%$, $11, 81 \pm 0.12 \%$ and $8.26 \pm 0.22 \%$.

We notice that higher is the initial concentration, lower is the percentage of removal. It seems to make sense because for the same mass of adsorbent evaluated, higher is the P-PO_4^{3-} concentration solution and faster the adsorption sites on the material may become saturated.

If we study in more details the adsorption kinetic (on the right) of LECA for the different concentrations tested, we find generally similar phenomena for the low and medium concentrations (0.25 and 0.5 mg L⁻¹) and slightly different phenomenon for the maximum concentration of 1 mg L⁻¹ (figure 22).

Indeed in particular for the phenomenon of desorption one notes that this one arrives more quickly for 0.25 and 0.5 mg/L (with 3h) than for 1 mg L⁻¹ (with 8h). Same thing for maximum sorption (defined when an asymptotic value is reached), it is respectively set at 8h for 0.25 mg/L and 5h for 0.5 mg L⁻¹ while for 1 mg L⁻¹, it is assumed to be reached after 48h.

Finally, the most interesting is to present the results in mg P-PO_4^{3-} g⁻¹ LECA as is most frequently done in the research papers. Thus, for initial concentrations of 0.25 mg L⁻¹, 0.5 mg L⁻¹ and 1 mg L⁻¹, the maximum adsorption is respectively 1.71×10^{-3} mg P-PO_4^{3-} g⁻¹, 2.79×10^{-3} mg P-PO_4^{3-} g⁻¹ and finally 3.6×10^{-3} mg P-PO_4^{3-} g⁻¹ (figure 22).

If we compare these results with the data obtained by the literature, our results show a significant adsorption of phosphorus on LECA unlike Karczmarczyk and Bus in 2014 for work carried out under the same experimental conditions. However, these results are still 10 times lower than those obtained by Zhu and al. in 1997.

4.4.2 Dolomite

Figure 22 shows the maximum percentage P-PO_4^{3-} removals achieved by dolomite A and dolomite B.

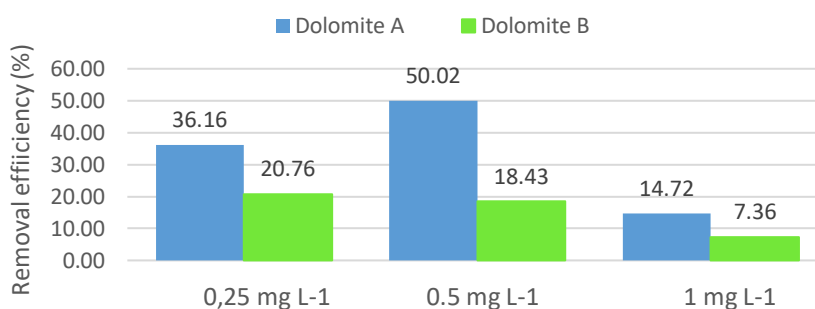


Figure 22: Maximum removal percentages achieved by dolomite A and dolomite B for different P concentrations tested

The experiment was performed at a temperature between 23.6 (end) and pH 8.16 (end) for dolomite A and 8.07 (end) for dolomite B.

Thus, the elimination percentage of dolomite A for a concentration of 0.25 mg L⁻¹, 0.5 mg L⁻¹ and 1 mg L⁻¹ is 36.16%, 50.02% and 14.72% respectively. For dolomite B, the results are lower: 20.76%, 18.43% and 7.36% respectively for the same concentrations tested.

We observe (Figure 22) that the maximum removal percentage of dolomite A is obtained for the middle concentration while for dolomite B, the maximum removal percentage is reached for the lowest

concentration, as for LECA. It would therefore appear that for dolomite A, the ideal phosphorus content for maximum phosphorus removal is 0.5 mg/L (commonly found in urban rivers).

If we study in more detail the adsorption kinetics of dolomite A and dolomite B for the different concentrations tested (Figure 24), we find generally similar adsorption mechanisms as a function of time. However, there is a slight difference.

Indeed, for dolomite A (high and medium concentration), at 5h, there is a desorption phenomenon until 8h, then at 8h a new adsorption phenomenon until 24h, then reached a balance at 48h. This phenomenon does not occur for dolomite B.

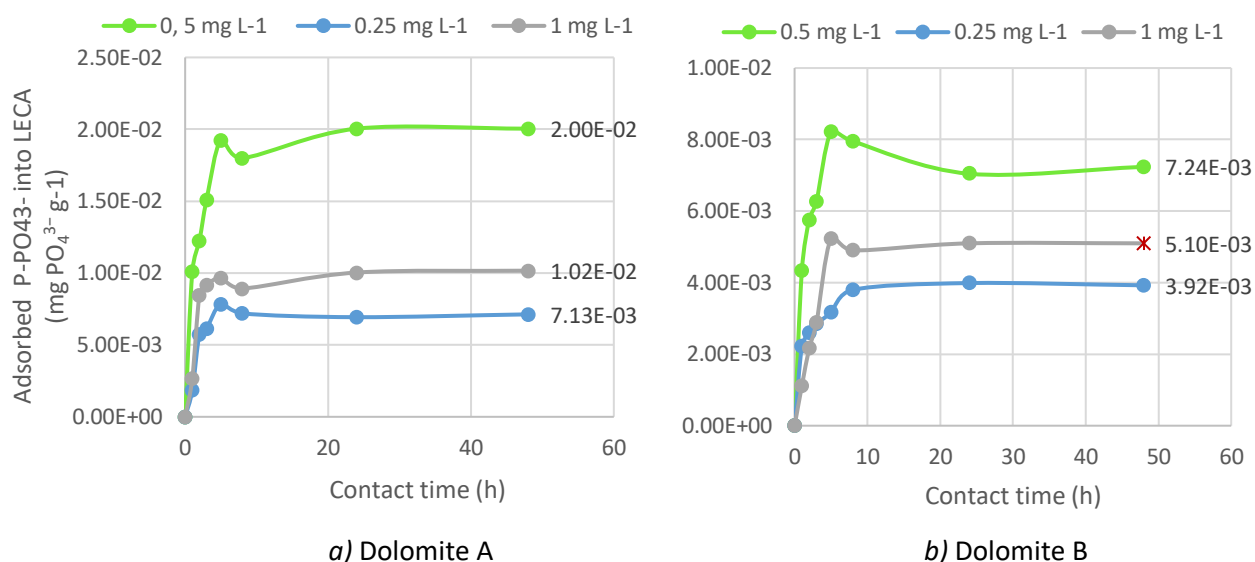


Figure 23: Kinetic adsorption of dolomite A and dolomite B as a function of initial PO_4^{3-} concentration

Thus, for initial concentrations of 0.25 mg L⁻¹, 0.5 mg L⁻¹ and 1 mg L⁻¹, the maximum adsorption of dolomite A is respectively 7.13×10^{-3} mg P- PO_4^{3-} g⁻¹, 2.00×10^{-2} mg P- PO_4^{3-} g⁻¹ and finally 1.02×10^{-2} mg P- PO_4^{3-} g⁻¹ (Figure 23).

For the same concentrations, the maximum adsorption of dolomite B is lower, respectively by 3.92×10^{-3} mg P- PO_4^{3-} g⁻¹, 7.24×10^{-3} mg P- PO_4^{3-} g⁻¹ and finally 5.10×10^{-3} mg P- PO_4^{3-} g⁻¹ (Figure 22).

Dolomite A therefore appears to have a maximum adsorption capacity almost three times greater than dolomite B for a concentration of 0.5 mg L⁻¹.

If we compare these results with the data obtained by the literature, our results for dolomite A are similar to those obtained by Karczmarczyk and Bus in 2014 (P sorption : 2×10^{-2} mg g⁻¹, material amount : 1g and a concentration P- PO_4^{3-} tested: 1.4 mg L⁻¹).

However, for dolomite B, the results are more similar to those obtained by Al-Anbari and al in 2008 (P sorption: 7×10^{-3} , material amount: 1g, concentration P- PO_4^{3-} tested 0.01 mg L⁻¹).

5. Conclusion and future work

In the first part, the complete diagnosis of the functioning of a biofiltration zone of a mature SSBS was carried out in order to better understand and evaluate the abiotic and biotic processes that can take place there. As this system is heterogeneous, the study focused on the entire SSBS biofiltration zone and on these two subparts: the surface flow system and the groundwater flow having a gravelly subsoil.

This diagnosis first made it possible to characterize the sediments in the biofiltration zone, very rich in organic matter, as black peat with a sapiric structure, then to evaluate the capacity of each part to eliminate nutrients and to better understand its process.

Thus, it can be concluded that the surface flow system is by far the most efficient for the removal of TN and TP with a removal capacity of $62.55 \pm 15.41\%$ and $46.74 \pm 33.14\%$ respectively. Indeed, the underground flow most certainly presents a fouling with episodes of elimination then episodes of release which makes it ineffective or very weakly effective concerning the elimination of TN and TP. It can even be assumed that fluctuations in TSS and VSS removal efficiency throughout the SSBS biofiltration zone are related to this plugging and unclogging process.

Furthermore, we did not identify any difference in the removal performance of the SSBS biofiltration zone and its two subsections over time (day - night) but we did identify a correlation between the TN concentration and the DO concentration in the surface runoff system.

In the second part, the batch experiment carried out classifies the three materials according to their maximum synthetic phosphorus adsorption capacity: dolomite A > dolomite B > LECA®. Indeed, dolomite A presents an adsorption evaluated as being almost three times more important than dolomite B and six times more important than LECA®.

These results are promising and with their addition to the Freundlich theoretical model (heterogeneous substrate), they could lead to the publication of future scientific articles. Indeed, if our experimental points fit the theoretical model, it could be very interesting because it would then be possible to determine theoretically the sorption of the substrate according only to a known phosphorus concentration.

Moreover, the easy and inexpensive evaluation technique used in this study has already shown its limitations. Its main disadvantage is that it overestimates the amount of phosphorus removed (Chazarenc and al. 2008). Thus, to obtain more reliable results, these materials could be tested with laboratory tests in columns or on a pilot scale.

Presented subject, was in my opinion, very interesting and rich in knowledge. I discovered the concept of ecohydrology and I learned how to use the abiotic and biotic processes to enhance the resilience and carrying capacity of the Nature-Based Solutions. I was able to apply multidisciplinary knowledge such as ecology (SSBS species recognition), chemistry (SSBS water analysis) and mathematics (selection of the appropriate statistical test).

The major objective of this internship was to learn how to work independently on this scientific project. To plan the project, I had to read many scientific articles in order to select information that could be useful to its realization. Then together with my tutor, we have planned the precise schedule for the project. Due to time constraints, not all of the experiments were conducted.

Finally, this first work experience abroad not only enriched my spoken and scientific English, but also gave me a strong insight into the professional work of environmental researcher. After the end of this internship, I am convinced of my attraction for scientific research, so I will looking for this aspect when I will apply for future work, perhaps working as a consulting engineer.

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APPENDICE I: Presentation of the host organization

The European Regional Centre for Ecohydrology (ERCE) under the auspices of UNESCO emerged from the International Centre for Ecology (ICE) in 2008 of the Polish Academy of Sciences. Indeed, since an agreement signed on the 8th of March 2006 in the UNESCO Headquarters in Paris, by the Director General of UNESCO, Koichiro Matsuura, and the Minister of Science and High Education of Poland, the ERCE centre was created in Lodz, in Poland (erce.unesco.lodz.pl).



The European Regional Centre for Ecohydrology
Source: <http://www.erce.unesco.lodz.pl/>

The centre has grown out of the ICE which was based Ecological Bioenergetics, developed in 1970 through the International Programme (IBP). One of the purpose of this programme was to provide fundamentals for dialogue between ecologist and hydrologist in the 1990s, and this, gradually led to the creation of the new ecohydrology paradigm by Professor Zalewski and these collaborators.

The international and national numerous research activities of the centre focused on interactions between hydrological, biogeochemical and ecological processes at the catchment scale for the purpose of water quality improvement, biodiversity enhancement and sustainable development by restoration or protection approaches.

Thus, the scientific profile of the Centre is focused on the development of ecohydrological science with the use of ecosystem properties as a tool for water management and restoration, and its implementation for restoring freshwater resources in the framework of the UNESCO International Hydrological Programme (alter-net.info/ERCE-UNESCO). One of the main priorities of the centre is to create tools for the implementation of the EU Water Framework Directive in the framework of Poland's national cooperation. The centre works on many projects:

- ❖ Three LIFE+ projects: “**EKOROB**: Ecotones for reducing diffusion pollution”, “**ENVEUROPE**: Environmental quality and pressures assessment across Europe: the LTER network as an integrated and shared system for ecosystem monitoring”, “**EH-REK**: Ecohydrologic rehabilitation of Arturowek recreational reservoirs (in Lodz) as a model approach to rehabilitation of urban reservoirs”
- ❖ Two IAP projects: “Towards Engineering Harmony Between Water, Ecosystem and Society. Strengthening the Collaboration between European Academies of Science in the IAP Water Programme” and “Water, Ecosystem Services and Society - establishing the Collaboration between European Academies of Science under the IAP Water Programme and European Long-Term Ecosystem Research Network”
- ❖ An European project: “EXPEER: Distributed Infrastructure for EXPERimentation in Ecosystem Research” and other national projects.

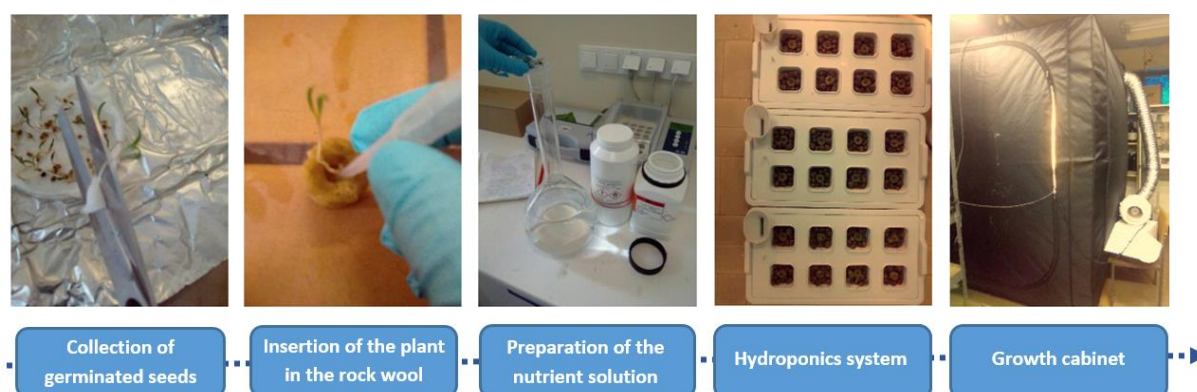
Consequently, Ecohydrology research therefore includes research sub-disciplines such as: hydrobiology, river ecology, fish ecology, environmental chemistry, landscape processes, socio-ecological systems, soil ecology, bioindication, phytotechnology (including remediation) and ecotoxicology



Illustration of some books published by the ERCE

APPENDICE II: Conditions for germination and cultivation of spinach

The different steps are summarized below:



For more details:

➤ Germination conditions

The germination of spinach seeds Matador, *Spinacia oleracea* lasted 8 days at a temperature of $25 \pm 0.5^\circ\text{C}$ or a duration comprised between 7 -14 days mentioned by www.seedsavers.org. Although the study by Katzman et al, 2001, aimed at improving the yield of hydroponic systems, recommends for an inhibitory growth temperature ($>20^\circ\text{C}$), a pre-treatment which consists of soaking the seeds for 4 hours in 0.5% NaOCl then for 15 hours in water and finally in 0.3% H₂O₂ in order to better stimulate germination by weakening the tissues covering the seed and thus improving gas exchanges between the seed and the outside; the pre-treatment of the seeds was simply carried out in our study by soaking the seeds for 6 hours in tap water.

After this pre-treatment, about 40 seeds were placed on a damp tissue on a Petri dish, and arranged so that they touched each other as little as possible. On the boxes, a second moistened tissue was placed over the seeds and the lid placed on top to maintain moisture. The beginning of the germination was carried out in the darkness to avoid that the roots, if they are dried, are weakened. As a result, all the petri dishes were wrapped in aluminium foil before being placed at 25°C .

Re-wetting of the tissues with 7ml of water was performed every 72 hours using a pipette. Then, after 5 days, when the radicle of the seed appears, the upper tissue is removed and the germinated seeds are placed under artificial light (unknown brand, 3000-4000 lumens). However, it should be noted that for another experiment of a researcher in the laboratory, the lamp could not be used and the seeds were placed for 48 hours in natural light.

➤ Growing conditions

Each Växer Hydroponic System (IKEA) has a size of 41×22 cm and 16 cultivation insert set that can be grown. Thus, the 24 sets (spinach shoots + rock wool "plug") were then deposited in 3 Växer hydroponic systems.

In each cultivation insert set, after placing the spinach shoot and its rockwool support, LECA®, also commonly used in hydroponics, was added as substrate (beads-clay-expandee). Although some sites recommend soaking LECA® in water with a basic pH, as was done for rockwool stoppers, LECA® was simply rinsed with tap water and kept dust free beforehand and incorporated into the system.

The same day, the hydroponic systems were then placed in a mountable growth chamber with an ambient temperature of about 26°C (Proietti 2013). Spinach being part of the Long Day Plants (LDP), this means that their growth requires at least 12 hours of light/day.

Thus, although some studies (Proietti 2013) used a 10 h light/14 h dark photoperiod for the growth of this same variety of spinach, a 16 h light/8 h dark photoperiod was applied in accordance with the instructions for use. Hence, a Phytolite High Pressure Sodium (HPS) Grow-Bloom Extreme full cycle 600W lamp with a luminous flux of 90000 lumens and a ventilation of 420 m³/h by the primaklima fan were applied to the crop.

➤ Composition of nutrient solutions

The three different nutrient solutions tested are listed in the following table:

Nutrient solution information	Author	Composition	Results
Homemade (pending delivery of the chemicals)		340 mg KH ₂ PO ₄ ³⁻ and 350 mg NH ₄ NO ₃ ³⁻ in 1.7 L of tap water to obtain a solution at 57.5 mg L ⁻¹ K and 36.25 mg/L nitrogen.	✓
Enshi (Full nutrient solution)	(Asaduzzaman and al., 2013) for hydroponic culture using perlite as substrate	1000 L-1 of tap water; 250 g of MgSO ₄ -7H ₂ O; 155 g of NH ₄ H ₂ PO ₄ ; 2 g of ZnSO ₄ -7H ₂ O; 0.05 g of CuSO ₄ -5H ₂ O; 0.02 g of NaMoO ₄ ; and 25 g of NaFe-EDTA	✗
Spinach specific	Used to grow Spinacia Oleracea in hydropony (SimonaProietti and al.,2013)	Full-strength nutrient solution containing: 5 mM NH ₄ NO ₃ , 4 mM CaCl ₂ , 1 mM K ₂ SO ₄ , 1.3 mM NaH ₂ PO ₄ · H ₂ O, 1.5 mM MgSO ₄ · H ₂ O and 2 µM MnSO ₄ , ZnSO ₄ , CuSO ₄ , 1 µM NaMoO ₄ , 100 µM H ₃ BO ₃ , 0.4 µM Co(NO ₃), 200 µM NaCl and 1 µM EDTA	✓

Approximately 1.1 to 1.4 L of these solutions were inserted into the system using the funnel provided, so that the water gauge indicates that the level of the solution is below the maximum line. The solutions were changed every week.

After the application of the full 25% Enshi solution (Asaduzzaman and al., 2013), the spinach leaves began to wilt and yellow:



APPENDICE III: Ascorbic acid method for the total phosphorus (TP) determination

Background:

This technique comes from the standard text for water analysis created in 1905: "Standards Methods for the Examination of Water and Wastewater" (Eaton and al, 2015). It contains about hundred procedures to analyze river water, wastewater and other parameters related to aquatic environments. This paper describes three possible ways to analyze TP (Total Phosphorus): the vanadomolybdophosphoric method, the stannous chloride method and finally, the ascorbic acid method. The techniques most commonly used is the ascorbic acid method (Doolittle, 2014).

Principle of the method:

The principle of the method lies in the fact that ammonium molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ and antimony potassium tartrate $\text{K}_2\text{Sb}_2(\text{C}_8\text{H}_4\text{O}_{12}) \cdot 3\text{H}_2\text{O}$ react in an acid medium with dilute solutions of orthophosphate to form a blue- colored antimony-phospho-molybdate complex. The absorbance of the complex is measured at $\lambda = 690 \text{ nm}$

Reagents preparation:



A, B, C and D reagents

(A) *Sulfuric acid*: Add 70 ml of concentrated sulfuric acid to approximate 400 ml H_2O slowly. Cool and make up to 500 ml with water.

(B) *Antimony potassium tartrate*: Dissolve 0.2743 g of antimony potassium tartrate in 100 ml H_2O . Store in the fridge.

(C) *Ammonium molybdate*: Dissolve 10g ammonium molybdate in 250 ml H_2O . Store in the fridge.

(D) *Ascorbic acid*: Dissolve 4.4 g ascorbic acid in 250 ml H_2O . Store in the fridge.

Procedure:

Mineralization:

- Measure 10 ml of samples (not filtrated) into the digestion vessel for TP
- Add 2 tea-spoons from the chemical Oxisolv (or 2.5 if the sample seems heavily polluted)
- Dispose the digestion vessel in the microwave and mineralize for 60 seconds (button P2 and Start)
- Cooling 3 minutes in cool water, then, shake the container and open the mineralization unit
- The sample should be clear and colorless. If it is not-repeat the mineralization by adding 2 teaspoons from the chemical Oxisolv.

Fresh reaction mixture:

For each series of analyses, prepare a fresh reaction mixture=20 parts

- 10 parts of sulfuric acid (A),
- 1 part of antimony potassium tartrate (B); mix
- 3 parts ammonium molybdate (C); mix
- 6 parts ascorbic acid solution (D); mix

Measure out 5 ml of the sample + 5 ml of deionised water and 10 ml deionised water for the control (C)

Add 1.75 ml reaction mixture, mix

After 15 min, read extinction in 5 cm cuvettes at $\lambda = 690\text{nm}$. The results in $\mu\text{g/L}$ should be read from the calibration curve.

APPENDICE III: Realization of the calibration curve

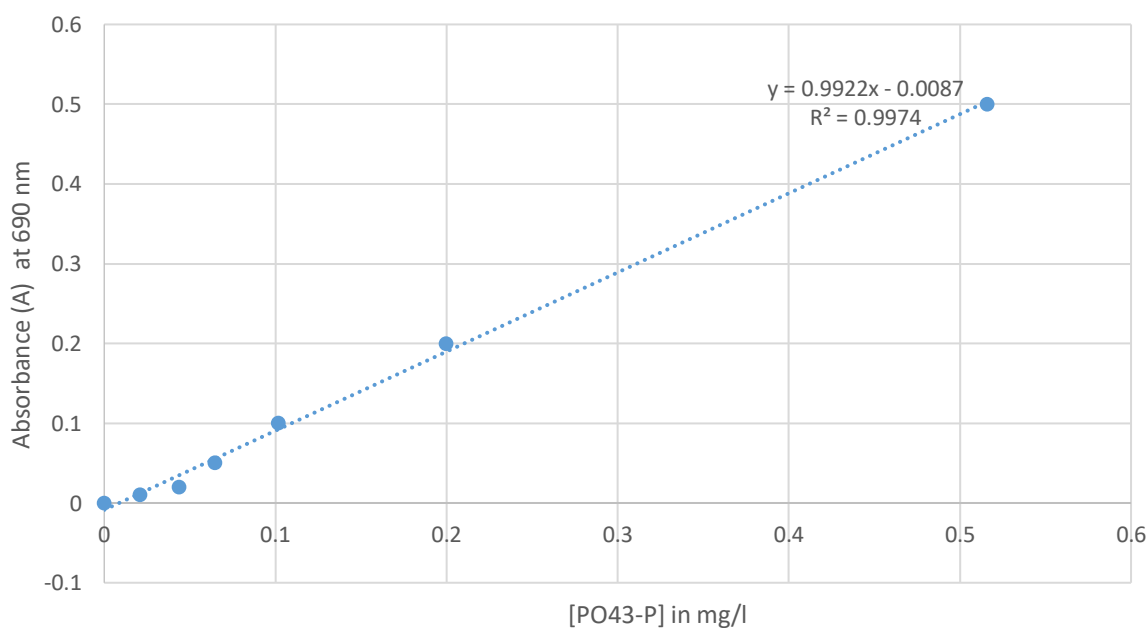
Preparation of a phosphorus stock solution (1000 mg P/L): Dissolve 2.197g anhydrous KH_2PO_4 and dilute to 500ml in a volumetric flask. Thus 1 ml = 1 mg $\text{PO}_4^{3-}\text{-P}$.

Preparation of a phosphorus intermediate solution (10mg/L): After stirring the phosphorus stock solution, take 5 ml and dilute in 500 ml. Thus 1ml = 0.010mg $\text{PO}_4^{3-}\text{-P}$.

Preparation of calibration standards for Total Phosphorus analysis

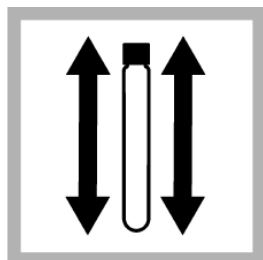
Solution	ml intermediate solution	Final ml	Concentration (mg $\text{PO}_4^{3-}\text{-P/L}$)
TP-1	0.10	100	0.010 mg $\text{PO}_4^{3-}\text{-P/L}$
TP-2	0.20	100	0.020 mg $\text{PO}_4^{3-}\text{-P/L}$
TP-3	0.50	100	0.050 mg $\text{PO}_4^{3-}\text{-P/L}$
TP-4	1.00	100	0.100 mg $\text{PO}_4^{3-}\text{-P/L}$
TP-5	2.00	100	0.200 mg $\text{PO}_4^{3-}\text{-P/L}$
TP-6	5.00	100	0.500 mg $\text{PO}_4^{3-}\text{-P/L}$

From this series of analyses, the following calibration curve can be made:



APPENDICE IV: Persulfate oxidation method for the total nitrogen (TN) determination

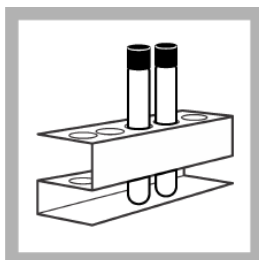
Different steps of method 10072:



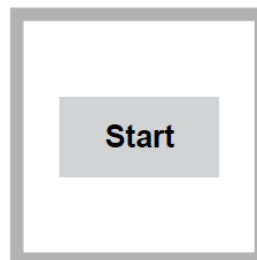
5. Put the caps on both vials. Shake vigorously for at least 30 seconds to mix. Undissolved powder will not affect the accuracy of the test.



6. Put the vials in the reactor and close the lid. Leave the vials in the reactor for exactly 30 minutes.

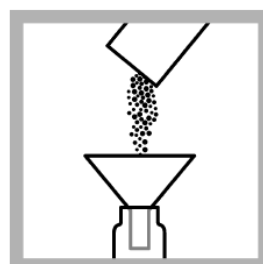


7. At 30 minutes, use finger cots to immediately remove the vials from the reactor. Let the vials cool to room temperature.

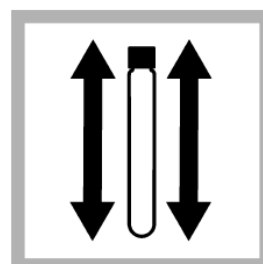


8. Start program **394 N, Total HR TNT**. For information about sample cells, adapters or light shields, refer to [Instrument-specific information](#) on page 1.

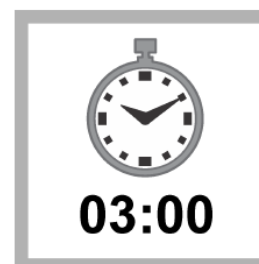
Note: Although the program name can be different between instruments, the program number does not change.



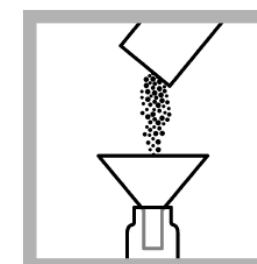
9. Add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.



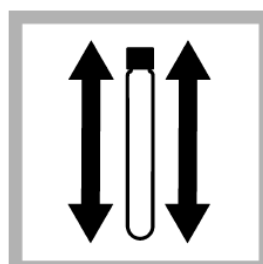
10. Put the caps on both vials. Shake for 30 seconds.



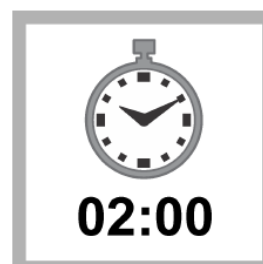
11. Start the instrument timer. A 3-minute reaction time starts.



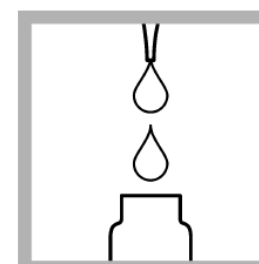
12. After the timer expires, remove the caps from the vials. Add one TN Reagent B Powder Pillow to each vial.



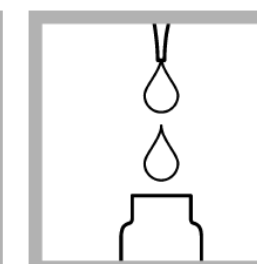
13. Put the caps on both vials. Shake vigorously for 15 seconds to mix. The reagent will not dissolve completely. Undissolved powder will not affect the accuracy of the test. The solution will start to turn yellow.



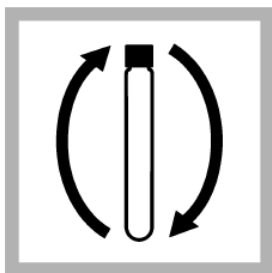
14. Start the instrument timer. A 2-minute reaction time starts.



15. Prepared sample: When the timer expires, use a pipet to put 2 mL of the digested, treated prepared **sample** into one TN Reagent C vial.



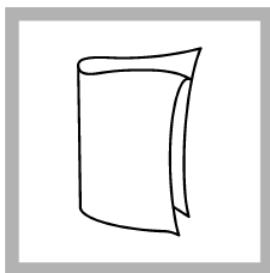
16. Blank: When the timer expires, use a pipet to put 2 mL of the digested, treated **blank** into the second TN Reagent C vial.



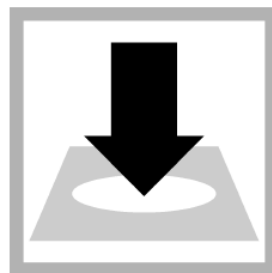
17. Put the caps on both vials. Invert 10 times to mix. Use slow, deliberation inversions for complete recovery. The vials will be warm to the touch.



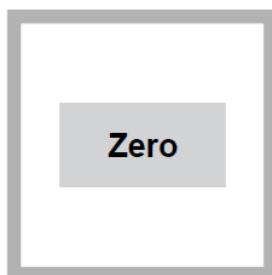
18. Start the instrument timer. A 5-minute, reaction time starts. The yellow color will intensify.



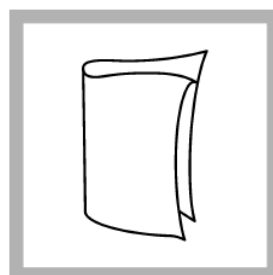
19. When the timer expires, clean the blank vial.



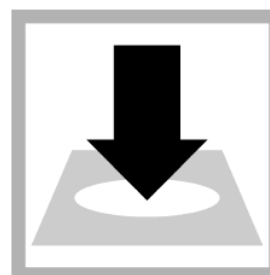
20. Insert the blank vial into the 16-mm cell holder.



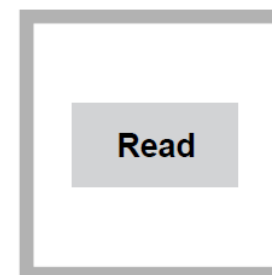
21. Push **ZERO**. The display shows 0 mg/L N.



22. Clean the sample vial.



23. Insert the sample vial into the 16-mm cell holder.



24. Push **READ**. Results show in mg/L N.

APPENDICE V: Critical values of Spearman's ranked correlation coefficient (r_s)

$\alpha(2):$	0.50	0.20	0.10	0.05	0.02	0.01	0.005	0.002	0.001
$\alpha(1):$	0.25	0.10	0.05	0.025	0.01	0.005	0.0025	0.001	0.0005
n									
4	0.600	1.000	1.000						
5	0.500	0.800	0.900	1.000	1.000				
6	0.371	0.657	0.829	0.886	0.943	1.000	1.000		
7	0.321	0.571	0.714	0.786	0.893	0.929	0.964	1.000	1.000
8	0.310	0.524	0.643	0.738	0.833	0.881	0.905	0.952	0.976
9	0.267	0.483	0.600	0.700	0.783	0.833	0.867	0.917	0.933
10	0.248	0.455	0.564	0.648	0.745	0.794	0.830	0.879	0.903
11	0.236	0.427	0.536	0.618	0.709	0.755	0.800	0.845	0.873
12	0.217	0.406	0.503	0.587	0.678	0.727	0.769	0.818	0.846
13	0.209	0.385	0.484	0.560	0.648	0.703	0.747	0.791	0.824
14	0.200	0.367	0.464	0.538	0.626	0.679	0.723	0.771	0.802
15	0.189	0.354	0.446	0.521	0.604	0.654	0.700	0.750	0.779
16	0.182	0.341	0.429	0.503	0.582	0.635	0.679	0.729	0.762
17	0.176	0.328	0.414	0.485	0.566	0.615	0.662	0.713	0.748
18	0.170	0.317	0.401	0.472	0.550	0.600	0.643	0.695	0.728
19	0.165	0.309	0.391	0.460	0.535	0.584	0.628	0.677	0.712
20	0.161	0.299	0.380	0.447	0.520	0.570	0.612	0.662	0.696
21	0.156	0.292	0.370	0.435	0.508	0.556	0.599	0.648	0.681
22	0.152	0.284	0.361	0.425	0.496	0.544	0.586	0.634	0.667
23	0.148	0.278	0.353	0.415	0.486	0.532	0.573	0.622	0.654
24	0.144	0.271	0.344	0.406	0.476	0.521	0.562	0.610	0.642
25	0.142	0.265	0.337	0.398	0.466	0.511	0.551	0.598	0.630
26	0.138	0.259	0.331	0.390	0.457	0.501	0.541	0.587	0.619
27	0.136	0.255	0.324	0.382	0.448	0.491	0.531	0.577	0.608
28	0.133	0.250	0.317	0.375	0.440	0.483	0.522	0.567	0.598
29	0.130	0.245	0.312	0.368	0.433	0.475	0.513	0.558	0.589
30	0.128	0.240	0.306	0.362	0.425	0.467	0.504	0.549	0.580
31	0.126	0.236	0.301	0.356	0.418	0.459	0.496	0.541	0.571
32	0.124	0.232	0.296	0.350	0.412	0.452	0.489	0.533	0.563
33	0.121	0.229	0.291	0.345	0.405	0.446	0.482	0.525	0.554
34	0.120	0.225	0.287	0.340	0.399	0.439	0.475	0.517	0.547
35	0.118	0.222	0.283	0.335	0.394	0.433	0.468	0.510	0.539
36	0.116	0.219	0.279	0.330	0.388	0.427	0.462	0.504	0.533
37	0.114	0.216	0.275	0.325	0.383	0.421	0.456	0.497	0.526
38	0.113	0.212	0.271	0.321	0.378	0.415	0.450	0.491	0.519
39	0.111	0.210	0.267	0.317	0.373	0.410	0.444	0.485	0.513
40	0.110	0.207	0.264	0.313	0.368	0.405	0.439	0.479	0.507
41	0.108	0.204	0.261	0.309	0.364	0.400	0.433	0.473	0.501
42	0.107	0.202	0.257	0.305	0.359	0.395	0.428	0.468	0.495
43	0.105	0.199	0.254	0.301	0.355	0.391	0.423	0.463	0.490
44	0.104	0.197	0.251	0.298	0.351	0.386	0.419	0.458	0.484
45	0.103	0.194	0.248	0.294	0.347	0.382	0.414	0.453	0.479
46	0.102	0.192	0.246	0.291	0.343	0.378	0.410	0.448	0.474
47	0.101	0.190	0.243	0.288	0.340	0.374	0.405	0.443	0.469
48	0.100	0.188	0.240	0.285	0.336	0.370	0.401	0.439	0.465
49	0.098	0.186	0.238	0.282	0.333	0.366	0.397	0.434	0.460
50	0.097	0.184	0.235	0.279	0.329	0.363	0.393	0.430	0.456

APPENDICE VI: Table of raw TSS and VSS removal efficiency data

code	weight of filtering paper [g]	volume of water [dm ³]	105°C	500°C	total contain [g/dm ³]	mineral forms	organic forms
04/06 4 pm St 1	0,09000	0,505	0,15600	0,12060	0,130693	0,0606	0,0701
04/06 4 pm St 5	0,09000	0,72	0,10350	0,09420	0,01875	0,0058	0,0129
04/06 8.30 pm St 1	0,09000	0,71	0,15060	0,11840	0,085352	0,0400	0,0454
04/06 8.30 pm St 1	0,09000	0,68	0,11580	0,10150	0,037941	0,0169	0,0210
05/06 12.30 am St 1	0,09000	0,54	0,16540	0,12280	0,13963	0,0607	0,0789
05/06 12.30 am St 5	0,09000	0,55	0,10150	0,09480	0,020909	0,0087	0,0122
05/06 4 am St 1	0,09000	0,49	0,11490	0,10080	0,050816	0,0220	0,0288
05/06 4 am St 5	0,09000	0,75	0,10660	0,09730	0,022133	0,009733	0,0124
05/06 8 am St 1	0,09000	0,35	0,11530	0,10020	0,072286	0,029143	0,043143
05/06 8 am St 5	0,09000	0,55	0,10680	0,09640	0,030545	0,011636	0,018909
05/06 12.30 pm St 1	0,09000	0,6	0,14270	0,11400	0,087833	0,04	0,047833
05/06 12.30 pm St 5	0,09000	0,4	0,09590	0,09040	0,01475	0,001	0,0138
05/06 4 pm St1	0,09000	0,5	0,13590	0,10936	0,0918	0,03872	0,0531
05/6 4 pm St 5	0,09000	0,49	0,09700	0,09200	0,014286	0,0041	0,0102



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2017-2018

Optimization of the Sequential Sedimentation-Biofiltration System (SSBS) with application of sorptive materials

Constructed wetlands (CWs) can be defined as engineering systems that use natural wetland ecology to remove impurities from sewage or rivers. Water treatment in these systems is achieved through a combination of complex processes: sedimentation of suspended matter; physical trapping by plant roots and rocks, biological oxidation of organic matter and ion exchange/adsorption with litter and organic sediments. Thus, one way to improve phosphorus reduction capacity in the wetland is to use more efficient filter materials than conventional materials generally used in the construction of constructed wetlands (sand, gravel).

Consequently, in this study, a complete diagnosis of the different biotic and abiotic processes taking place in the biofiltration zone of an old Sequential Sedimentation-Biofiltration System (SSBS) will be realized. In addition, three reactive materials will be tested in a batch sorption experiment: a modified material which is LECA® and two natural materials which are different types of dolomite. Finally, all this work therefore represents a major interest for research on improving the operational efficiency of constructed wetlands.

Keywords: constructed wetlands – SSBS - biofiltration zone – surface flow – subsurface flow – removal mechanisms – phosphorus sorption – LECA® – dolomite

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